(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 May 2023 (11.05.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/078273 A1

(51) International Patent Classification:
C07K 16/00 (2006.01) *A61K 47/68* (2017.01)
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/CN2022/129122

(22) International Filing Date:
02 November 2022 (02.11.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/CN2021/128453
03 November 2021 (03.11.2021) CN
PCT/CN2022/123901
08 October 2022 (08.10.2022) CN

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report (Art. 21(3))*

WO 2023/078273 A1

(54) **Title:** SPECIFIC CONJUGATION FOR AN ANTIBODY-DRUG CONJUGATE

(57) **Abstract:** Provided herein is a process for preparing a homogeneous conjugate of an antibody or antibody-like protein via linkage of cysteine sites between heavy-light chains in the IgG antibody or antibody-like protein. Provided herein also are methods of making the conjugates in a specific manner comprising either generation of specific thiols of an antibody or antibody-like protein agent, followed by reaction with drug/linker complexes, or generation of specific thiols of an antibody or antibody-like protein agent and conjugation of a synthetic linker-drug assembly with the thiols simultaneously in one pot reaction, to provide conjugates with over 75%, in most cases more than 80%of payloads linked at the specific cysteine sites between heavy-light chains of the IgG antibody or antibody-like protein. It also relates to methods of using the homogeneous conjugate in targeted prophylaxis or treatment of cancer, infection and immunological disorders.

SPECIFIC CONJUGATION FOR AN ANTIBODY-DRUG CONJUGATE

DESCRIPTION

INFORMATION OF PRIORITY

The present application claims the benefit of PCT/CN2021/128453 (WO/2022/078524), filed on November 3rd, 2021, which is incorporated herein by reference entirely. This application is also claims the benefit of PCT/CN2022/123901, filed on October 8, 2022, which is incorporated herein by reference entirely. Thus, the present application claims the benefit of both PCT/CN2021/128453 and PCT/CN2022/123901.

FIELD OF THE INVENTION

The present invention relates to a process for preparing a homogeneous conjugate of an antibody via linkage of certain sulphurs of cysteine sites in the antibody. The present invention also relates to methods of making the conjugates in a specific manner comprising either generation of specific thiols of an antibody or antibody-like protein agent, followed by reaction with drug/linker complexes, or generation of specific thiols of an antibody or antibody-like protein agent and conjugation of a synthetic linker-drug assembly to the protein molecule simultaneously in one pot reaction. It also relates to methods of using the homogeneous conjugate in targeted prophylaxis or treatment of cancer, infection and immunological disorders.

BACKGROUND OF THE INVENTION

Nowadays, the pace of ADC development is accelerating, with the number of the clinical trials having more than tripled over the past 5 years and 9 of 14 marketed ADCs were approved by US FDA in last 3 years, underscoring the enthusiasm for this transformative approach to cancer treatment. The conjugation of payload and antibody via a linker is a critical aspect that defines ADC quality, safety, efficacy and the overall success (M. Acchione, H. Kwon, et al, 2012, mAbs 4:3, 362-372; M. J. Birrer, K. N. Moore, et al, 2019, J. National Cancer Inst., 111(6), 538-549). A report published by US FDA indicated that for most ADCs currently in clinical development, dose-limiting toxicities (DLT) appear to be unrelated to the targeted antigen, but driven by the payload/linker complexes (H. Saber and J. K. Leighton, Regulatory Toxicology and Pharmacology 71 (2015) 444–452); It also known that ADC linkers and the conjugation sites play critical roles in an ADCs' stability during preparation and storage, as well extent of systemic toxicity in the blood circulation in vivo (J. R. McCombs and S. C. Owen, 2015, AAPS Journal 17, 339–351).

Thus research and development into ADC chemistry and design are now expanding the scopes of the linker-payload compartments and conjugate chemistry to address the challenge of dose-limiting toxicities (DLT) of ADCs toward target diseases (Lambert, J. M. 2016, Ther. Deliv. 7, 279-82; Zhao, R. Y. et al, 2011, J. Med. Chem. 54, 3606-23). Nowadays, one of the extensive R&D effort for broader therapeutic windows (TW) of ADCs is to establish novel reliable methods for site-specific ADC conjugation, which seem to have longer circulation half-life, higher efficacy, potentially decreased off-target toxicity, and a narrow range of in vivo pharmacokinetic (PK) properties of ADCs as well as better batch-to-batch consistency in ADC production (Hussain, A. F., et al, Pharmaceuticals (Basel), 2021, 14(4), 343; Sadiki, A., et al, Antib Ther. 2020, 3(4), 271-284; Wolska-Washer, A.; Robak, T., Drug Saf, 2019, 42(2), 295-314; Tsuchikama, K., An, Z., Protein Cell. 2018, 9(1), 33-46; Thomas, A. et al, Lancet Oncol. 2016, 17(6), e254-e262; Strop, P., et al 2013 Chem. Biol. 20, 161−67; Wakankar, A. mAbs, 2011, 3, 161–172; Zhao, R. Y. et al, 2011, J. Med. Chem. 54, 3606-23).

There are several approaches developed in recent years for the site selective ADC preparation (Panowski, S, 2014, mAbs 6, 34). They include incorporation of unpaired cysteines, e.g. engineered reactive cysteine residues, called THIOMAB from Genentech (Junutula, J. R., et al 2010 Clin. Cancer Res. 16, 4769; Junutula, J. R., et al 2008 Nat Biotechnol. 26, 925-32; US Patents 8, 309, 300; 7, 855, 275; 7, 521, 541; 7, 723, 485, WO2008/141044), genetically introduced glutamine tag with Streptoverticillium mobaraense transglutaminase (mTG) (Strop, P., Bioconjugate Chem., 2014, 25, 855–862; Strop, P., et al., 2013, Chem. Biol. 20, 161–167; US Patent 8, 871, 908 for Rinat-Pfizer) or with Microbial transglutaminase (MTGase) (Dennler, P., et al, 2014, Bioconjug. Chem. 25, 569–578. US pat appl 20130189287 for Innate Pharma; US Pat 7, 893, 019 for Bio-Ker S.r.l. (IT)), incorporation of thiolfucose (Okeley, N. M., et al 2013 Bioconjugate Chem. 24, 1650), incorporation of unnatural amino acids through mutagenesis (Axup, J.Y., et al., 2012, Proc. Natl. Acad. Sci. 109, 16101–16106; Zimmerman, E.S., et al., 2014, Bioconjug. Chem. 25, 351–361; Wu, P., et al, 2009 Proc. Natl. Acad. Sci. 106, 3000-5; Rabuka, D., et al, 2012 Nat. Protoc. 7, 1052−67; US Pattent 8, 778, 631, and US Pat Appl. 20100184135, WO2010/081110 for Sutro Biopharma; WO2006/069246, 2007/059312, US Patents 7, 332, 571, 7, 696, 312, and 7, 638, 299 for Ambrx; WO2007/130453, US patents 7, 632, 492 and 7, 829, 659 for Allozyne), incorporation of selenocysteine into antibodies (Hofer, T., et al 2009, Biochemistry 48, 12047–12057; US Patent 8, 916, 159 for US National Cancer Institute), Conversion of cysteines located in the CXPXR consensus sequence to formylglycine (FGly) with formylglycine generating enzyme (FGE) (Drake, P.M., et al., 2014, Bioconjug. Chem. 25, 1331–1341; Carrico, I. S. et al 7, 985, 783; 8, 097, 701; 8, 349, 910, and US Pat Appl 20140141025, 20100210543 for Redwood Bioscience); via glycoengineeringly

introduction of sialic acid with the use of galactosyl- and sialytransferases (Zhou, Q., et al 2014, Bioconjug.Chem., 25, 510-520, US9,580,511/US Pat Appl 20140294867 for Sanofi-Genzyme); the incorporation of a cyclopropene derivative of lysine, followed by Diels-Alder cycloaddition with tetrazine derivatives to yield 1, 4-dihydropyridazines in conjugation (Oller-Salvia, B. et al, 2018 Angew Chem Int Ed Engl, 57, 2831-2834). However, the above methods are required antibody-engineering processes and reoptimization of cell culture conditions, often suffer from low protein expression, low conjugation yields, or are limited to specific conjugation sites. Therefore, some simple homogeneous conjugation methods were practically studied through rebridging the reduced inter chain disulfide bonds of a native antibody, such as, using bromo or dibromo-maleimides, called next generation maleimides (NGMs) (Schumacher, F.F., et al 2014, Org. Biomol. Chem. 12, 7261–69; UCL Cancer Institute), or using dibromopyri-dazinediones (A. Maruani, et al, Nat. Commun., 2015, 6, 6645; M. T. Lee, et al, Chem. Sci., 2017, 8, 2056), or applying bis-alkylating reagents via a three-carbon bridge (Badescu, G., et al., 2014, Bioconjug. Chem. 25, 1124–36; WO2013/190272, WO2014/064424 for PolyTherics Ltd), or via arylenedipropiolonitrile (ADPN) molecule (Koniev, O., et al, 2018 MedChemComm. 2018, 9, 827-830). Recently, Coumans et al used 2-(diphenylphosphino)-benzenesulfonic acid (diPPBS) as the reducing agent that only reduced the engineered cysteines without harming the interchain disulfides in an antibody for preparation of ADCs (R. G. E. Coumans, et al, Bioconjugate Chem. 2020, 31, 2136−2146). Wuxi Biologics Co., applied Zn^{2+} ions ($ZnCl_2$ salt in a buffer) for selected control the cystine reduction in an antibody, followed by reaction with a payload containing maleimide linker to improve homogeneity in production of ADCs (PCT/CN2020/075162). However, as most of antibodies are stored in a phosphate based buffer, e. g. called PBS buffer, Zn cation may swiftly precipitate in a phosphate buffer to form zinc phosphate, since the solubility constant of zinc phosphate is about 9.1×10^{-33} at neutral or base pH conditions (Martin, R. Bruce. "Solubility and Solubility Products (J. Chem. Educ. 1998, 75, 1179-1181 and J. Chem. Educ. 1998, 75, 1182-1185) "J. Chem. Educ. 2000, 77, 1558; Dupuis, V., et al, 1992, Biomaterials, 13 (7), 467-470; https://www.chm.uri.edu/weuler/chm112/refmater/KspTable.html). Therefore, when using $ZnCl_2$ in the reduction of an antibody, the amount of phosphate anions and the pH of the buffers have to be precisely controlled to avoid formation of the precipitate of zinc phosphate.

We have disclosed several conjugation methods of rebridging a pair of thiols of the reduced inter chain disulfide bonds of a native antibody, such as using bromo maleimide and dibromomaleimide linkers (WO2014/009774), 2, 3-disubstituted succinic /2-monosubstituted / 2, 3-disubstituted fumaric or maleic linkers (WO2015/155753, WO2016/596228), acetylenedicarboxylic linkers (WO2015/151080, WO2016/596228), hydrazine linkers (WO2015/151081), acryloyl or

propiolyl linkers (WO2018/086139), bis-linker (WO2018185526), and 2, 3-diaminosuccinyl linker (WO2020/073345). In this patent application, we extend the scopes of our earlier patent application in production of more homogeneous conjugates via selective control reduction of interchains of disulfide bonds of an antibody follow by or simultaneous conjugation under coordinative help by a zinc amino complex / chelate. Zinc amino complexes have more advantages over $ZnCl_2$ in coordination of reduction of disulfide bond in an antibody. First, zinc amino complexes are much bulkier than $ZnCl_2$ and can be more 3-D space selectively inserted in certain positions (e. g. more specifically in the positions of the disulfide bonds between heavy-light chains of an IgG1 antibody); Second, zinc amino complexes are more stable in a water based solution, for instance, the stability constant of zinc ammonia complex ion is 2.9×10^9 (https://chempedia. info/info/stability_constants/), which in turn, slow the precipitation in a neutral pH phosphate buffer. More importantly, by uses of zinc amino complexes to stoichiometrically control reduction of the disulfur bonds between the light chain and heavy chain of an IgG antibody without hurting the disulfide bonds between the heavy-heavy chains (in the hinge region) of the antibody, a bis-alkylation of the two geometrically adjacent thiols with a linker, particularly with a bis-linker can be well specifically achieved. In a word, the conjugation strategy of this invention has robust manufacturability to yield highly homogeneous ADCs without antibody engineering and can successfully tackle an important shortcoming in current ADC preparation methods, in particular for bridge-linkage of di-thiol conjugation of an antibody. This conjugation strategy can be applied directly to other antibody likes of proteins. The resulting homogeneous ADCs demonstrate improved pharmacokinetics, superior efficacy, and reduced toxicity in vivo compared to analogous conventional heterogeneous ADCs.

SUMMARY OF THE INVENTION

The present invention provides conjugation process with improved homogeneity of an antibody conjugate, or antibody-like protein conjugate, in particular, an antibody –drug conjugate (ADC), wherein over 75% of payloads (drugs) are specifically conjugated to the disulfide bond sites between heavy-light chains of an antibody.

The homogenous conjugation process comprises the following three key steps:

(a) incubating an antibody-like protein, in particular, an IgG antibody in the presence of an effective amount of transition metal cation-amino chelate/complex ($Zn(NR_1R_2R_3)_{m1}^{2+}$) and a reductant (e.g. Tris(2-carboxyethyl)phosphine (TCEP)) in a buffer system (e. g. PBS, Mes, Bis-Tris, Bis-Tris Propane, Pipes, Aces, Mopso, Bes, Mops, Hepes, Tes, Pipps, Dipso, Tapso, Heppso, Tris-up, Tris-HCl, Tricine, Hepps, Gly-Gly, Bicine, Taps, Hepee, Acetates, Histidine, Citrates, MES, or Borates, etc.) to selectively reduce interchain disulfide bonds within the antibody, or antibody-like protein to generate thiols;

(b). introducing an effective amount of linker or payload/linker complex/assembly bearing thiol reactive groups (e.g., a drug containing maleimide terminal) to react with the thiol groups resulted from step (a); and

(c). optionally adding an effective amount of oxidant (e. g. dehydroascorbic acid (DHAA)) to re-oxidize unreacted thiol groups, or adding a thiol reactive compound (e.g. N-ethyl maleimide (NEM) or N-ethyl-maleimide-3-sulfonate) to quench the unreacted thiols in the protein, and then purifying the resulted conjugates;

(d). the step (c) can be optionally replaced by: adding an effective amount of cystine to quench the excessive conjugation linker or linker/payload complex containing thiol reactive groups (e. g. maleimide); and simultaneously or sequentially adding an azido compound (e. g. 4-(azidomethyl)-benzoic acid) or a disulfide compound (e. g. cystine) to quench the unreacted reductant (e. g. TCEP or Tris(hydroxypropyl)phosphine). The addition of cystine to quench the unreacted reductant (e. g. TCEP) can form a cysteine which can simultaneously quench the excessive conjugation linker or linker/payload complex containing thiol reactive groups (e. g. maleimide).

wherein R_1, R_2 and R_3 in the formula of $Zn(NR_1R_2R_3)_{m1}^{2+}$ are independently selected from C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; m1 is selected from 1, 2, 3, 4, 5, 6, 7 or 8; Proferably m1 is 1, 2, 3 or 4.

In addition, $(NR_1R_2R_3)_{m1}$ can be form a dimer, trimer, tetramer, pentamer, or hexamer wherein these polymers are covalently linked among N, R_1, R_2 and R_3; and N, R_1, R_2 or R_3 themselves or together can form heterocyclic, carbocyclic, diheterocyclic, or dicarbocyclic rings.

The Zinc cation-amino chelate/complex, Zn $(NR_1R_2R_3)_{m1}^{2+}$, used in step (a) is 0.01 mM — 1.0mM in concentration, or 0.5 ~ 20 equivalents in moles of the protein, and it can be added to the reaction solution with a water-soluble organic solvent, selected from, ethanol, methanol, propanol, propandiol, DMA, DMF, DMSO, THF, CH_3CN.

The reductant is an organic phosphine, preferably selected from Tris(2-carboxyethyl)-phosphine (TECP) or Tris(hydroxypropyl)phosphine and its use in the reaction solution is 0.02 mM — 1.0mM in concentration, or 1.0 – 20 equivalents in moles of the protein. The oxidant to be added in step (c) may be DHAA, Fe^{3+}, I_2, Cu^{2+}, Mn^{3+}, MnO_2, or a mixture of Fe^{3+}/I^- or DHAA/Fe^{3+}. The oxidant used in the reaction solution is 0.02 mM – 1.0 mM in concentration, or 0.2 -100 equivalents in moles of the protein. The optimum pH in the conjugation reaction is typically between about 5.0 to 8.0, and preferably, about 5.5 to 7.5. The optimum temperature in the conjugation reaction is typically between about - 5 to about 40 °C, and preferably, about 0 to

37 °C; more preferably about 2 to 8 °C; further preferably about 2 to 6 °C. The optimum time of the conjugation reaction is typically between about 15 min to about 48 hours and preferably, about 30 min to overnight (10 ~ 16 h), more preferably about 2 h ~ 6 h. The optimal reaction conditions (e. g. pH, temeperature, buffer, concentrations of the reactants) of course are depended upon specifically an antibody-like protein, a payload/linker complex, a reductant and/or $Zn(NR_1R_2R_3)_{m1}^{2+}$ used.

The antibody or antibody-like protein in the conjugation process can be any types of antibodies or proteins as long as they have two or more disulfide bonds in the protein for differentiation of reduction. And the payload/linker complex may be any types or formats as long as it has a thiol reactive group.

In a word, the ADCs prepared by the process of the present application have more than 70% (and many of them can be over 85%) of payloads conjugated in the Fab region of an antibody, in contrast to the conventional process wherein around 40% of the payloads are in the Fab region of an antibody. The advantages of the application along with the improved conjugation process for homogeneity of ADCs will become more apparent from the following detailed description of several embodiments, experimentals and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the general synthesis of components of a bis-linker.

Fig. 2 shows the synthesis of a camptothecin analog and its conjugate containing a bis-conjugate linker.

Fig. 3 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 4 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 5 shows the general synthesis of components of a bis-linker.

Fig. 6 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 7 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 8 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 9 shows the general synthesis of components of a bis-linker.

Fig. 10 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 11 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 12 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 13 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 14 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 15 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 16 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 17 shows the synthesis of components of a tubulysin B analogs.

Fig. 18 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 19 shows the synthesis of a tubulysin B analog containing a bis-conjugate linker.

Fig. 20 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 21 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 22 shows the synthesis of a camptothecin analog and its conjugate containing a bis-conjugate linker.

Fig. 23 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 24 shows the synthesis of a camptothecin analog containing a bis-conjugate linker.

Fig. 25 shows the synthesis of a camptothecin analog containing a bis-conjugate linker and components of amanitin analogs.

Fig. 26 shows the synthesis of a conjugate of an amanitin containing a bis-conjugate linker.

Fig. 27 shows the synthesis of a conjugate of an amanitin containing a bis-conjugate linker.

Fig. 28 shows the synthesis of a conjugate of an amanitin containing a bis-conjugate linker.

Fig. 29 shows the synthesis of a conjugate of an amanitin containing a bis-conjugate linker.

Fig. 30 shows the synthesis of a conjugate of a tubulysin B analog containing a linker.

Fig. 31 shows the synthesis of a conjugate of a tubulysin B analog containing a linker.

Fig. 32 shows the synthesis of a conjugate of a tubulysin B analog containing a linker.

Fig. 33 shows the synthesis of a conjugate of a tubulysin B analog containing a bis-linker.

Fig. 34 shows the synthesis of a conjugate of a tubulysin B analog containing a bis-linker.

Fig. 35 shows the synthesis of a conjugate of a tubulysin B analog containing a bis-linker.

Fig. 36 shows the synthesis of a conjugate of a tubulysin B analog containing a bis-linker.

Fig. 37 shows the synthesis of a conjugate of a tubulysin B analog containing a bis-linker.

Fig. 38 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 39 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 40 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 41 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 42 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 43 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 44 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 45 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 46 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 47 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 48 shows the synthesis of a conjugate of an amanitin containing a linker.

Fig. 49 shows the synthesis of a PBD dimer containing a bis-linker.

Fig. 50 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 51 shows the synthesis of a camptothecin analog containing a bis-linker.

Fig. 52 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 53 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 54 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 55 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 56 shows the synthesis of a conjugate of a camptothecin analog containing a linker.

Fig. 57 shows the synthesis of a conjugate of a camptothecin analog containing a bis-linker.

Fig. 58 shows middle-level characterization of BCMA-Tubulysin B analog ADC (C-390) after N-deglycosylation and reduction. (a) rpHPLC chromatogram of ADC fragments obtained after deglycosylation and DTT reduction. Light chains (LC) with zero or one drug molecule attached (L0 and L1), (b) heavy chains with zero, one, two, or three drug molecules attached (H0, H1, H2 and H3). It demonstrated that the drug was mainly (>85%) conjugated at Fab region.

Fig. 59 shows the Percentage of Drug Loaded Peptides of a BCMA ADC (C-390). (a): LC Peptide [GEC] with zero or one drug molecule attached (D0 and D1); (b): HC Peptide [SCDK] at the arm with zero or one drug molecule attached (D0 and D1); (c): HC Peptide [THTCPPCPAPELLGGPSVFLFPPKPK] at the hinge with zero, one or two drug molecules attached (D0, D1 and D2).

Fig. 60 shows middle-level characterization of a conjugate of Trop2-C608 ADC after reduction. Light chains (LC) with zero or one drug molecule attached (L0 and L1), and heavy chains with zero, one, two, or three drug molecules attached (H0, H1, H2 and H3). It demonstrated that the drug was mainly (>85%) conjugated at Fab region.

Fig. 61 shows middle-level characterization of bridged cysteine conjugations (a conjugate of Muc-1-C606) after reduction. Light chains(LC), heavy chain(HC) and cysteine-bridged HC and LC with one drug molecule (LC+HC+LP). It demonstrated that the drug was mainly (>85%) conjugated at Fab region.

Fig. 62 shows middle-level characterization of bridged cysteine conjugations (a conjugate of Muc-1-C72) after reduction. Light chains(LC), heavy chain(HC) and cysteine-bridged HC and LC with one drug molecule (LC+HC+LP). It demonstrated that the drug was mainly (>85%) conjugated at Fab region.

Fig. 63 shows middle-level characterization of bridged cysteine conjugations (a conjugate of Muc-1-C45) after reduction. Light chains(LC), heavy chain(HC) and cysteine-bridged HC and LC with one drug molecule (LC+HC+LP).

Fig. 64 shows middle-level characterization of bridged cysteine conjugations (a conjugate of Trop2-C140) after reduction. Light chains(LC), heavy chain(HC) and cysteine-bridged HC and LC with one drug molecule (LC+HC+LP).

Fig. 65 shows change in tumor volume in a NCI-H929 cell xenograft mouse model of multiple myeloma in response to a serial of single dose (3 mg/Kg) treatment with BCMA (hu5D2) ADCs (C-68a, C-115, C-192, C-202, C-221, C-290, C-306, C-385, C-390, C-399, C-402, C-417), in comparison to BCMA-mcMMAF(belantamab-mcMMAF) and PBS buffer (the control).The figure indicates that all the 13 conjugates had antitumor activity, and the orders of the antitumor activity are: C-385 < C-306 < C-290 < C-68a < C-115 < BCMA-mcMMAF< C-192 < C-202 < C-399 < C-390 < C-417 < C-402 < C-221.

Fig. 66 shows change in tumor volume in a JJN-3 cell xenograft mouse model of multiple myeloma in response to a serial of single dose (5 mg/Kg) treatment with hu5D2-ADC in comparison to belantamab-mcMMAF and PBS buffer (the control). The figure indicates that all the 9 conjugates had antitumor activity, and the orders of the antitumor activity are: Paclitaxel < C-385 < belantamab-mcMMAF < C-195 < C-137 < C-181b < C-126 < C-83 < C-277 < C-258.

Fig. 67 shows change in tumor volume in NCI-H929 cell xenograft mouse model of multiple myeloma in response to a serial of single dose (2 mg/Kg) treatment with hu5D2-ADC in comparison to belantamab-mcMMAF and PBS buffer (the control). The figure indicates that all the 7 conjugates had antitumor activity, and the orders of the antitumor activity are: belantamab-mcMMAF < C-406 < C-396 < C-399 < C-400 < C-221b < C-402.

Figure 68 shows the comparison of the anti-tumor effect of B7H3 antibody conjugate of C-550, C-570-T (with traditional conjugation at DAR =4.0), C-543, C-595, C-570-H (with homogeneous conjugation of this invention), C-581, C-582, C-560, C-562, and C-561, at dose of 6 mg/Kg, single injection, along with Paclitaxel at dose of 15 mg/Kg, QWx3, against Calu-6 cells. The figure indicates that all the 10 conjugates had better antitumor activity than paclitaxel. And the conjugate C-570 prepared with the method of this invention had better in vivo activity than that prepared by the regular method.

Figure 69 shows the comparison of the anti-tumor effect of Muc1 antibody conjugate of C-553, C-484, C-490, C-450, C-432-T (with traditional conjugation at DAR =4.0), C-501, C-519, C-432-H (with homogeneous conjugation of this invention), C-417, and C-560, at dose of 6 mg/Kg, single injection, along with Paclitaxel at dose of 15 mg/Kg, QWx3, against Calu-6 cells. The figure indicates that all the 10 conjugates had better antitumor activity than paclitaxel. And the conjugate C-432 prepared with the method of this invention had better in vivo activity than that prepared by the regular method.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

"Alkyl" refers to an aliphatic hydrocarbon group or univalent groups derived from alkane by removal of one or two hydrogen atoms from carbon atoms. It may be straight or branched having C_1-C_8 (1 to 8 carbon atoms) in the chain. "Branched" means that one or more lower C numbers of alkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. Exemplary alkyl groups include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl, *n*-pentyl, 3-pentyl, octyl, nonyl, decyl, cyclopentyl, cyclohexyl, 2, 2-dimethylbutyl, 2, 3-dimethylbutyl, 2, 2-dimethylpentyl, 2, 3-dimethylpentyl, 3, 3-dimethylpentyl, 2, 3, 4-trimethylpentyl, 3-methyl-hexyl, 2, 2-dimethylhexyl, 2, 4-dimethylhexyl, 2, 5-dimethylhexyl, 3, 5-dimethylhexyl, 2, 4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, *n*-heptyl, isoheptyl, *n*-octyl, and isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from -C_1-C_8 alkyl and aryl.

"Halogen" refers to fluorine, chlorine, bromine or iodine atom; preferably fluorine and chlorine atom.

"Heteroalkyl" refers to C_2-C_8 alkyl in which one to four carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N.

"Carbocycle" refers to a saturated or unsaturated ring having 3 to 8 carbon atoms as a monocycle or 7 to 13 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, arranged as a bicycle [4, 5], [5, 5], [5, 6] or [6, 6] system, or 9 or 10 ring atoms arranged as a bicycle [5, 6] or [6, 6] system. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1, 3-cyclohexadienyl, -1, 4-cyclohexadienyl, -cycloheptyl, -1, 3-cycloheptadienyl, -1, 3, 5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl.

A "C_3-C_8 carbocycle" refers to a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated nonaromatic carbocyclic ring. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -S(O)R', -$S(O)_2$R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from -C_1-C_8 alkyl and aryl.

"Alkenyl" refers to an aliphatic hydrocarbon group containing a carbon-carbon double bond

which may be straight or branched having 2 to 8 carbon atoms in the chain. Exemplary alkenyl groups include ethenyl, propenyl, n-butenyl, i-butenyl, 3-methylbut-2-enyl, n-pentenyl, hexylenyl, heptenyl, octenyl.

"Alkynyl" refers to an aliphatic hydrocarbon group containing a carbon-carbon triple bond which may be straight or branched having 2 to 8 carbon atoms in the chain. Exemplary alkynyl groups include ethynyl, propynyl, *n*-butynyl, 2-butynyl, 3-methylbutynyl, 5-pentynyl, *n*-pentynyl, hexylynyl, heptynyl, and octynyl.

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH_2-), 1, 2-ethyl (-CH_2CH_2-), 1, 3-propyl (-$CH_2CH_2CH_2$-), 1, 4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1, 2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene, propargyl and 4-pentynyl.

"Aryl" or Ar refers to an aromatic or hetero aromatic group, composed of one or several rings, comprising three to fourteen carbon atoms, preferentially six to ten carbon atoms. The term of "hetero aromatic group" refers one or several carbons on aromatic group, preferentially one, two, three or four carbon atoms are replaced by O, N, Si, Se, P or S, preferentially by O, S, and N. The term aryl or Ar also refers to an aromatic group, wherein one or several H atoms are replaced independently by -R', -halogen, -OR', or -SR', -NR'R'', -N=NR', -N=R', -NR'R'', -NO_2, -S(O)R', -$S(O)_2$R', -$S(O)_2$OR', -$OS(O)_2$OR', -PR'R'', -P(O)R'R'', -P(OR')(OR''), -P(O)(OR')(OR'') or -OP(O)(OR')(OR'') wherein R', R'' are independently H, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, arylalkyl, carbonyl, or pharmaceutical salts.

"Heterocycle" refers to a ring system in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group of O, N, S, Se, B, Si and P. Preferable heteroatoms are O, N and S. Heterocycles are also described in The Handbook of Chemistry and Physics, 78th Edition, CRC Press, Inc., 1997-1998, p. 225 to 226, the disclosure of which is hereby incorporated by reference. Preferred nonaromatic heterocyclic include epoxy, aziridinyl, thiiranyl,

pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxiranyl, tetrahydrofuranyl, dioxolanyl, tetrahydropyranyl, dioxanyl, dioxolanyl, piperidyl, piperazinyl, morpholinyl, pyranyl, imidazolinyl, pyrrolinyl, pyrazolinyl, thiazolidinyl, tetrahydrothiopyranyl, dithianyl, thiomorpholinyl, dihydropyranyl, tetrahydropyranyl, dihydropyranyl, tetrahydropyridyl, dihydropyridyl, tetrahydropyrimidinyl, dihydrothiopyranyl, azepanyl, as well as the fused systems resulting from the condensation with a phenyl group.

The term "heteroaryl" or aromatic heterocycles refers to a 3 to 14, preferably 5 to 10 membered aromatic hetero, mono-, bi-, or multi-cyclic ring. Examples include pyrrolyl, pyridyl, pyrazolyl, thienyl, pyrimidinyl, pyrazinyl, tetrazolyl, indolyl, quinolinyl, purinyl, imidazolyl, thienyl, thiazolyl, benzothiazolyl, furanyl, benzofuranyl, 1, 2, 4-thiadiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, carbazolyl, benzimidazolyl, isoxazolyl, pyridyl-*N*-oxide, as well as the fused systems resulting from the condensation with a phenyl group.

"Alkyl", "cycloalkyl", "alkenyl", "alkynyl", "aryl", "heteroaryl", "heterocyclic" and the like refer also to the corresponding "alkylene", "cycloalkylene", "alkenylene", "alkynylene", "arylene", "heteroarylene", "heterocyclene" and the likes which are formed by the removal of two hydrogen atoms.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Examples of heteroarylalkyl groups are 2-benzimidazolylmethyl, 2-furylethyl.

Examples of a "hydroxyl protecting group" include, methoxymethyl ether, 2-methoxyethoxy-methyl ether, tetrahydropyranyl ether, benzyl ether, *p*-methoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, triisopropylsilyl ether, *t*-butyldimethylsilyl ether, triphenylmethylsilyl ether, acetate ester, substituted acetate esters, pivaloate, benzoate, methanesulfonate and *p*-toluenesulfonate.

"Leaving group" refers to a functional group that can be substituted by another functional group. Such leaving groups are well known in the art, and examples include, a halide (e.g., chloride, bromide, and iodide), methanesulfonyl (mesyl), *p*-toluenesulfonyl (tosyl), trifluoro-methylsulfonyl (triflate), and trifluoromethylsulfonate. A preferred leaving group is selected from nitrophenol; N-hydroxysuccinimide (NHS); phenol; dinitrophenol; pentafluorophenol; tetrafluorophenol; difluorophenol; monofluorophenol; pentachlorophenol; triflate; imidazole; dichlorophenol; tetrachlorophenol; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3′-

sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions or for Mitsunobu reactions.

The following abbreviations may be used herein and have the indicated definitions: Boc, tert-butoxy carbonyl; BroP, bromotrispyrrolidinophosphonium hexafluorophosphate; CDI, 1, 1'-carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; DCE, dichloroethane; DCM, dichloromethane; DIAD, diisopropylazodicarboxylate; DIBAL-H, diisobutyl-aluminium hydride; DIPEA, diisopropylethylamine; DEPC, diethyl phosphorocyanidate; DMA, N, N-dimethyl acetamide; DMAP, 4-(N, N-dimethylamino)pyridine; DMF, N, N-dimethylformamide; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; ESI-MS, electrospray mass spectrometry; HATU, O-(7-azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high pressure liquid chromatography; NHS, N-Hydroxysuc-cinimide; MMP, 4-methylmorpholine; PAB, p-aminobenzyl; PBS, phosphate-buffered saline (pH 7.0~7.5); PEG, polyethylene glycol; SEC, size-exclusion chromatography; TCEP, tris(2-carboxyethyl)phosphine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Val, valine.

The "amino acid(s)" can be natural and/or unnatural amino acids, preferably alpha-amino acids. Natural amino acids are those encoded by the genetic code, which are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine. tryptophan and valine. The unnatural amino acids are derived forms of proteinogenic amino acids. Examples include hydroxyproline, lanthionine, 2-aminoisobutyric acid, dehydroalanine, gamma-aminobutyric acid (the neurotransmitter), ornithine, citrulline, beta alanine (3-aminopropanoic acid), gamma-carboxyglutamate, selenocysteine (present in many noneukaryotes as well as most eukaryotes, but not coded directly by DNA), pyrrolysine (found only in some archaea and one bacterium), N-formylmethionine (which is often the initial amino acid of proteins in bacteria, mitochondria, and chloroplasts), 5-hydroxytryptophan, L-dihydroxyphenylalanine, triiodothyronine, L-3, 4-dihydroxyphenylalanine (DOPA), and O-phosphoserine. The term amino acid also includes amino acid analogs and mimetics. Analogs are compounds having the same general $H_2N(R)CHCO_2H$ structure of a natural amino acid, except that the R group is not one found among the natural amino acids. Examples of analogs include homoserine, norleucine, methionine-sulfoxide, and methionine methyl sulfonium. Preferably, an amino acid mimetic is a compound that has a structure different from the general chemical structure of an alpha-amino acid but functions in a manner similar to one. The term "unnatural amino acid" is intended to represent the "D" stereochemical form, the natural

amino acids being of the "L" form. When 1~8 amino acids are used in this patent application, amino acid sequence is then preferably a cleavage recognition sequence for a protease. Many cleavage recognition sequences are known in the art. See, e.g., Matayoshi et al. Science 247: 954 (1990); Dunn et al. Meth. Enzymol. 241: 254 (1994); Seidah et al. Meth. Enzymol. 244: 175 (1994); Thornberry, Meth. Enzymol. 244: 615 (1994); Weber et al. Meth. Enzymol. 244: 595 (1994); Smith et al. Meth. Enzymol. 244: 412 (1994); and Bouvier et al. Meth. Enzymol. 248: 614 (1995); the disclosures of which are incorporated herein by reference. In particular, the sequence is selected from the group consisting of Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Ala, Gly-Gly-Phe-Gly, Ala-Arg, Val-Arg, Phe-Arg, Ala-Cit, Phe-Cit, Cit-Cit, Val-Lys, Ala-Ala-Asn, Lys, Cit, Ser, and Glu.

"Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate.

"Pharmaceutically acceptable solvate" or "solvate" refer to an association of one or more solvent molecules and a disclosed compound. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid and ethanolamine.

"Pharmaceutically acceptable excipient" includes any carriers, diluents, adjuvants, or vehicles, such as preserving or antioxidant agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions as suitable therapeutic combinations.

As used herein, "pharmaceutical salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, tartaric, citric, methanesulfonic, benzenesulfonic, glucuronic, glutamic, benzoic, salicylic, toluenesulfonic, oxalic, fumaric, maleic, lactic and the like.

Further addition salts include ammonium salts such as tromethamine, meglumine, epolamine, etc., metal salts such as sodium, potassium, calcium, zinc or magnesium.

The pharmaceutical salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared via reaction the free acidic or basic forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

"Administering" or "administration" refers to any mode of transferring, delivering, introducing or transporting a pharmaceutical drug or other agent to a subject. Such modes include oral administration, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intranasal, subcutaneous or intrathecal administration. Also contemplated by the present invention is utilization of a device or instrument in administering an agent. Such device may utilize active or passive transport and may be slow-release or fast-release delivery device.

The abbreviations of biological buffers and their chemical names are listed below:

ACES (N-(2-Acetamido)-2-aminoethanesulfonic acid) is used to buffer at pH = 6.1 - 7.5 (pKa = 6.88)

ADA (N-(2-Acetamido)iminodiacetic acid, N-(Carbamoylmethyl)iminodiacetic acid) is useful to buffer at pH = 6.0 - 7.2 (pKa = 6.65).

AMPD (2-amino-2-methyl-1, 3-propanediol)) is a useful buffer at pH = 7.8 - 9.7.

AMPSO (N-(1, 1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid).

BES (N, N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid).

Bicine (N, N-Bis(2-hydroxyethyl)glycine], Bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane) is used to buffer at pH = 5.8 - 7.2 (pKa = 8.35).

BisTris (Bis-(2-Hydroxyethyl)amino-tris(Hydroxymethyl)Methane).

BisTris propane (1, 3-Bis[tris(hydroxymethyl)methylamino]propane).

DIPSO (N, N-Bis(2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid) is used to buffer at pH = 7.0 - 8.2.

Gly-Gly (Diglycine; Glycyl-glycine) is used to buffer at pH = 7.5 - 8.9 (pKa = 8.30).

HEBPS (N-(2-Hydroxyethyl)piperazine-N′-(4-butanesulfonic acid)) is an homolog of HEPES and EPPS with higher pKa (pKa= 8.30), used to buffer at pH = 7.6 - 9.0

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid ; 2-morpholinoethanesulfonic acid;

2-(4-morpholino)ethanesulphonic acid; 2-(N-morpholino)ethanesulfonic acid; morpholine-4-ethanesulfonic acid hydrate) is widely used to buffer at pH = 6.8 - 8.2; pKa at 20°C: 7.45 - 7.65)

HEPPS or EPPS (3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid hydrate; 4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) Hydrate) is used as a buffering agent at pH 7.3-8.7 (pKa= 8.00/piperazine ring).

HEPPSO (4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) hydrate).

MES (2-(N-morpholino)ethanesulfonic acid, monohydrate) is used as buffering agent at pH = 5.2 - 7.1 (pKa = 6.16).

MOBS (4-Morpholinebutanesulfonic acid; 3-(N-Morpholino)butanesulfonic acid hemisodium salt) is an homolog of MES and MOPS with higher pKa/ It is used to buffer solution at pH = 6.9 - 8.3 (pKa = 7.6).

MOPS (4-Morpholinepropanesulfonic acid Sodium salt).

MOPSO (β-Hydroxy-4-morpholinepropanesulfonic acid, 3-Morpholino-2-hydroxypropanesulfonic acid).

PIPES (Piperazine-1, 4-bis(2-ethanesulfonic acid) is used to buffer at pH 6.1-7.5 (pKa = 6.80).

POPSO (Piperazine-1, 4-bis(2-hydroxypropanesulfonic acid) dihydrate).

TAPS ([(2-Hydroxy-1, 1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid).

TAPSO (2-Hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid).

TES (2-[(2-Hydroxy-1, 1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid).

Tricine (Piperazine-N, N'-bis[2-Hydroxypropanesulfonic Acid)] is used to buffer at pH = 7.4 - 8.8 (pKa = 8.16).

The term “antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and my, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., J. Immunol. 150: 880-887 (1993); Clarkson et al., Nature 352: 624-628 (1991).

As used herein, “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256:495, 1975, or may be made by

recombinant DNA methods such as described in U.S. Pat. No. 4, 816, 567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., Nature 348:552-554, 1990, for example.

As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Preferred are antibodies having Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., Nature Biotechnology, 14:309-314, 1996; Sheets et al., Proc. Natl. Acad. Sci. (USA) 95:6157-6162, 1998; Hoogenboom and Winter, J. Mol. Biol., 227:381, 1991; Marks et al., J. Mol. Biol., 222:581, 1991). Human antibodies can also be made by immunization of animals into which human

immunoglobulin loci have been transgenically introduced in place of the endogenous loci, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Pat. Nos. 5, 545, 807; 5, 545, 806; 5, 569, 825; 5, 625, 126; 5, 633, 425; and 5, 661, 016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or from single cell cloning of the cDNA, or may have been immunized in vitro). See, e.g., Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, 1985; Boerner et al., J. Immunol., 147 (1):86-95, 1991; and U.S. Pat. No. 5, 750, 373.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to chains of amino acids of any length, preferably, relatively short (e.g., 10-100 amino acids). The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

A "monovalent antibody" comprises one antigen binding site per molecule (e.g., IgG or Fab). In some instances, a monovalent antibody can have more than one antigen binding sites, but the binding sites are from different antigens.

A "monospecific antibody" comprises two identical antigen binding sites per molecule (e.g. IgG) such that the two binding sites bind identical epitope on the antigen. Thus, they compete with each other on binding to one antigen molecule. Most antibodies found in nature are monospecific. In some instances, a monospecific antibody can also be a monovalent antibody (e.g. Fab).

A "bivalent antibody" comprises two antigen binding sites per molecule (e.g., IgG). In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

A "bispecific" or "dual-specific" is a hybrid antibody having two different antigen binding sites. The two antigen binding sites of a bispecific antibody bind to two different epitopes, which may

reside on the same or different protein targets.

A “bifunctional” is antibody is an antibody having identical antigen binding sites (i.e., identical amino acid sequences) in the two arms but each binding site can recognize two different antigens.

A “heteromultimer”, “heteromultimeric complex”, or “heteromultimeric polypeptide” is a molecule comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromultimer can comprise a “heterodimer” formed by the first and second polypeptide or can form higher order tertiary structures where polypeptides in addition to the first and second polypeptide are present.

A “heterodimer”, “heterodimeric protein”, “heterodimeric complex”, or “heteromultimeric polypeptide” is a molecule comprising a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue.

The “hinge region”, “hinge sequence”, and variations thereof, as used herein, includes the meaning known in the art, which is illustrated in, for example, Janeway et al., ImmunoBiology: the immune system in health and disease, (Elsevier Science Ltd., NY) (4th ed., 1999); Bloom et al., Protein Science (1997), 6:407-415; Humphreys et al., J. Immunol. Methods (1997), 209:193-202.

The “immunoglobulin-like hinge region”, “immunoglobulin-like hinge sequence, ” and variations thereof, as used herein, refer to the hinge region and hinge sequence of an immunoglobulin-like or an antibody-like molecule (e.g., immunoadhesins). In some embodiments, the immunoglobulin-like hinge region can be from or derived from any IgG1, IgG2, IgG3, or IgG4 subtype, or from IgA, IgE, IgD or IgM, including chimeric forms thereof, e.g., a chimeric IgG1/2 hinge region.

The term “immune effector cell” or “effector cell” as used herein refers to a cell within the natural repertoire of cells in the human immune system which can be activated to affect the viability of a target cell. The viability of a target cell can include cell survival, proliferation, and/or ability to interact with other cells.

Antibodies of the invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art (see, for example, Jayasena, S. D., Clin. Chem., 45: 1628-50, 1999 and Fellouse, F. A., et al, J. Mol. Biol., 373(4): 924-40, 2007).

The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At211, I131, I125, Y90, In111, Re186, Re188, Sm153, Bi212, P32,

Pb212, Zr89, F18, and radioactive isotopes of Lu, e.g. Lu177); chemotherapeutic agents or drugs (e.g., tubulysin, maytansin, auristatin, DNA minor groove binders (such as PBD dimers), ducarmysin, topoisomerase I or II inhibitor, RNA polymerase inhibitors, DNA alkylators, methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed throughout the application.

"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: --$(CR_2)_nO(CR_2)$ n--, repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide. In various embodiments, linkers can comprise one or more amino acid residues, such as valine, phenylalanine, lysine, and homolysine.

The words "comprise", "comprising", "include", "including" and "includes" when used in this specification and claims are intended to specify the presence of stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more other features, integers, components, steps, or groups thereof. The novel conjugates disclosed herein use the bridge linkers. Examples of some suitable linkers and their synthesis are shown in the examples 1- 468 below of the specification.

THE CONJUGATION PROCESS OF THE PRESENT INVENTION

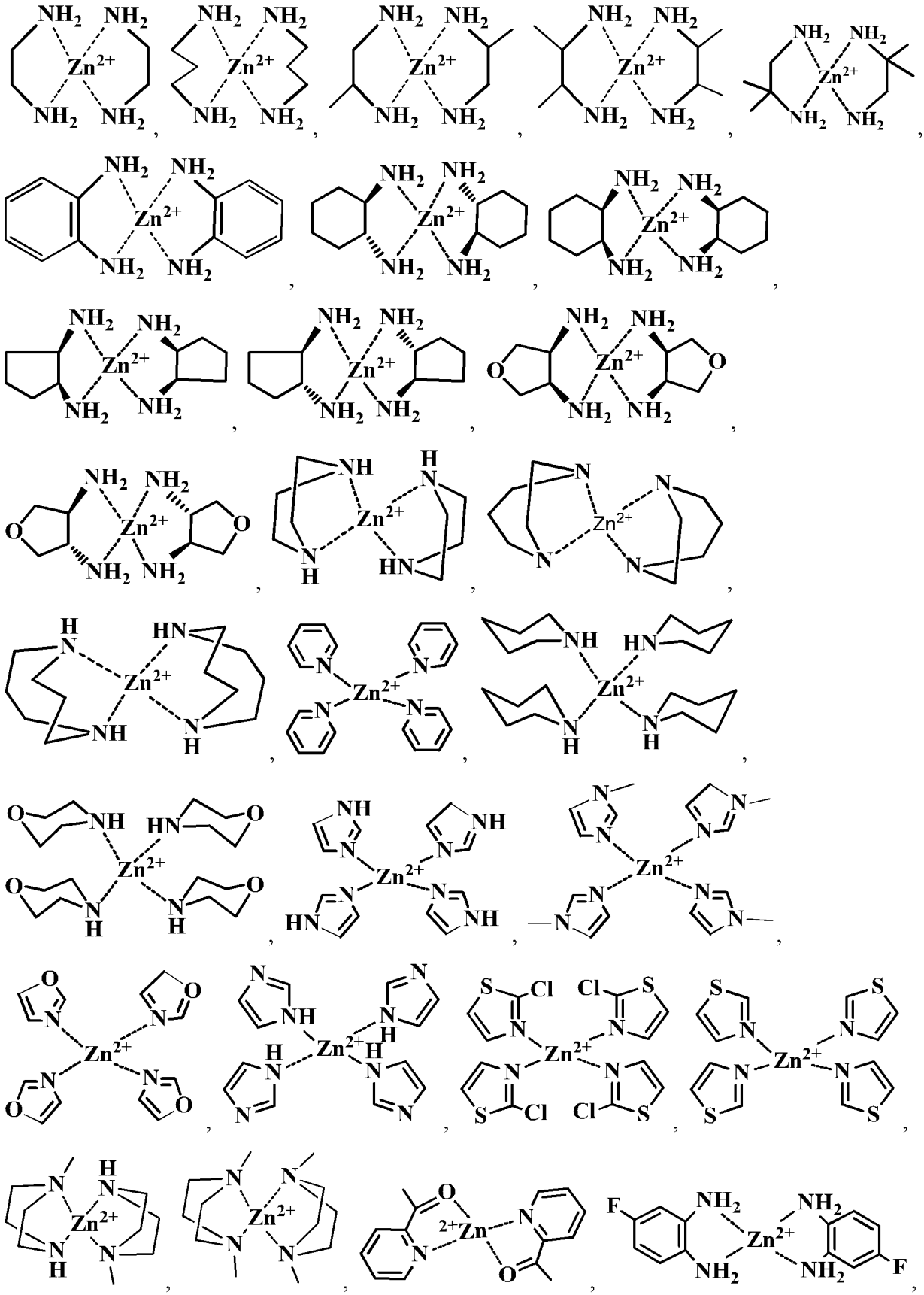
As disclosed above, the key factor of the invention in the conjugation process is the transition zinc cation-amino complex, $Zn(NR_1R_2R_3)_{m1}^{2+}$, which coordinate the selective reduction of certain chain of disulfide bonds of biomolecule. Preferably the certain inter-chain disulfide bonds in an antibody. Previous study by LC-MS indicated that inter chain disulfide bonds of IgG antibodies are more susceptible to reduction than intra chain disulfide bonds, and the disulfide bonds between the light chain and heavy chain were more susceptible than disulfide bonds between the two heavy chains. The upper disulfide bond of the two inter heavy chain disulfide bonds of IgG antibodies was more susceptible than the lower one. Furthermore, disulfide bonds in the CH2 domain were the most susceptible to reduction. Disulfide bonds in VL, CL, VH, and CH1 domains had similar and moderate susceptibility, while disulfide bonds in the CH3 domain were the least susceptible to reduction (Liu, H, et al Anal. Chem., 2010, *82*, 5219–5226). The using of $ZnCl_2$ salt at low

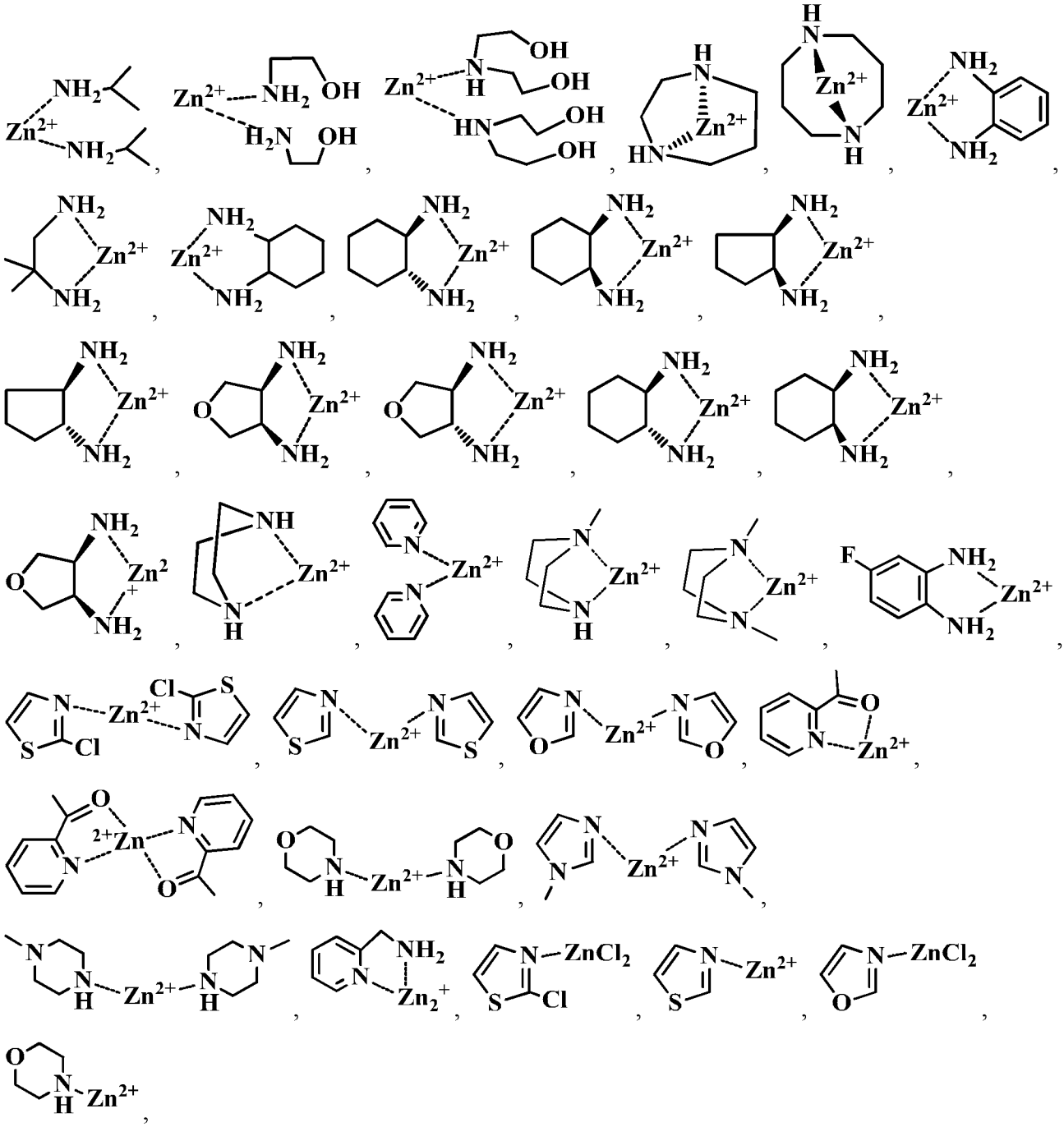
temperatures of 2 ~ 8 °C in coordination the reduction of the disulfide bonds of a IgG antibody of the invention WO2020164561 made practically possible of the distinguishable reduction above. Here, the transition zinc cation-amino complex, $Zn(NR_1R_2R_3)_{m1}^{2+}$, which is used in the conjugation process of the invention, is much bulky, not only can coordinate the disulfide reduction, but also stereoscopically hinders the reductant (such as TCEP) to access to disulfide bonds between the two heavy chains of an IgG antibody, thus results in much better selective reduction and following by conjugation with a drug/linker complex.

The transition Zinc cation-amino chelate/complex, $Zn(NR_1R_2R_3)_{m1}^{2+}$, wherein R_1, R_2 and R_3 are independently selected from C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heteroaryl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; m1 is selected from 1, 2, 3, 4, 5, 6, 7, or 8; In preference, M is selected from Zn^{2+}, m_1 is selected from 1, 2, 3, or 4, and m_1 is more preferably 2.

In addition, $(NR_1R_2R_3)_{m1}$ can form a dimer, trimer, tetramer, pentamer, or hexamer wherein these polymers are covalently linked among N, R_1, R_2 and R_3; and N, R_1, R_2 and/or R_3 themselves can form heterocyclic, carbocyclic, diheterocyclic, or dicarbocyclic rings.

The preferred $Zn(NR_1R_2R_3)_{m1}^{2+}$ are exampled as following: $Zn(NH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2CH_2CH_3)_2^{2+}$, $Zn(NH_2CH(CH_3)_2)_2^{2+}$, $Zn(NH_2C(CH_3)_3)_2^{2+}$, $Zn(NH_2CH_2C(CH_3)_3)_2^{2+}$, $Zn(NH(CH_3)_2)_2^{2+}$, $Zn(NH(CH_2CH_3)_2)_2^{2+}$, $Zn(NH(CH(CH_3)_2)_2)_2^{2+}$, $Zn(NH(C(CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH(CH_2CH_3)_2)_2)_2^{2+}$, $Zn(NH(CH_2C(CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH_2C(CH_2CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH_2CH_2C(CH_3)_3)_2)_2^{2+}$, $Zn(NH_2CH_2CH_2OH)_2^{2+}$, $Zn(NH(CH_2CH_2OH)_2)_2^{2+}$, $Zn(N(CH_2CH_2OH)_3)_2^{2+}$, $Zn(NH_2CH_2COOH)_2^{2+}$, $Zn(NH_2CH_2CONH_2)_2^{2+}$, $Zn(NH_2CH_2COOCH_3)_2^{2+}$, $Zn(NH_2CH_2COOCH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2COOC(CH_3)_3)_2^{2+}$, $Zn(NH_2CH_2COOCH(CH_3)_2)_2^{2+}$, $Zn(NH_2CH_2CH_2COOH)_2^{2+}$, $Zn(NH(CH_2COOH)_2)_2^{2+}$, $Zn(N(CH_2CH_2COOH)_3)_2^{2+}$, $Zn(NH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2CH_2CH_3)_4^{2+}$, $Zn(NH_2CH(CH_3)_2)_4^{2+}$, $Zn(NH_2C(CH_3)_3)_4^{2+}$, $Zn(NH_2CH_2C(CH_3)_3)_4^{2+}$, $Zn(NH(CH_3)_2)_4^{2+}$, $Zn(NH(CH_2CH_3)_2)_4^{2+}$, $Zn(NH(CH(CH_3)_2)_2)_4^{2+}$, $Zn(NH(C(CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH(CH_2CH_3)_2)_2)_4^{2+}$, $Zn(NH(CH_2C(CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH_2C(CH_2CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH_2CH_2C(CH_3)_3)_2)_4^{2+}$, $Zn(NH_2CH_2CH_2OH)_4^{2+}$, $Zn(NH(CH_2CH_2OH)_2)_4^{2+}$, $Zn(N(CH_2CH_2OH)_3)_4^{2+}$, $Zn(NH_2CH_2COOH)_4^{2+}$, $Zn(NH_2CH_2CONH_2)_4^{2+}$, $Zn(NH_2CH_2COOCH_3)_4^{2+}$, $Zn(NH_2CH_2COOCH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2COOC(CH_3)_3)_4^{2+}$, $Zn(NH_2CH_2COOCH(CH_3)_2)_4^{2+}$, $Zn(NH_2CH_2CH_2COOH)_4^{2+}$, $Zn(NH(CH_2COOH)_2)_4^{2+}$, $Zn(N(CH_2CH_2COOH)_3)_4^{2+}$,





All the complex cations above can be formed with an anion, selected from, but not limited, Cl^-, Br^-, I^-, SO_4^{2-}, HSO_4^-, NO_3^-, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, CO_3^{2-}, HCO_3^-, $HCOO^-$, CH_3COO^-, F_3CCOO^-, Cl_3CCOO^-, FCH_2COO^-, $ClCH_2COO^-$, F_2CHCOO^-, Cl_2CHCOO^-, BF_4^-, SO_3^{2-}, HSO_3^-, CH_3SO^{3-}, $C_6H_5CH_2SO^{3-}$, $C_6H_5SO^{3-}$, $C_6H_5COO^-$, $C_6H_5CH_2COO^-$, $C_6F_5O^-$, $C_6H_4(OH)COO^-$, $C_6H_2F_3O^-$, $C_6H_4(NO_2)O^-$, $C_6H_2(NO_2)_3O^-$, etc.

The zinc cation-amino complex in the reaction solution are 0.5 ~ 20 equivalents of the antibody, preferably 1.0 -5.0 equivalents of the antibody, more preferably 1.5 -3.0 equivalents of the antibody. The transition metal cation-amino complex can be added to the reaction solution

with a water- miscible organic solvent, selected from, but not limited, ethanol, methanol, propanol, propandiol, DMA, DMF, DMSO, THF, or CH_3CN.

The reductant used in the reaction solution with the transition metal cation-amino complex is selected from Tris(2-carboxyethyl)phosphine (TCEP), ($P(CH_2CH_2COOH)_3$). It can be other reductants, such as Tris(hydroxypropyl)-phosphine ($P(CH_2CH_2CH_2OH)_3$), $P(CH_2CH_3)_3$, $P(CH_2CH_2CH_3)_3$, $P(CH_2CH_2.CH_2CH_3)_3$, $P(CH(CH_3)_2)_3$, $P(CH_2CH=CH_2)_3$, $P(CH_2CH_2CN)_3$, $P(CH(CH_3)_2)_2(CH_2CH_2NH_2)$, $P(CH_2CH_2CONH_2)_3$, $P(CH_2CH_2CONHCH_3)_3$, $P(CH_2CH_2CH_2NHCOCH_3)_3$, $NaB(CN)H_3$, $(C_6H_{11})_2P(CH_2)_4P(C_6H_{11})_2$, $(C_6H_{11})_2P(CH_2)_3P$-$(C_6H_{11})_2$, Dicyclohexyl(ethyl)phosphine, Bis[2-(di-*tert*-butylphosphino)ethyl]amine, Tricyclohexylphosphine, 1, 2-Ethanediylbis[dicyclohexyl]-phosphine, Bis[2-(dicyclohexylphosphino)ethyl]amine, Tris[2-(diphenylphosphino)ethyl]-phosphine ($[(C_6H_5)_2PCH_2CH_2]_3P$), triphenylphosphine, sulfonylated triphenylphosphines (2-(diphenylphosphino)benzenesulfonic acid (diPPBS), 3-(diphenylphosphino)benzenesulfonic acid, 4-(diphenylphosphino)benzenesulfonic acid, 3, 3', 3''-phosphinetriyltribenzenesulfonic acid). Preferably the reductant is selected from TECP or $P(CH_2CH_2CH_2OH)_3$, and more preferably the reductant is selected from TECP. And the concentration of the reductant in the reaction solution may be 0.04 mM – 0.4 mM, or 1.0 - 10.0 equivalents of antibody used in the reaction. Preferably the reductant is used at 2.0 - 4.0 equivalents of an antibody.

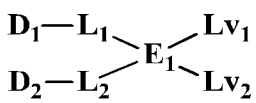
The optimum buffer for conduction of the selective reduction is selected from, but not limited, PBS, Mes, Bis-Tris, Bis-Tris Propane, Pipes, Aces, Mopso, Bes, Mops, Hepes, Tes, Pipps, Dipso, Tapso, Heppso, Tris-up, Tris-HCl, Tricine, Hepps, Gly-Gly, Bicine, Taps, Hepee, Acetates, Histidine, Citrates, MES, Borates, or combinations two, three or four buffer components from above. And the pH of the buffer is selected 4.0 -9.0, preferred 5.0 -7.5, more preferred 5.5 -7.5. The concentration of the buffer in the reaction is 0.02 – 1.0 M, preferably 20 – 200 mM, more preferably 20 – 100 mM. And up to 30% of water mixable (miscible) organic solvents, selected from DMA, DMF, ethanol, methanol, acetone, acetonitrile, THF, isopropanol, dioxane, propylene glycol, or ethylene diol can be added as the co-solvent in water based buffer solution;

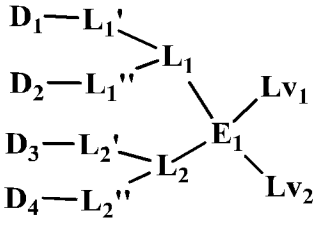
The optimum temperature for the reduction reaction is typically controlled between about -5 and 40 °C, and the reaction time is 15 minutes to 48 hours. But it is well-understandable in the field of protein conjugation that the reaction time and temperature can be determined by those skilled in the art based on the specific protein, in particular, the antibody to be conjugated. For example a preferable reduction reaction can be controlled at a temperature typically between about -5 to about 40 °C, and preferably, about 0 to 37 °C; more preferably

about 2 to 8 °C, and more precisely 4 ± 2 °C. The process of the conjugation is 15 min to 12 hours, and more preferably at a temperature between about 2 and 8 °C, and the process time is about 30 min to 15 hours (overnight).

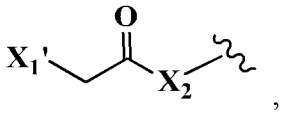
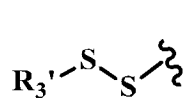
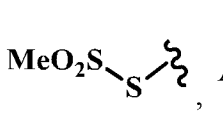
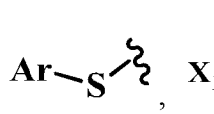
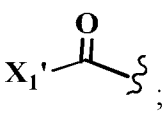
During the reduction, or after the reduction, a Drug/linker complex/assembly is directly added to the solution of the reduction reaction for conjugation. The Drug/linker complex/assembly, having a formula (I) or (II) represented as:

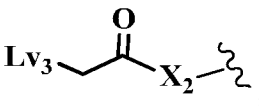
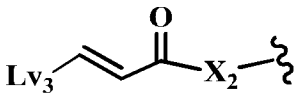
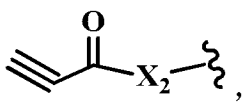
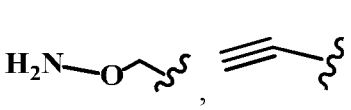

(I), or

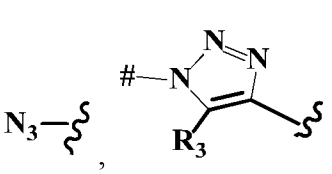
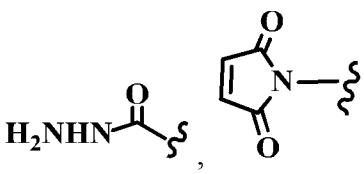
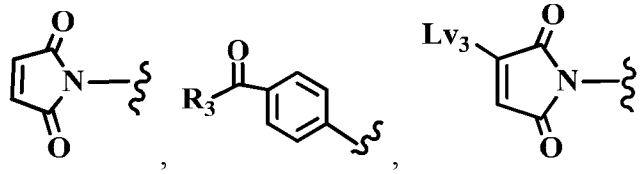
(II), or

(III), or

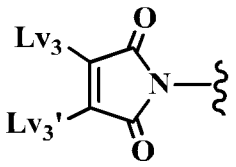
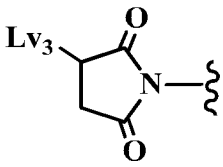

(IV),

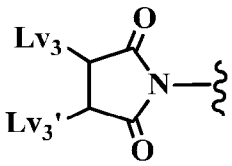
wherein: Lv_1 and Lv_2 are a thiol reaction group, and are independently or jointly selected from:

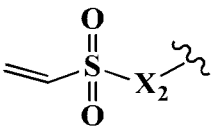
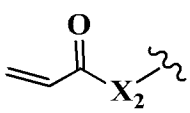
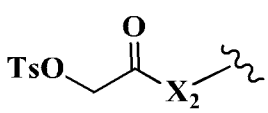
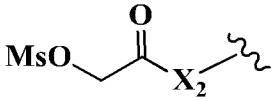
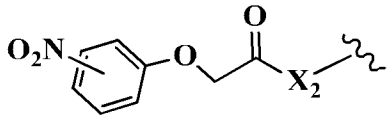

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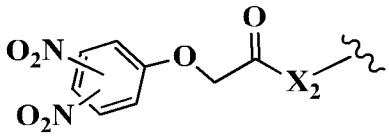

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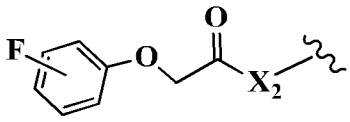
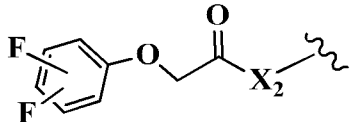
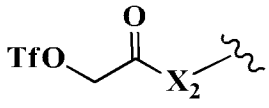

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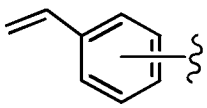
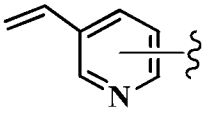
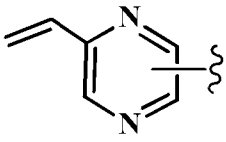
monosubstituted maleimide;

disubstituted maleimide;


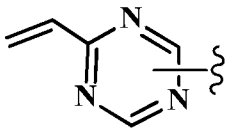
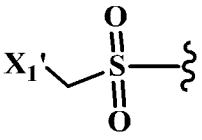
monosubstituted succinimide;

disubstituted succinimide; -CHO aldehyde;

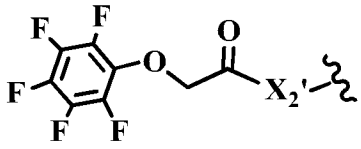
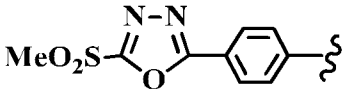

ethenesulfonyl;

acryl (acryloyl);

2-(tosyloxy)acetyl;

2-(mesyloxy)acetyl;

2-

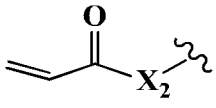
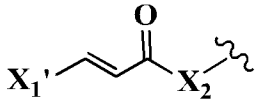
(nitrophenoxy)acetyl;  2-(dinitrophenoxy)acetyl;

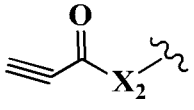
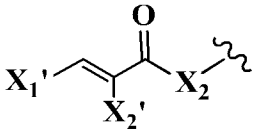
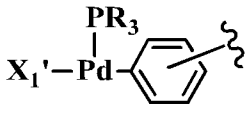
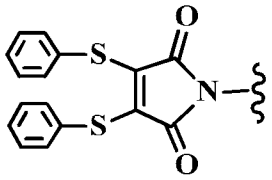
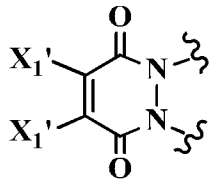
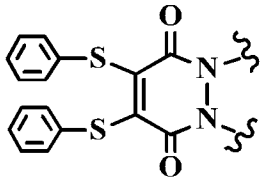
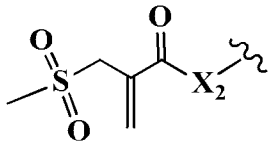
 2-(fluorophenoxy)-acetyl;  2-(difluorophenoxy)-acetyl;  2-(((trifluoromethyl)-sulfonyl)oxy)acetyl;

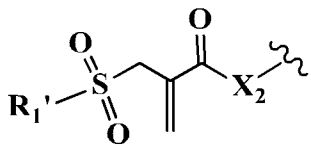
 styrene,  vinylpyridine,  vinylpyrazine,

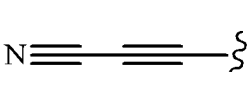
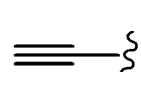
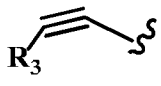
 vinyl-1, 3, 5-triazine,  substituted methylsulfonyl,

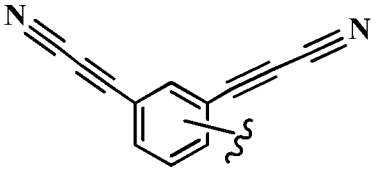
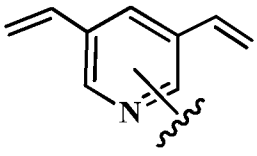
 2-(pentafluorophenoxy)acetyl;  ,

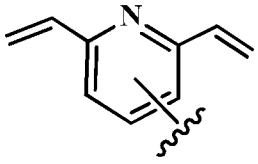
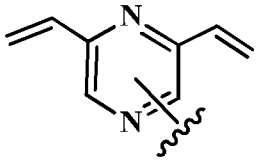
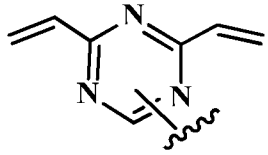
methylsulfonephenyloxadiazole (ODA);  acryl,  halo acryl,

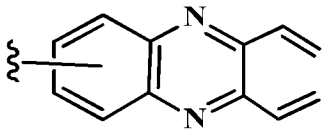
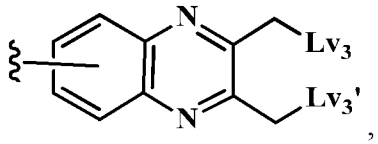
 propiol,  2, 3-dihaloacryl,  Aryl-palladium complex,  bis(phenylsulfanyl)-maleimides,  bis-halide-pyridazinediones,  bis-phenylsulfanyl-pyridazinedione, 

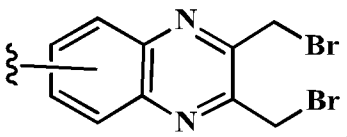
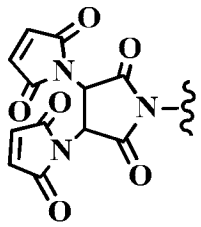
 2-((methylsulfonyl)methyl)acryl, 2-((alkyl or aryl-sulfonyl)methyl)acryl,

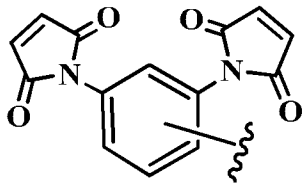
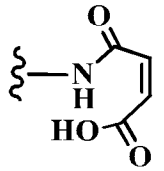
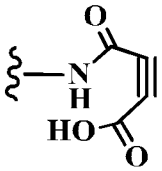
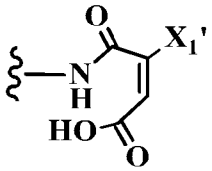
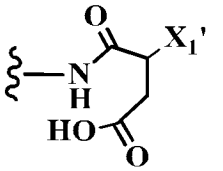
 cyanoethynyl,  ethynyl;  alkynyl,

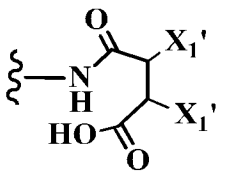
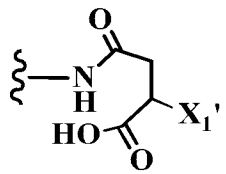
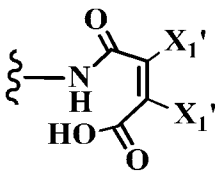
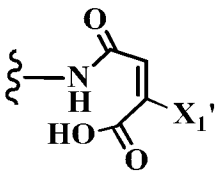
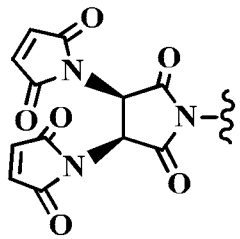
 arylenedipropiolonitrile (ADPN),  or

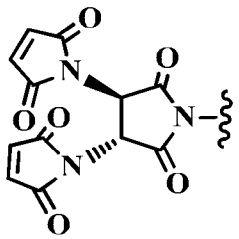
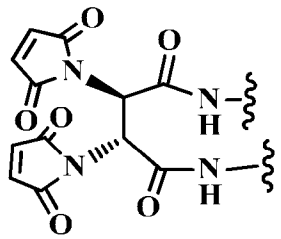
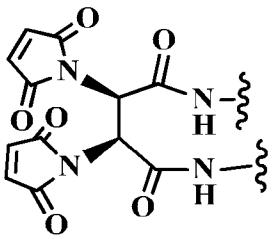
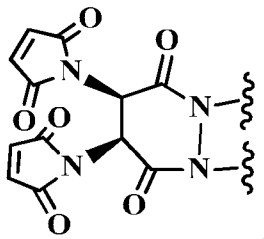
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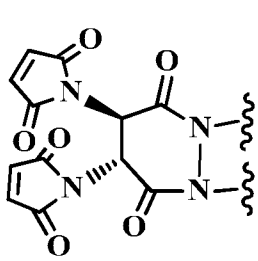
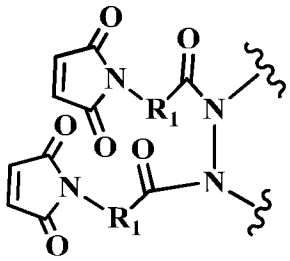
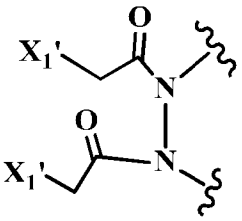
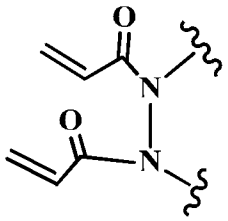
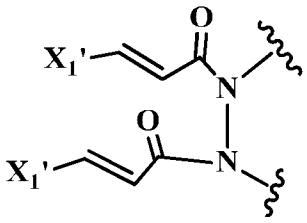
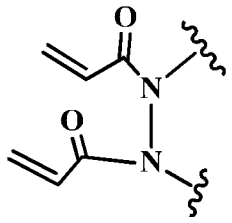
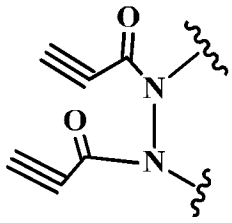
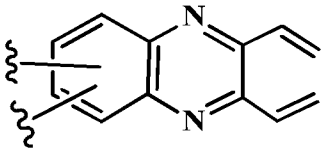
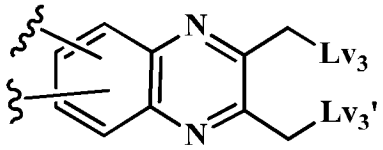
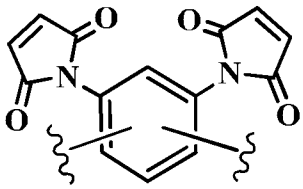
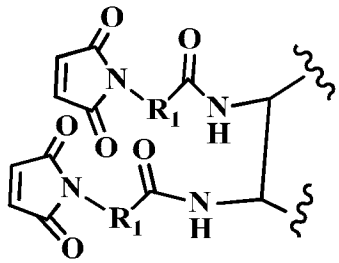
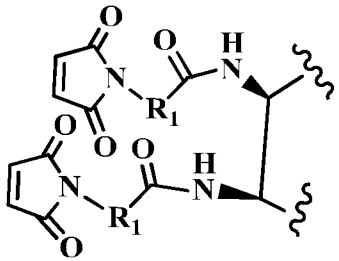
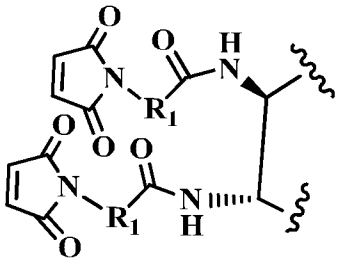
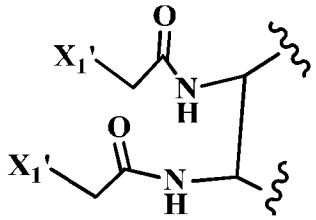
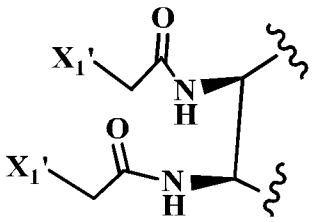
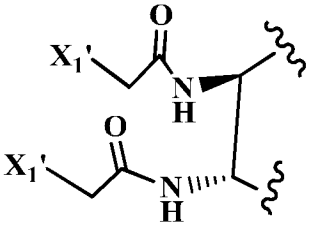
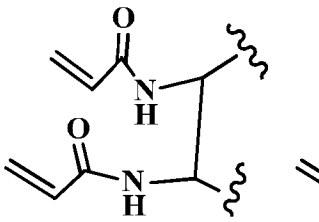
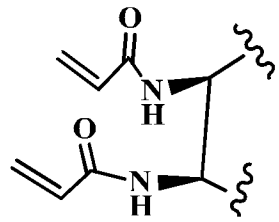
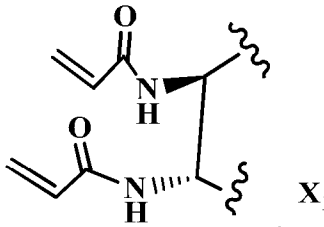
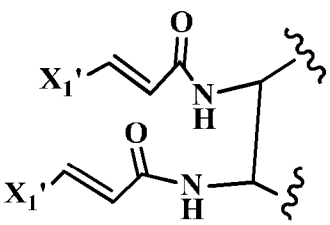
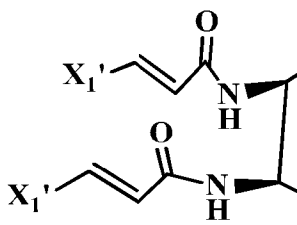
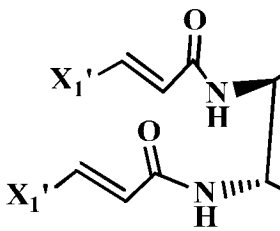
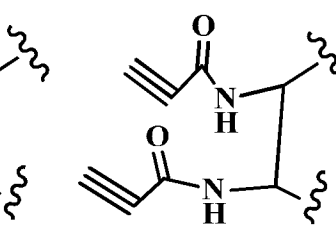
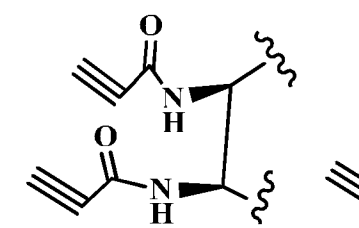
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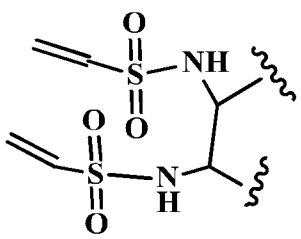
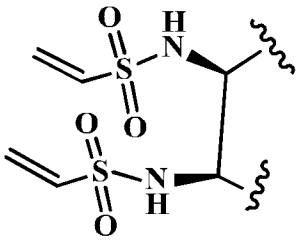
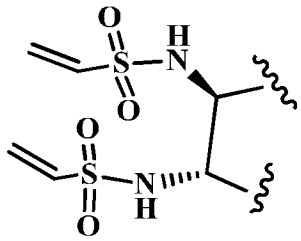
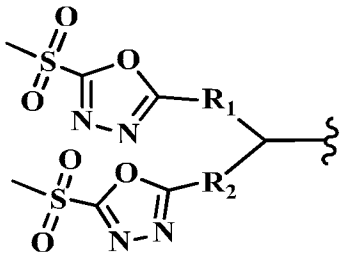
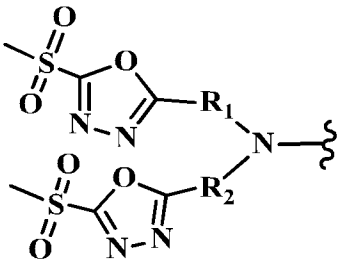
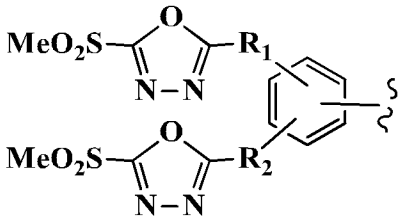
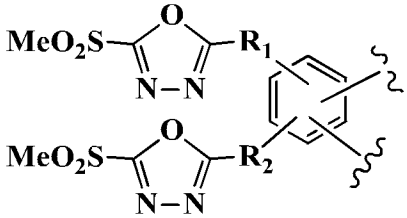
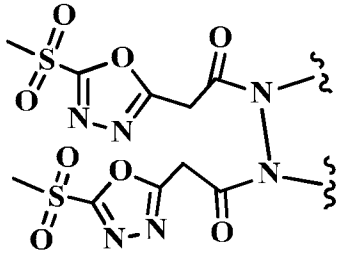
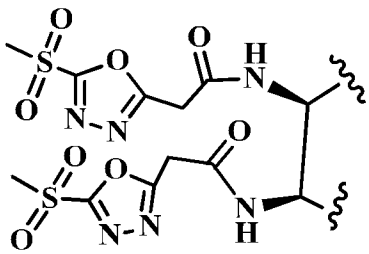
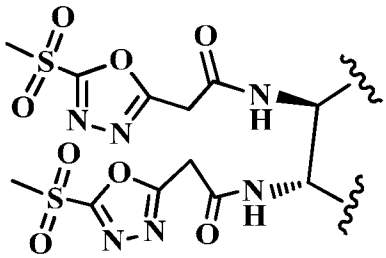
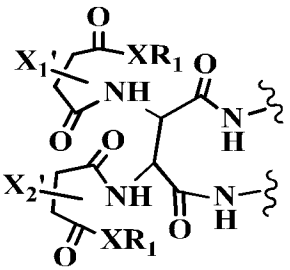
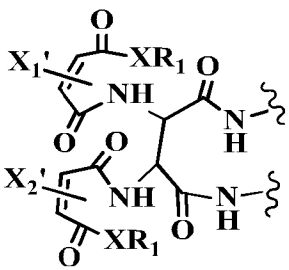
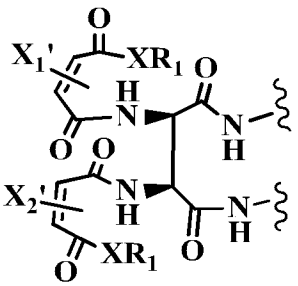
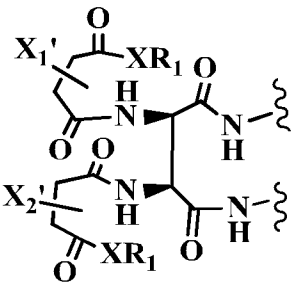
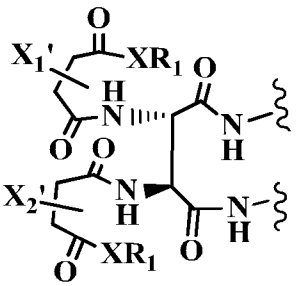
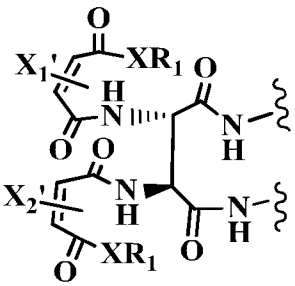
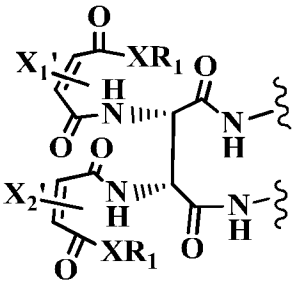
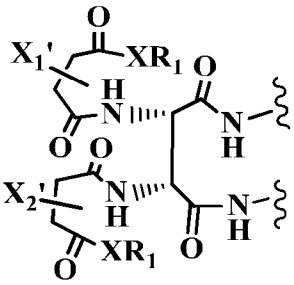
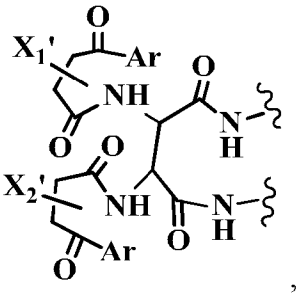
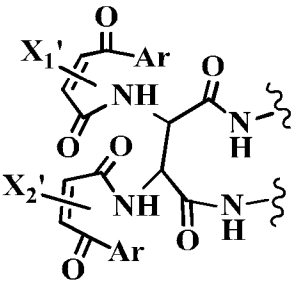

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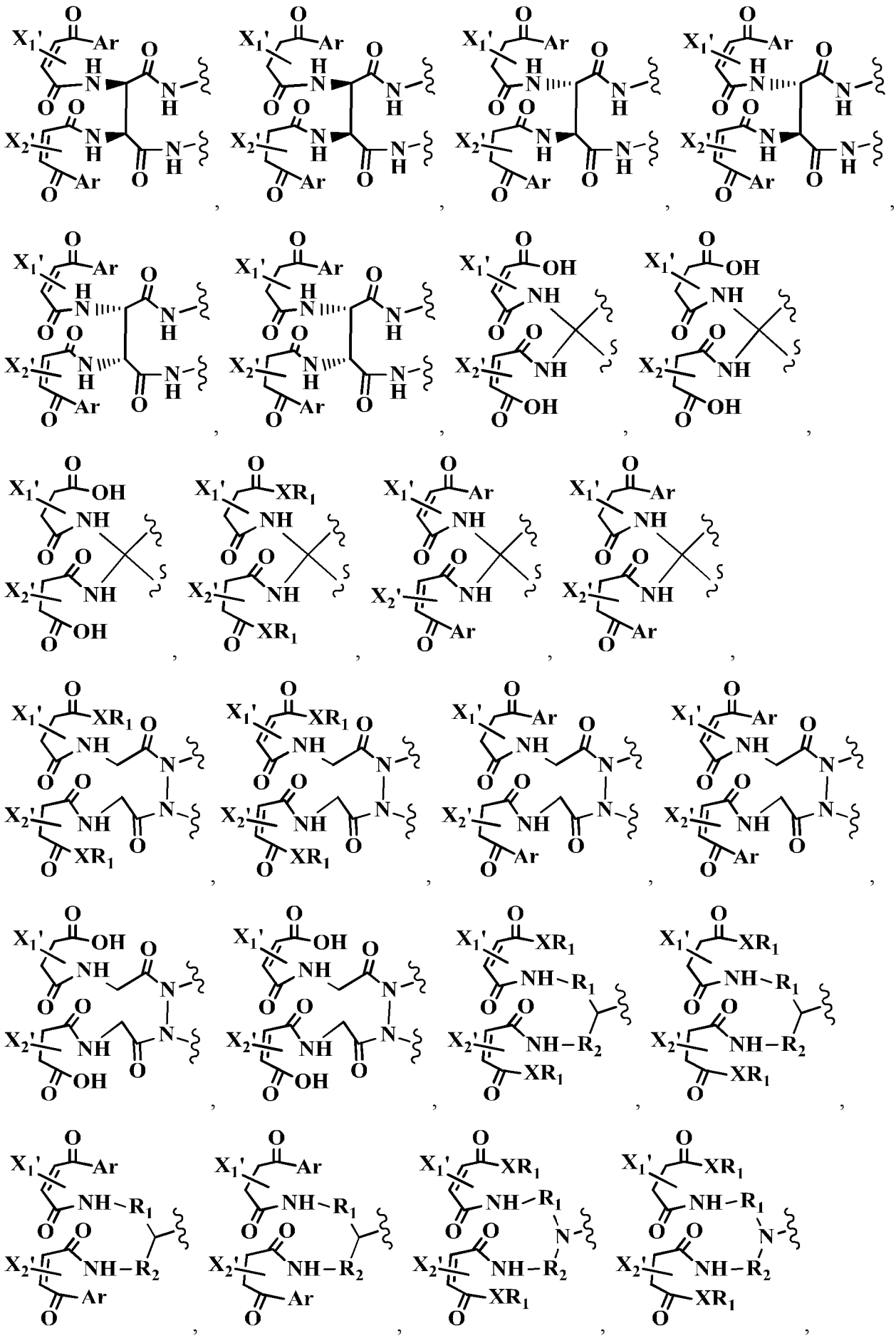
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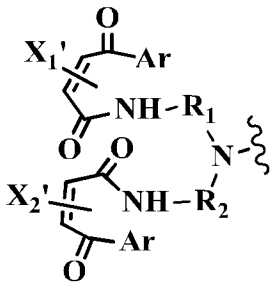
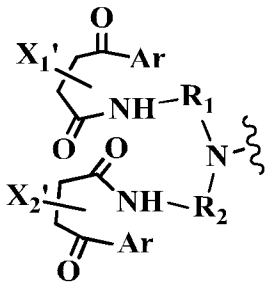
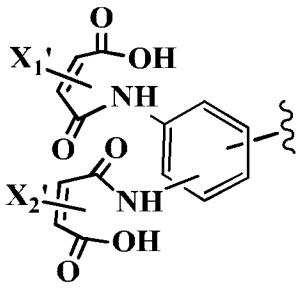
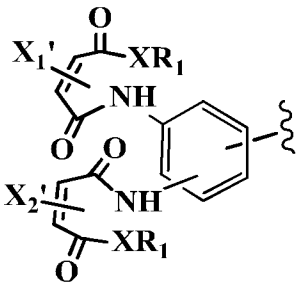
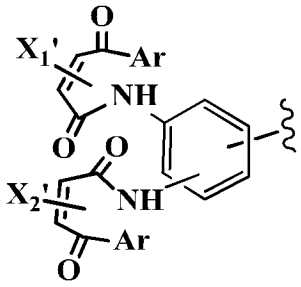
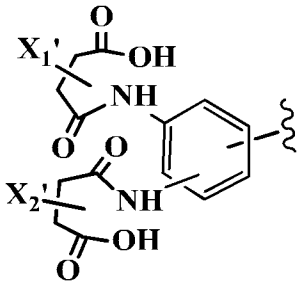
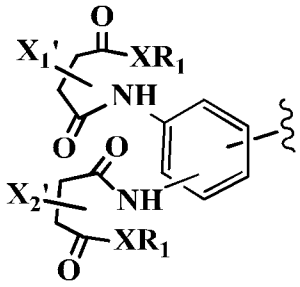
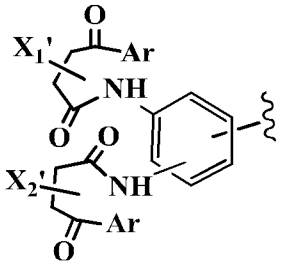
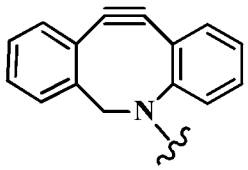
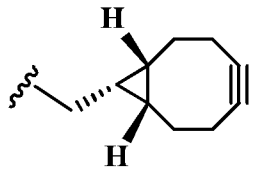
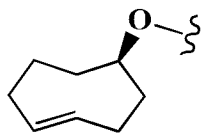
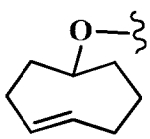
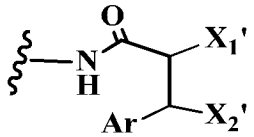
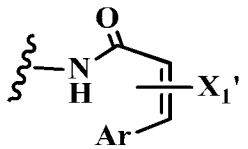
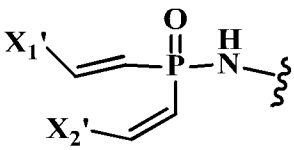
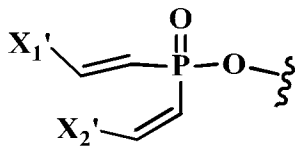
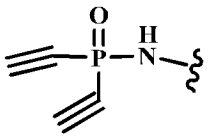
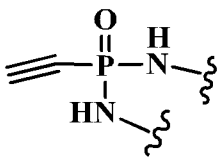
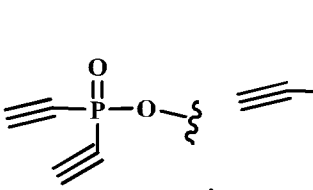
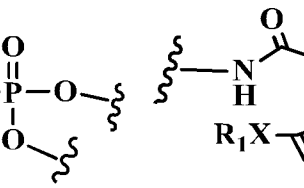
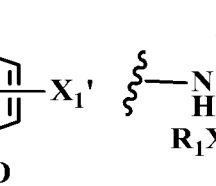
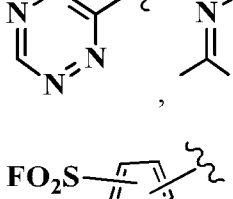
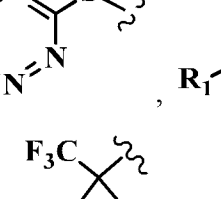
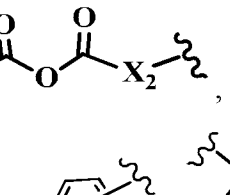
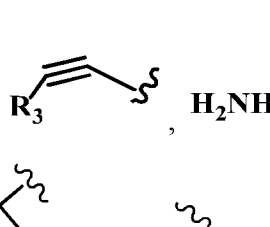
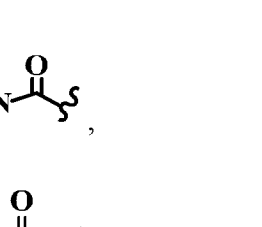
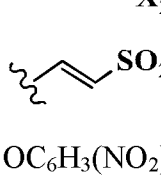
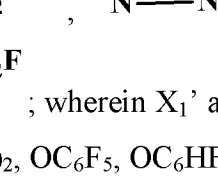
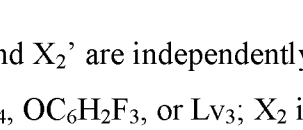
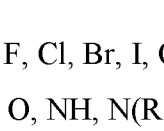
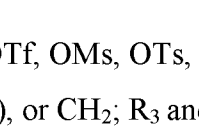
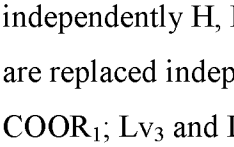
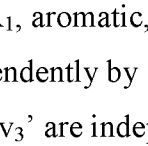
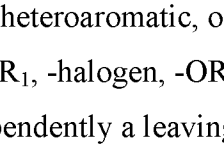
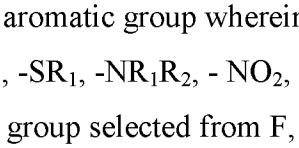
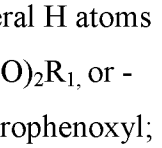
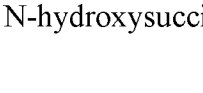
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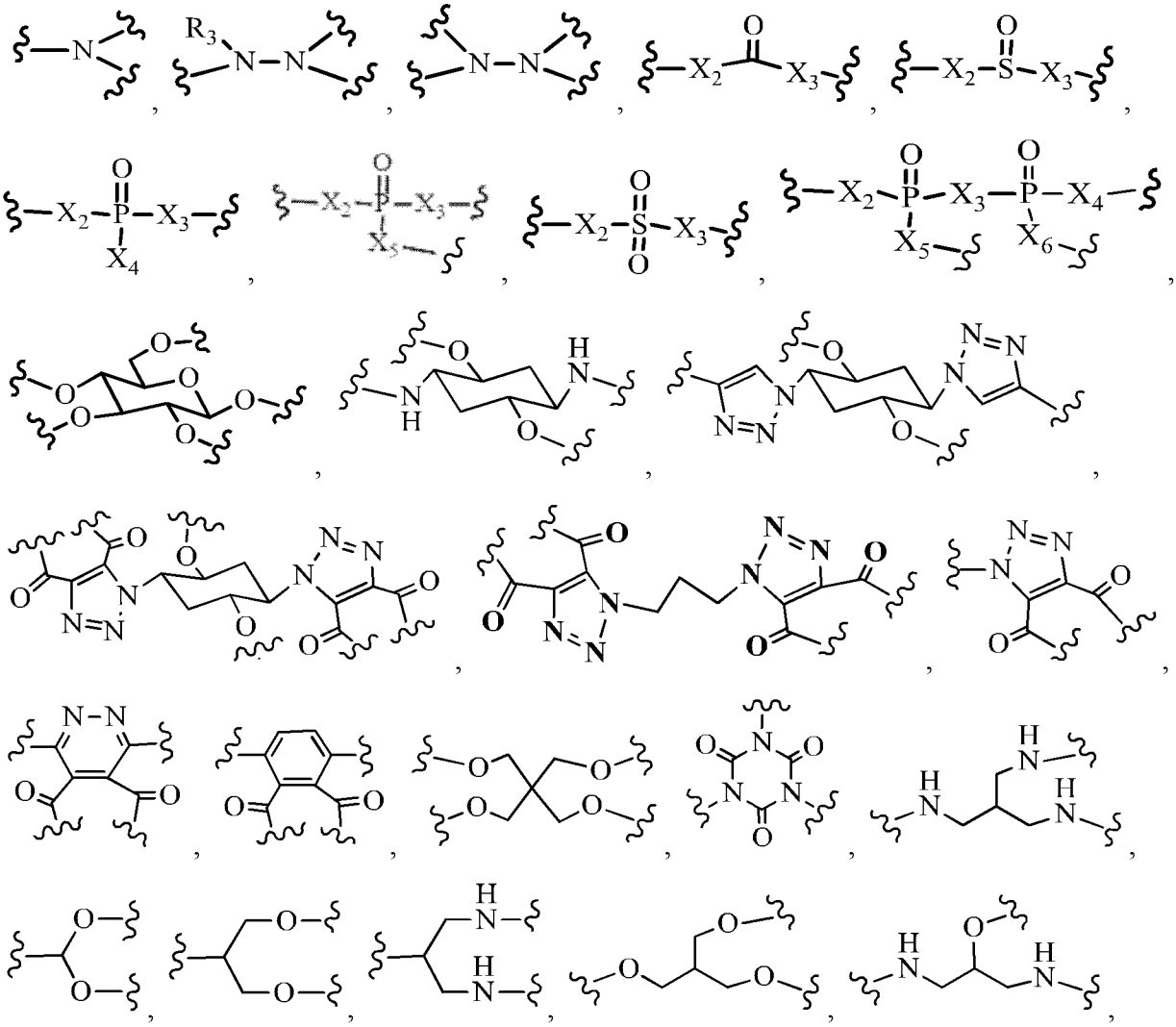


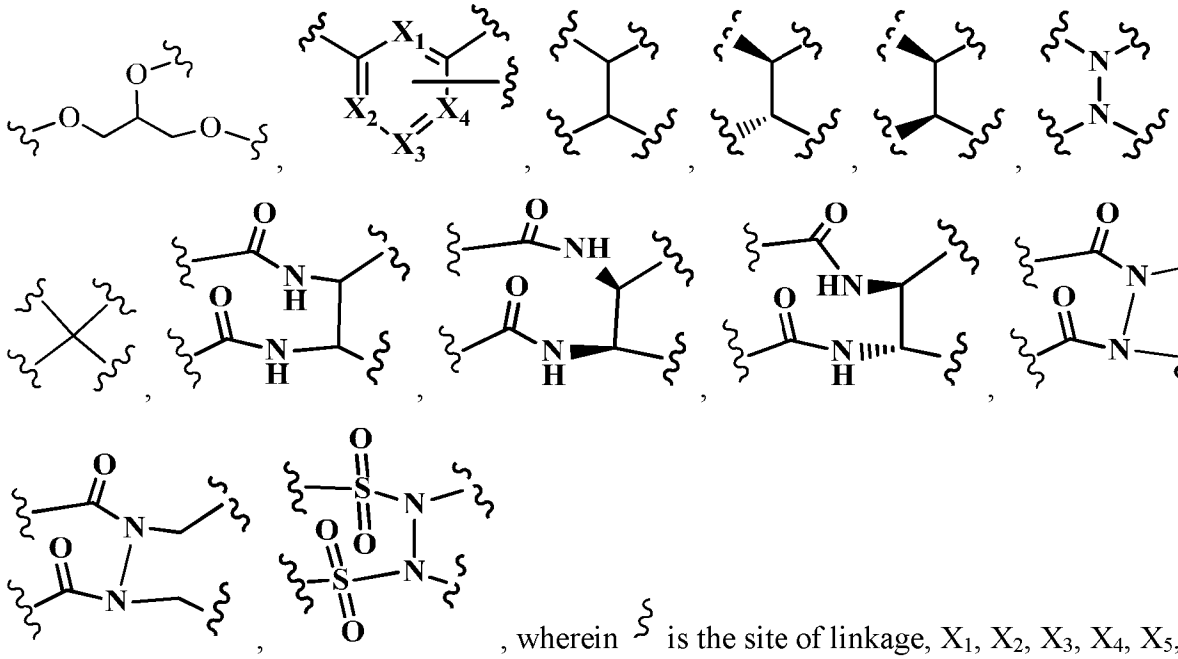






































; wherein X_1' and X_2' are independently F, Cl, Br, I, OTf, OMs, OTs, $OC_6H_4(NO_2)$, $OC_6H_3(NO_2)_2$, OC_6F_5, OC_6HF_4, $OC_6H_2F_3$, or Lv_3; X_2 is O, NH, N(R_1), or CH_2; R_3 and R_5 are independently H, R_1, aromatic, heteroaromatic, or aromatic group wherein one or several H atoms are replaced independently by -R_1, -halogen, -OR_1, -SR_1, -NR_1R_2, - NO_2, -$S(O)R_1$, -$S(O)_2R_1$, or -$COOR_1$; Lv_3 and Lv_3' are independently a leaving group selected from F, Cl, Br, I, nitrophenoxyl; N-hydroxysuccinimide (NHS); phenoxyl; benzenethiol, dinitrophenoxyl; pentafluorophenoxyl;

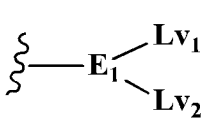
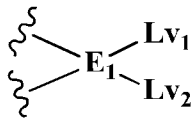
tetrafluorophenoxyl; difluorophenoxyl; monofluorophenoxyl; pentachlorophenoxyl; triflate; imidazole; dichlorophenoxyl; tetrachlorophenoxyl; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3′-sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions or for Mitsunobu reactions;

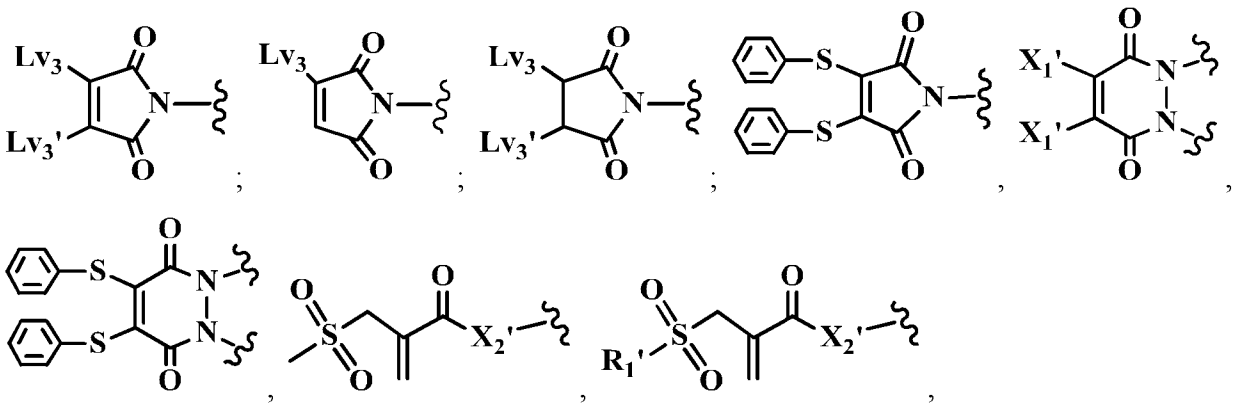
E_1 is a joint group that link two thiol reactive groups of Lv_1 and Lv_2. E_1 is selected from CH, CH_2, NH, NHNH, $N(R_3)$, $N(R_3)N(R_{3'})$, N=N, N-N, P, P(=O), S, Si, C_2-C_8 of alkyl, heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; a peptide containing1~4 units of aminoacids, preferably selected from aspartic acid, glutamic acid, arginine, histidine, lysine, serine, threonine, asparagine, glutamine, cysteine, selenocysteine, tyrosine, phenylalanine, glycine, proline, tryptophan, alanine; or one of the following structures:

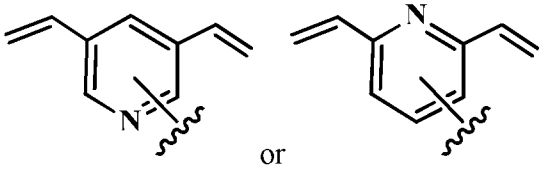


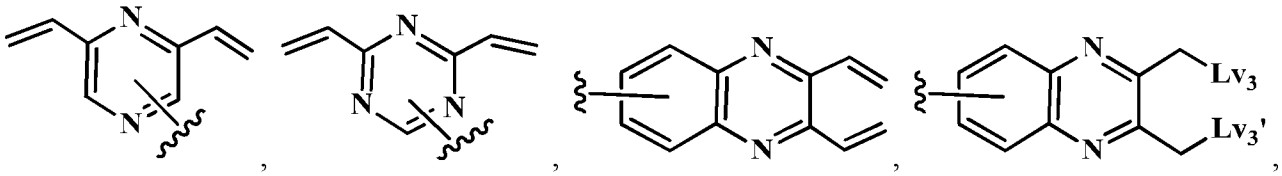


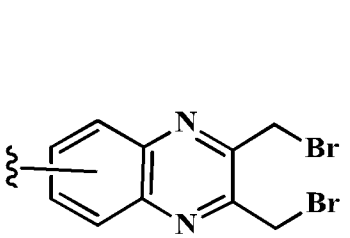
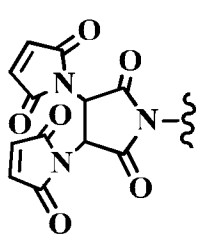
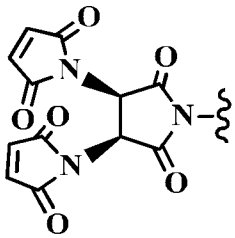
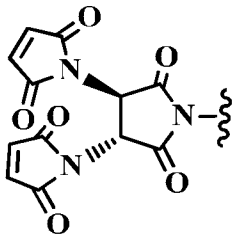
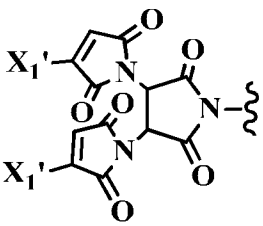
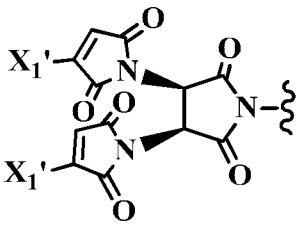
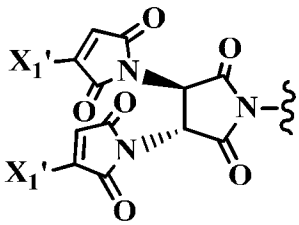
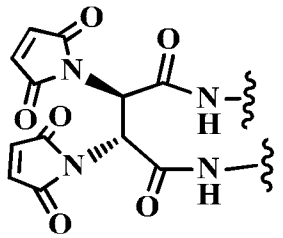
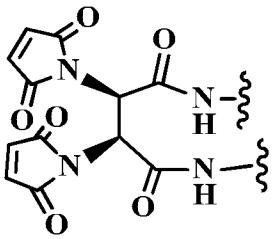
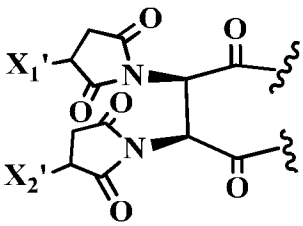
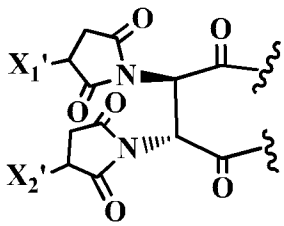
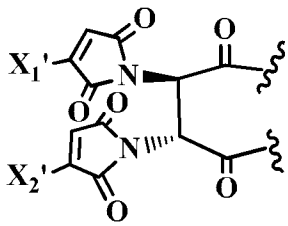
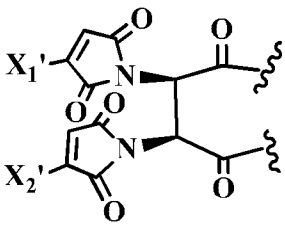
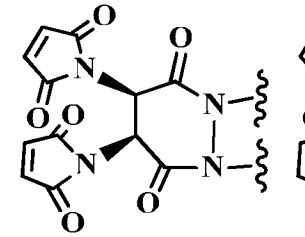
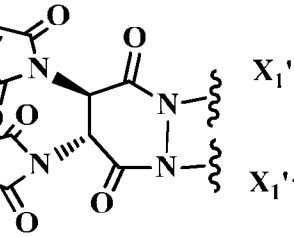
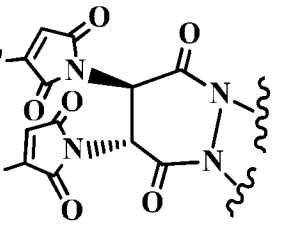
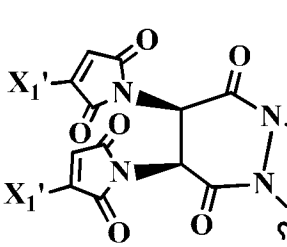
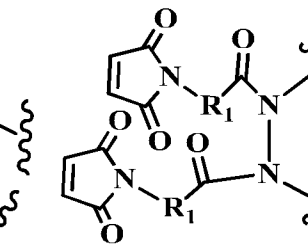
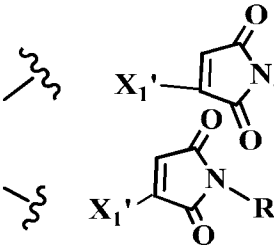
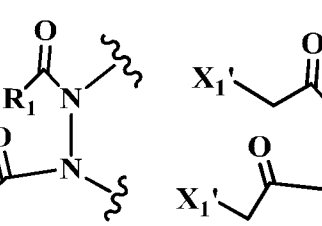
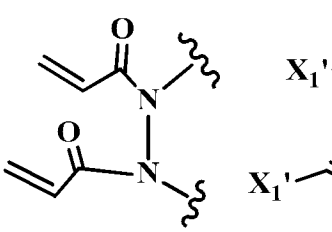
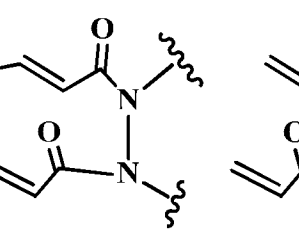
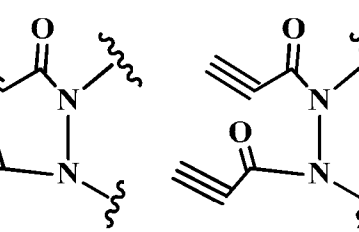
, wherein ~ is the site of linkage, X_1, X_2, X_3, X_4, X_5, and X_6 are independently selected from O, NH, S, N(R_3), CH_2; R_3 is C_1-C_6 alkyl.

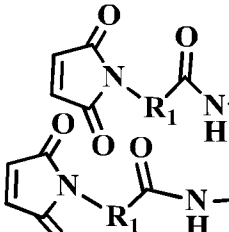
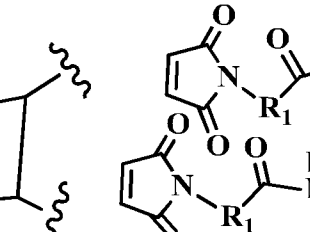
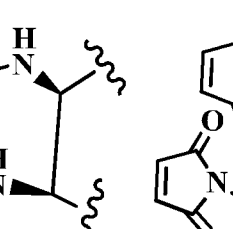
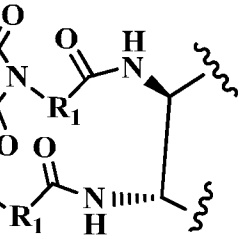
In the formula (II), formula (III) and formula (IV) wherein  and  can be selected from:

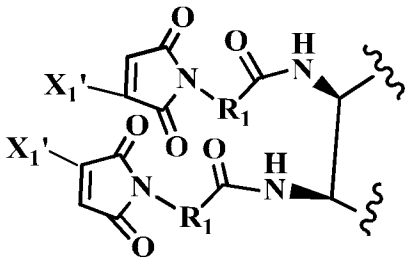
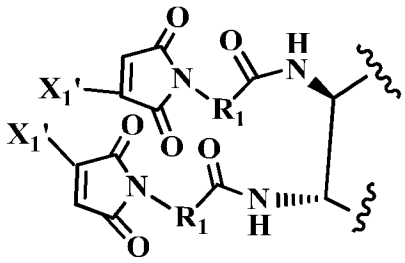
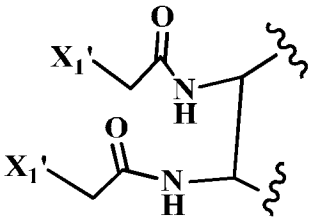
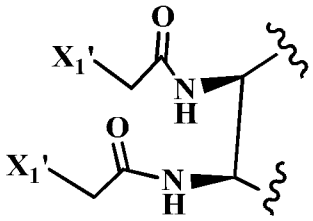
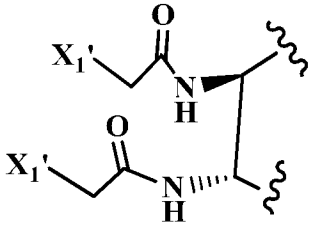
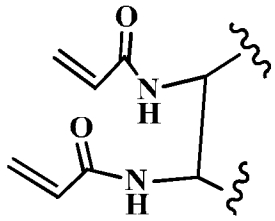
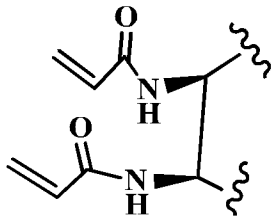
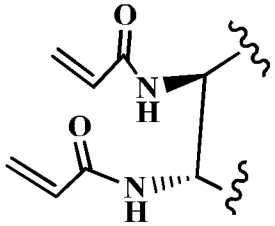
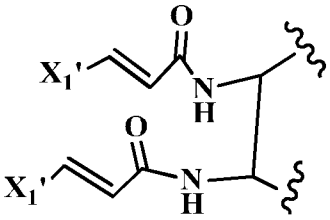
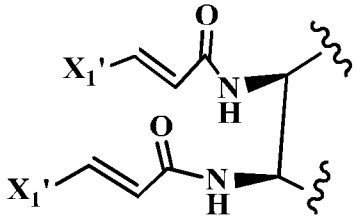
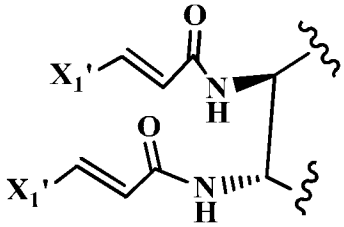
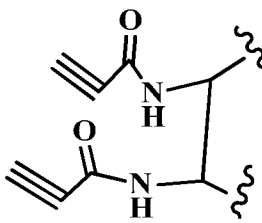
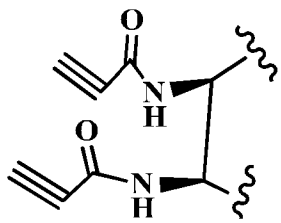
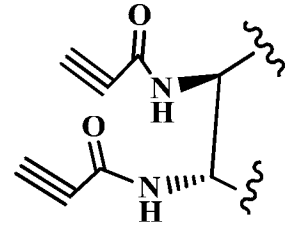
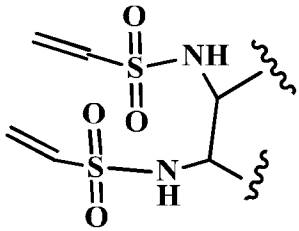
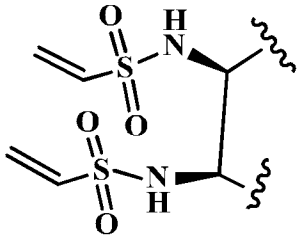
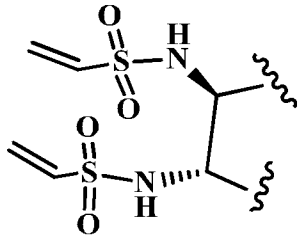
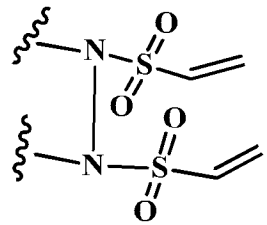
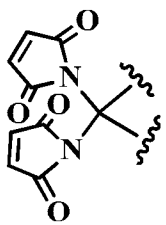
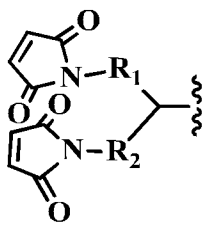
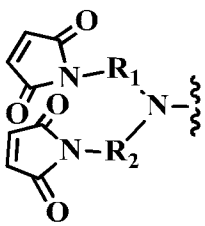
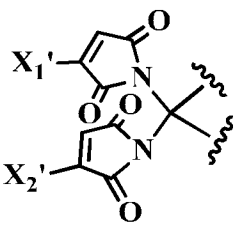
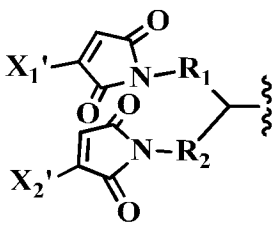
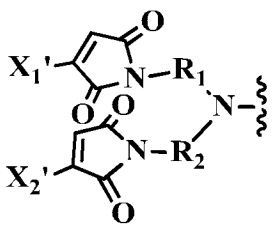
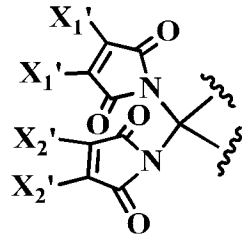
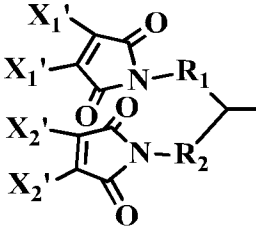
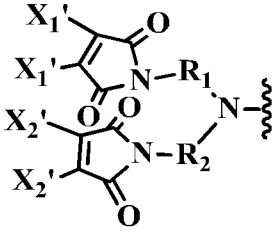


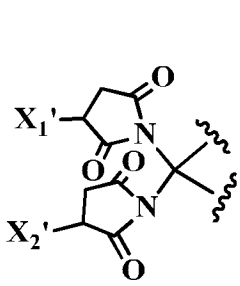
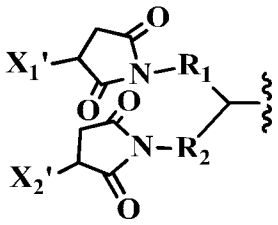
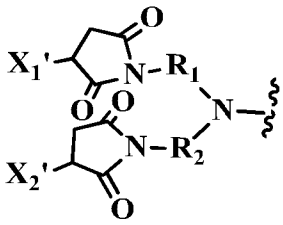
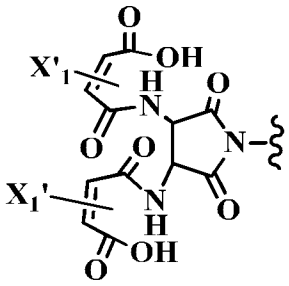
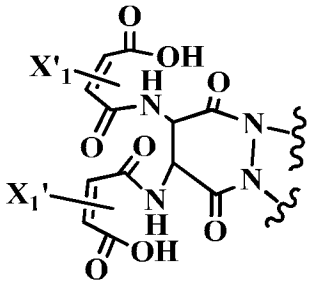
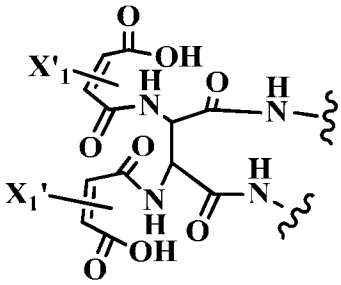
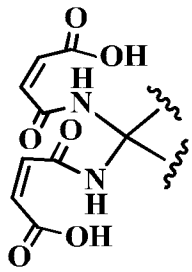
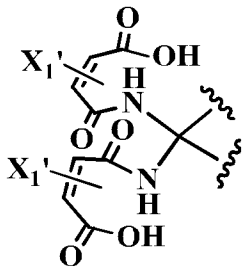
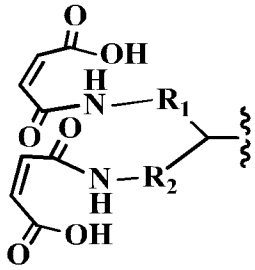
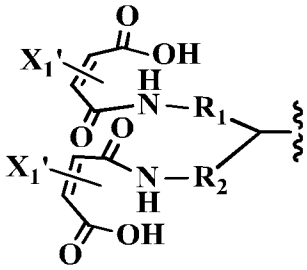
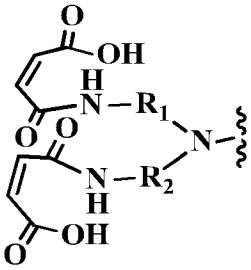
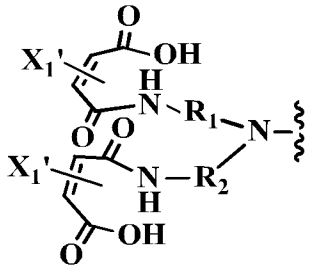
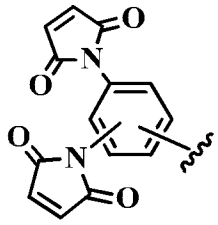
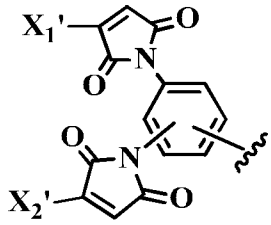
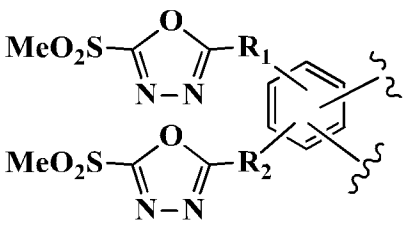
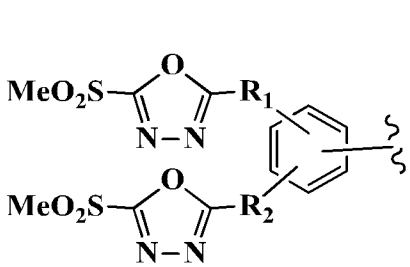
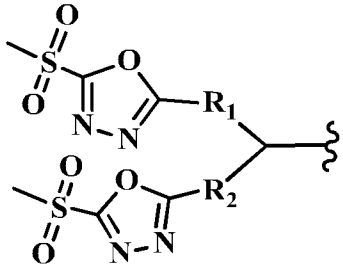
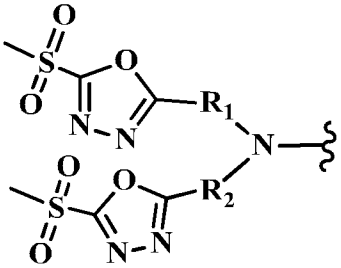
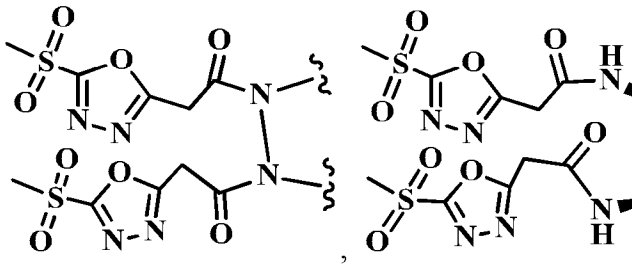
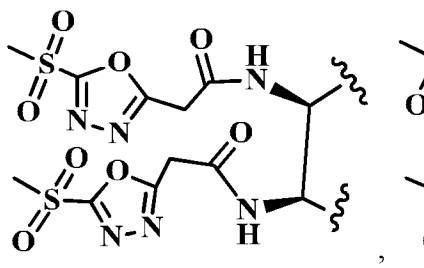
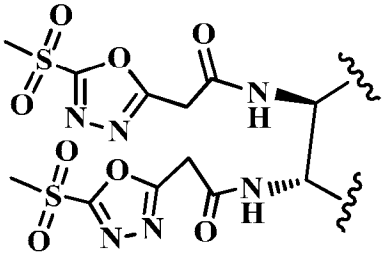
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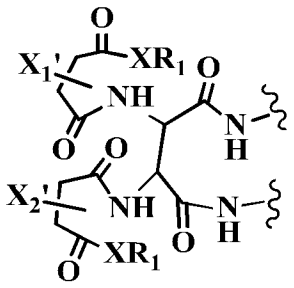
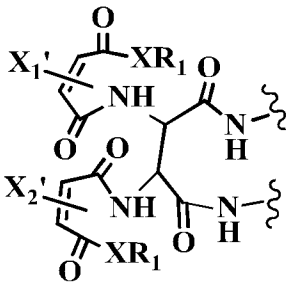
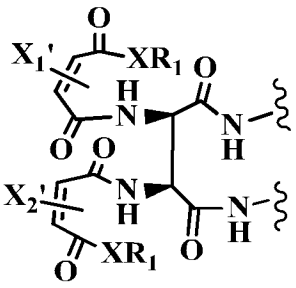
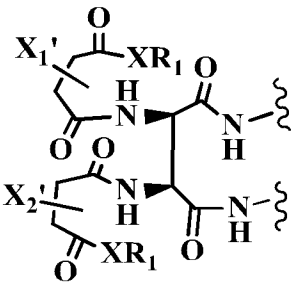
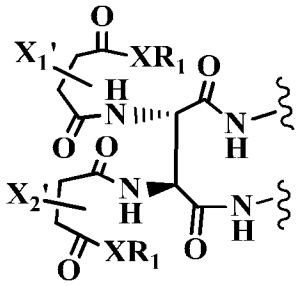
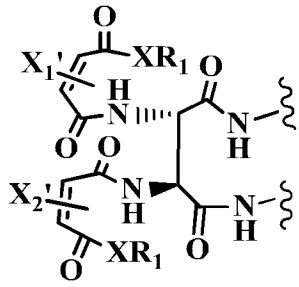
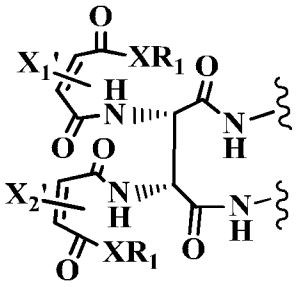
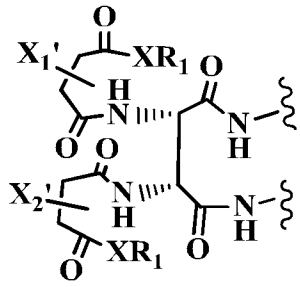
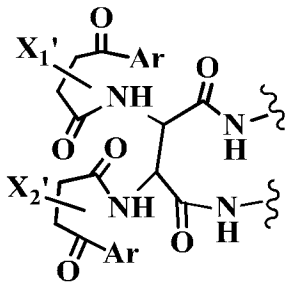
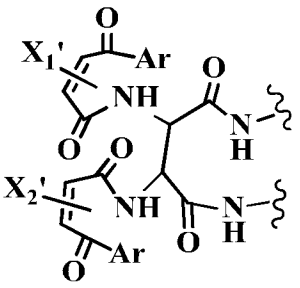
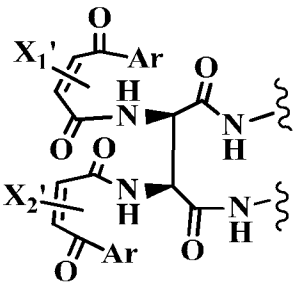
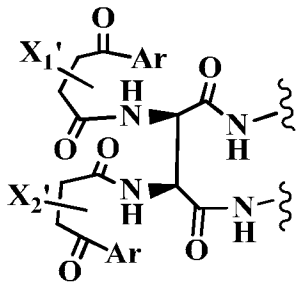
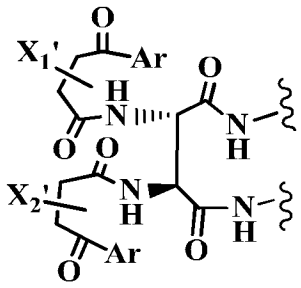
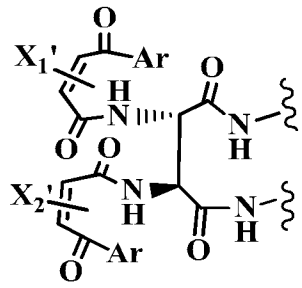
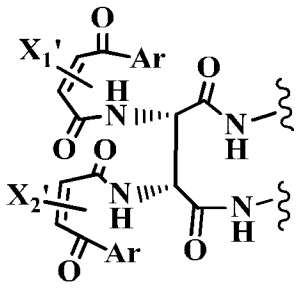
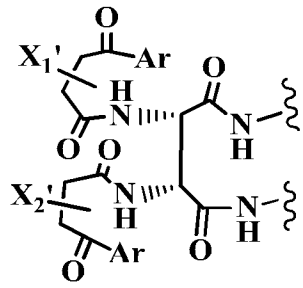
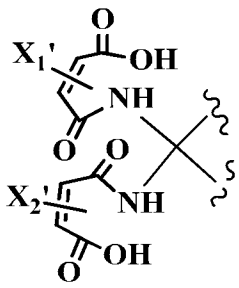
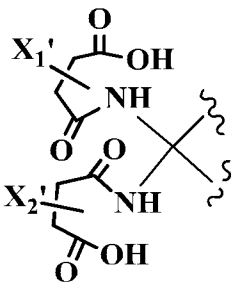
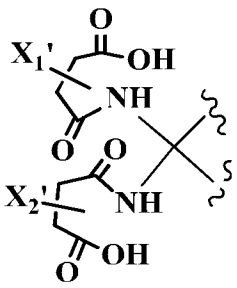
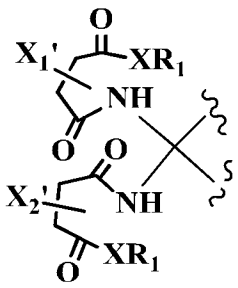
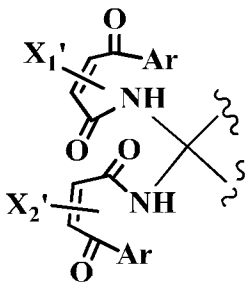
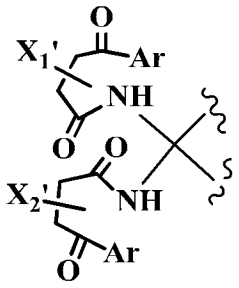
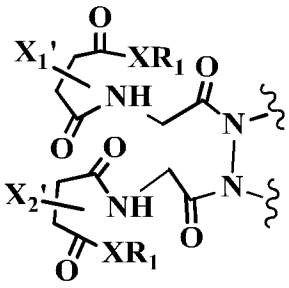
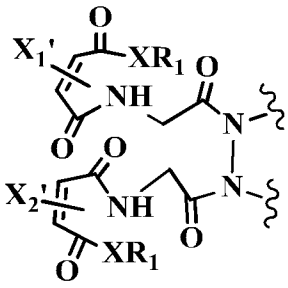
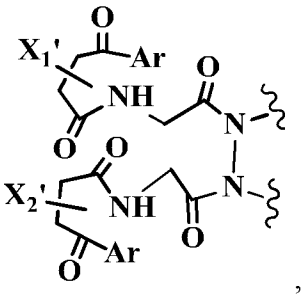


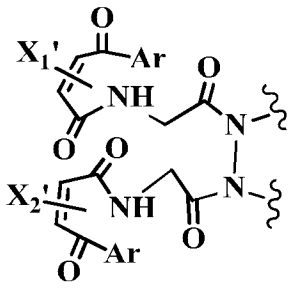
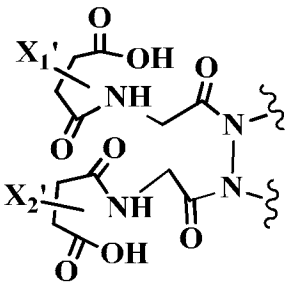
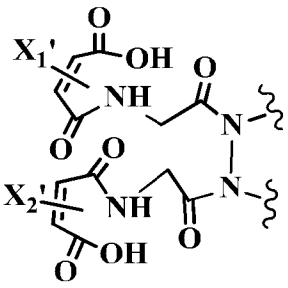
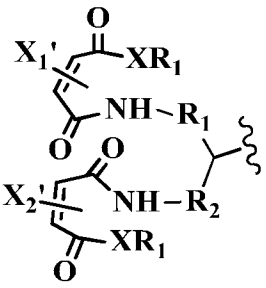
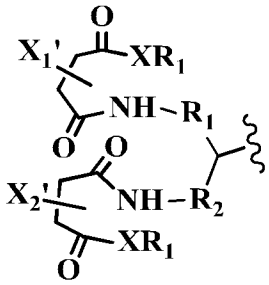
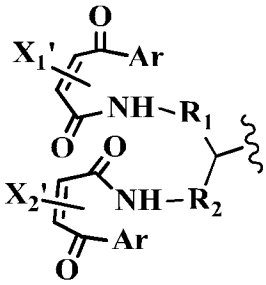
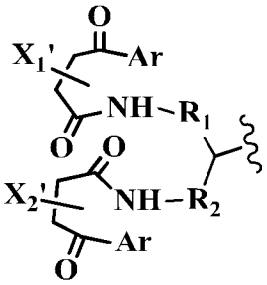
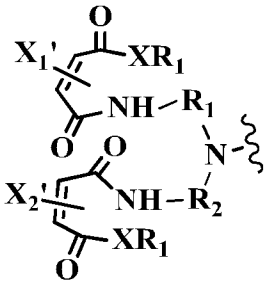
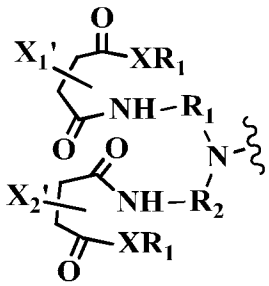
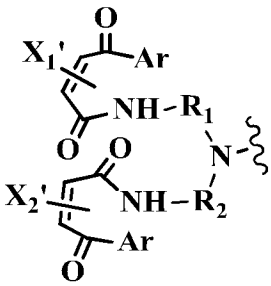
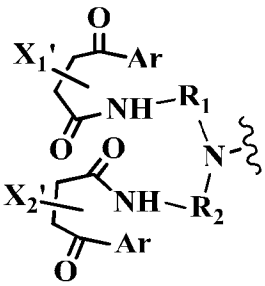
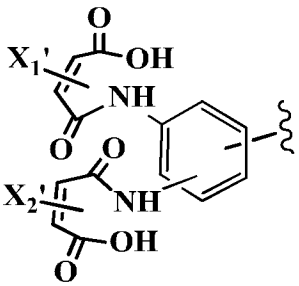
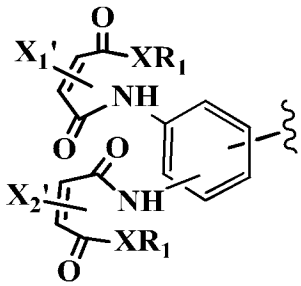
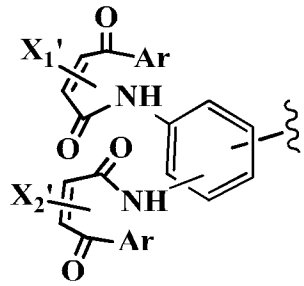
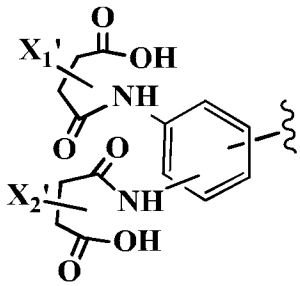
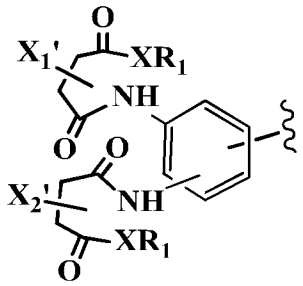
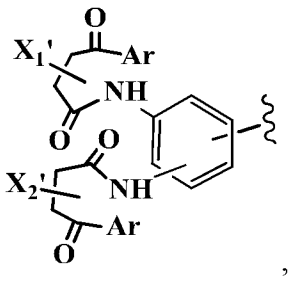
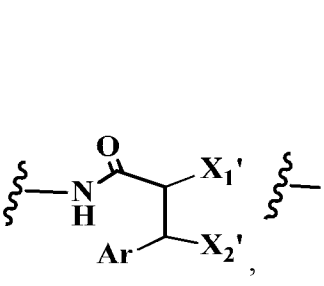
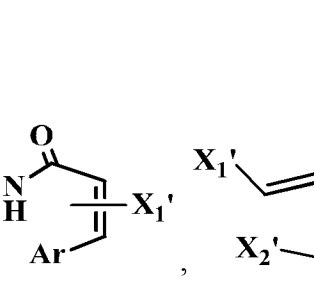
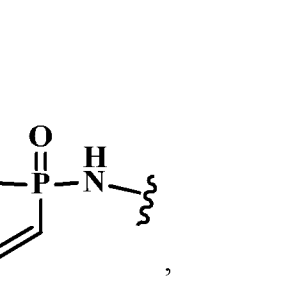
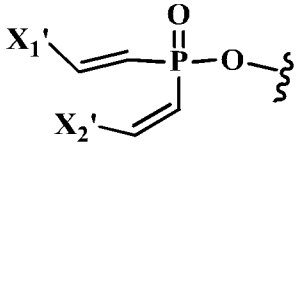
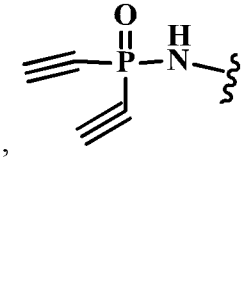
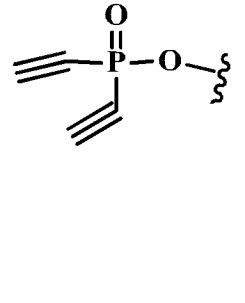
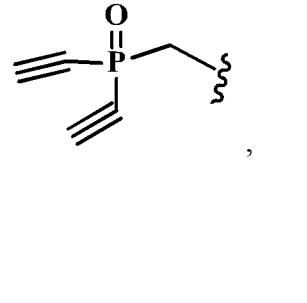

























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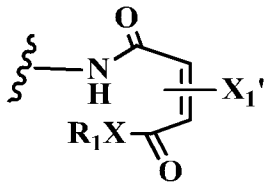
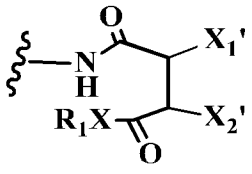

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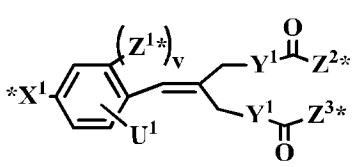
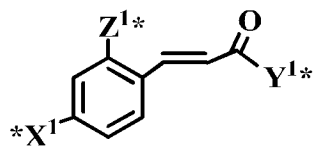
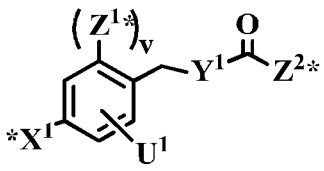



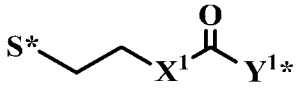
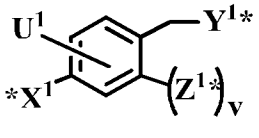
, wherein Lv_3, Lv_3', X_1' and X_2'are described above; the connecting bond "—" in the middle of the two atoms means it can link either one of the two atoms;

Wherein L_1 and L_2 are, the same or different, independently selected from O, NH, S, NHNH, $N(R_3)$, $N(R_3)N(R_{3'})$, polyethyleneoxy unit of formula $(OCH_2CH_2)_pOR_3$, or $(OCH_2CH(CH_3))_pOR_3$, or $NH(CH_2CH_2O)_pR_3$, or $NH(CH_2CH(CH_3)O)_pR_3$, or $N[(CH_2CH_2O)_pR_3][(CH_2CH_2O)_{p'}R_{3'}]$, or $(OCH_2CH_2)_pCOOR_3$, or $CH_2CH_2(OCH_2CH_2)_pCOOR_3$, wherein p and p' are independently an integer selected from 0 to about 1000, or combination thereof; C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; Wherein R_3 and $R_{3'}$ are independently H; C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 1-8 carbon atoms of esters, ether, amide or aminoalkylcarboxyl; or 1~8 natural or unnatural amino acids or peptides described in the definition; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000, or combination above thereof.

Wherein L_1 or L_2 may contain a self-immolative or a non-self-immolative component, peptidyl units, a hydrazone bond, a disulfide, an ester, an oxime, an amide, or a thioether bond. The self-immolative unit includes, but is not limited to, aromatic compounds that are electronically similar to the para-aminobenzylcarbamoyl (PAB) groups such as 2-aminoimidazol-5-methanol derivatives, heterocyclic PAB analogs, beta-glucuronide, and ortho or para-aminobenzylacetals.

Preferably, the self-immolative linker component has one of the following structures:

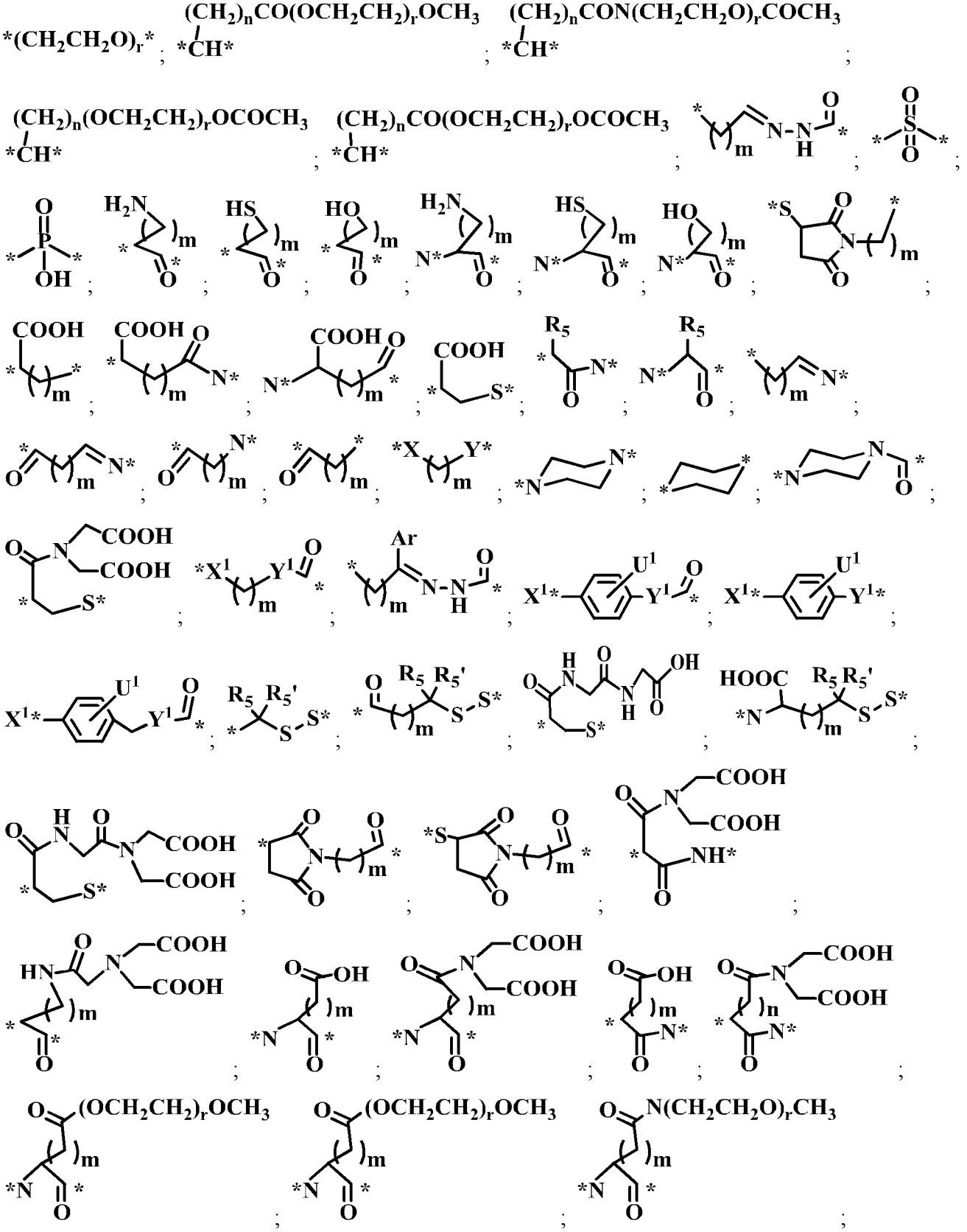


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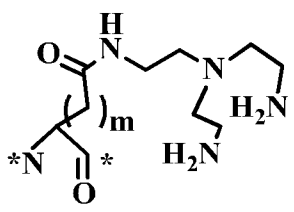
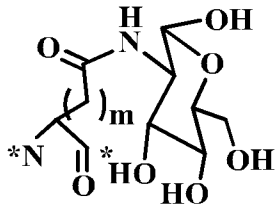
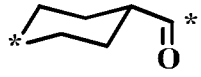
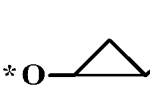
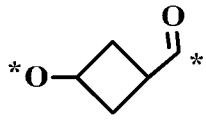
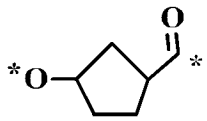
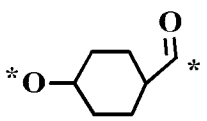
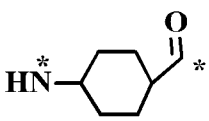
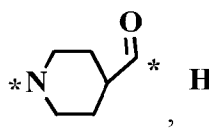
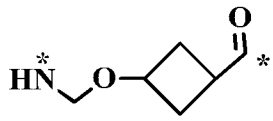
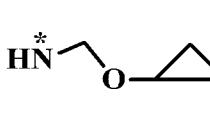
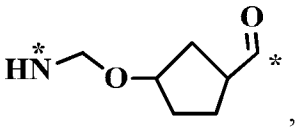
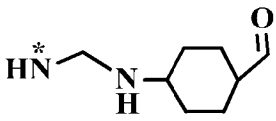
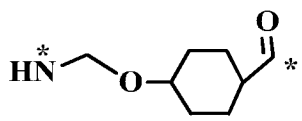
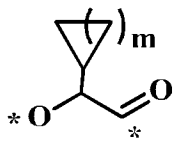
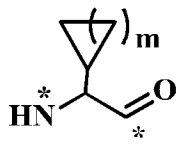
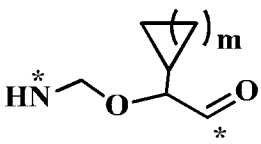
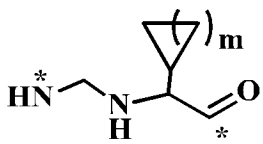
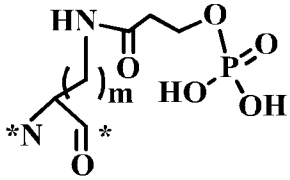
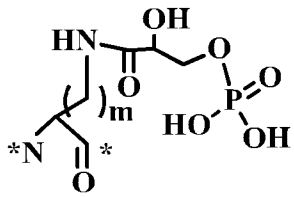
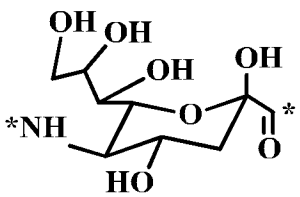
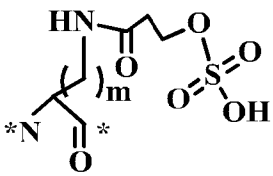
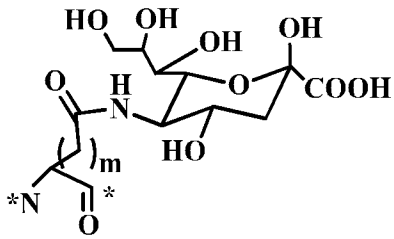
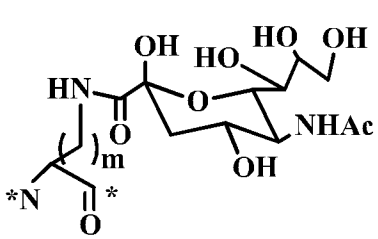
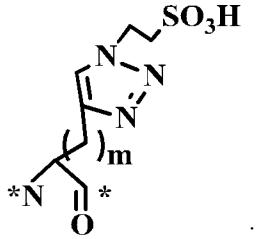
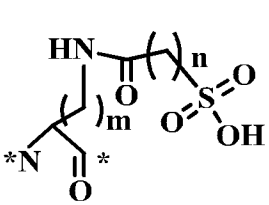
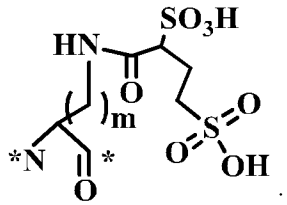
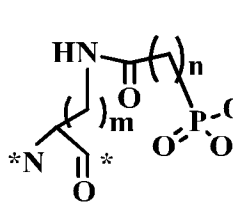

; or


wherein the (*) atom is the point of attachment of additional spacer or releasable linker units, or the cytotoxic agent, and/or the binding molecule (CBA); X^1, Y^1, Z^2 and Z^3 are independently NH, O, or S; Z^1 is independently H, NH, O or S; v is 0 or 1; U^1 is independently H, OH, C_1~C_6 alkyl, $(OCH_2CH_2)_nF$, Cl, Br, I, OR_5, SR_5, NR_5R_5', N=NR_5, N=R_5, NR_5R_5', NO_2, SOR_5R_5', SO_2R_5, SO_3R_5, OSO_3R_5, PR_5R_5', POR_5R_5', $PO_2R_5R_5$', $OPO(OR_5)(OR_5')$, or $OCH_2PO(OR_5(OR_5')$ wherein R_5 and R_5'

are as defined above; preferably R_5 and R_5' are independently selected from H, C_1~C_8 alkyl; C_2~C_8 alkenyl, alkynyl, heteroalkyl; C_3~C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, heterocycloalkyl, heteroaralkyl, alkylcarbonyl; or pharmaceutical cation salts.

The non-self-immolative linker component is one of the following structures:




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Wherein the (*) atom is the point of attachment of additional spacer R_1 or releasable linkers, the cytotoxic agents, and/or the binding molecules; X^1, Y^1, U^1, R_1, R_5, R_5' are defined as above; r is 0~100; m and n are 0~6 independently.

More preferably, L_1 or L_2 may be composed of one or more linker components of 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe" or "af"), p-aminobenzyloxycarbonyl ("PAB"), 4-thiopentanoate ("SPP"), 4-(N-maleimidomethyl)cyclohexane-1 carboxylate ("MCC"), (4-acetyl)amino-benzoate

("SIAB"), 4-thio-butyrate (SPDB), 4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB), or natural or unnatural peptides having 1~8 natural or unnatural amino acid unites.

Further preferably, L_1 or L_2 may be a releasable linker. The term releasable linker refers to a linker that includes at least one bond that can be broken under physiological conditions, such as a pH-labile, acid-labile, base-labile, oxidatively labile, metabolically labile, biochemically labile, or enzyme-labile bond. It is appreciated that such physiological conditions resulting in bond breaking do not necessarily include a biological or metabolic process, and instead may include a standard chemical reaction, such as a hydrolysis or substitution reaction, for example, an endosome having a lower pH than cytosolic pH, and/or disulfide bond exchange reaction with an intracellular thiol, such as a millimolar range of abundant of glutathione inside the malignant cells.

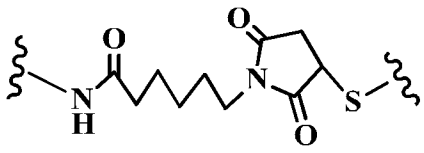
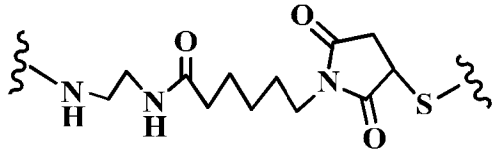
Examples of the releasable linkers (L, L_1, L_1', L_2 or L_2') include, but not limited:

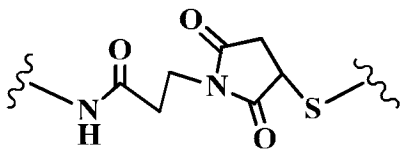
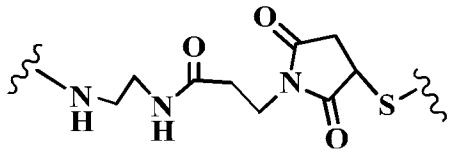
$-(CR_5R_6)_m(Aa)r(CR_7R_8)_n(OCH_2CH_2)_t-$, $-(CR_5R_6)_m(CR_7R_8)_n(Aa)_r(OCH_2CH_2)_t-$, $-(Aa)_r(CR_5R_6)_m-(CR_7R_8)_n(OCH_2CH_2)_t-$, $-(CR_5R_6)_m(CR_7R_8)_n(OCH_2CH_2)_r(Aa)_t-$, $-(CR_5R_6)_m(CR_7{=}CR_8)(CR_9R_{10})_n(Aa)_t-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(NR_{11}CO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(Aa)_t(NR_{11}CO)-(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(OCO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(OCNR_7)(Aa)_t-(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(CO)(Aa)_t-(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(NR_{11}CO)(Aa)_t-(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m-(OCO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(OCNR_7)(Aa)_t-(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m(CO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-(CR_5R_6)_m$-phenyl-CO-$(Aa)_t(CR_7R_8)_n-$, $-(CR_5R_6)_m$-furyl-CO$(Aa)_t(CR_7R_8)_n-$, $-(CR_5R_6)_m$-oxazolyl-CO$(Aa)_t(CR_7R_8)_n-$, $-(CR_5R_6)_m$thiazolyl-CO-$(Aa)_t(CCR_7R_8)_n-$, $-(CR_5R_6)_t$-thienyl-CO-$(CR_7R_8)_n-$, $-(CR_5R_6)_t$-imidazolyl-CO-$(CR_7R_8)_n-$, $-(CR_5R_6)_t$-morpholino-CO$(Aa)_t-(CR_7R_8)_n-$, $-(CR_5R_6)_t$piperazino-CO$(Aa)_t(CR_7R_8)_n-$, $-(CR_5R_6)_t$-N-methyl-piperazin-CO$(Aa)_t-(CR_7R_8)_n-$, $-(CR_5R)_m-(Aa)_t$phenyl-, $-(CR_5R_6)_m-(Aa)_t$furyl-, $-(CR_5R_6)_m$-oxazolyl$(Aa)_t-$, $-(CR_5R_6)_m$-thiazolyl$(Aa)_t-$, $-(CR_5R_6)_m$-thienyl-$(Aa)_t-$, $-(CR_5R_6)_m$-imidazolyl$(Aa)_t-$, $-(C\ R_5R_6)_m$-morpholino-$(Aa)_t-$, $-(CR_5R_6)_m$-piperazino-$(Aa)_t-$, $-(CR_5R_6)_m$N-methylpiperazino-$(Aa)_t-$, $-K(CR_5R_6)_m(Aa)r(CR_7R_8)_n(OCH_2CH_2)_t-$, $-K(CR_5R_6)_m-(CR_7R_8)_n-(Aa)_r(OCH_2CH_2)_t-$, $-K(Aa)_r(CR_5R_6)_m-(CR_7R_8)_n(OCH_2CH_2)_t-$, $-K(CR_5R_6)_m(CR_7R_8)_n-(OCH_2CH_2)_r(Aa)_t-$, $-K(CR_5R_6)_m(CR_7{=}CR_8)(CR_9R_{10})_n-(Aa)_t(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m-(NR_{11}CO)(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(Aa)_t(NR_{11}CO)-(CR_9R_{10})_n(OCH_2-CH_2)_r-$, $-K(CR_5R_6)_m(OCO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(OCNR_7)(Aa)_t-(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(CO)(Aa)_t-(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(NR_{11}CO)-(Aa)_t-(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m-(OCO)(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(OCNR_7)-(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m(CO)(Aa)_t(CR_9R_{10})_n-(OCH_2CH_2)_r-$, $-K(CR_5R_6)_m-$phenyl-CO-$(Aa)_t(CR_7R_8)_n-$, $-K-(CR_5R_6)_m$-furyl-CO$(Aa)_t-(CR_7R_8)_n-$, $-K(CR_5R_6)_m$-oxazolyl-CO-$(Aa)_t(CR_7R_8)_n-$, $-K(CR_5R_6)_m$thiazolyl-CO$(Aa)_t-(CR_7R_8)_n-$, $-K(CR_5R_6)_t$-thienyl-CO$(CR_7R_8)_n-$, -K-

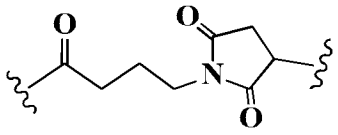
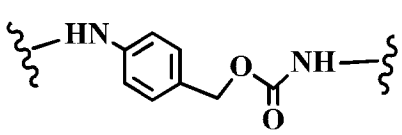
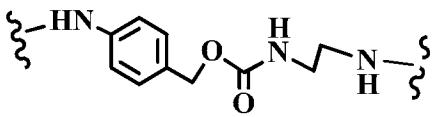
$(CR_5R_6)_t$imidazolyl-CO-$(CR_7R_8)_n$-, -K$(CR_5R_6)_t$morpholino-CO$(Aa)_t(CR_7R_8)_n$-, -K$(CR_5R_6)_t$-piperazino-CO$(Aa)_t$-$(CR_7R_8)_n$-, -K$(CR_5R_6)_t$-N-methylpiperazinCO$(Aa)_t(CR_7R_8)_n$-, -K$(CR_5R)_m(Aa)_t$-phenyl, -K-$(CR_5R_6)_m$-$(Aa)_t$furyl-, -K$(CR_5R_6)_m$-oxazolyl$(Aa)_t$-, -K$(CR_5R_6)_m$-thiazolyl$(Aa)_t$-, -K-$(CR_5R_6)_m$-thienyl-$(Aa)_t$-, -K$(CR_5R_6)_m$-imidazolyl$(Aa)_t$-, -K$(CR_5R_6)_m$-morpholino$(Aa)_t$-, -K$(CR_5R_6)_m$-piperazino-$(Aa)_t$G, -K$(CR_5R_6)_m$N-methylpiperazino-$(Aa)_t$-;

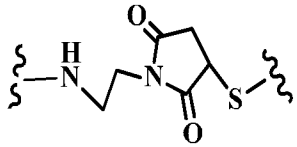
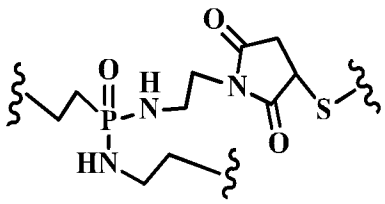
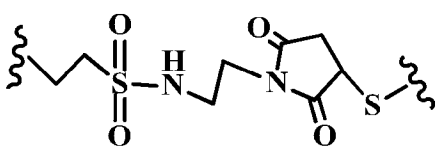
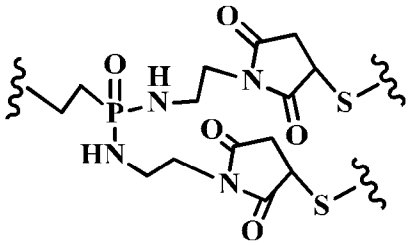
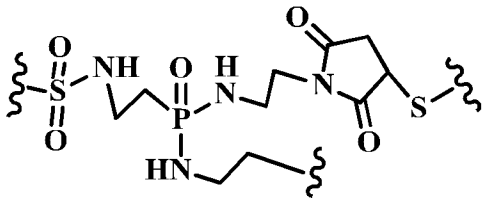
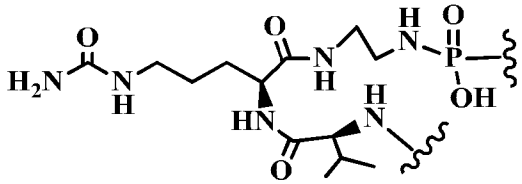
werein m, Aa, m, n, R_3, R_4, and R_5 are described above; t and r are 0 – 100 independently; R_6, R_7, and R_8 are independently chosen from H; halide; C_1~C_8 of alkyl, aryl, alkenyl, alkynyl, ether, ester, amine or amide, which optionally substituted by one or more halide, CN, NR_1R_2, CF_3, OR_1, Aryl, heterocycle, $S(O)R_1$, SO_2R_1, $-CO_2H$, $-SO_3H$, $-OR_1$, $-CO_2R_1$, -CONR$_1$, $-PO_2R_1R_2$, $-PO_3H$ or $P(O)R_1R_2R_3$; K is NR_1, -SS-, -C(=O)-, -C(=O)NH-, -C(=O)O-, -C=NH-O-, -C=N-NH-, -C(=O)NH-NH-, O, S, Se, B or C_3-C_6 heteroaromatic group.

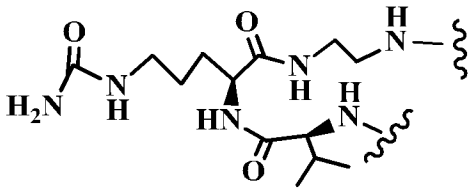
Example structures of the components of the linker L_1 and L_2 may contain:



(containing MC, 6-maleimidocaproyl),

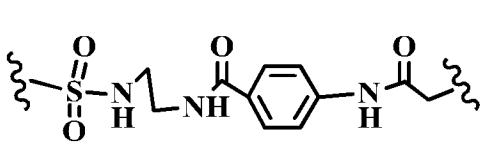


(MP, maleimidopropanoyl),




(PAB, p-aminobenzyloxycarbonyl),

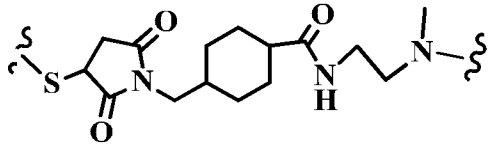









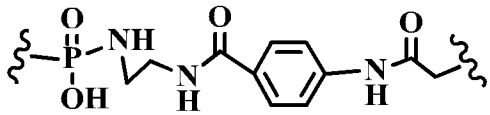
(containing valine-citrulline (VC)),



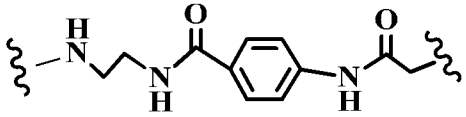
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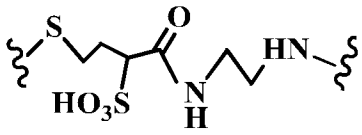
(MCC, 4-(N-maleimidomethyl)cyclohexane-1 carboxylate),



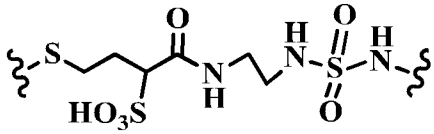
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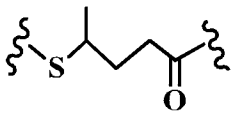
((4-acetyl)aminobenzoate),



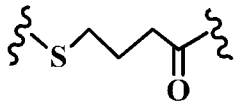
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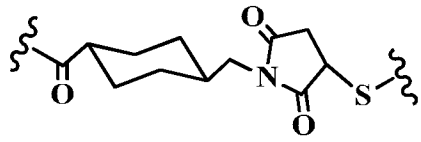
(4-thio-2-hydroxysulfonyl-butyrate, 2-sulfo-SPDB), (PAB),



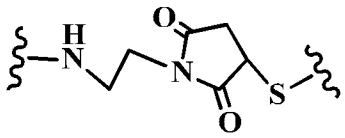
4-thio-pentanoate (SPP),



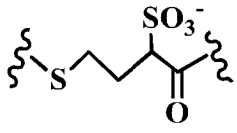
4-thio-butyrate (SPDB),



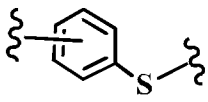
4-(N-maleimidomethyl)cyclo-hexane-1-carboxylate (MCC),



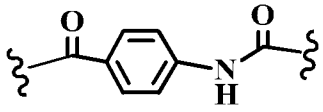
maleimidoethyl (ME),



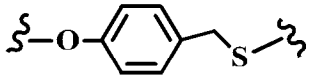
4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB),



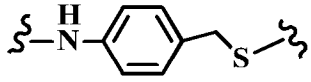
aryl-thiol (PhSS),



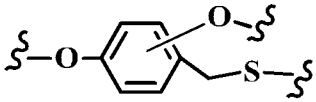
(4-acetyl)amino-benzoate (SIAB),



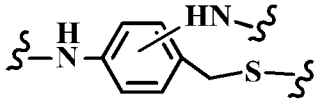
, oxylbenzylthio,



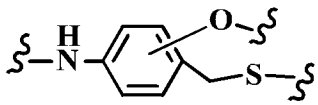
aminobenzylthio,



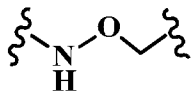
dioxylbenzylthio,



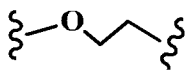
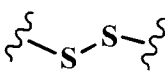
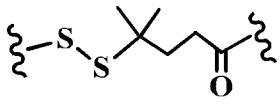
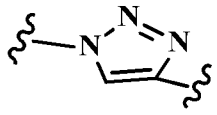
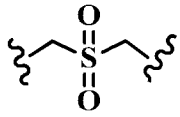
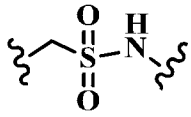
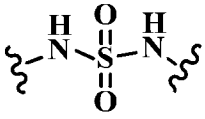
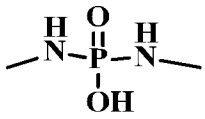
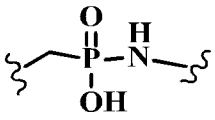
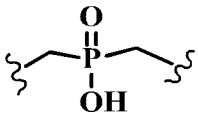
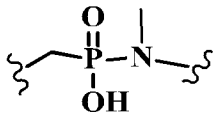
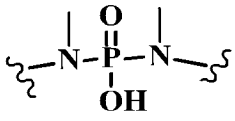
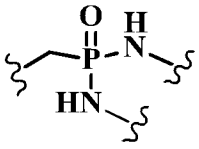
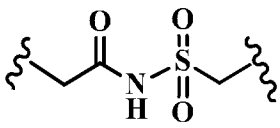
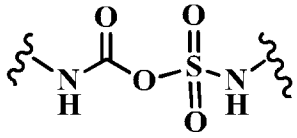
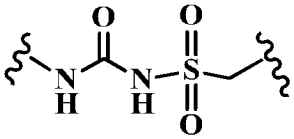
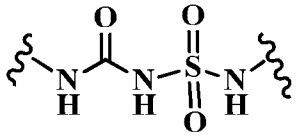
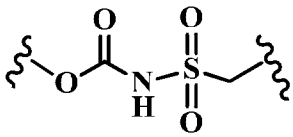
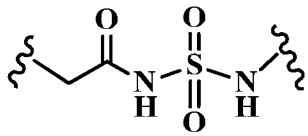
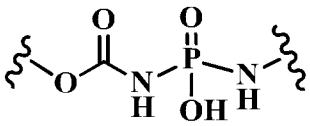
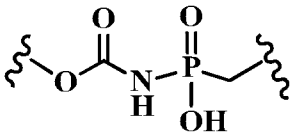
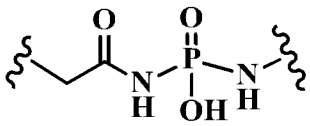
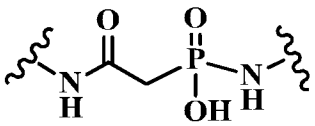
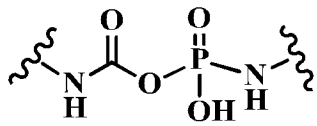
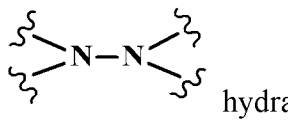
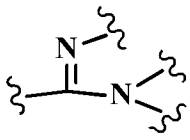
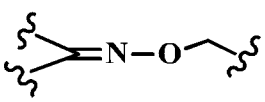
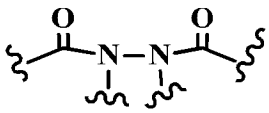
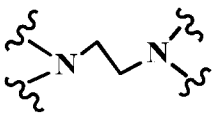
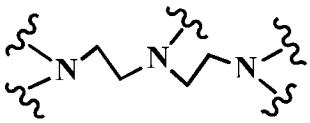
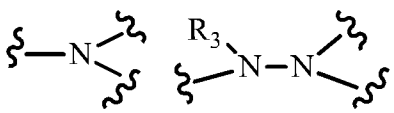
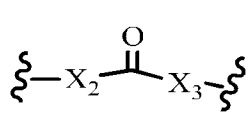
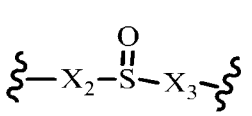
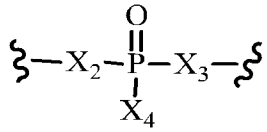
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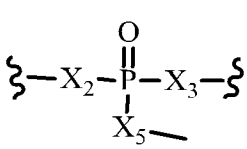
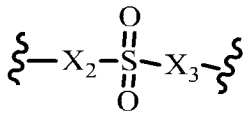
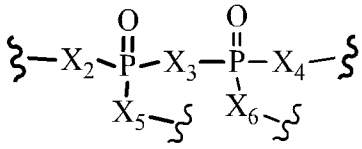
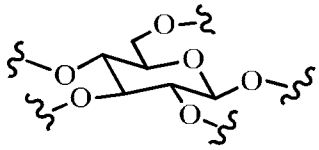
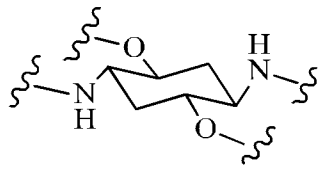
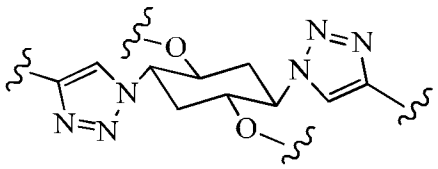
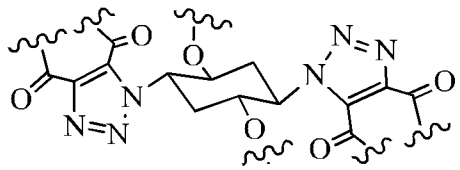
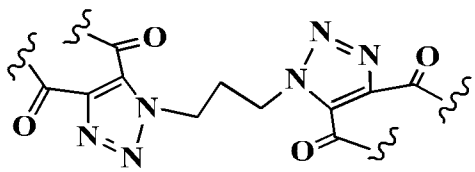
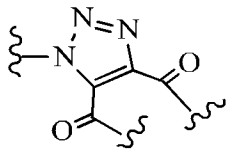
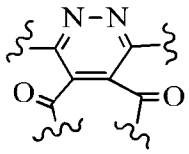
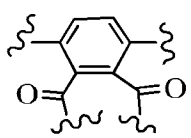
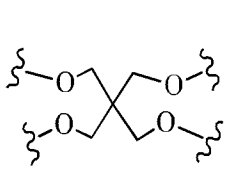
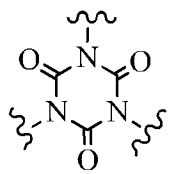
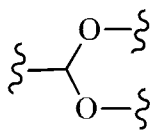
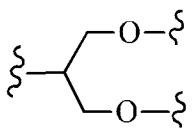
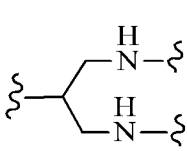
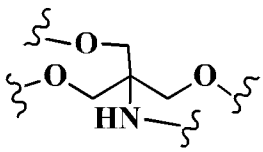
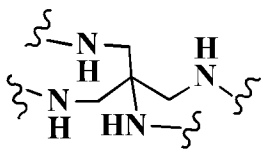
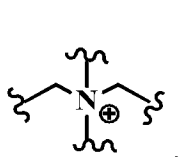
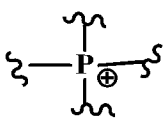
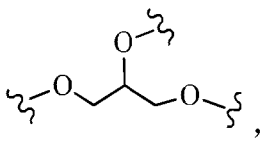
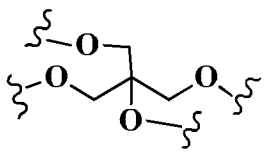
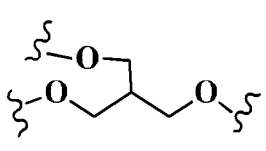
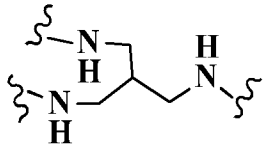
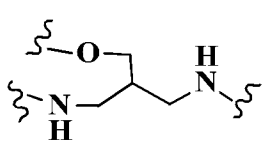
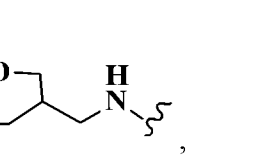
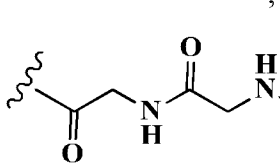
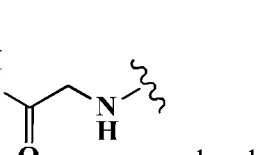
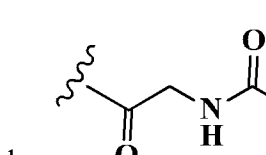
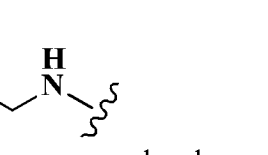
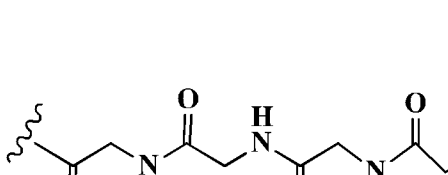
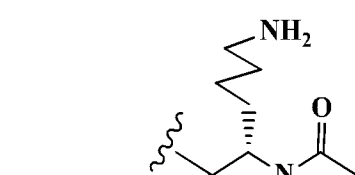
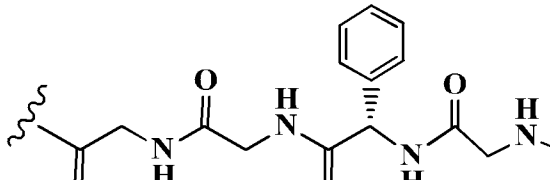
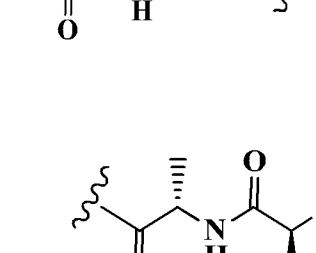


amino-oxylbenzylthio,



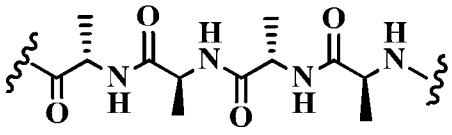
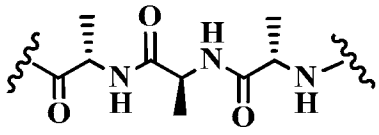
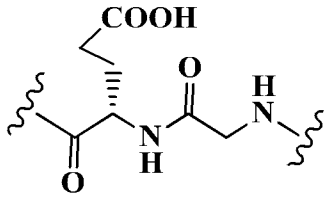
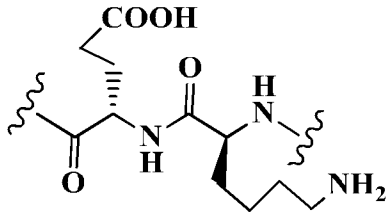
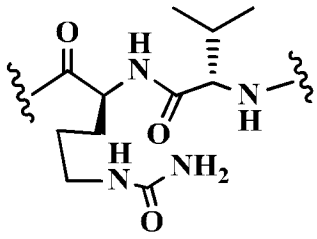
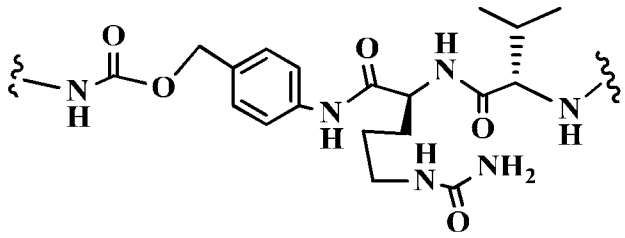
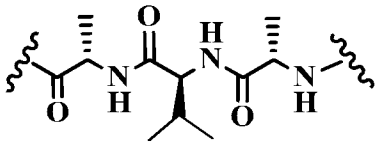
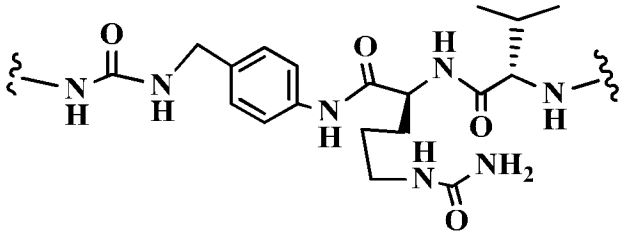
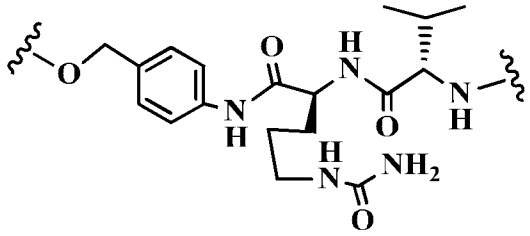
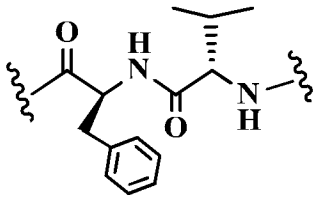
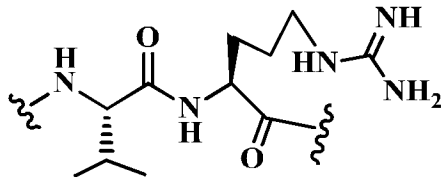
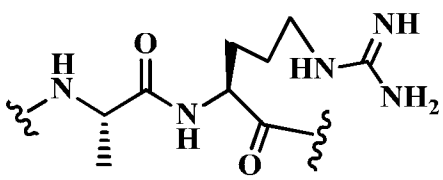
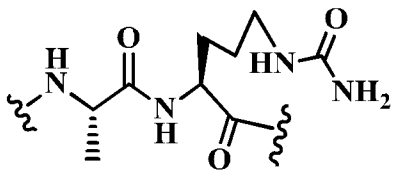
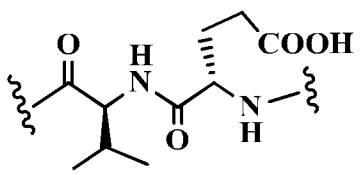
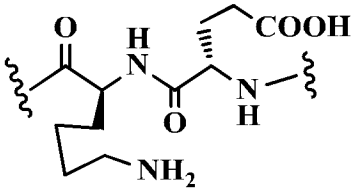
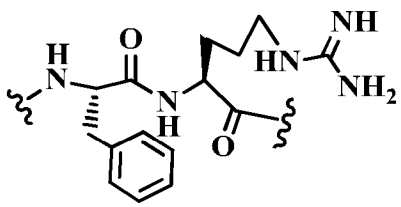
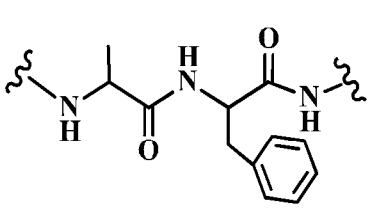
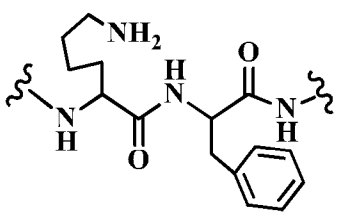
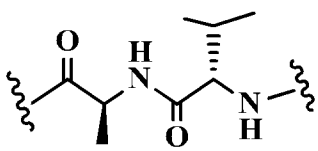
alkoxy amino

(AOA),  ethyleneoxy(EO),  dithio,  4-methyl-4-dithio-pentanoic (MPDP),  triazole,  alkylsulfonyl,  alkylsulfonamide,  sulfon-bisamide,  Phosphondiamide,  alkylphosphonamide,  phosphinic acid,  N-methylphosphonamidic acid,  N, N'-dimethylphosphonamidic acid,  N, N'-dimethylphosphondiamide,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  hydrazine,  acetimidamide,  oxime,  acetylacetohydrazide,  aminoethyl-amine,  aminoethyl-aminoethyl-amine,  ,  ,  ,  ,


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gly-gly-gly,

gly-gly,

gly-gly-gly-gly,

Lys-gly,

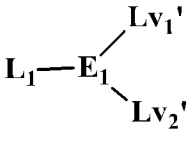
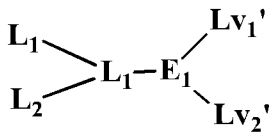
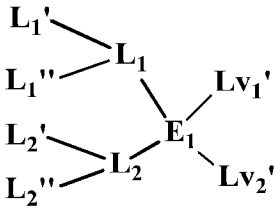
gly-gly-phe-gly,

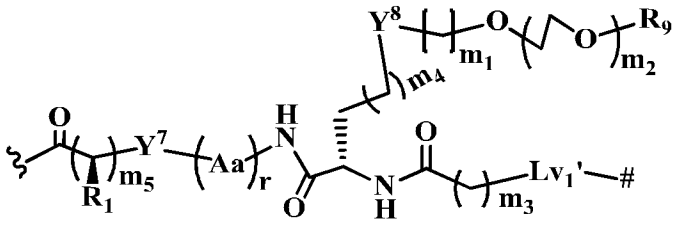
ala-ala,


ala-ala-ala-ala,

ala-ala-ala,

glu-gly,

glu-lys,


(VC),

ala-val-ala,

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, (lys-phe), or a combination above thereof; wherein is the site of linkage; X_2, X_3, X_4, X_5, or X_6, are independently selected from NH; NHNH; $N(R_{12})$; $N(R_{12})N(R_{12'})$; O; S; C_1-C_6 of alkyl; C_2-C_6 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl,

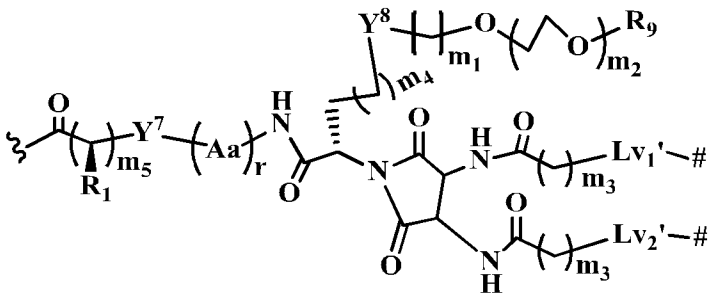
heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; CH_2OR_{12}, CH_2SR_{12}, CH_2NHR_{12}, or 1~8 amino acids; wherein R_{12} and $R_{12'}$ are independently H;C_1-C_8 of alkyl; C_2-C_8 of hetero-alkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 1-8 carbon atoms of esters, ether, or amide; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 100.

In addition, L_1, L_2, X_1, X_2, X_3, $X_{1'}$, $X_{2'}$ and $X_{3'}$ can be independently absent.

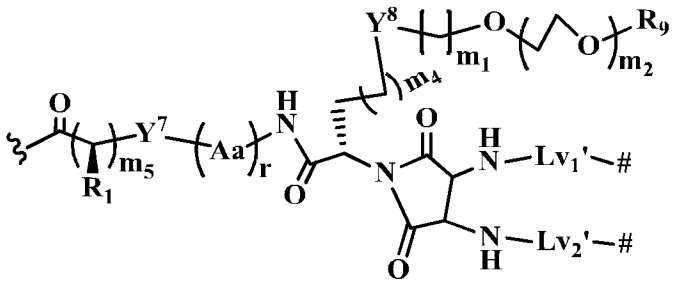
In other embodiments, the conjugate of formula (VI), (VII), (VIII) or (IX), wherein the components of $L_1—E_1—Lv_1'$ in formula (VI),  in formula (VII),  in formula (VIII), and  in formula (IX), are preferably selected from the following structures accordingly:



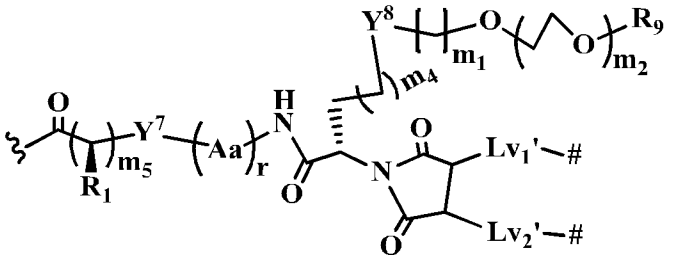
(I-a),



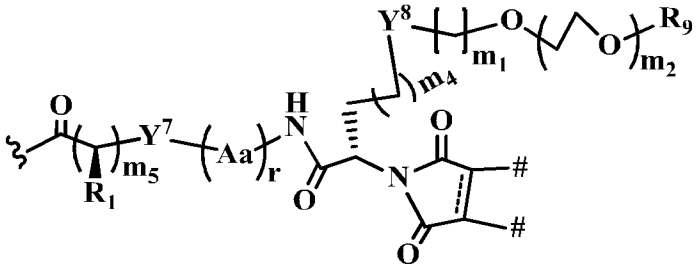
(I-b),



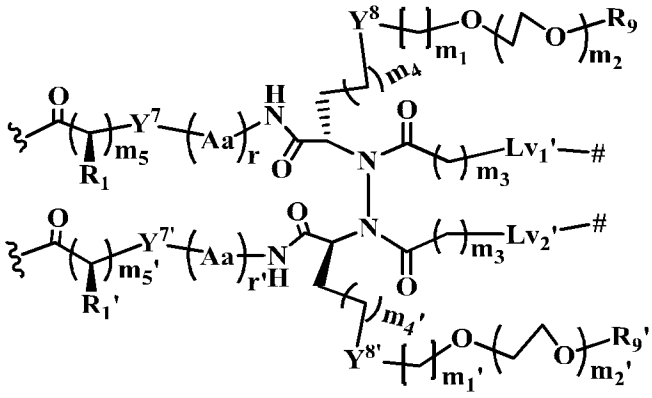
(I-c),



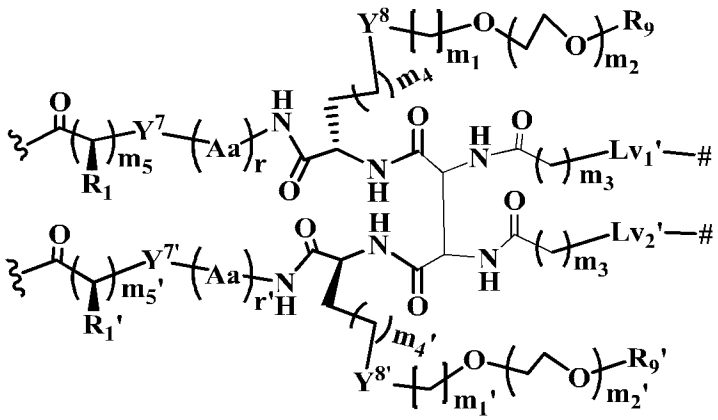
(I-d),



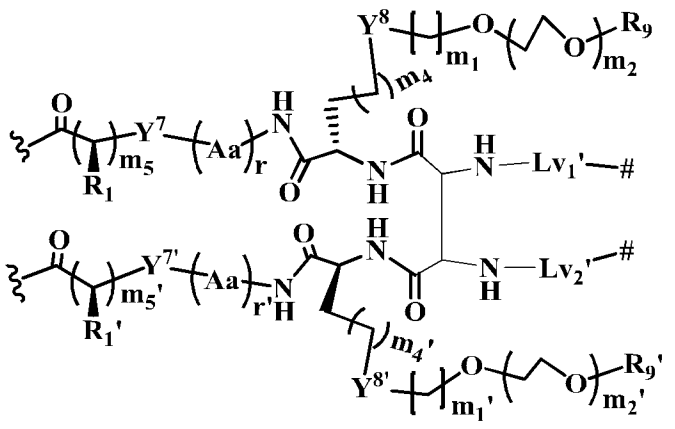
(I-e),



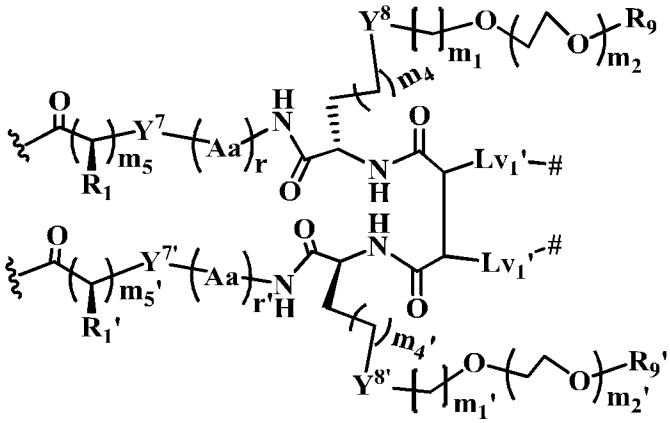
(I-f),



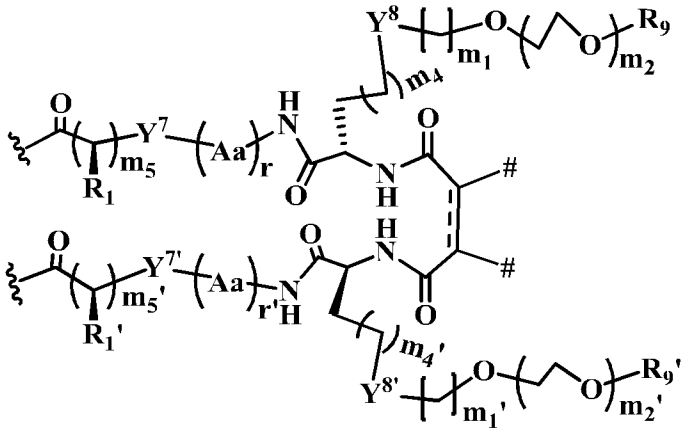
(I-g),



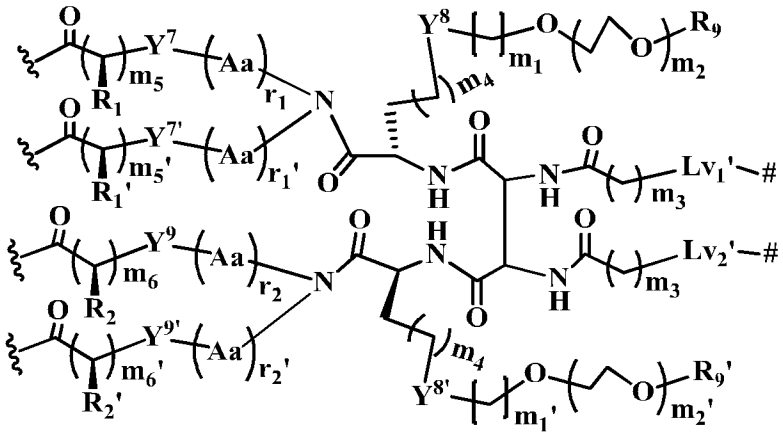
(I-h),



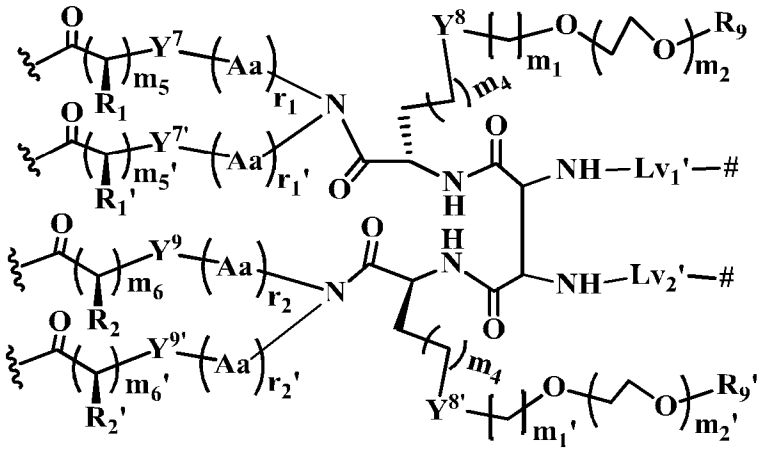
(I-i),



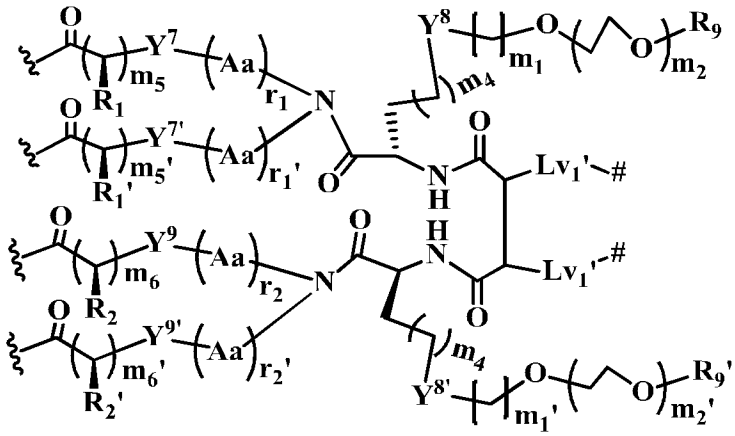
(I-j),



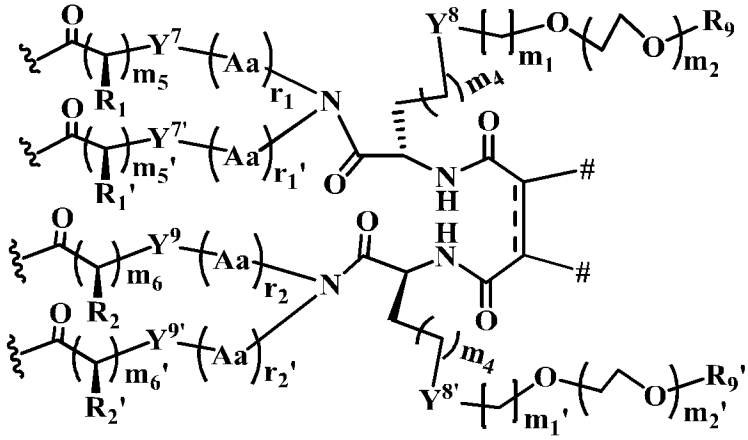
(I-k),



(I-l),



(I-m),



(I-n),

Wherein "$\sim$" is a site that links a drug or a site of linker L_1, L_1', L_2, or L_2'; "#" is a site that links a S (thiol), O (phenol), NH (amino), CHO (aldehyde), C(=O) (ketone), C(O)(NH) (amide) and C(O)(OH) (carboxylate) of an antibody; Aa is L-or D- natural or unnatural amino acids;

Wherein R_1, R_1', R_2, and R_2' are independently selected from H, C_1-C_8 alkyl, OH, CH_2OH, CH_2CH_2OH, NH_2, SH, SCH_3, CH_2COOH, CH_2CH_2COOH, $CH_2CH_2CH_2CH_2NH_2$, C_6H_5, $CH_2C_6H_5$, $CH_2C_6H_4OH$, $CH(OH)CH_3$, $CH_2C(O)NH_2$, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2NHC(=NH)NH_2$;

Wherein r, r_1, r_1', r_2, and r_2' are independently selected from is 0-12; when r is not 0, $(Aa)_r$ is the same or different amino acids or peptide units;

Wherein m_1 and m_1' are independently 1 - 18; m_2 and m_2' are independently 1-100; m_3 and m_3' are independently 1 - 8; m_4 and m_4' are independently 0 - 8; m_5 and m_5' are independently 1 - 8;

Wherein Y^7 and $Y^{7'}$ are independently NH, OCH_2NH, NHC(=O), NHNH, C(=O)NH, $N(R_1)$, SO_2, P(O)(OH), $NHS(O)_2$, $NHS(O)_2NH$, $NHS(O)_2NHC(O)$, $NHS(O)_2NHC(O)O$, $NHS(O)_2NHC(O)NH$, NHP(O)(OH), NHP(O)(OH)NH, OP(O)(OH)O, NHP(O)(OH)O, OP(O)(OH)NH, S, O, OP(O)(OH)OP(O)(OH)NH, NHP(O)(OH)OP(O)(OH)NH, NHP(O)(OH)OP(O)(OH)O, OCH_2CH_2O, OCH_2CH_2NH,

$N(CH_2CH_2)_2N$, NHC_6H_4NH, CH_2;

Y^8 and $Y^{8'}$ are independently NHC(=O), $NHS(O_2)$, NH(SO), $NHS(O_2)NH$, NHP(O)(OH)NH or C(O)NH;

Y^9 and $Y^{9'}$ are independently H, $(O=)CR_1$, $(O=)CNHR_1$, R_1COOH, $R_1(COCH_2NH)_{m2}H$, $R_1(Aa)_r$ or $R_1(COCH_2NCH_3)_{m2}H$, wherein R_1 is defined above;

In other embodiments, D, D_1, D_2, D_3, and D_4 are independently a cytotoxic drug, or a therapeutic drug, or an immunotherapeutical protein, or a function molecule for enhancement of binding or stabilization of the cell-binding protein agent, or a cell-surface receptor binding ligand, such as an antibody or an antibody fragment, or siRNA or DNA molecule.

The cytotoxic drug is selected from, but not limited to:

1). Chemotherapeutic agents: a). Alkylating agents: such as Nitrogen mustards: chlorambucil, chlornaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiotepa, trofosfamide, uracil mustard; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); Duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); Benzodiazepine dimers (e.g., dimmers of pyrrolobenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidino-benzodiazepines); Nitrosoureas: (carmustine, lomustine, chlorozotocin, fotemustine, nimustine, ranimustine); Alkylsulphonates: (busulfan, treosulfan, improsulfan and piposulfan); Triazenes: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin); aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemel-amine, trietylenephosphoramide, triethylenethio-phosphaoramide and trimethylolomel-amine]; b). Plant Alkaloids: such as Vinca alkaloids: (vincristine, vinblastine, vindesine, vinorelbine, navelbin); Taxoids: (paclitaxel, docetaxol) and their analogs, Maytansinoids (DM1, DM2, DM3, DM4, DM5, DM21, maytansine and ansamitocins) and their analogs, cryptophycins (particularly cryptophycin 1 and cryptophycin 8); epothilones, eleutherobin, discodermo-lide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins; pancratistatin; a sarcodictyin; spongistatin; c). DNA Topoisomerase Inhibitors: such as [Epipodophyllins: (9-aminocamptothecin, camptothecin, crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, retinoic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000)); mitomycins: (mitomycin C)]; d). Anti-metabolites: such as {[Anti-folate: DHFR inhibitors: (methotrexate, trimetrexate, denopterin, pteropterin, aminopterin (4-aminopteroic acid) or the other folic acid analogues); IMP dehydrogenase Inhibitors: (mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide

reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs: (ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Fluorouracil, floxuridine, ratitrexed (Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine)]; folic acid replenisher, such as frolinic acid}; e). Hormonal therapies: such as {Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen); LHRH agonists: (goscrclin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, trilostane and other androgens inhibitors)]; Retinoids/Deltoids: [Vitamin D3 analogs: (CB 1093, EB 1089 KH 1060, cholecalciferol, ergocalciferol); Photodynamic therapies: (verteporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A); Cytokines: (Interferon-alpha, Interferon-gamma, tumor necrosis factor (TNFs), human proteins containing a TNF domain)]}; f). Kinase inhibitors, such as BIBW 2992 (anti-EGFR/Erb2), imatinib, gefitinib, pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, axitinib, pazopanib. vandetanib, E7080 (anti-VEGFR2), mubritinib, ponatinib (AP24534), bafetinib (INNO-406), bosutinib (SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib, bevacizumab, cetuximab, Trastuzumab, Ranibizumab, Panitumumab, ispinesib; g). antibiotics, such as the enediyne antibiotics (e.g. calicheamicins, especially calicheamicin γ1, δ1, α1 and β1, see, e.g., *J. Med. Chem.*, 39 (11), 2103–2117 (1996), Angew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; f). Others: such as Polyketides (acetogenins), especially bullatacin and bullatacinone; gemcitabine, epoxomicins (e. g. carfilzomib), bortezomib, thalidomide, lenalidomide, pomalidomide, tosedostat, zybrestat, PLX4032, STA-9090, Stimuvax, allovectin-7, Xegeva, Provenge, Yervoy, Isoprenylation inhibitors (such as Lovastatin), Dopaminergic neurotoxins (such as 1-methyl-4-phenylpyridinium ion), Cell cycle inhibitors (such as staurosporine), Actinomycins (such as Actinomycin D, dactinomycin), Bleomycins (such as bleomycin A2, bleomycin B2, peplomycin), Anthracyclines (such as daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin,

pirarubicin, zorubicin, mtoxantrone, MDR inhibitors (such as verapamil), Ca^{2+}ATPase inhibitors (such as thapsigargin), Histone deacetylase inhibitors (Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A); Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.; Anti-adrenals, such as aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; eflornithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine, razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucarin A, roridin A and anguidine); urethane, siRNA, antisense drugs, and a nucleolytic enzyme.

2). An anti-autoimmune disease agent includes, but is not limited to, cyclosporine, cyclosporine A, aminocaproic acid, azathioprine, bromocriptine, chlorambucil, chloroquine, cyclophosphamide, corticosteroids (e.g. amcinonide, betamethasone, budesonide, hydrocortisone, flunisolide, fluticasone propionate, fluocortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate), DHEA, enanercept, hydroxychloroquine, infliximab, meloxicam, methotrexate, mofetil, mycophenylate, prednisone, sirolimus, tacrolimus.

3). An anti-infectious disease agent includes, but is not limited to, a). Aminoglycosides: amikacin, astromicin, gentamicin (netilmicin, sisomicin, isepamicin), hygromycin B, kanamycin (amikacin, arbekacin, bekanamycin, dibekacin, tobramycin), neomycin (framycetin, paromomycin, ribostamycin), netilmicin, spectinomycin, streptomycin, tobramycin, verdamicin; b). Amphenicols: azidamfenicol, chloramphenicol, florfenicol, thiamphenicol; c). Ansamycins: geldanamycin, herbimycin; d). Carbapenems: biapenem, doripenem, ertapenem, imipenem/cilastatin, meropenem, panipenem; e). Cephems: carbacephem (loracarbef), cefacetrile, cefaclor, cefradine, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalexin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, cephamycin (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef); f). Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin),

teicoplanin (dalbavancin), ramoplanin; g). Glycylcyclines: e. g. tigecycline; g). β-Lactamase inhibitors: penam (sulbactam, tazobactam), clavam (clavulanic acid); i). Lincosamides: clindamycin, lincomycin; j). Lipopeptides: daptomycin, A54145, calcium-dependent antibiotics (CDA); k). Macrolides: azithromycin, cethromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, josamycin, ketolide (telithromycin, cethromycin), midecamycin, miocamycin, oleandomycin, rifamycins (rifampicin, rifampin, rifabutin, rifapentine), rokitamycin, roxithromycin, spectinomycin, spiramycin, tacrolimus (FK506), troleandomycin, telithromycin; l). Monobactams: aztreonam, tigemonam; m). Oxazolidinones: linezolid; n). Penicillins: amoxicillin, ampicillin (pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin), azidocillin, azlocillin, benzylpenicillin, benzathine benzylpenicillin, benzathine phenoxymethyl-penicillin, clometocillin, procaine benzylpenicillin, carbenicillin (carindacillin), cloxacillin, dicloxacillin, epicillin, flucloxacillin, mecillinam (pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin, penamecillin, penicillin, pheneticillin, phenoxymethylpenicillin, piperacillin, propicillin, sulbenicillin, temocillin, ticarcillin; o). Polypeptides: bacitracin, colistin, polymyxin B; p). Quinolones: alatrofloxacin, balofloxacin, ciprofloxacin, clinafloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, floxin, garenoxacin, gatifloxacin, gemifloxacin, grepafloxacin, kano trovafloxacin, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, orbifloxacin, ofloxacin, pefloxacin, trovafloxacin, grepafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin; q). Streptogramins: pristinamycin, quinupristin/dalfopristin); r). Sulfonamides: mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (co-trimoxazole); s). Steroid antibacterials: e.g. fusidic acid; t). Tetracyclines: doxycycline, chlortetracycline, clomocycline, demeclocycline, lymecycline, meclocycline, metacycline, minocycline, oxytetracycline, penimepicycline, rolitetracycline, tetracycline, glycylcyclines (e.g. tigecycline); u). Other types of antibiotics: annonacin, arsphenamine, bactoprenol inhibitors (Bacitracin), DADAL/AR inhibitors (cycloserine), dictyostatin, discodermolide, eleutherobin, epothilone, ethambutol, etoposide, faropenem, fusidic acid, furazolidone, isoniazid, laulimalide, metronidazole, mupirocin, mycolactone, NAM synthesis inhibitors (e. g. fosfomycin), nitrofurantoin, paclitaxel, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampicin (rifampin), tazobactam tinidazole, uvaricin;

4). Anti-viral drugs: a). Entry/fusion inhibitors: aplaviroc, maraviroc, vicriviroc, gp41 (enfuvirtide), PRO 140, CD4 (ibalizumab); b). Integrase inhibitors: raltegravir, elvitegravir, globoidnan A; c). Maturation inhibitors: bevirimat, vivecon; d). Neuraminidase inhibitors: oseltamivir, zanamivir, peramivir; e). Nucleosides &nucleotides: abacavir, aciclovir, adefovir, amdoxovir, apricitabine, brivudine, cidofovir, clevudine, dexelvucitabine, didanosine (ddI),

elvucitabine, emtricitabine (FTC), entecavir, famciclovir, fluorouracil (5-FU), 3'-fluoro-substituted 2', 3'-dideoxynucleoside analogues (e.g. 3'-fluoro-2', 3'-dideoxythymidine (FLT) and 3'-fluoro-2', 3'-dideoxyguanosine (FLG), fomivirsen, ganciclovir, idoxuridine, lamivudine (3TC), l-nucleosides (e.g. β-l-thymidine and β-l-2'-deoxycytidine), penciclovir, racivir, ribavirin, stampidine, stavudine (d4T), taribavirin (viramidine), telbivudine, tenofovir, trifluridine valaciclovir, valganciclovir, zalcitabine (ddC), zidovudine (AZT); f). Non-nucleosides: amantadine, ateviridine, capravirine, diarylpyrimidines (etravirine, rilpivirine), delavirdine, docosanol, emivirine, efavirenz, foscarnet (phosphonoformic acid), imiquimod, interferon alfa, loviride, lodenosine, methisazone, nevirapine, NOV-205, peginterferon alfa, podophyllotoxin, rifampicin, rimantadine, resiquimod (R-848), tromantadine; g). Protease inhibitors: amprenavir, atazanavir, boceprevir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, pleconaril, ritonavir, saquinavir, telaprevir (VX-950), tipranavir; h). Other types of anti-virus drugs: abzyme, arbidol, calanolide a, ceragenin, cyanovirin-n, diarylpyrimidines, epigallocatechin gallate (EGCG), foscarnet, griffithsin, taribavirin (viramidine), hydroxyurea, KP-1461, miltefosine, pleconaril, portmanteau inhibitors, ribavirin, seliciclib.

5). The drugs used for conjugates via a bridge linker of the present invention also include radioisotopes. Examples of radioisotopes (radionuclides) are ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Radioisotope labeled antibodies are useful in receptor targeted imaging experiments or can be for targeted treatment such as with the antibody-drug conjugates of the invention (Wu et al (2005) Nature Biotechnology 23(9): 1137-46). The cell binding molecules, e.g. an antibody can be labeled with ligand reagents through the bridge linkers of the present patent that bind, chelate or otherwise complex a radioisotope metal, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, Tex. USA).

6). The pharmaceutically acceptable salts, acids or derivatives of any of the above drugs.

In another embodiment, the drug D can be a chromophore molecule, for which the conjugate can be used for detection, monitoring, or study the interaction of the cell binding molecule with a target cell. Chromophore molecules are a compound that have the ability to absorb a kind of light, such as UV light, florescent light, IR light, near IR light, visual light; A chromatophore molecule includes a class or subclass of xanthophores, erythrophores, iridophores, leucophores, melanophores, and cyanophores; a class or subclass of fluorophore molecules which are fluorescent chemical compounds re-emitting light upon light; a class or subclass of visual phototransduction molecules; a class or subclass of photophore molecules; a class or subclass of luminescence molecules; and a class or subclass of luciferin compounds.

The chromophore molecule can be selected from, but not limited, non-protein organic fluorophores, such as: Xanthene derivatives (fluorescein, rhodamine, Oregon green, eosin, and Texas red); Cyanine derivatives: (cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, and merocyanine); Squaraine derivatives and ring-substituted squaraines, including Seta, SeTau, and Square dyes; Naphthalene derivatives (dansyl and prodan derivatives); Coumarin derivatives; Oxadiazole derivatives (pyridyloxazole, nitrobenzoxadiazole and benzoxadiazole); Anthracene derivatives (anthraquinones, including DRAQ5, DRAQ7 and CyTRAK Orange); Pyrene derivatives (cascade blue, etc); Oxazine derivatives (Nile red, Nile blue, cresyl violet, oxazine 170 etc). Acridine derivatives (proflavin, acridine orange, acridine yellow etc). Arylmethine derivatives (auramine, crystal violet, malachite green). Tetrapyrrole derivatives (porphin, phthalocyanine, bilirubin).

Or a chromophore molecule can be selected from any analogs and derivatives of the following fluorophore compounds: CF dye (Biotium), DRAQ and CyTRAK probes (BioStatus), BODIPY (Invitrogen), Alexa Fluor (Invitrogen), DyLight Fluor (Thermo Scientific, Pierce), Atto and Tracy (Sigma Aldrich), FluoProbes (Interchim), Abberior Dyes (Abberior), DY and MegaStokes Dyes (Dyomics), Sulfo Cy dyes (Cyandye), HiLyte Fluor (AnaSpec), Seta, SeTau and Square Dyes (SETA BioMedicals), Quasar and Cal Fluor dyes (Biosearch Technologies), SureLight Dyes (APC, RPEPerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phyco-Biotech).

Examples of the widely used fluorophore compounds which are reactive or conjugatable with the linkers of the invention are: Allophycocyanin (APC), Aminocoumarin, APC-Cy7 conjugates, BODIPY-FL, Cascade Blue, Cy2, Cy3, Cy3.5, Cy3B, Cy5, Cy5.5, Cy7, Fluorescein, FluorX, Hydroxycoumarin, IR-783, Lissamine Rhodamine B, Lucifer yellow, Methoxycoumarin, NBD, Pacific Blue, Pacific Orange, PE-Cy5 conjugates, PE-Cy7 conjugates, PerCP, R-Phycoerythrin (PE), Red 613, Seta-555-Azide, Seta-555-DBCO, Seta-555-NHS, Seta-580-NHS, Seta-680-NHS, Seta-780-NHS, Seta-APC-780, Seta-PerCP-680, Seta-R-PE-670, SeTau-380-NHS, SeTau-405-Maleimide, SeTau-405-NHS, SeTau-425-NHS, SeTau-647-NHS, Texas Red, TRITC, TruRed, X-Rhodamine.

The fluorophore compounds that can be linked to the linkers of the invention for study of nucleic acids or proteins are selected from the following compounds or their derivatives: 7-AAD (7-aminoactinomycin D, CG-selective), Acridine Orange, Chromomycin A3, CyTRAK Orange (Biostatus, red excitation dark), DAPI, DRAQ5, DRAQ7, Ethidium Bromide, Hoechst33258, Hoechst33342, LDS 751, Mithramycin, PropidiumIodide (PI), SYTOX Blue, SYTOX Green, SYTOX Orange, Thiazole Orange, TO-PRO: Cyanine Monomer, TOTO-1, TO-PRO-1, TOTO-3, TO-PRO-3, YOSeta-1, YOYO-1. The fluorophore compounds that can be linked to the linkers of the invention for study cells are selected from the following compounds or their derivatives: DCFH

(2'7'Dichorodihydro-fluorescein, oxidized form), DHR (Dihydrorhodamine 123, oxidized form, light catalyzes oxidation), Fluo-3 (AM ester. pH > 6), Fluo-4 (AM ester. pH 7.2), Indo-1 (AM ester, low/high calcium (Ca2+)), and SNARF (pH 6/9). The preferred fluorophore compounds that can be linked to the linkers of the invention for study proteins/antibodies are selected from the following compounds or their derivatives: Allophycocyanin (APC), AmCyan1 (tetramer, Clontech), AsRed2 (tetramer, Clontech), Azami Green (monomer, MBL), Azurite, B-phycoerythrin (BPE), Cerulean, CyPet, DsRed monomer (Clontech), DsRed2 ("RFP", Clontech), EBFP, EBFP2, ECFP, EGFP (weak dimer, Clontech), Emerald (weak dimer, Invitrogen), EYFP (weak dimer, Clontech), GFP (S65A mutation), GFP (S65C mutation), GFP (S65L mutation), GFP (S65T mutation), GFP (Y66F mutation), GFP (Y66H mutation), GFP (Y66W mutation), GFPuv, HcRed1, J-Red, Katusha, Kusabira Orange (monomer, MBL), mCFP, mCherry, mCitrine, Midoriishi Cyan (dimer, MBL), mKate (TagFP635, monomer, Evrogen), mKeima-Red (monomer, MBL), mKO, mOrange, mPlum, mRaspberry, mRFP1 (monomer, Tsien lab), mStrawberry, mTFP1, mTurquoise2, P3 (phycobilisome complex), Peridinin Chlorophyll (PerCP), R-phycoerythrin(RPE), T-Sapphire, TagCFP (dimer, Evrogen), TagGFP (dimer, Evrogen), TagRFP (dimer, Evrogen), TagYFP (dimer, Evrogen), tdTomato (tandem dimer), Topaz, TurboFP602 (dimer, Evrogen), TurboFP635 (dimer, Evrogen), TurboGFP (dimer, Evrogen), TurboRFP (dimer, Evrogen), TurboYFP (dimer, Evrogen), Venus, Wild Type GFP, YPet, ZsGreen1 (tetramer, Clontech), ZsYellow1 (tetramer, Clontech).

In another embodiment, the drug D can be polyalkylene glycols that are used for extending the half-life of the cell-binding antibody, or antibody-like protein molecule when administered to a mammal. Polyalkylene glycols include, but are not limited to, poly(ethylene glycols) (PEGs), poly(propylene glycol) and copolymers of ethylene oxide and propylene oxide; particularly preferred are PEGs, and more particularly preferred are monofunctionally activated hydroxyPEGs (e.g., hydroxyl PEGs activated at a single terminus, including reactive esters of hydroxyPEG-monocarboxylic acids, hydroxyPEG-monoaldehydes, hydroxyPEG-monoamines, hydroxyPEG-monohydrazides, hydroxyPEG-monocarbazates, hydroxyl PEG-monoiodoacetamides, hydroxyl PEG-monomaleimides, hydroxyl PEG-monoorthopyridyl disulfides, hydroxyPEG-monooximes, hydroxyPEG-monophenyl carbonates, hydroxyl PEG-monophenyl glyoxals, hydroxyl PEG-monothiazolidine-2-thiones, hydroxyl PEG-monothioesters, hydroxyl PEG-monothiols, hydroxyl PEG-monotriazines and hydroxyl PEG-monovinylsulfones).

In certain such embodiments, the polyalkylene glycol has a molecular weight of from about 10 Daltons to about 200 kDa, preferably about 88 Da to about 40 kDa; two branches each with a molecular weight of about 88 Da to about 40 kDa; and more preferably two branches, each of about 88 Da to about 20 kDa. In one particular embodiment, the polyalkylene glycol is poly(ethylene)

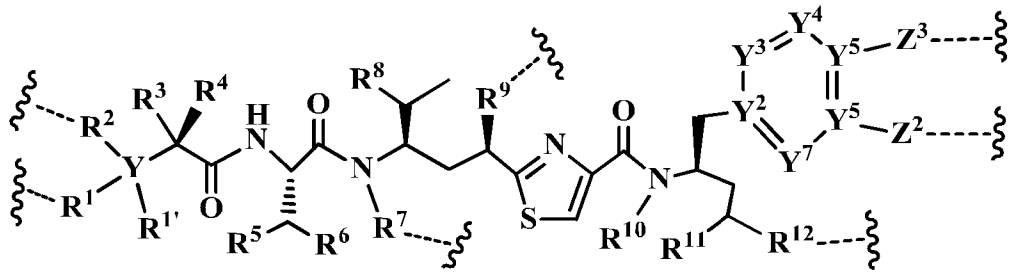
glycol and has a molecular weight of about 10 kDa; about 20 kDa, or about 40 kDa. In specific embodiments, the PEG is a PEG 10 kDa (linear or branched), a PEG 20 kDa (linear or branched), or a PEG 40 kDa (linear or branched). A number of US patents have disclosed the preparation of linear or branched "non-antigenic" PEG polymers and derivatives or conjugates thereof, e.g., U.S. Pat. Nos. 5, 428, 128; 5, 621, 039; 5, 622, 986; 5, 643, 575; 5, 728, 560; 5, 730, 990; 5, 738, 846; 5, 811, 076; 5, 824, 701; 5, 840, 900; 5, 880, 131; 5, 900, 402; 5, 902, 588; 5, 919, 455; 5, 951, 974; 5, 965, 119; 5, 965, 566; 5, 969, 040; 5, 981, 709; 6, 011, 042; 6, 042, 822; 6, 113, 906; 6, 127, 355; 6, 132, 713; 6, 177, 087, and 6, 180, 095.

In yet another embodiment, D is more preferably a potent cytotoxic agent, selected from a tubulysin and its analogs, a maytansinoid and its analogs, a taxanoid (taxane) and its analogs, a CC-1065 and its analogs, a daunorubicin or doxorubicin and its analogs, an amatoxin and its analogs, a benzodiazepine dimer (e.g., dimers of pyrrolobenzodiazepine (PBD), tomaymycin, anthramycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzo-diazepines) and their analogs, a calicheamicin and the enediyne antibiotic and their analogs, an actinomycin and its analogs, an azaserine and its analogs, a bleomycin and its analogs, an epirubicin and its analogs, a tamoxifen and its analogs, an idarubicin and its analogs, a dolastatin and its analogs, an auristatin (including monomethyl auristatin E (MMAE), MMAF, auristatin PYE, auristatin TP, Auristatins 2-AQ, 6-AQ, EB (AEB), and EFP (AEFP)) and its analogs, a combretastatin, a duocarmycin and its analogs, a camptothecin, a geldanamycin and its analogs, a methotrexate and its analogs, a thiotepa and its analogs, a vindesine and its analogs, a vincristine and its analogs, a hemiasterlin and its analogs, a nazumamide and its analogs, a spliceostatin, a pladienolide, a microginin and its analogs, a radiosumin and its analogs, an alterobactin and its analogs, a microsclerodermin and its analogs, a theonellamide and its analogs, an esperamicin and its analogs, PNU-159682 and its analogs, a protein kinase inhibitor, a MEK inhibitor, a KSP inhibitor, a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor, a topoisomerase I or II inhibitor, an immunotoxin, and stereoisomers, isosteres, analogs, or derivatives above thereof.

Tubulysin and its analogs are well known in the art and can be isolated from natural sources according to known methods or prepared synthetically according to known methods (e. g. Balasubramanian, R., et al. J. Med. Chem., 2009, 52, 238–40; Wipf, P., et al. Org. Lett., 2004, 6, 4057–60; Pando, O., et al. J. Am. Chem. Soc., 2011, 133, 7692–5; Reddy, J. A., et al. Mol. Pharmaceutics, 2009, 6, 1518–25; Raghavan, B., et al. J. Med. Chem., 2008, 51, 1530–33; Patterson, A. W., et al. J. Org. Chem., 2008, 73, 4362–9; Pando, O., et al. Org. Lett., 2009, 11 (24), 5567–9; Wipf, P., et al. Org. Lett., 2007, 9 (8), 1605–7; Friestad, G. K., Org. Lett., 2004, 6, 3249–52; Peltier, H. M., et al. J. Am. Chem. Soc., 2006, 128, 16018–9; Chandrasekhar, S., et al J. Org. Chem., 2009,

74, 9531–4; Liu, Y., et al. Mol. Pharmaceutics, 2012, 9, 168–75; Friestad, G. K., et al. Org. Lett., 2009, 11, 1095–8; Kubicek, K., et al., Angew Chem Int Ed Engl, 2010.49: 4809-12; Chai, Y., et al., Chem Biol, 2010, 17: 296-309; Ullrich, A., et al., Angew Chem Int Ed Engl, 2009, 48, 4422-5; Sani, M., et al. Angew Chem Int Ed Engl, 2007, 46, 3526-9; Domling, A., et al., Angew Chem Int Ed Engl, 2006, 45, 7235-9; Patent applications: Zanda, M., et al, Can. Pat. Appl. CA 2710693 (2011); Chai, Y., et al. Eur. Pat. Appl. 2174947 (2010), WO 2010034724; Leamon, C. et al, WO2010033733, WO 2009002993; Ellman, J., et al, PCT WO2009134279; WO 2009012958, US appl. 20110263650, 20110021568; Matschiner, G., et al, WO2009095447; Vlahov, I., et al, WO2009055562, WO 2008112873; Low, P., et al, WO2009026177; Richter, W., WO2008138561; Kjems, J., et al, WO 2008125116; Davis, M.; et al, WO2008076333; Diener, J.; et al, U.S. Pat.Appl. 20070041901, WO2006096754; Matschiner, G., et al, WO2006056464; Vaghefi, F., et al, WO2006033913; Doemling, A., Ger. Offen. DE102004030227, WO2004005327, WO2004005326, WO2004005269; Stanton, M., et al, U.S. Pat. Appl. Publ. 20040249130; Hoefle, G., et al, Ger. Offen. DE10254439, DE10241152, DE10008089; Leung, D., et al, WO2002077036; Reichenbach, H., et al, Ger. Offen. DE19638870; Wolfgang, R., US20120129779; Chen, H., US appl. 20110027274. The preferred structures of tubulysins for conjugation of cell binding molecules through process of the present patent application are described in the patent application of PCT/IB2012/053554.

Tubulysin analog having the following formula (V):



(V)

or a pharmaceutically acceptable salt, hydrates, or hydrated salt; or a polymorphic crystalline structure; or an optical isomer, racemate, diastereomer or enantiomer thereof,

wherein ∿∿ is a linkage site that either one or two of them can link to L_1 and/or L_2 independently; when two of "∿∿" link to both L_1 and L_2, R^1 and R^2, or Z^2 and Z^3 are preferably the dual linkage sites;

wherein R^1, $R^{1'}$, R^2, R^3, and R^4 are independently H, C_1~C_8 alkyl; C_2~C_8 heteroalkyl, or heterocyclic; C_3~C_8 aryl, Ar-alkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, carbocyclic, or alkylcarbonyl; or R^1R^2, R^1R^3, R^2R^3, R^3R^4, or R^5R^6 form a 3~7 membered carbocyclic, cycloalkyl, heterocyclic, heterocycloalkyl, aromatic or heteroaromatic ring system; R^1 and R^2 can be independently absent when they link to L_1 or L_2 independently or simultaneously, Y^1 is N or CH;

wherein R^5, R^6, R^8, R^{10} and R^{11} are independently H, or C_1~C_4 alkyl or heteroalkyl;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$; or $-R^{14}X^1R^{15}$; X^1 is O, S, S-S, NH, CH_2 or NR^{14};

wherein R^9 is selected from H, OH, =O, $-OR^{14}$, $-OC(=O)R^{14}$, $-OC(=O)NHR^{14}$, $-OC(=O)NR^{14}R^{15}$, $OP(=O)(OR^{14})_2$, $-OC(=O)NR^{14}R^{15}$, or $OR^{14}OP(=O)(OR^{15})_2$; when R^9 links L_1 or L_2, R^9 is , -O-, $-OC(=O)NH-$ or $-OC(=O)N(R^{14})-$;

wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{15}$, $-R^{14}C(=O)X^2R^{15}$, wherein X^2 is -O-, -S-, -NH-, or $-N(R^{14})-$;

wherein R^{12} is -COOH, -COSH, $-CONH_2$, $CONHNH_2$, $CONHNHR^{15}$, $-CONH(R^{15})$, $-COOR^{15}$, $-R^{15}COR^{16}$, $-R^{15}COOR^{16}$, $-R^{15}C(O)NH_2$, $-R^{15}C(O)NHR^{16}$, $-COSR^{15}$, $R^{15}S(=O)_2R^{16}$, $-R^{15}P(=O)(OR^{17})_2$, $-R^{15}OP(=O)(OR^{17})_2$, $-COOCH_2OP(=O)(OR^{17})_2$, $-COX^2SO_2R^{17}$, $-COOR^{15}X^2R^{16}$, tetrazole, imidazole, or triazole, where X^2 is -O-, -S-, -NH-, $-N(R^{15})-$, $-O-R^{15}-$, $-S-R^{15}-$, CH_2 or $-NHR^{15}-$; when R^{12} links L_1 or L_2, R^{12} is $-C(O)O-$, $-C(O)NH-$, $-C(=O)NHS(O)_2R^{15}-$ or $-C(=O)N(R^{15})-$;

$R^{13}$and R^{14} are independently C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl;

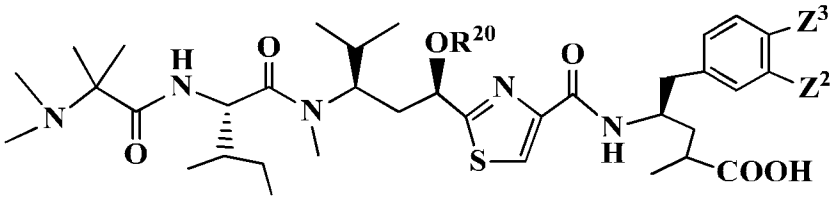
Z^2 and Z^3 are independently H, O, S, NH, $N(R^{15})$, NHNH, -OH, -SH, $-NH_2$, NH, $NHNH_2$, $-NH(R^{15})$, $-OR^{15}$, CO, $-COX^2$, $-COX^2R^{16}$, R^{17}, F, Cl, Br, I, SR^{16}, $NR^{16}R^{17}$, $N=NR^{16}$, $N=R^{16}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$, $OCH_2OP(O)(OR^{17})_2$, $OC(O)R^{17}$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{17})OP(O)(OR^{17})_2$, $OC(O)NHR^{17}$;-O-(C_4-C_{12} glycoside), -N-(C_4-C_{12} glycoside); C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl, or 2- 8 carbon atoms of esters, ether, or amide; or peptides containing 1-8 amino acids $(NH(Aa)_{1\sim8}$, or $CO(Aa)_{1\sim8}$ (which are respectively N-terminal or C-terminal 1 - 8 the same or different amino acids)), or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000, or combination of above groups thereof; X^2 is O, S, S-S, NH, CH_2, OH, SH, NH_2, CHR^{15} or NR^{15};

R^{15}, R^{16} and R^{17} are independently H, C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl, alkylcarbonyl, or Na^+, K^+, Cs^+, Li^+, Ca^{2+}, Mg^+, Zn^{2+}, $N^+(R^1)(R^2)(R^3)(R^4)$, $HN^+(C_2H_5OH)_3$ salt;

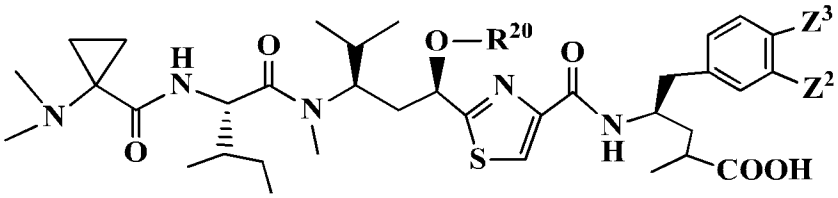
Y^1 and Y^2 are independently N or CH; q is 0 or 1; when q=0, Y^3 does not exist, Y^4, Y^5, Y^6 and Y^7 are independently CH, N, NH, O, S, or N (R1), thus Y^2, Y^4, Y^5, Y^6 and Y^7form a heteroaromatic ring of furan, pyrrole thiophene, thiazole, oxazole and imidazole, pyrazole, triazole, tetrazole, thiadiazole; when q=1, Y^3, Y^4, Y^5, Y^6 and Y^7 are independently CH or N, thus Y^2, Y^3, Y^4, Y^5, Y^6

and Y^7 form aromatic ring of benzene, pyridine, pyridazine, pyrimidine, pyrazine, triazine, tetrazine, pentazine;

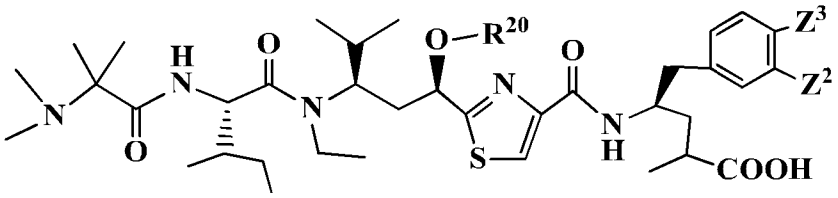
Examples of the structures of the tubulysin analogs are shown below:



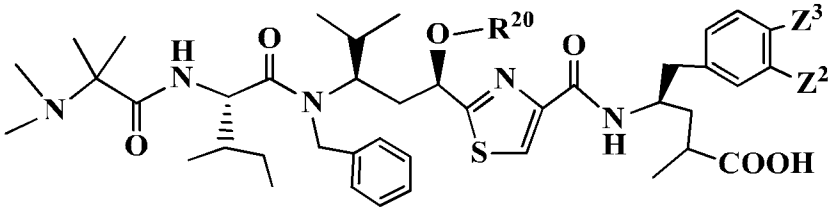
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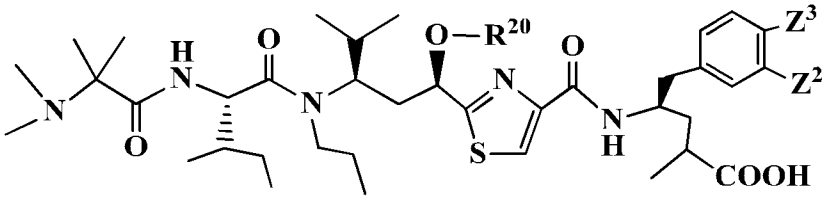
V-02,



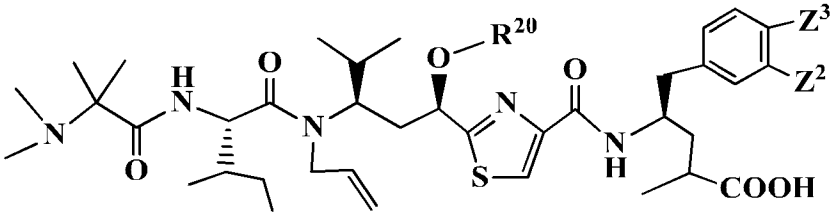
V-03,



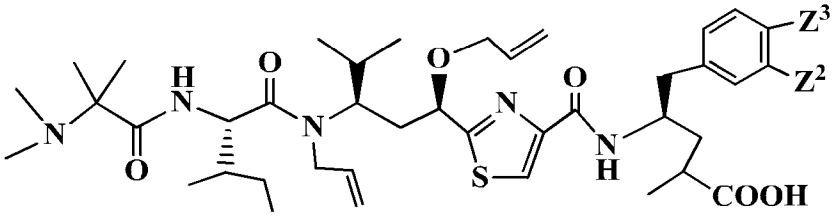
V-04,



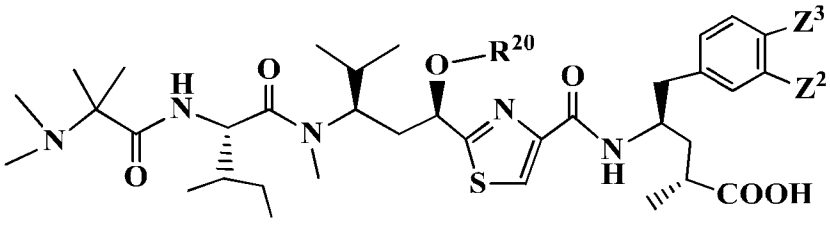
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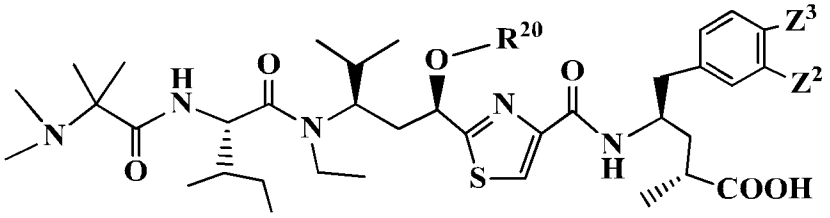


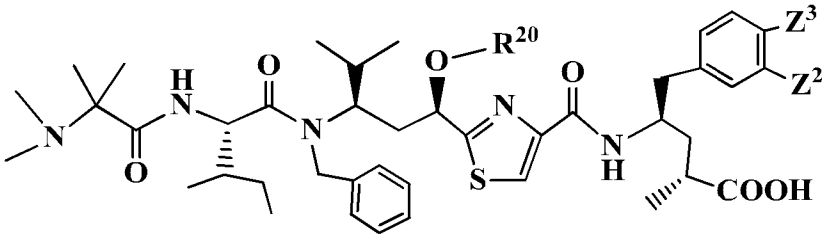
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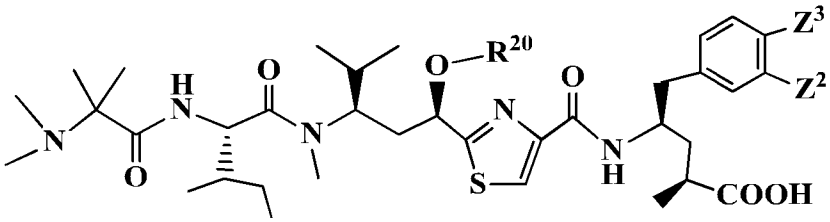


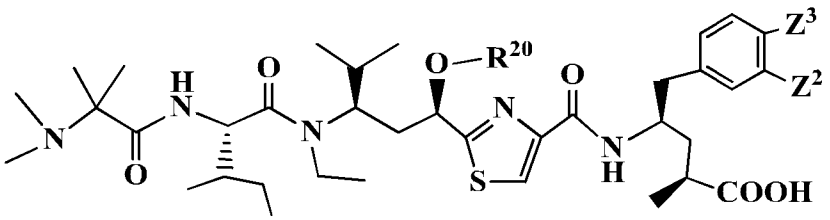
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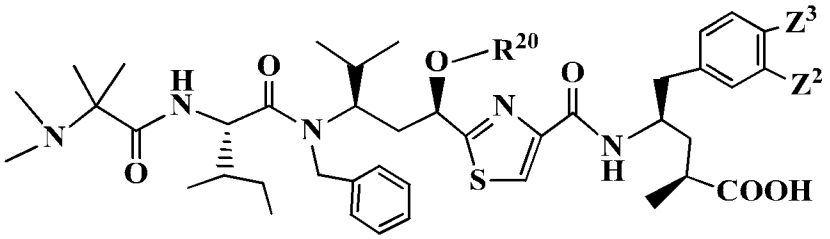

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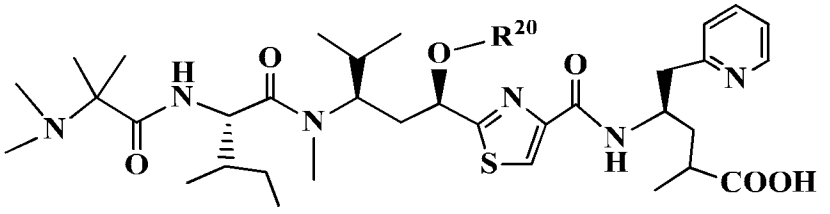

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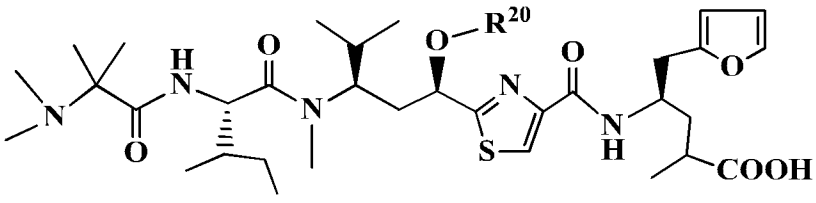

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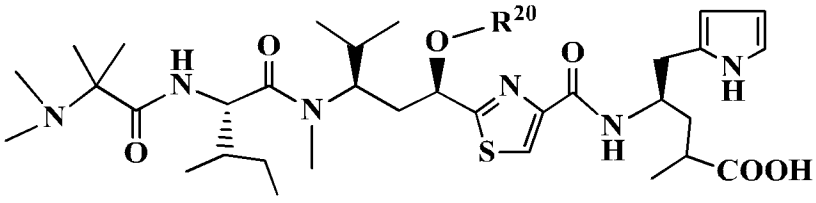

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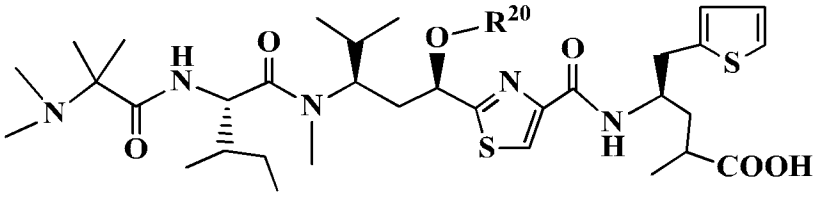

V-12,

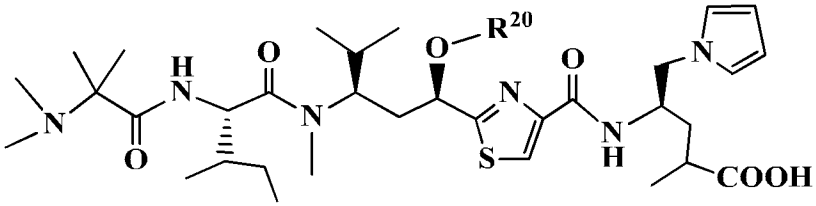

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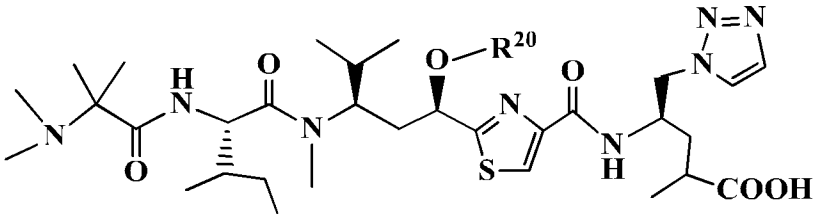

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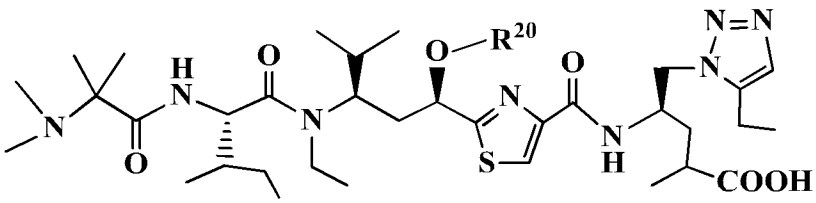

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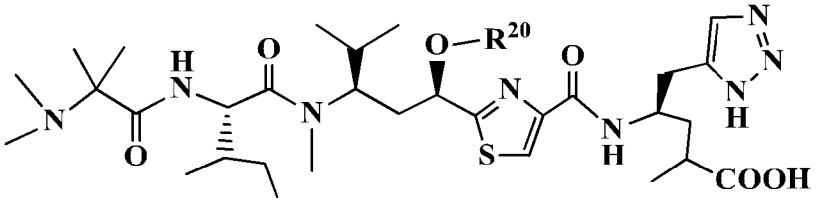

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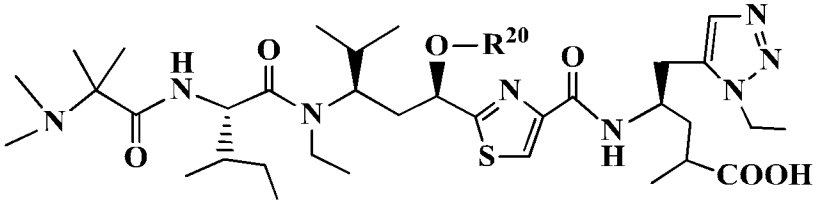

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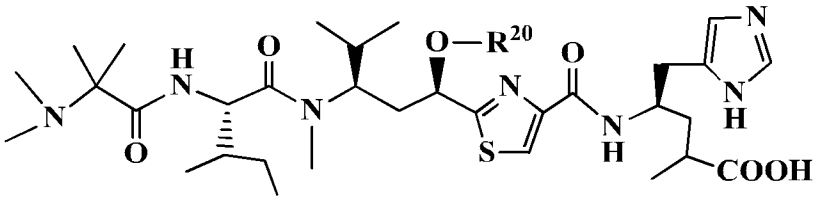

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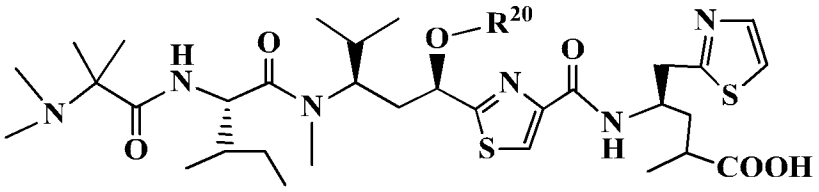

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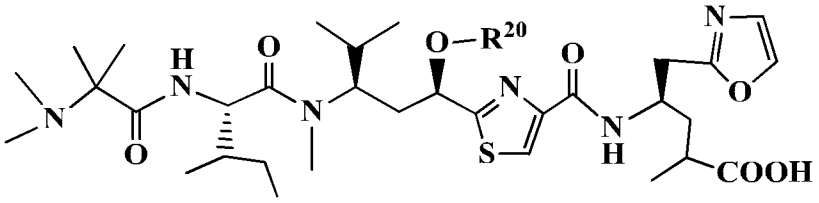

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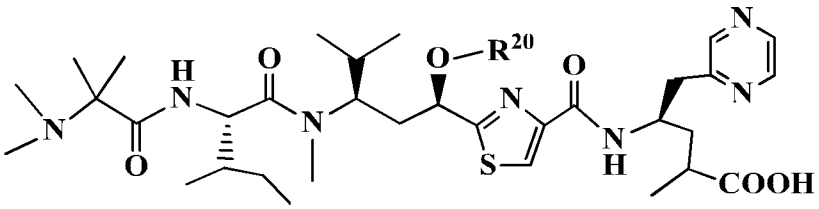

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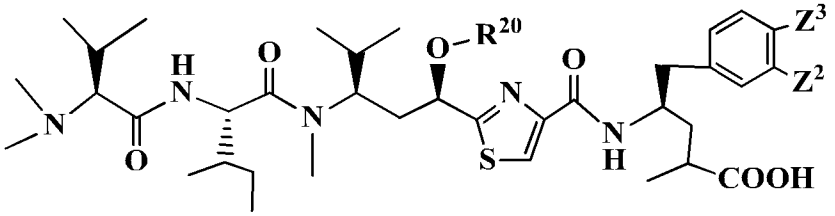

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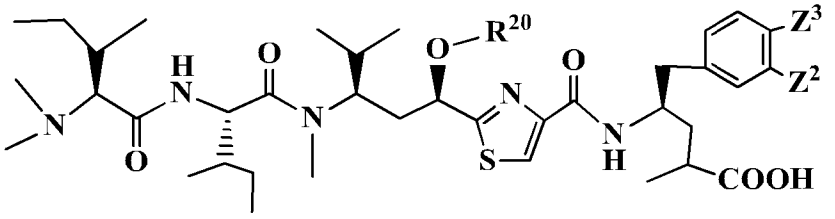

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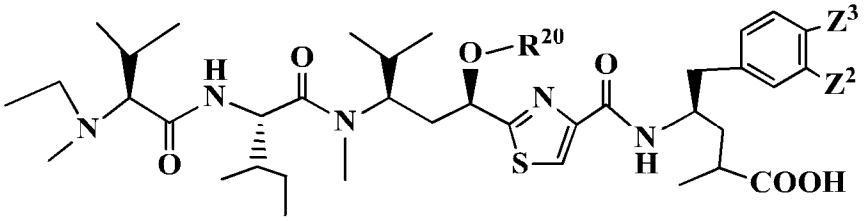

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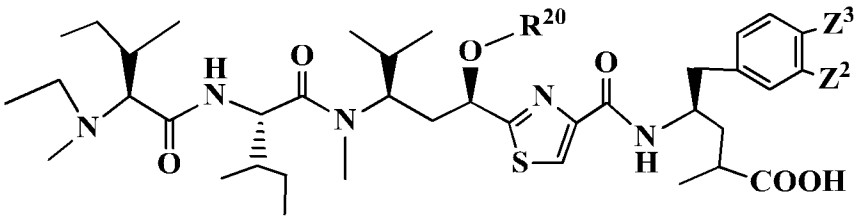

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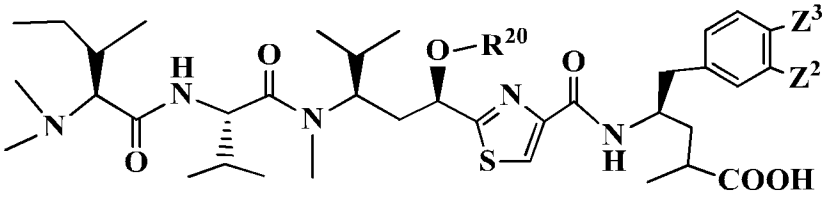

V-26,

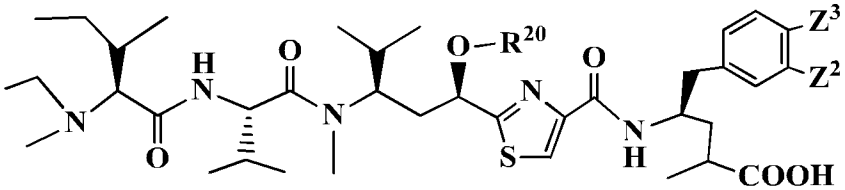

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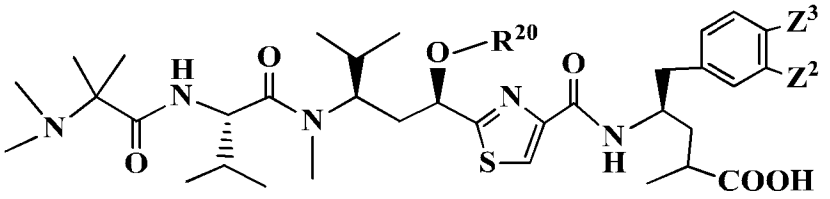

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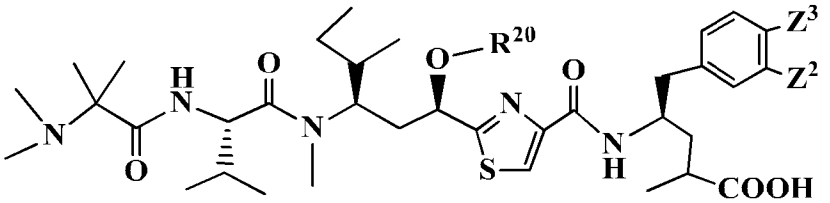

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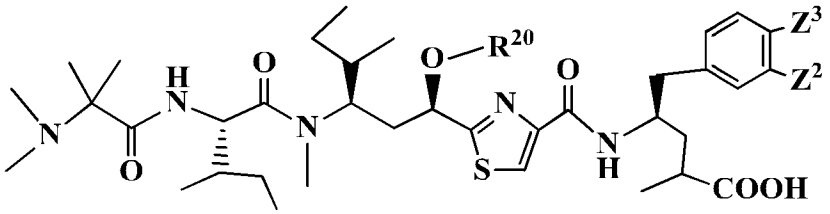

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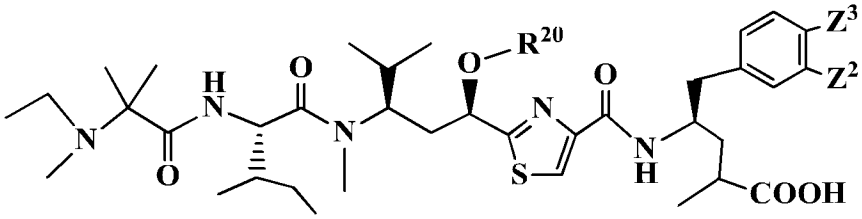

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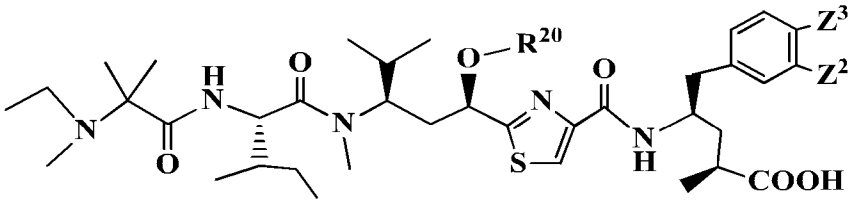

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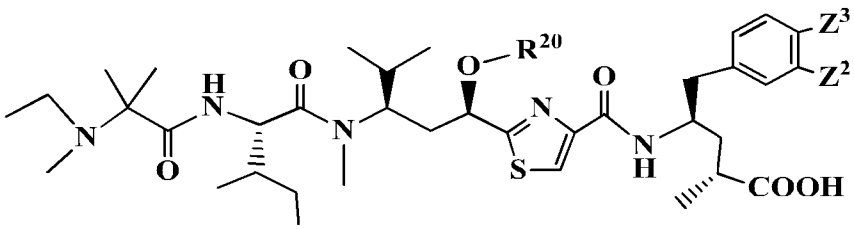

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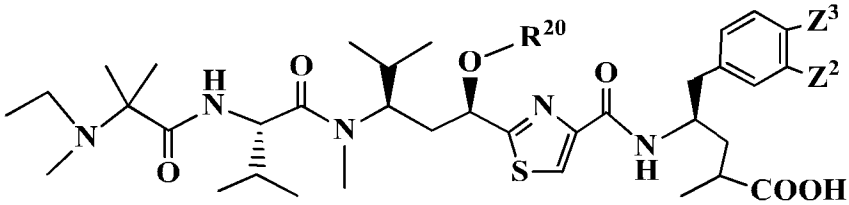

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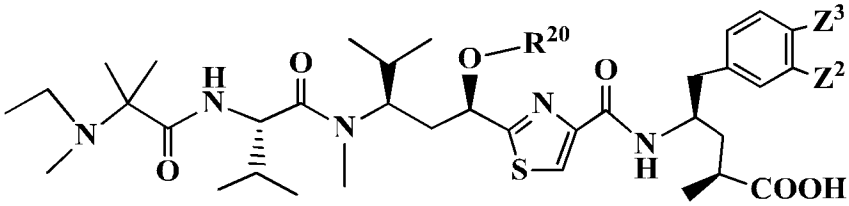

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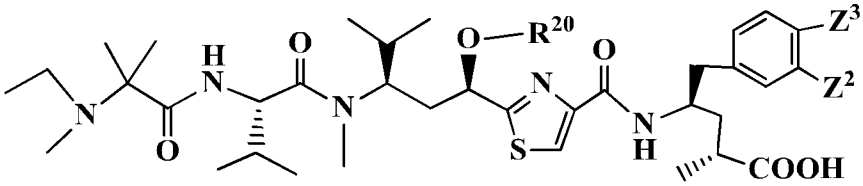

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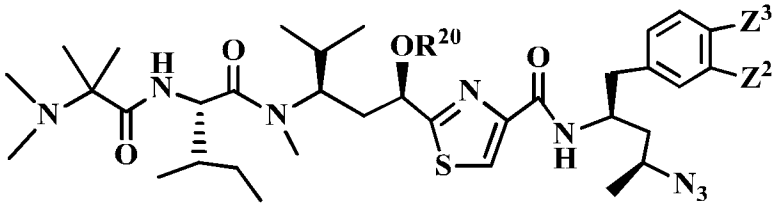

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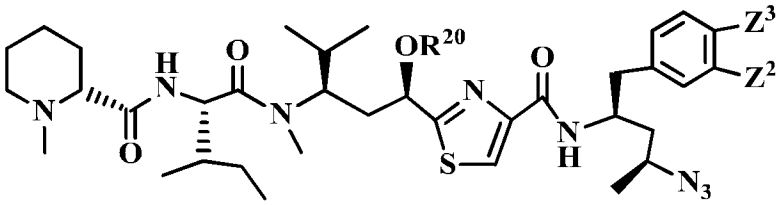

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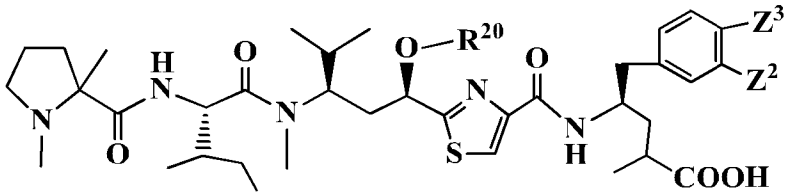

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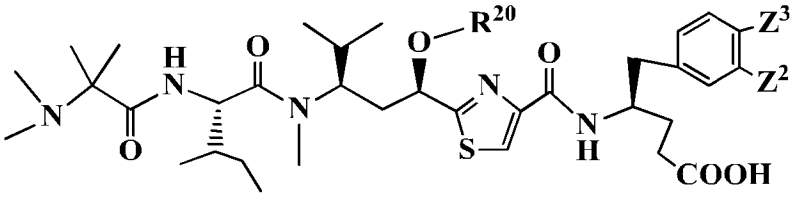

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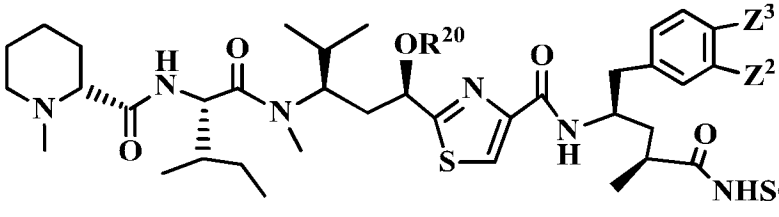

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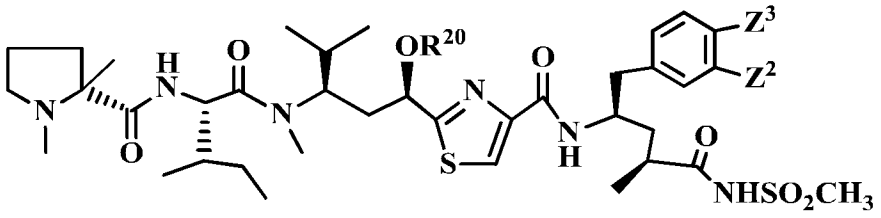

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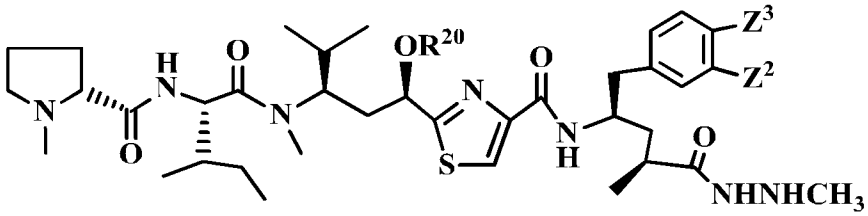

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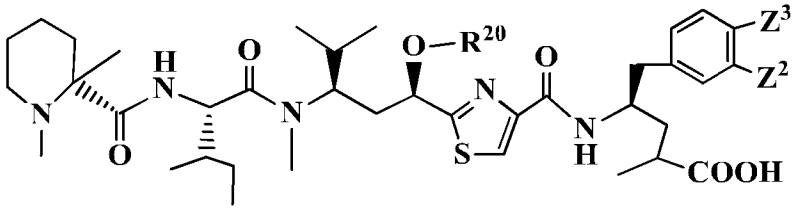

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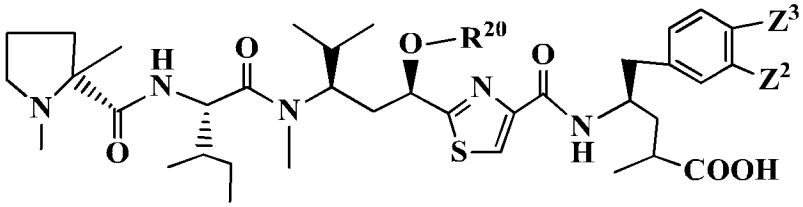

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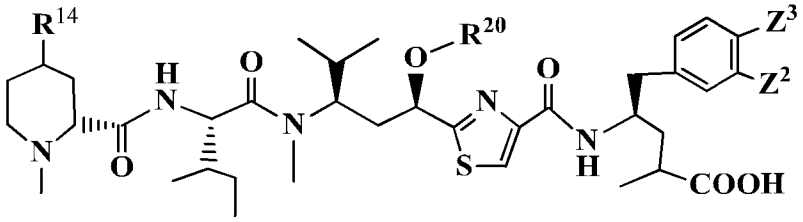

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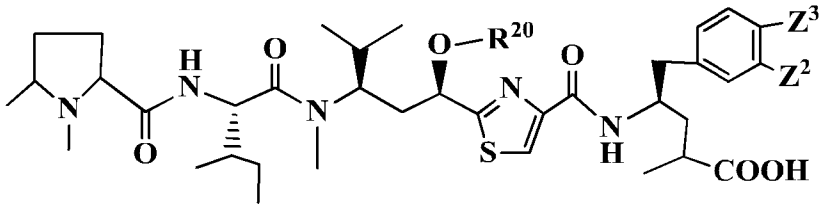

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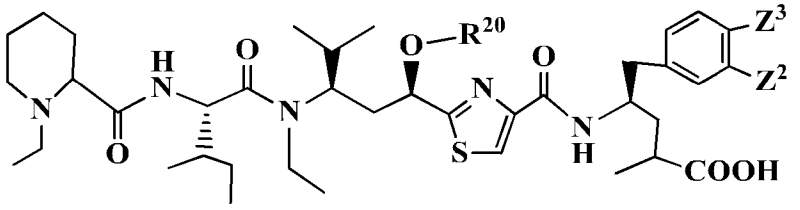

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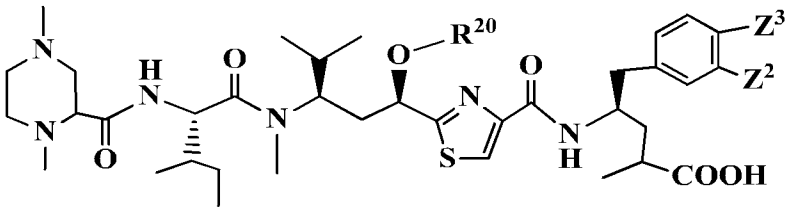

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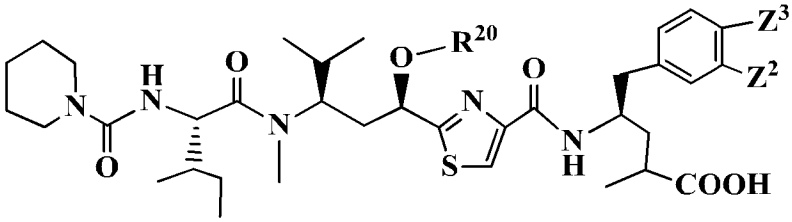

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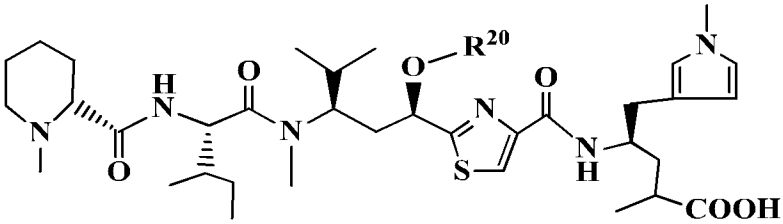

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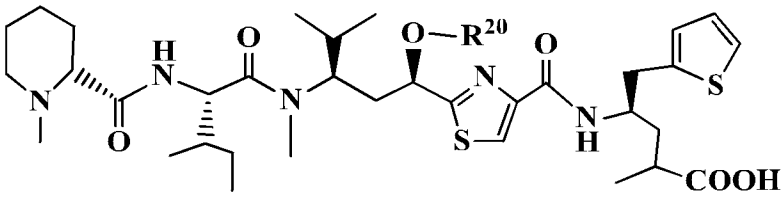

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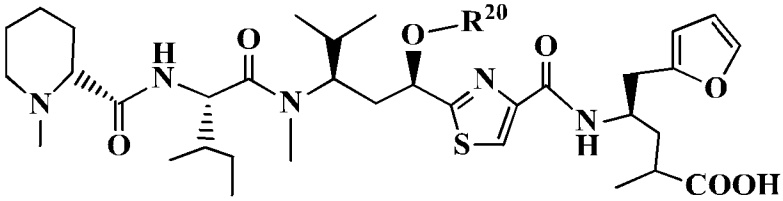

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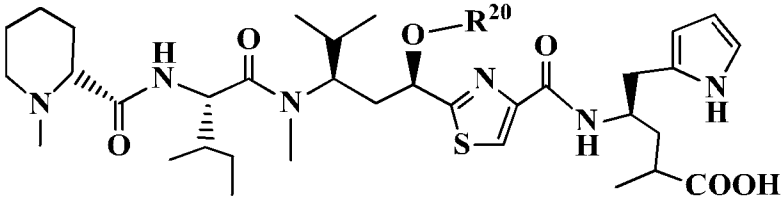

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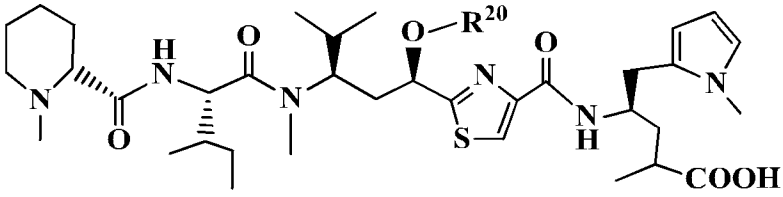

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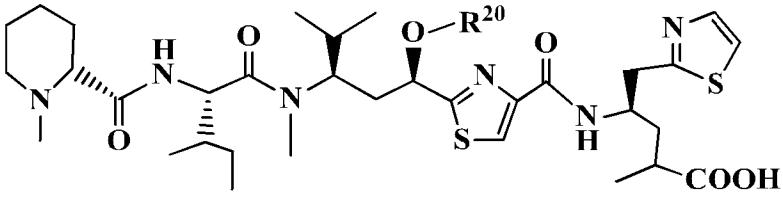

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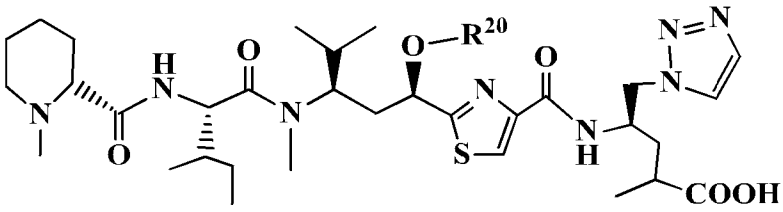

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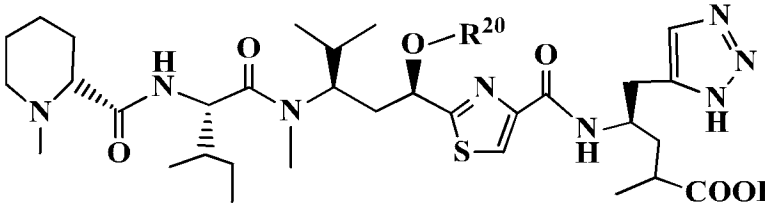

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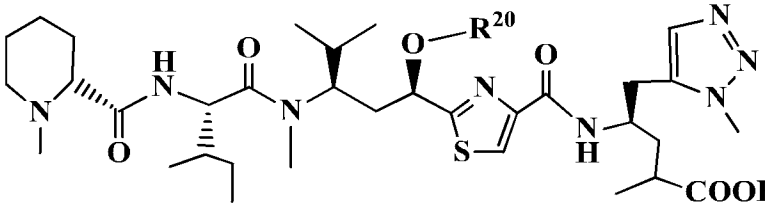

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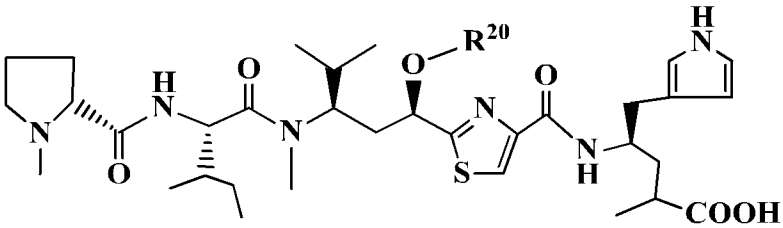

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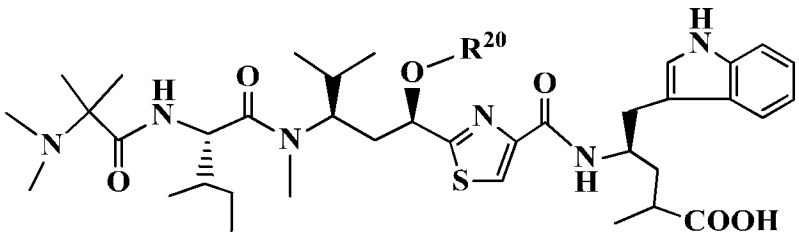

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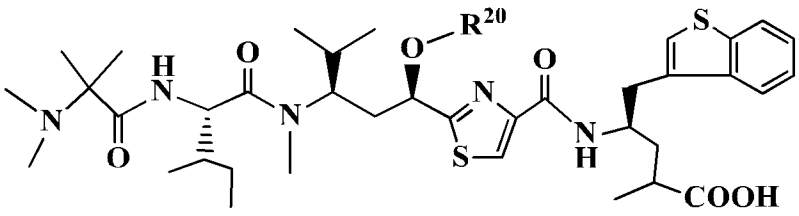

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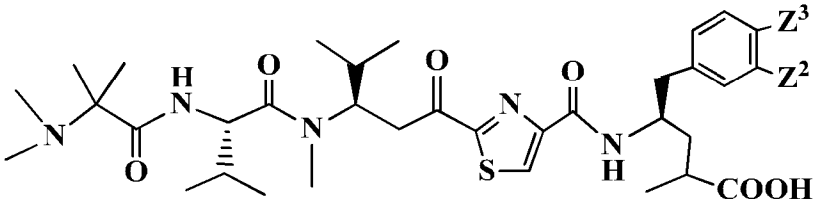

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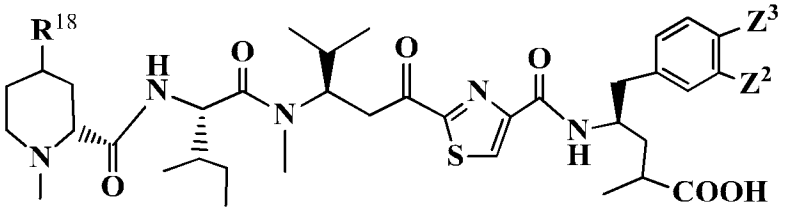

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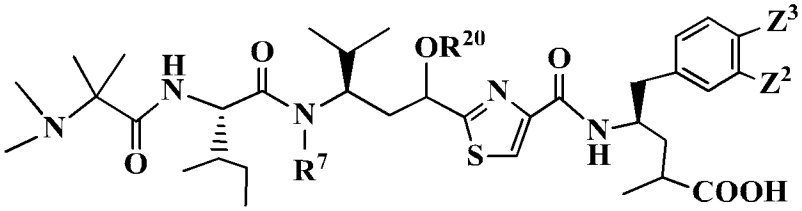

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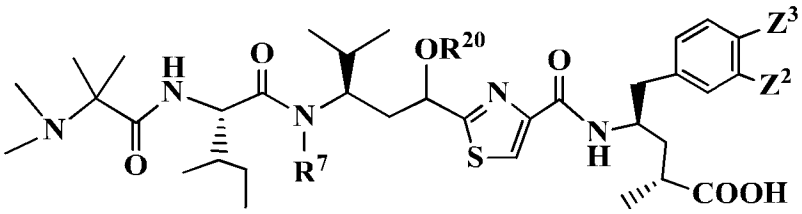

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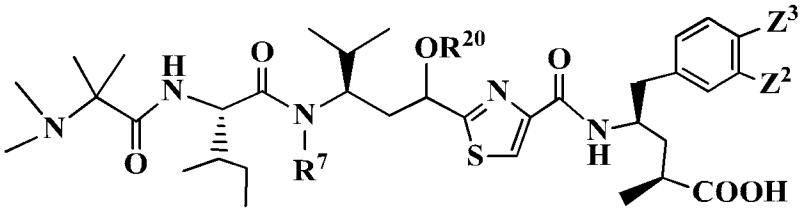

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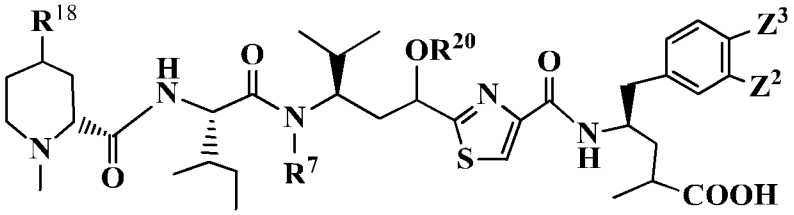

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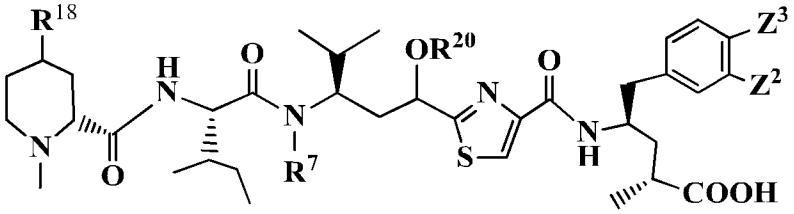

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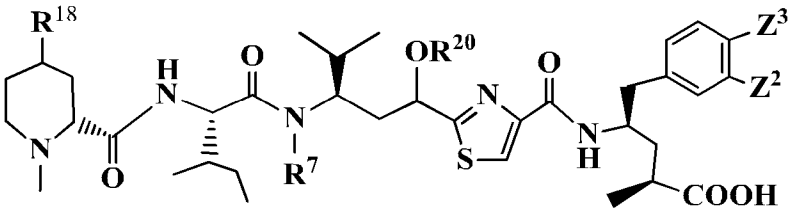

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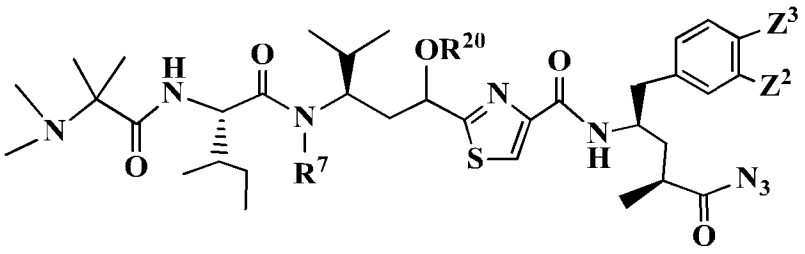

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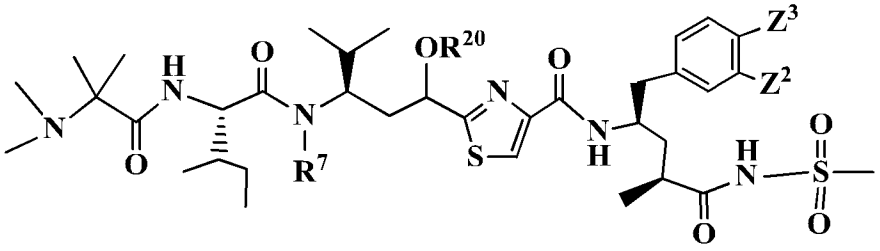

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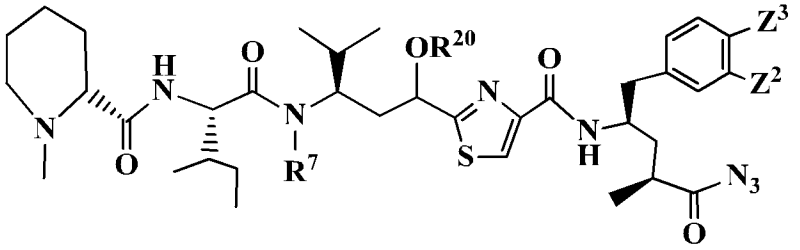

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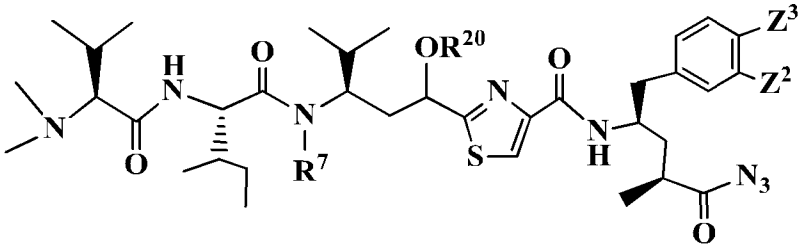
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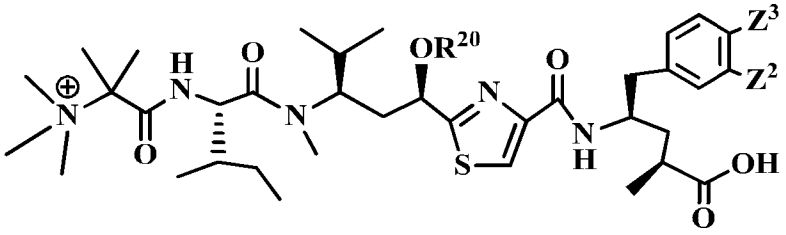
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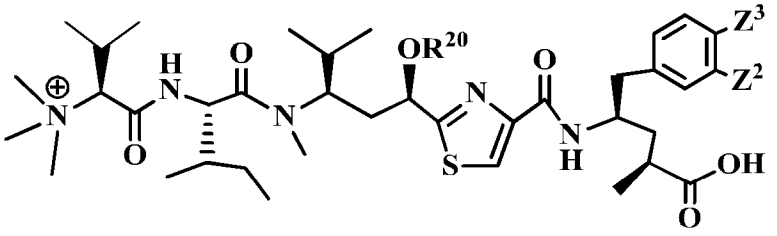
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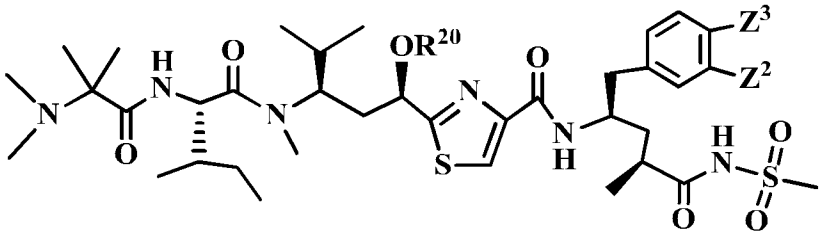
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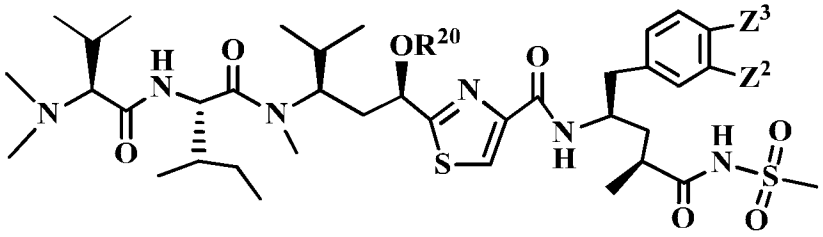
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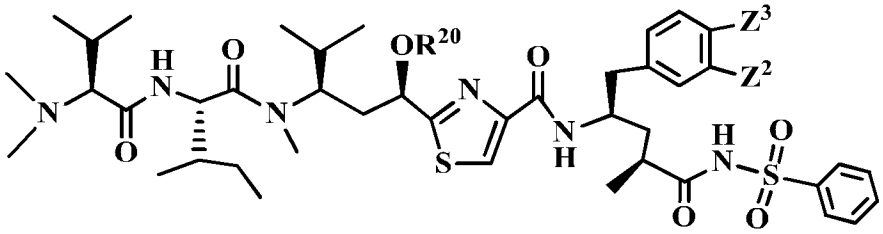
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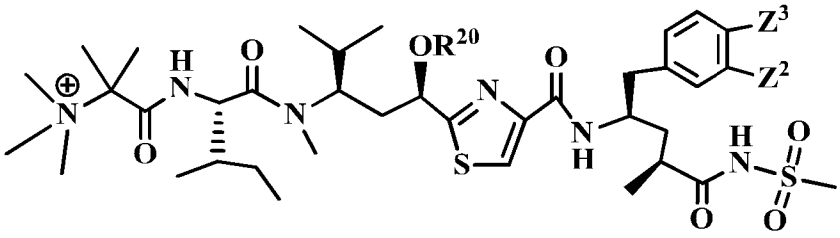
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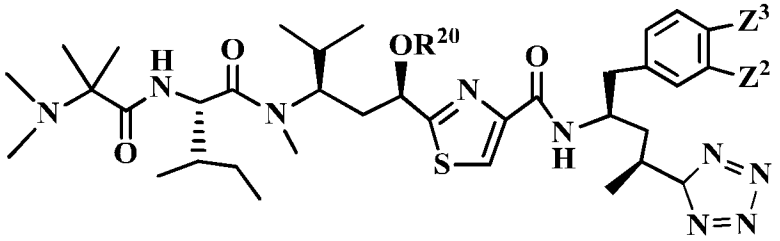
V-83,



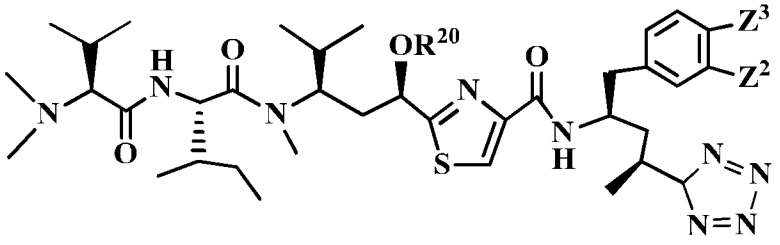
V-84,



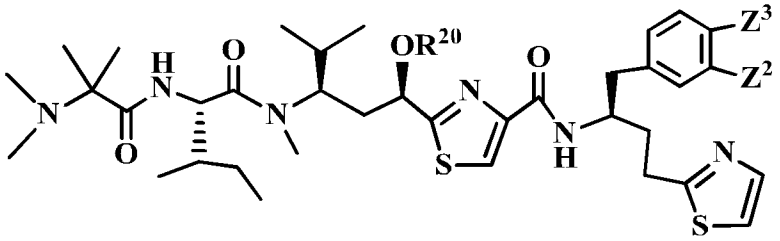
V-85,



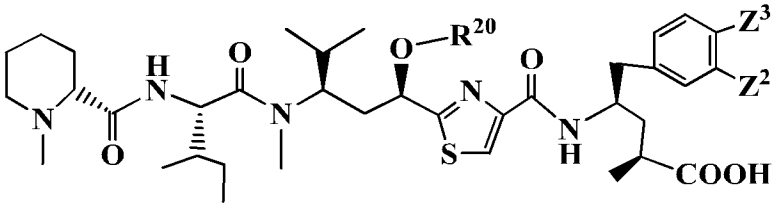
V-86,



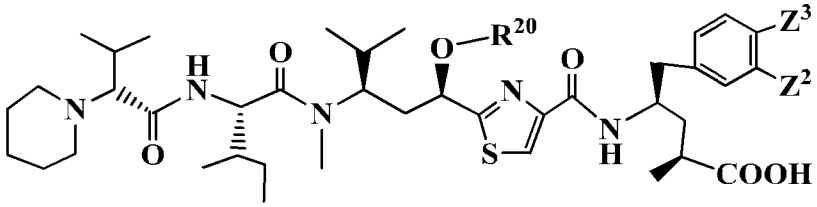
V-87,

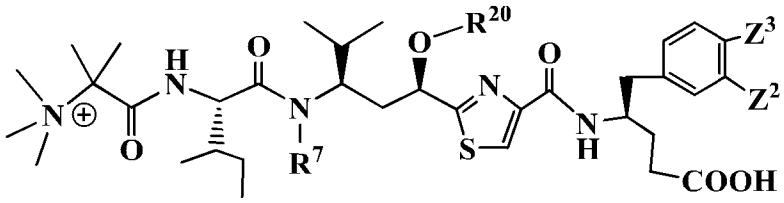


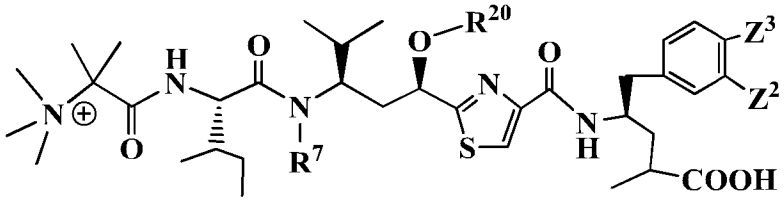
V-88,

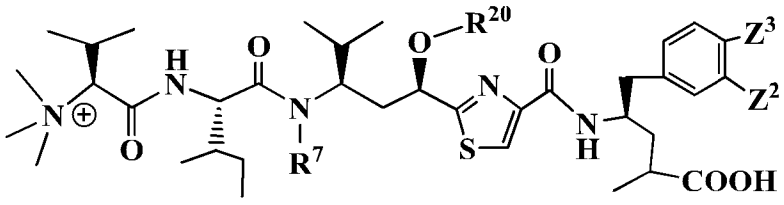


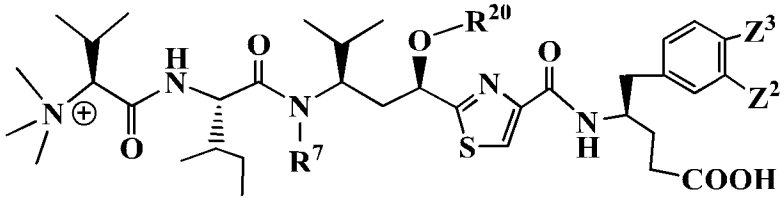
V-89,


V-90,


V-91,


V-92,

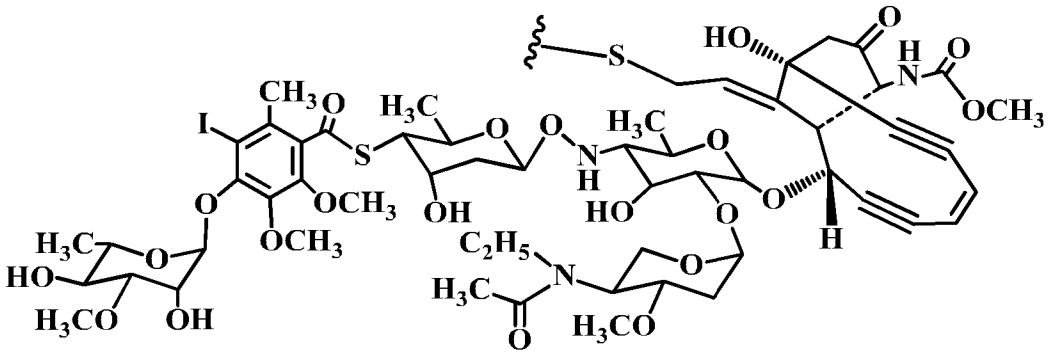

V-93,


V-94,

wherein R^{20} is H; C_1-C_8 of linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-$C(O)OR^{17}$), carbamate (-$C(O)NR^{17}R^{18}$); or 1-8 carbon atoms of carboxylate, esters, ether, or amide; or 1~8 amino acids; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000; or R^{20} is absent and the oxygen forms a ketone, or combination above thereof; $Z^3$and Z^3 are independently H, OH, NH_2, O, NH, COOH, COO, C(O), C(O), C(O)NH, $C(O)NH_2$, R^{18}, $OCH_2OP(O)(OR^{18})_2$, $OC(O)OP(O)(OR^{18})_2$, $OPO(OR^{18})_2$, $NHPO(OR^{18})_2$, $OP(O)(OR^{18})OP(O)(OR^{18})_2$, $OC(O)R^{18}$, $OC(O)NHR^{18}$, $OSO_2(OR^{18})$, O-(C_4-C_{12}-glycoside), of linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-$C(O)OR^{17}$), carbamate (-$C(O)NR^{17}R^{18}$); $R^{17}$and R^{18} are independently H, linear or branched alkyl or heteroalkyl;

C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-C(O)OR^{17}), carbamate (-C(O)$NR^{17}R^{18}$); $R^{19}$is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, C_1~C_8 alkyl or carboxylate; C_2~C_8 alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3~C_8 aryl or alkylcarbonyl; or pharmaceutical salts; X is O, S, NH, NHNH, or CH_2; R^7 is H, R^{14}, -$R^{14}C(=O)X^1R^{15}$; or -$R^{14}X^1R^{15}$; X^1 is O, S, S-S, NH, CH_2 or NR^{14}, wherein R^{14} and R^{15} are independently C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl; wherein the linkage sites, " ∿∿ " in formula IV-01- IV-94 are the same indication according to formula (IV).

Calicheamicins and their related enediyne antibiotics that are described in: Nicolaou, K. C. et al, Science 1992, 256, 1172-1178; Proc. Natl. Acad. Sci USA. 1993, 90, 5881-8), U.S. Patent Nos. 4, 970, 198; 5, 053, 394; 5, 108, 912; 5, 264, 586; 5, 384, 412; 5, 606, 040; 5, 712, 374; 5, 714, 586; 5, 739, 116; 5, 770, 701; 5, 770, 710; 5, 773, 001; 5, 877, 296; 6, 015, 562; 6, 124, 310; 8, 153, 768. Exemplary enediynes include, but are not limited to, calicheamicin, esperamicin, uncialamicin, dynemicin, and their derivatives. The structure of calicheamicins is preferred the following formula:

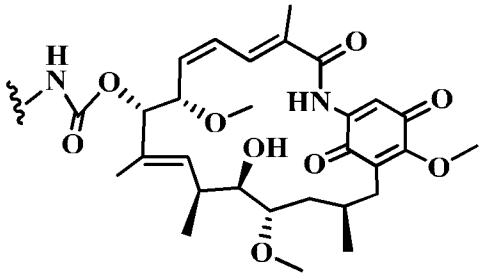


(Ia),

or an isotope of a chemical element, or a pharmaceutically acceptable salt, hydrates, or hydrated salt; or a polymorphic crystalline structure; or an optical isomer, racemate, diastereomer or enantiomer thereof,

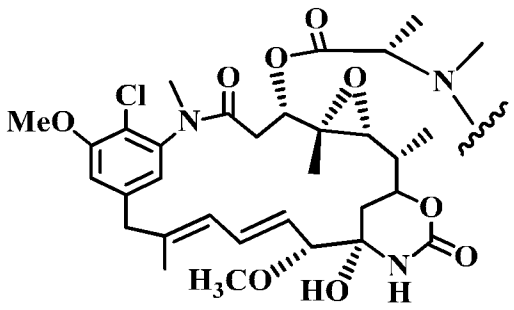
wherein ∿∿ is the site linked to L_1 or L_2;

Geldanamycins are benzoquinone ansamycin antibiotic that bind to Hsp90 (Heat Shock Protein 90) and have been used antitumor drugs. Exemplary geldanamycins include, but are not limited to, 17-AAG (17-N-Allylamino-17-Demethoxygeldanamycin) and 17-DMAG (17-dimethylamino-ethylamino-17-demethoxygeldanamycin). One of the geldanamycins structures is shown below:



wherein ~~~ is the site linked to L_1 or L_2.

Maytansines or their derivatives maytansinoids inhibit cell proliferation by inhibiting the mcirotubules formation during mitosis through inhibition of polymerization of tubulin. See Remillard et al., Science 189:1002-1005 (1975). Exemplary maytansines and maytansinoids include, but are not limited to, mertansines (DM1, DM4), maytansinol and its derivatives as well as ansamitocin. Maytansinoids are described in U.S. Patent Nos. 4, 256, 746, 4, 361, 650, 4, 307, 016, 4, 294, 757, 4, 294, 757, 4, 371, 533, 4, 424, 219, 4, 331, 598, 4, 450, 254, 4, 364, 866, 4, 313, 946, 4, 315, 929 4, 362, 663, 4, 322, 348, 4, 371, 533, 4, 424, 219, 5, 208, 020, 5, 416, 064, 5, 208, 020; 5, 416, 064; 6, 333.410; 6, 441, 163; 6, 716, 821, 7, 276, 497, 7, 301, 019, 7, 303, 749, 7, 368, 565, 7, 411, 063, 7, 851, 432, and 8, 163, 888. The structure of maytansinoids is preferred the following formula:



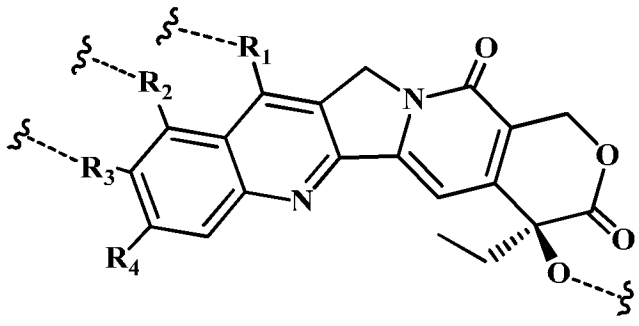
(Ib),

wherein ~~~ is the site linked to L_1 or L_2.

A camptothecin (CPTs) and its derivatives, which are topoisomerase inhibitors to prevent DNA re-ligation and therefore to causes DNA damage resulting in apoptosis, are described in: Shang, X. F. et al, Med Res Rev. 2018, 38(3):775-828; Botella, P. and Rivero-Buceta, E. J Control Release. 2017, 247: 28-54; Martino, E. et al, Bioorg Med Chem Lett. 2017, 27(4):701-707; Lu, A., et al, Acta Pharmacol Sin 2007, 28(2): 307–314. It includes SN-38, Topotecan, Irinotecan (CPT-11), Silatecan (DB-67, AR-67), Cositecan (BNP-1350), Etirinotecan, Exatecan, Lurtotecan, Gimatecan (ST1481), Belotecan (CKD-602), Rubitecan and several others (Shang, X. F. et al, Med Res Rev. 2018, 38(3):775-828). So far three CPT analogues, topotecan, irinotecan, and belotecan, have been approved and are used in cancer chemotherapy (Palakurthi, S., Expert Opin Drug Deliv. 2015;12(12):1911-21; Shang, X. F. et al, Med Res Rev. 2018, 38(3):775-828) and both SN-38 and Exatecan have been successfully used as payloads for ADC conjugates in the clinical trials (Ocean, A. J. et al, Cancer. 2017, 123(19): 3843-3854; Starodub, A. N., et al, Clin Cancer Res. 2015, 21(17):

3870-8; Cardillo, T. M., et al, Bioconjug Chem. 2015, 26(5): 919-31; Ogitani, Y. et al, Bioorg Med Chem Lett. 2016, 26(20): 5069-5072; Takegawa, N. et al, Int J Cancer. 2017 Oct 15;141(8):1682-1689. US patents 7, 591, 994; 7, 999, 083, 8, 080, 250, 8, 268, 317; US patent applications 20130090458, 20140099258, 20150297748, 20160279259).

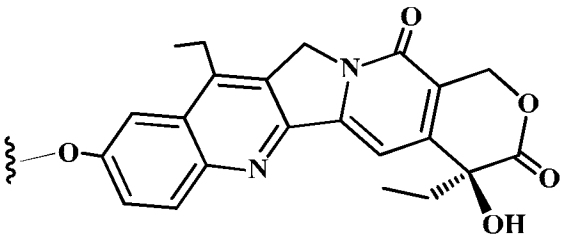
The structure of Camptothecin (CPT) is illustrated below formula:



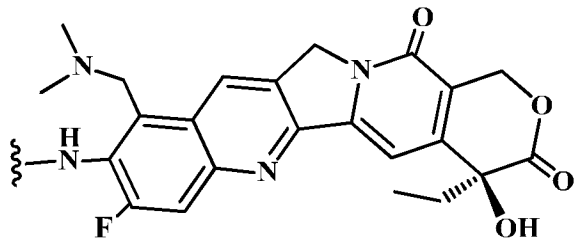
(Ic)

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein R_1, R_2 and R_4 are independently selected from H, F, Cl, Br, CN, NO_2, C_1~C_8 alkyl; O-C_1~C_8 alkyl; NH-C_1~C_8 alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 2-8 carbon atoms of esters, ether, amide, carbonate, urea, or carbamate; R_3 is H, OH, NH_2, C_1~C_8 alkyl; O-C_1~C_8 alkyl; NH-C_1~C_8 alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; or 2-8 carbon atoms of esters, ether, amide, carbonate, urea, or carbamate; or R_1R_2, R_2R_3 and R_3R_4 independently form a 5~7 membered carbocyclic, heterocyclic, heterocycloalkyl, aromatic or heteroaromatic ring system; ∿∿ is the site in the molecule that can be linked to L_1 or L_2.

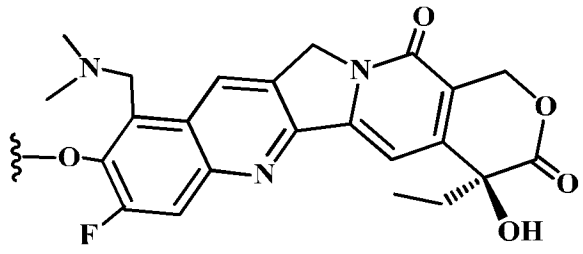
The structures of camptothecins are preferred the following formula:



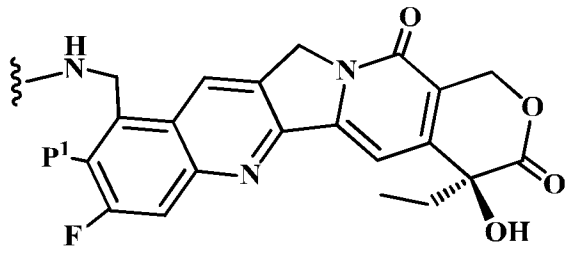
(Ic-01), SN-38,



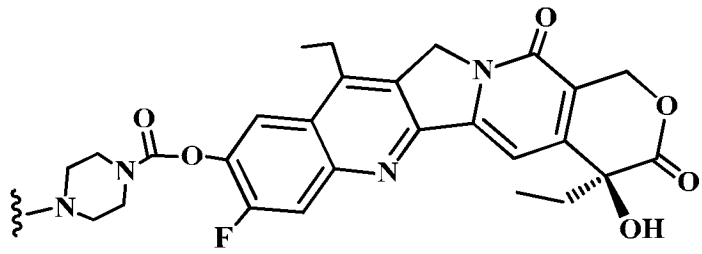
(Ic-02)



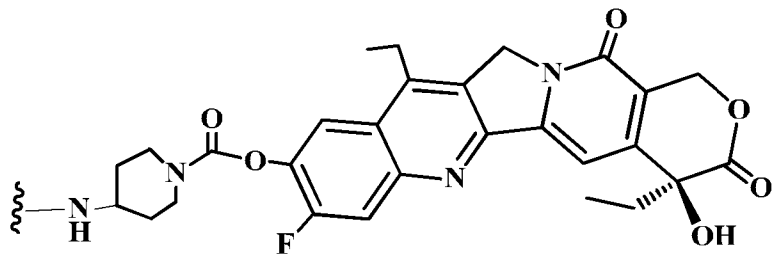
(Ic-03), Topotecan analog,



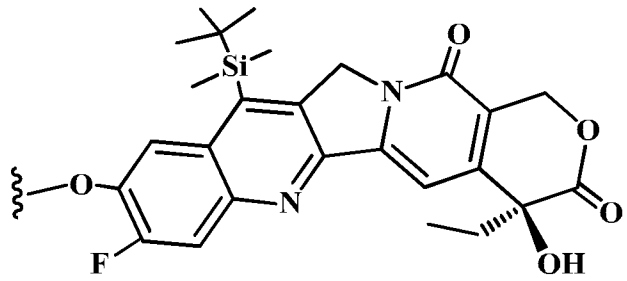
(Ic-04),



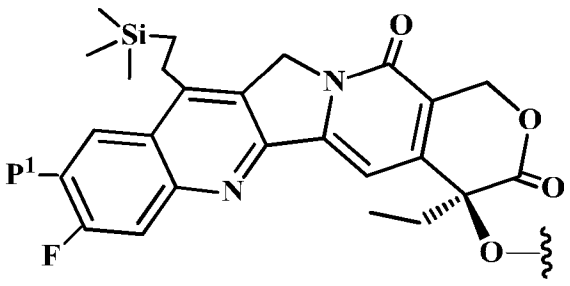
(Ic-05), Irinotecan analog,



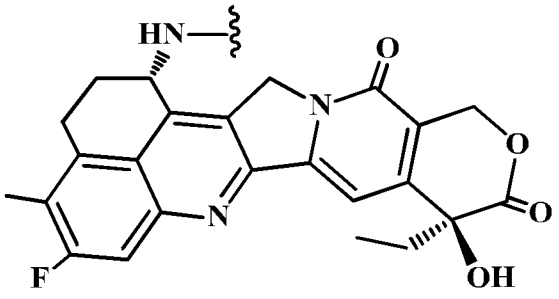
(Ic-06), Irinotecan analog,



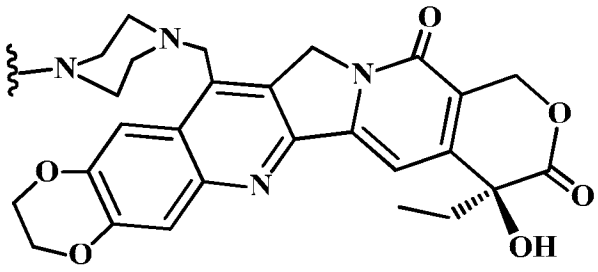
(Ic-07), Silatecan analog,



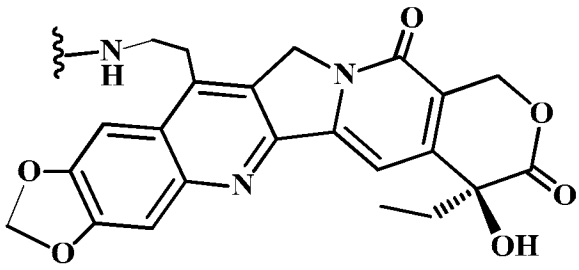
(Ic-08), Cositecan, analog



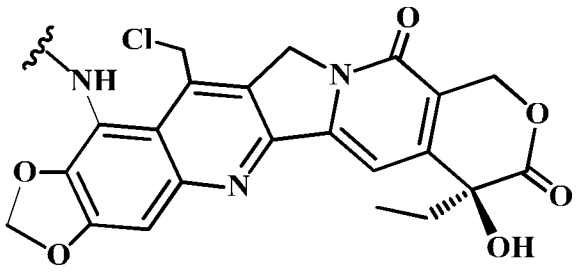
(Ic-09), Exatecan,



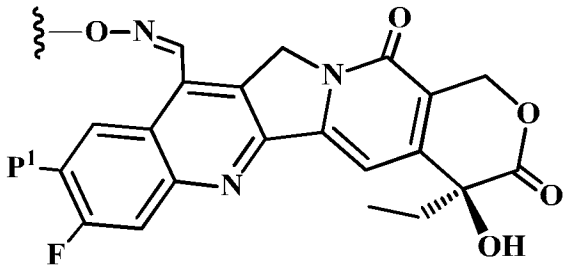
(Ic-10), Lurtotecan,



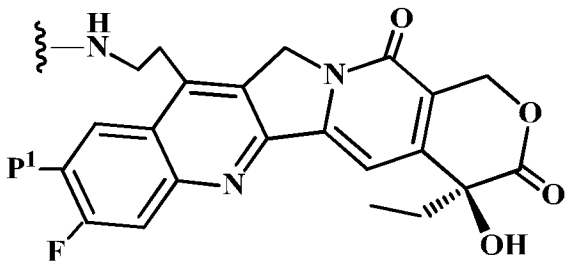
(Ic-11),



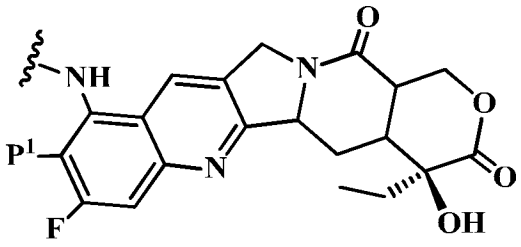
(Ic-12), GI-149893 analog,



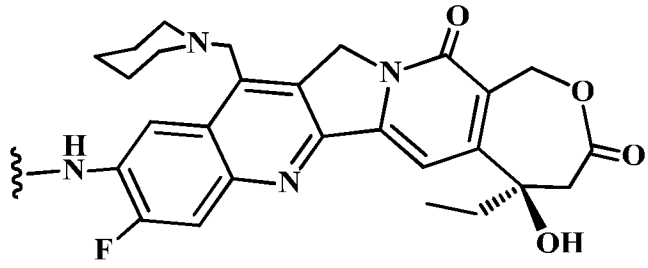
(Ic-13), Gimatecan analog,



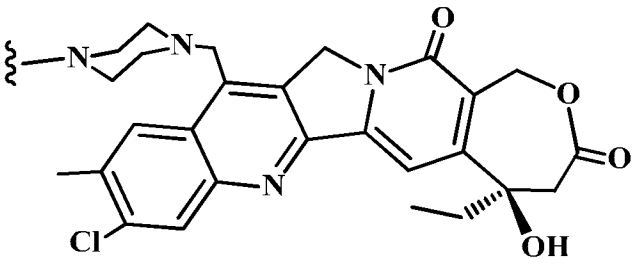
(Ic-14), Belotecan analog,



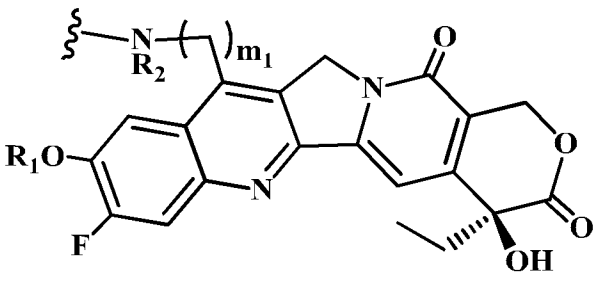
(Ic-15), Rubitecan or IDEC-132 analog, ,



(Ic-16), BN-80927 analog,



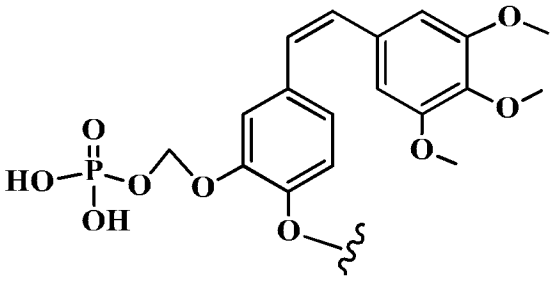
(Ic-17), BN-80927 analog,



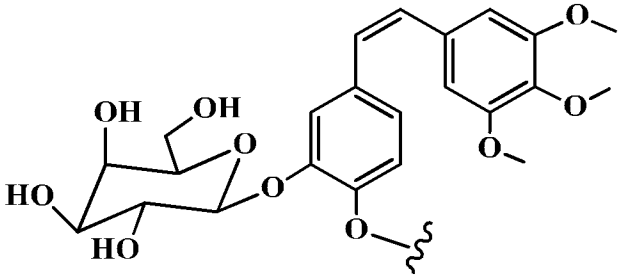
(Ic-18),

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein ~~~ is the site linked to L_1, L_1', L_2 or L_2'; P^1 is H, OH, NH_2, COOH, $C(O)NH_2$, $OCH_2OP(O)(OR^{18})_2$, $OC(O)OP(O)(OR^{18})_2$, $OPO(OR^{18})_2$, $NHPO(OR^{18})_2$, $OC(O)R^{18}$, $OP(O)(OR^{18})OP(O)(OR^{18})_2$, $OC(O)NHR^{18}$, $OC(O)N(C_2H_4)_2NCH_3$, $OSO_2(OR^{18})$, O-(C_4-C_{12}-glycoside), $OC(O)N(C_2H_4)_2CH_2N(C_2H_4)_2CH_3$, O-($C_1$-$C_8$ of linear or branched alkyl), C_1-C_8 of linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-$C(O)OR^{17}$), carbamate (-$C(O)NR^{17}R^{18}$); $R^{17}$and R^{18} are independently H, linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl;

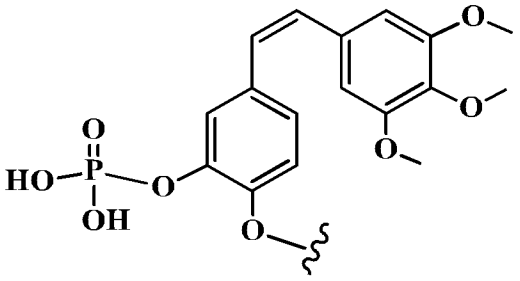
Combretastatins are natural phenols with vascular disruption properties in tumors. Exemplary combretastatins and their derivatives include, but are not limited to, combretastatin A-4 (CA-4), CA4-βGals, CA-4PD, CA4-NPs and ombrabulin.



CA-01



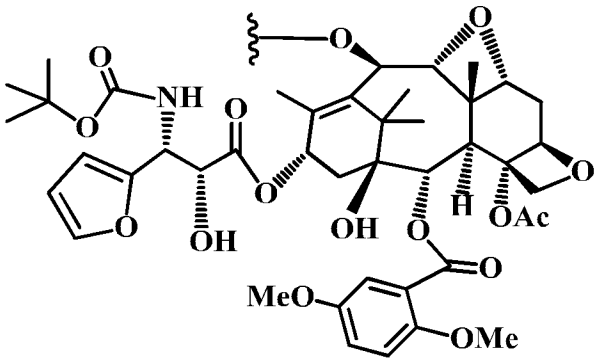
CA-02 (CA4-βGal),



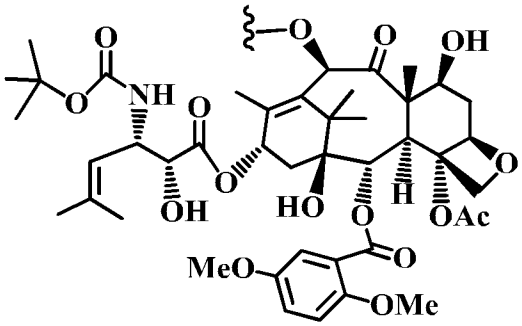
CA-03,

〰 is a site in the molecule that can be linked to L_1, L_1', L_2 or L_2';

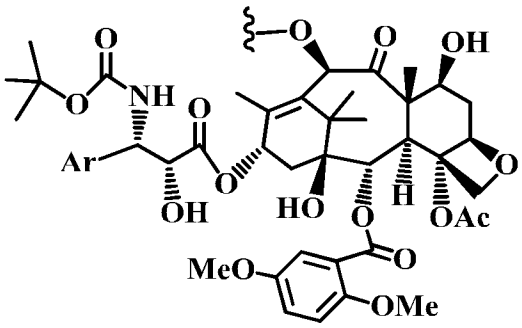
Taxanes, which includes Paclitaxel (Taxol), a cytotoxic natural product, and docetaxel (Taxotere), a semi-synthetic derivative, and their analogs which are preferred for conjugation are exampled in: K C. Nicolaou et al., J. Am. Chem. Soc. 117, 2409-20, (1995); Ojima et al, J. Med. Chem. 39:3889-3896 (1996); 40:267-78 (1997); 45, 5620-3 (2002); Ojima et al., Proc. Natl. Acad. Sci., 96:4256-61 (1999); Kim et al., Bull. Korean Chem. Soc., 20, 1389-90 (1999); Miller, et al. J. Med. Chem., 47, 4802-5 (2004); U.S. Patent No. 5, 475, 011 5, 728, 849, 5, 811, 452; 6, 340, 701; 6, 372, 738; 6, 391, 913, 6.436, 931; 6, 589, 979; 6, 596, 757; 6, 706, 708; 7, 008, 942; 7, 186, 851; 7, 217, 819; 7, 276, 499; 7, 598, 290; and 7, 667, 054. The structures of taxanes are preferred the following formula:



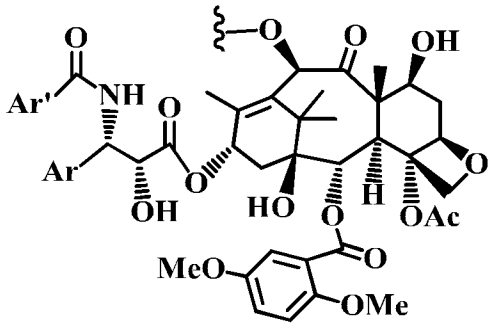
(Id-01),



(Id-02),



(Id-03),

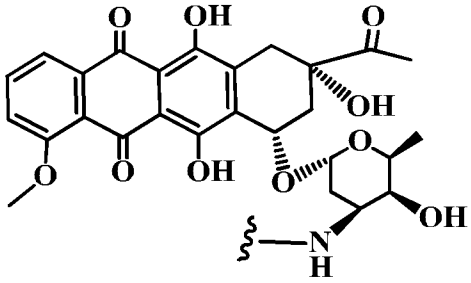


(Id-04),

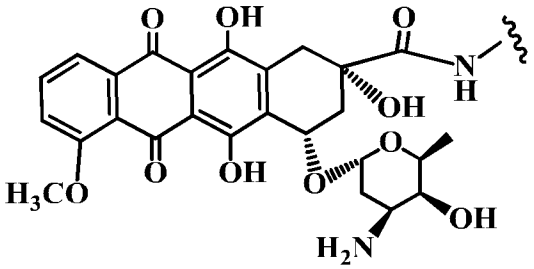
wherein ∿∿∿ is the site linked to L_1 or L_2; Ar and Ar' are independently aryl or heteroaryl.

Anthracyclines are mammalian DNA topoisomerases II inhibitors that are able to stabilize enzyme-DNA complexes wherein DNA strands are cut and covalently linked to the antibody-like protein. These anticancer agents maintain a prominent role in treating many forms of solid tumors and acute leukemias during the last several decades. However, anthracyclines cause cardiovascular morbidity and mortality (Sagi, J. C., et al, Pharmacogenomics. 2016, 17(9), 1075-87; McGowan, J. V., et al, Cardiovasc Drugs Ther. 2017, 31(1), 63-75). Thus, to enhance specific activity of such molecules while reducing the cardiotoxicity, researchers actively are using the conjugation of

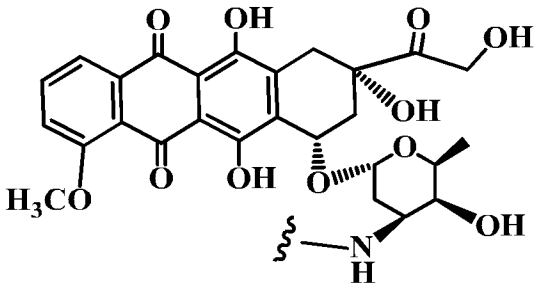
anthracyclines to a cell-binding antibody, or antibody-like protein molecule as a general approach for improving the therapeutic index of these drugs, (Mollaev, M. et al, Int J Pharm. 2018 Dec 29. pii: S0378-5173(18) 30991-8; Rossin, R., et al, Bioconjug Chem. 2016, 27(7):1697-706; Dal Corso, A., et al, J Control Release. 2017, 264:211-218). Exemplary anthracyclines include, but are not limited to, daunorubicin, doxorubicin (i.e., adriamycin), epirubicin, idarubicin, valrubicin, and mitoxantrone. The structures of anthracyclines used for the present application are preferred the following formula:



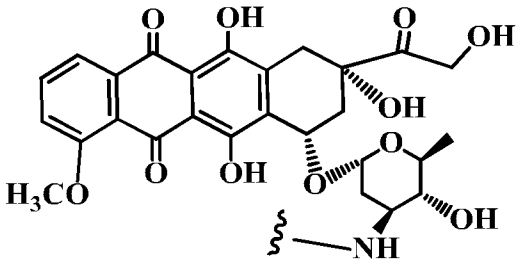
(Ie-01), Daunorubicin analog,



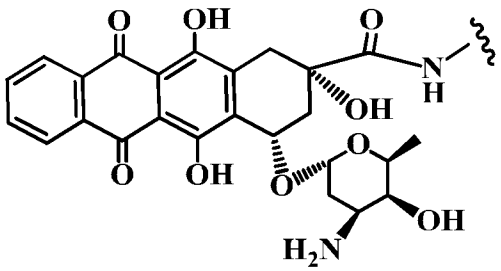
(Ie-02), Daunorubicin analog,



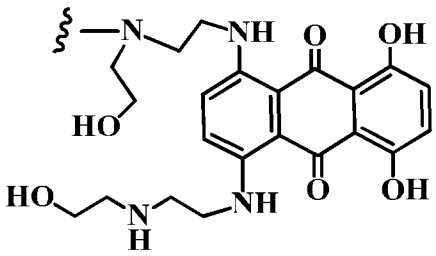
(Ie-03), Doxorubicin analog,



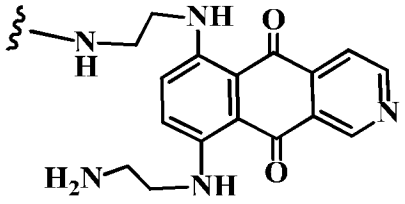
(Ie-04), Epirubicin analog,



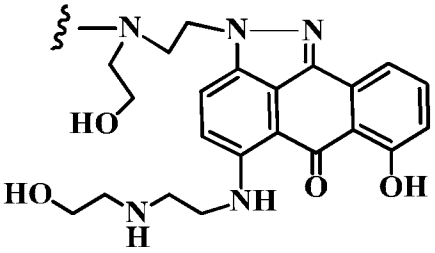
(Ie-05), Idarubicin analog,



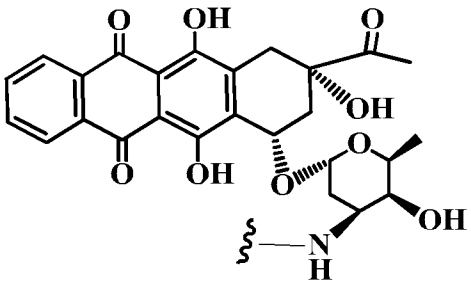
(Ie-06), Mitoxantrone analog,



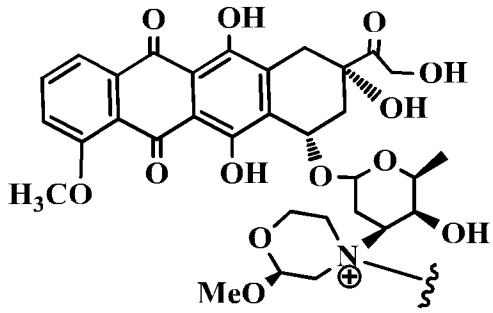
(Ie-07), Pixantrone analog,



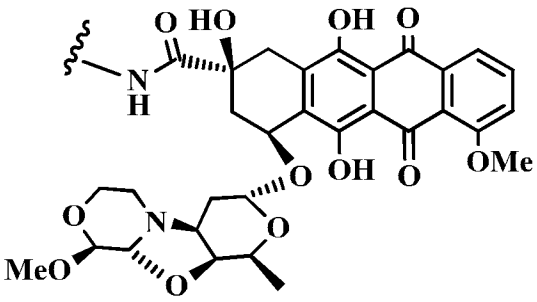
(Ie-08), Losoxantrone analog,



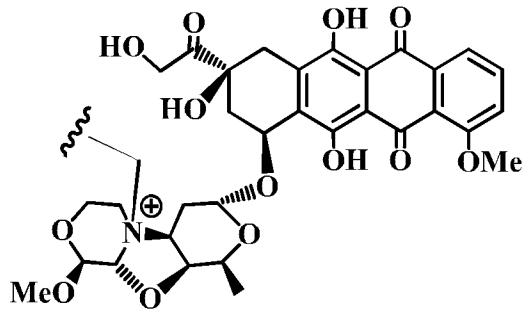
(Ie-09), analog,



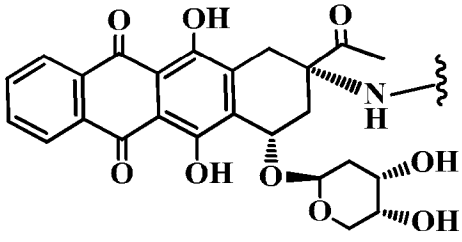
(Ie-10),



(Ie-11),



(Ie-12),

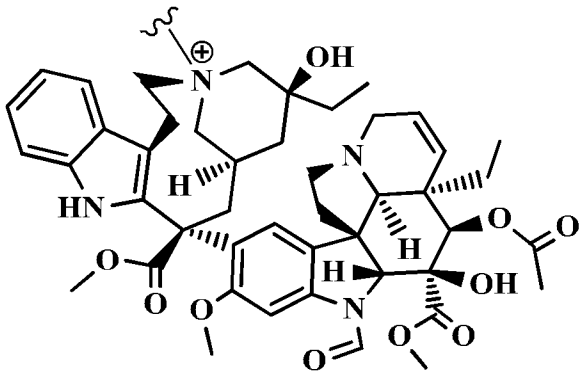


(Ie-13), Amrubicin analog.

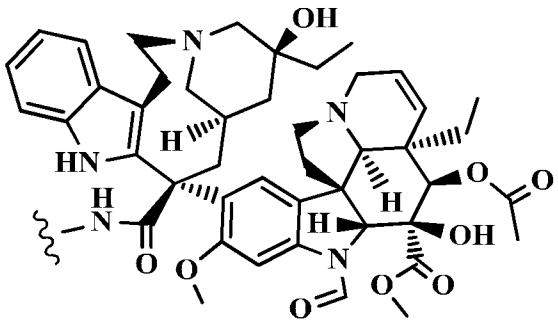
wherein ~~~ is the site that links to L_1 or L_2.

Vinca alkaloids are a set of anti-mitotic and anti-microtubule alkaloid agents that work by inhibiting the ability of cancer cells to divide. Vinca alkaloids include vinblastine, vincristine,

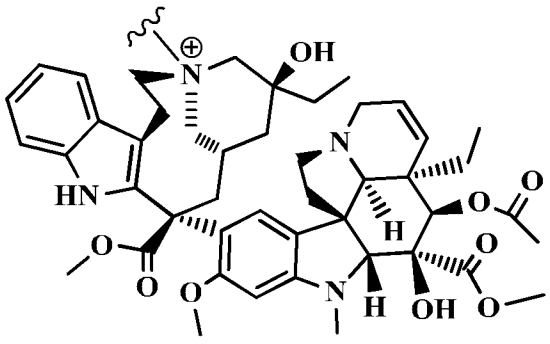
vindesine, leurosine, vinorelbine, catharanthine, vindoline, vincaminol, vineridine, minovincine, methoxyminovincine, minovincinine, vincadifformine, desoxyvincaminol, vincamajine, vincamine, vinpocetine, and vinburnine. The structures of vinca alkaloids are preferred vinblastine, vincristine having the following formula:



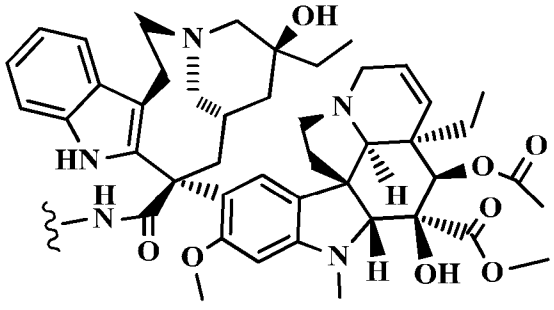
(If-01), vincristine (leurocristine),



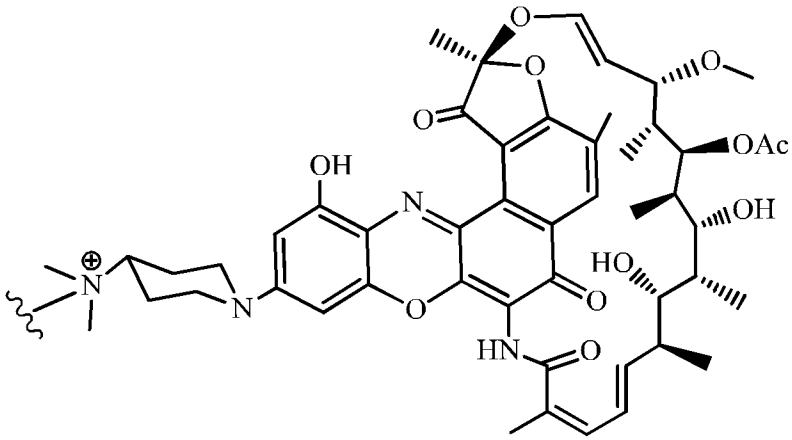
(If-02), vincristine (leurocristine),



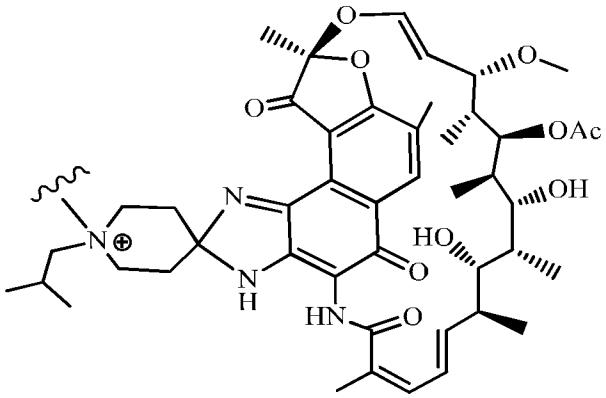
(If-03), vinblastine,



(If-04), vinblastine;



(If-05), Rifabutin analog,

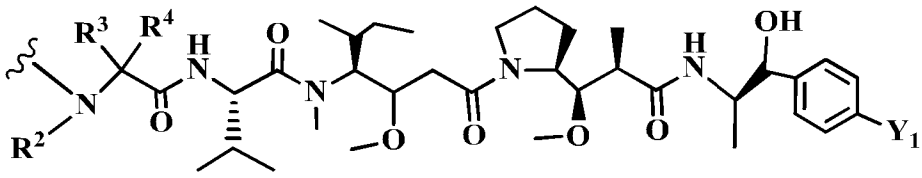


(If-06), rifabutin analog,

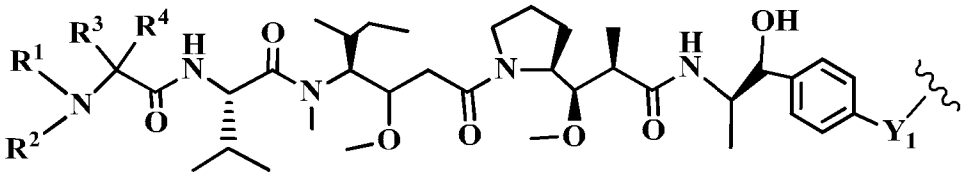
~~~ is a site in the molecule that can be linked to L_1, L_1', L_2 or L_2';

Dolastatins and their peptidic analogs and derivatives, auristatins, are highly potent antimitotic agents that have been shown to have anticancer and antifungal activity. See, e.g., U.S. Pat. No. 5, 663, 149 and Pettit et al., Antimicrob. Agents Chemother. 42:2961-2965, 1998. Exemplary dolastatins and auristatins include, but are not limited to, dolastatin 10, auristatin E (AE), auristatin EB (AEB), auristatin EFP (AEFP), MMAD (Monomethyl Auristatin D or monomethyl dolastatin 10), MMAF (Monomethyl Auristatin F or N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine), MMAE (Monomethyl Auristatin E or N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine), 5-benzoylvaleric acid-AE ester (AEVB), Auristatin F phenylene diamine (AFP) and other novel auristatins. The auristatins are described in Int. J. Oncol. 15: 367-72 (1999); Molecular Cancer Therapeutics, vol. 3, No. 8, pp. 921-32 (2004); U.S. Application Nos. 11134826, 20060074008, 2006022925. U.S. Patent Nos. 4414205, 4753894, 4764368, 4816444, 4879278, 4943628, 4978744, 5122368, 5165923, 5169774, 5286637, 5410024, 5521284, 5530097, 5554725, 5585089, 5599902, 5629197, 5635483, 5654399, 5663149, 5665860, 5708146, 5714586, 5741892, 5767236, 5767237, 5780588, 5821337, 5840699, 5965537, 6004934, 6033876, 6034065, 6048720, 6054297, 6054561, 6124431, 6143721, 6162930, 6214345, 6239104, 6323315, 6342219, 6342221, 6407213, 6569834, 6620911, 6639055, 6884869, 6913748, 7090843, 7091186, 7097840, 7098305, 7098308, 7498298, 7375078, 7462352, 7553816, 7659241, 7662387, 7745394, 7754681, 7829531,
~~~

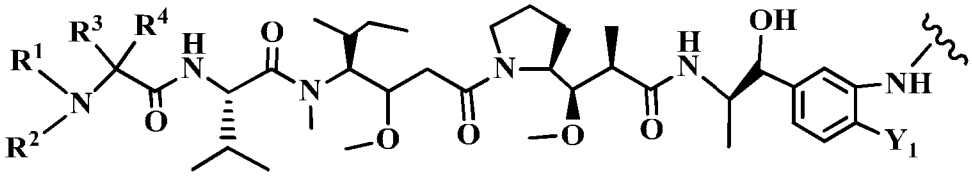
7837980, 7837995, 7902338, 7964566, 7964567, 7851437, 7994135. The structures of auristatin analogs are preferred the following formula (Ih-01), (Ih-02), (Ih-03), (Ih-04), (Ih-05), (Ih-06), (Ih-07), (Ih-08), (Ih-09), (Ih-10), and (Ih-11):



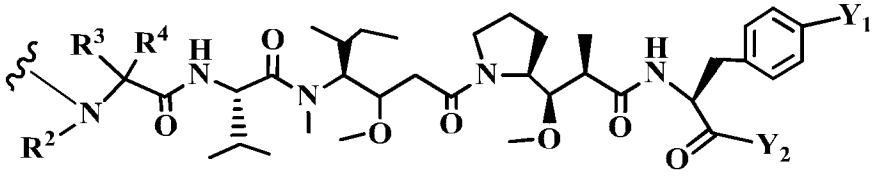
(Ih-01),



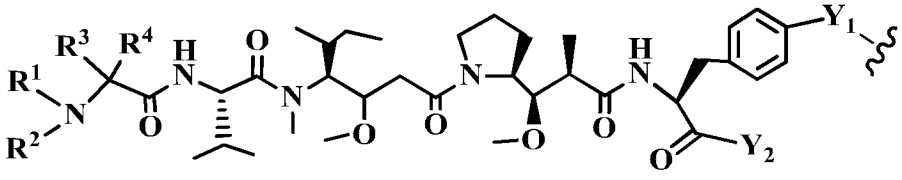
(Ih-02),



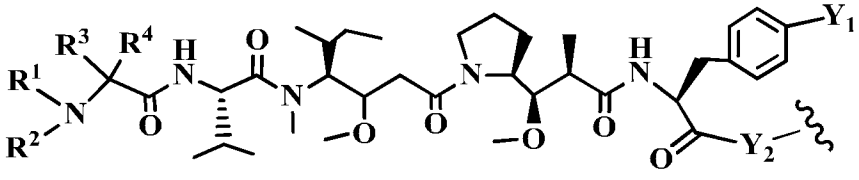
(Ih-03),



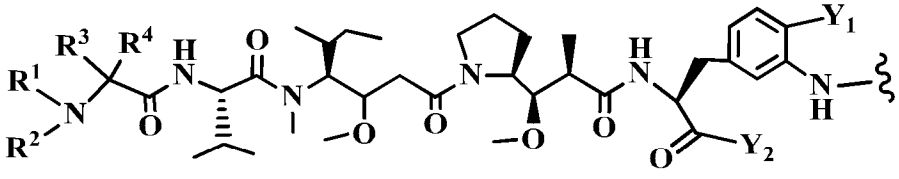
(Ih-04),



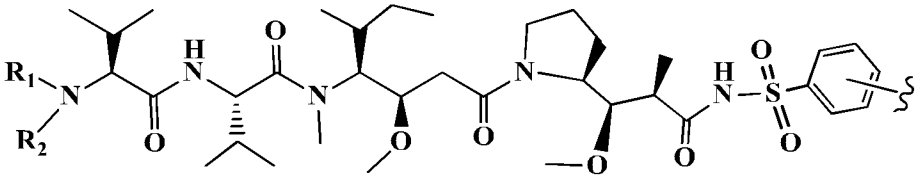
(Ih-05),



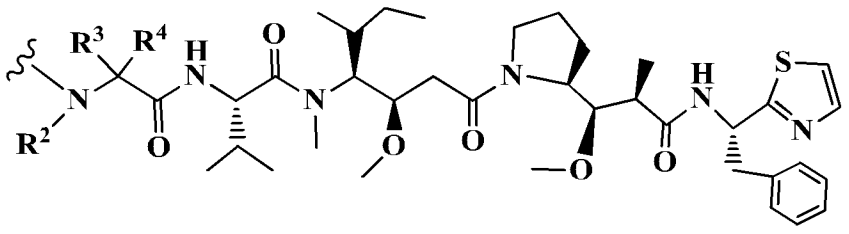
(Ih-06),



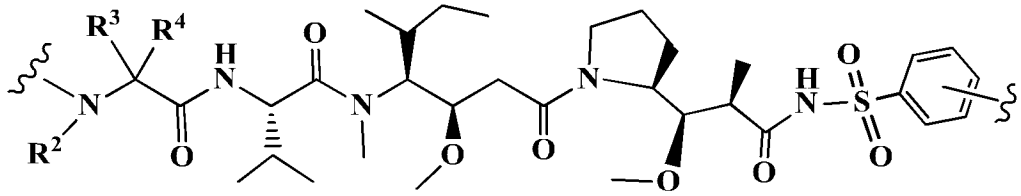
(Ih-07),



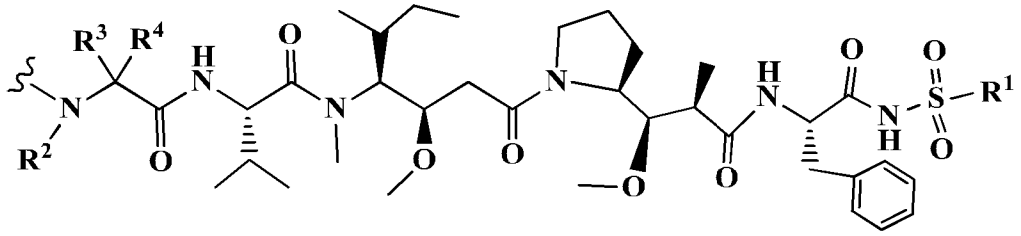
(Ih-08)



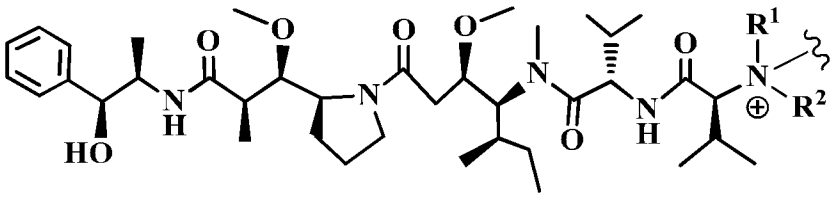
(Ih-09),



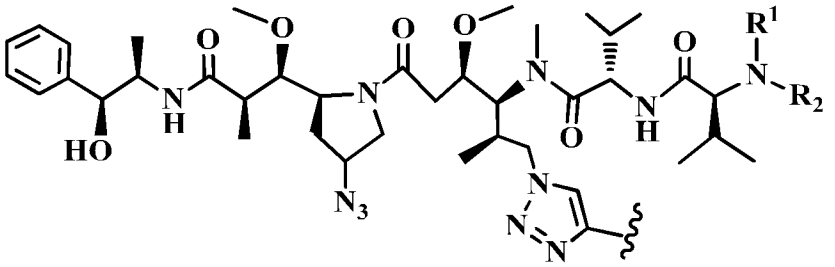
(Ih-10),



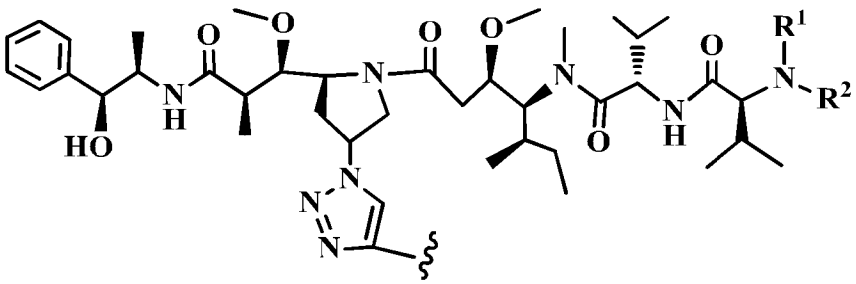
(Ih-11),



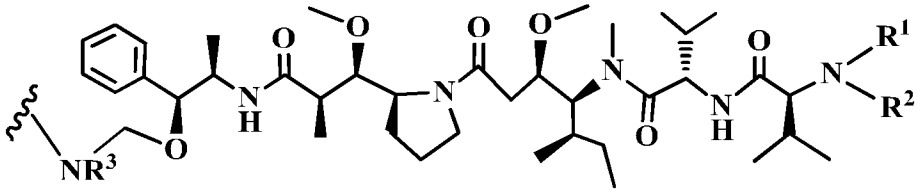
(Ih-12),



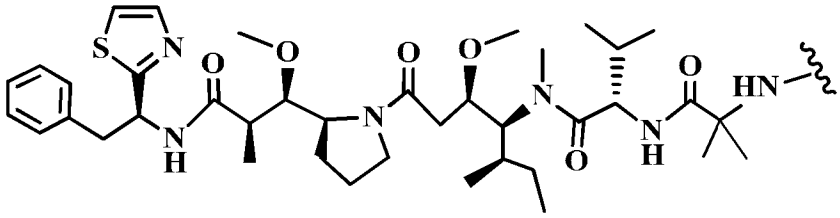
(Ih-13),



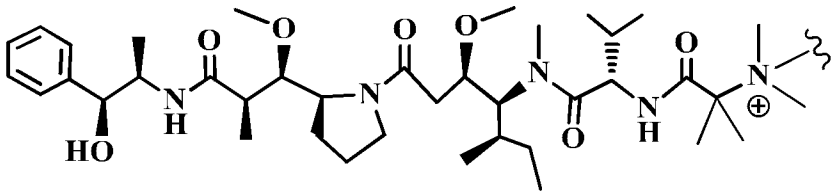
(Ih-14),



(Ih-15),



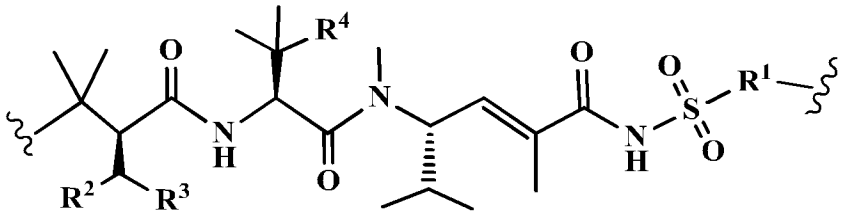
(Ih-16),



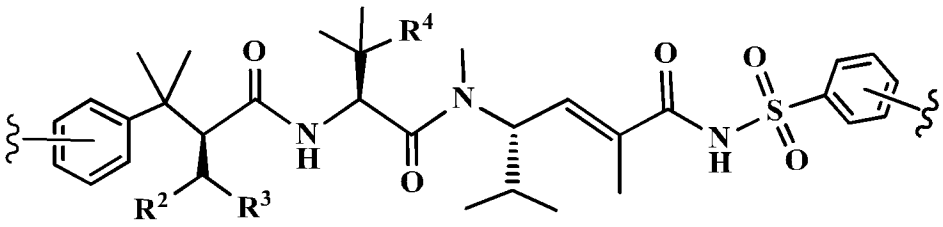
(Ih-17),

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein R^1, R^2, R^3, R^4 and R^5 are independently H; C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester, ether, amide, amines, heterocycloalkyl, or acyloxylamines; or peptides containing 1-8 aminoacids, or polyethyleneoxy unit having formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 1000. The two Rs: R^1R^2, R^2R^3, R^1R^3 or R^3R^4 together can form 3~8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group; Y_1 and Y_2 are independently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, $OC(O)N(R_1)$, $N(R_1)C(O)N(R_2)$, C(O)NHNHC(O) and $C(O)NR_1$ when linked to the connecting site " ∿∿ " (that links to L_1 and/or L_2 independently); or OH, NH_2, $NHNH_2$, NHR_5, SH, C(O)OH, $C(O)NH_2$, $OC(O)NH_2$, OC(O)OH, $NHC(O)NH_2$, NHC(O)SH, $OC(O)NH(R_1)$, $N(R_1)C(O)NH(R_2)$, C(O)NHNHC(O)OH and $C(O)NHR_1$ when not linked to the connecting site " ∿∿ "; R_{12} is OH, NH_2, NHR_1, $NHNH_2$, NHNHCOOH, O-R_1-COOH, NH-R_1-COOH, NH-$(Aa)_n$COOH, $O(CH_2CH_2O)_pCH_2CH_2OH$, $O(CH_2CH_2O)_pCH_2CH_2NH_2$, $NH(CH_2CH_2O)_pCH_2CH_2NH_2$, NR_1R_1', NHOH, $NHOR_1$, $O(CH_2CH_2O)_pCH_2CH_2COOH$, $NH(CH_2CH_2O)_pCH_2CH_2COOH$, NH-Ar-COOH, NH-Ar-$NH_2$, $O(CH_2CH_2O)_pCH_2CH_2NH$-SO_3H, $NH(CH_2CH_2O)_pCH_2CH_2NHSO_3H$, R_1-$NHSO_3H$, NH-R_1-$NHSO_3H$, $O(CH_2CH_2O)_pCH_2$-$CH_2NHPO_3H_2$, $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, OR_1, R_1-$NHPO_3H_2$, R_1-OPO_3H_2, $O(CH_2CH_2O)_pCH_2CH_2OPO_3H_2$, OR_1-$NHPO_3H_2$, NH-R_1-$NHPO_3H_2$, $NH(CH_2CH_2NH)_pCH_2$-CH_2NH_2, $NH(CH_2CH_2S)_pCH_2CH_2NH_2$, $NH(CH_2CH_2NH)_pCH_2CH_2OH$, $NH(CH_2CH_2S)_pCH_2$-CH_2OH, NH-R_1-NH_2, or $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, wherein Aa is 1-8 the same or different aminoacids; p is 1 -1000; R_1, R_2, R_3, R_4, R_5, R_5', Z_1, Z_2, and n are defined the same above.

Hemiasterlin and its analogues (e.g., HTI-286) bind to the tubulin, disrupt normal microtubule dynamics, and, at stoichiometric amounts, depolymerize microtubules. The structure of maytansinoids is preferred the following formula:



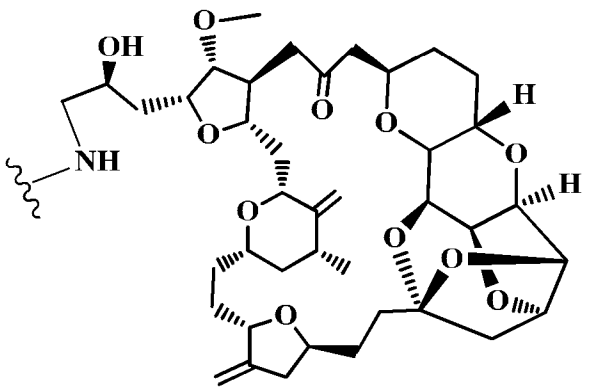
(Hs-01)



(Hs-02)

wherein wherein R^1, R^2, R^3, R^4 and R^5 are independently H; C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester, ether, amide, amines, heterocycloalkyl, or acyloxylamines; or peptides containing 1-8 aminoacids, or polyethyleneoxy unit having formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 5000; In addition, R^2R^3 can form 3~8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group.

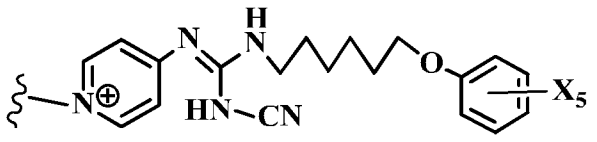
Eribulin which is binding predominantly to a small number of high affinity sites at the plus ends of existing microtubules has both cytotoxic and non-cytotoxic mechanisms of action. Its cytotoxic effects are related to its antimitotic activities, wherein apoptosis of cancer cells is induced following prolonged and irreversible mitotic blockade (Kuznetsov, G. et al, Cancer Research. 2004, 64 (16): 5760–6.; Towle, M. J, et al, Cancer Research. 2010, 71 (2): 496–505). In addition to its cytotoxic, antimitotic-based mechanisms, preclinical studies in human breast cancer models have shown that eribulin also exerts complex effects on the biology of surviving cancer cells and residual tumors that appear unrelated to its antimitotic effects. Eribulin has been approved by US FDA for the treatment of metastatic breast cancer who have received at least two prior chemotherapy regimens for late-stage disease, including both anthracycline- and taxane-based chemotherapies, as well as for the treatment of liposarcoma (a specific type of soft tissue sarcoma) that cannot be removed by surgery (unresectable) or is advanced (metastatic). Eribulin has been used as payload for ADC conjugates (US20170252458). The structure of Eribulin is preferred the following formula, Eb01:



Eb01,

is a linkage site that links to L_1, L_1', L_2 and/or L_2' independently;

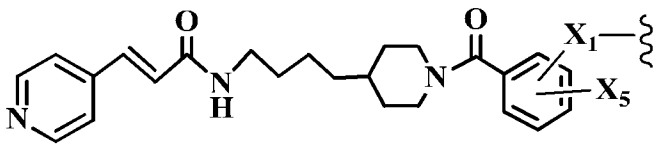
An Inhibitor of nicotinamide phosphoribosyltransferases (NAMPT) can be an interesting ADC payload due to their unique mechanisms of high potent activity (Sampath D, et al, Pharmacol Ther **2015**; 151, 16-31). NAMPT regulates nicotinamide adenine dinucleotide (NAD) levels in cells wherein NAD plays as an essential redox cofactor to support energy and anabolic metabolism. NAD has several essential roles in metabolism. It acts as a coenzyme in redox reactions, as a donor of ADP-ribose moieties in ADP-ribosylation reactions, as a precursor of the second messenger molecule cyclic ADP-ribose, as well as acting as a substrate for bacterial DNA ligases and a group of enzymes called sirtuins that use NAD^+ to remove acetyl groups from proteins. In addition to these metabolic functions, NAD^+ emerges as an adenine nucleotide that can be released from cells spontaneously and by regulated mechanisms (Smyth L. M, et al, J. Biol. Chem. 2004, 279 (47), 48893–903; Billington R. A, et al, Mol Med. 2006, 12, 324–7), and can therefore have important extracellular roles (Billington R. A, et al, Mol Med. 2006, 12, 324–7). When inhibitors of NAMPT present, NAD levels decline below the level needed for metabolism resulting in energy crisis and therefore cell death. So far, clinical NAMPT inhibitor candidates FK-866, CHS-828, and GMX-1777 advanced to clinical trials but each encountered dose-limiting toxicities prior to any objective responses (Holen K., et al, Invest New Drugs 2008, 26, 45-51; Hovstadius, P., et al, Clin Cancer Res 2002, 8, 2843-50; Pishvaian, M. J., et al, J Clin Oncol 2009, 27, 3581). Thus using ADCs for targeting delivery of NAMPT inhibitors might circumvent the systemic toxicities to achieve much broader therapeutic index. The structures of NAMPT inhibitors are preferred the following formula, NP01, NP02, NP03, NP04, NP05, NP06, NP07, NP08, and NP09:



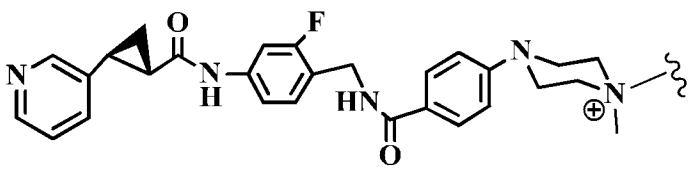
NP01,



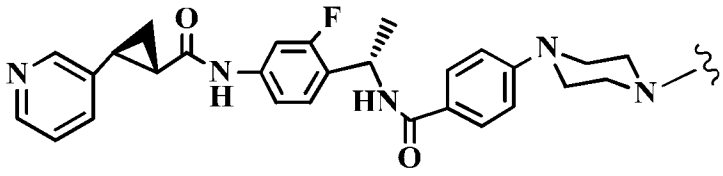
NP02,



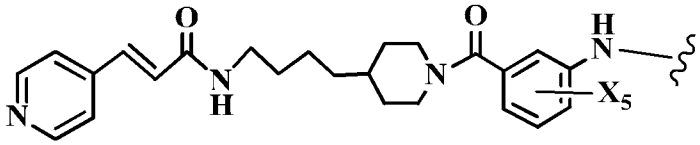
NP03,



NP04,



NP05,



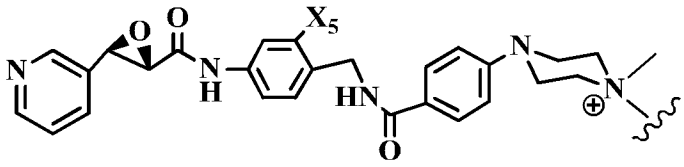
NP06,



NP07,



NP08,

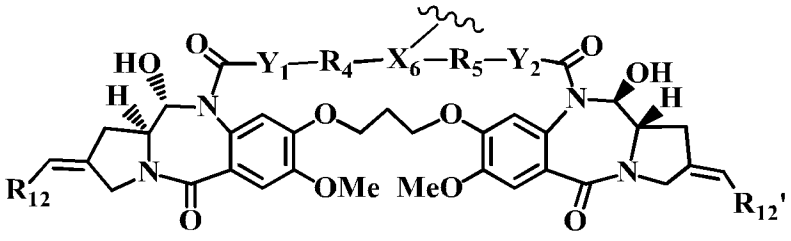


NP09,

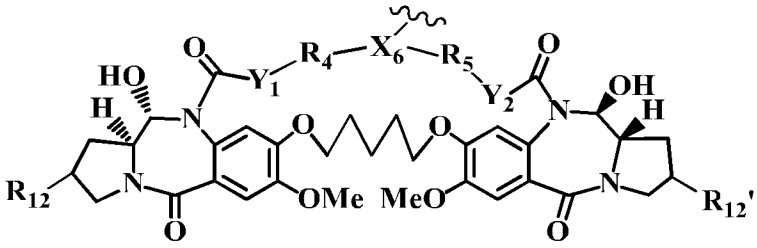
or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein " ∿∿ " is the same above; X_5 is F, Cl, Br, I, OH, OR_1, R_1, OPO_3H_2, OSO_3H, NHR_1, $OCOR_1$, $NHCOR_1$, wherein R_1 is defined the same above, and is preferred H, C_1-C_8 linear or branched alkyl.

A benzodiazepine dimer and its analogs: (e. g. a dimer of pyrrolobenzodiazepine (PBD) or (tomaymycin), a dimer of indolinobenzodiazepine (IGN), a dimer of imidazobenzothiadiazepine, or a dimer of oxazolidinobenzodiazepines) are anti-tumor agents that contain one or more immine functional groups, or their equivalents, that bind to duplex DNA. PBD and IGN molecules are based on the natural product athramycin, and interact with DNA in a sequence-selective manner, with a preference for purine-guanine-purine sequences. The preferred benzodiazepine dimers according to the present invention are exampled in: US Patent Nos. 8, 163, 736; 8, 153, 627; 8, 034, 808; 7, 834, 005; 7, 741, 319; 7, 704, 924; 7, 691, 848; 7, 678, 787; 7, 612, 062; 7, 608, 615; 7, 557, 099; 7, 528, 128; 7, 528, 126; 7, 511, 032; 7, 429, 658; 7, 407, 951; 7, 326, 700; 7, 312, 210; 7, 265, 105; 7, 202, 239; 7, 189, 710; 7, 173, 026; 7, 109, 193; 7, 067, 511; 7, 064, 120; 7, 056, 913; 7, 049, 311; 7, 022, 699; 7, 015, 215; 6, 979, 684; 6, 951, 853; 6, 884, 799; 6, 800, 622; 6, 747, 144; 6, 660, 856; 6, 608, 192; 6, 562, 806; 6, 977, 254; 6, 951, 853; 6, 909, 006; 6, 344, 451; 5, 880, 122; 4, 935, 362; 4, 764, 616; 4, 761, 412; 4, 723, 007; 4, 723, 003; 4, 683, 230; 4, 663, 453; 4, 508, 647; 4, 464, 467; 4, 427,

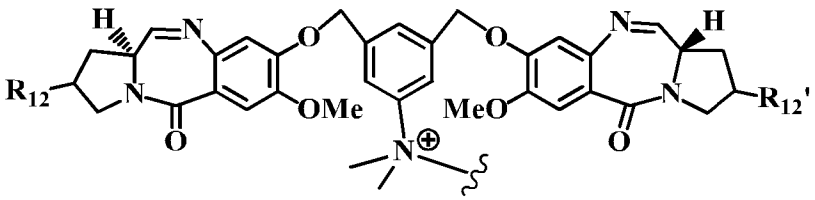
587; 4, 000, 304; US patent appl. 20100203007, 20100316656, 20030195196. Examples of the structures of the conjugate of the antibody- benzodiazepine dimers are illustrated below PB01, PB02, PB03, PB04, PB05, PB06, PB07, PB08, PB09, PB10, PB11, PB12, PB13, PB14, PB15, PB16, PB17, PB18, PB19, PB20, PB21, PB22, PB23, PB24, PB25, PB26, PB27 and PB28:



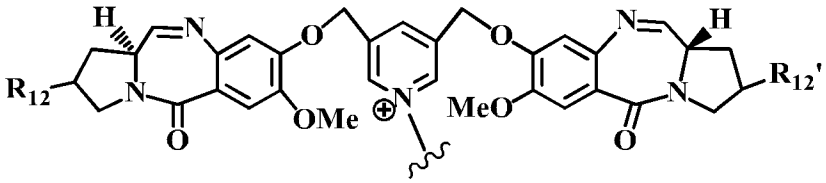
PB01,



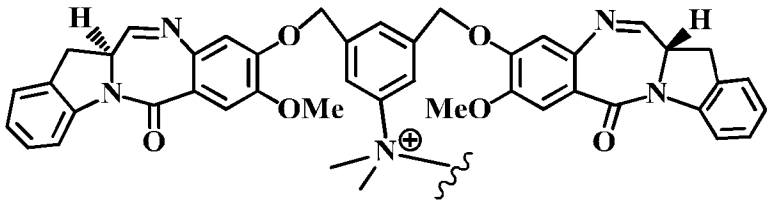
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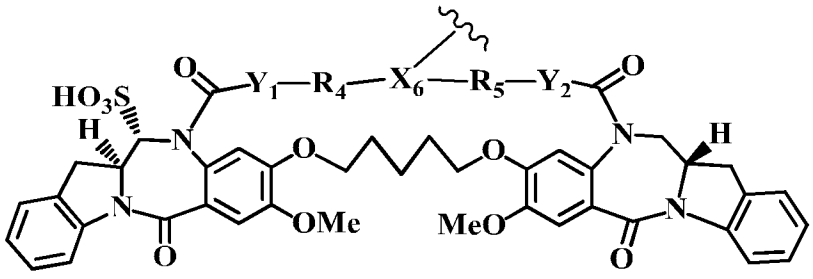
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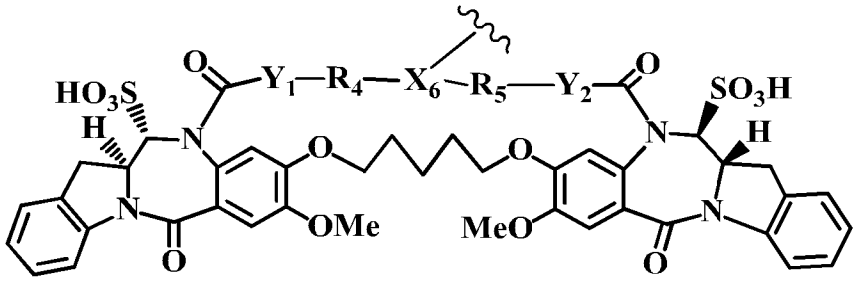
PB04,



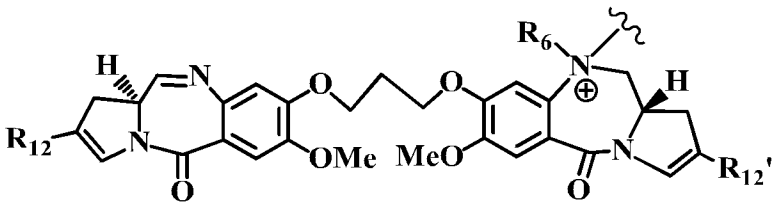
PB05,



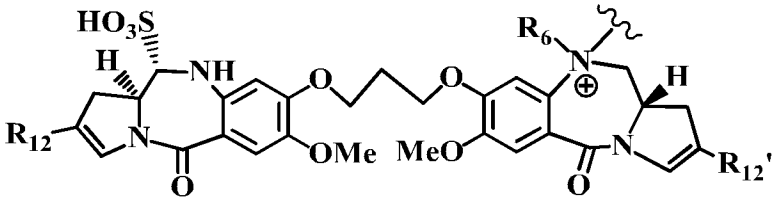
PB06,



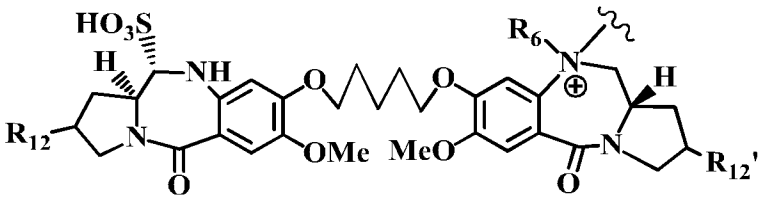
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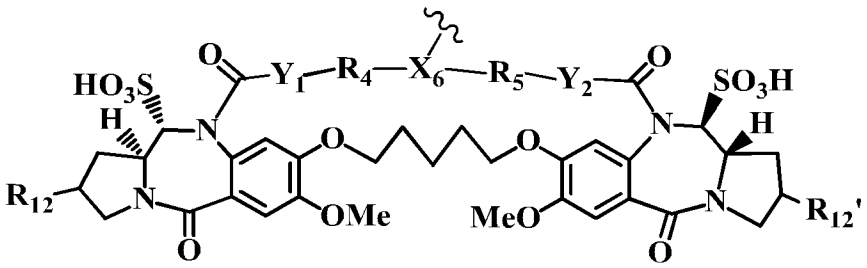
PB08,



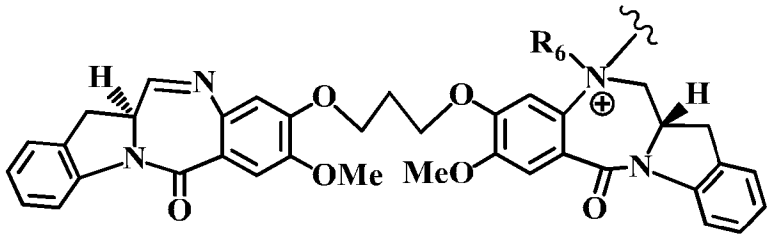
PB09,



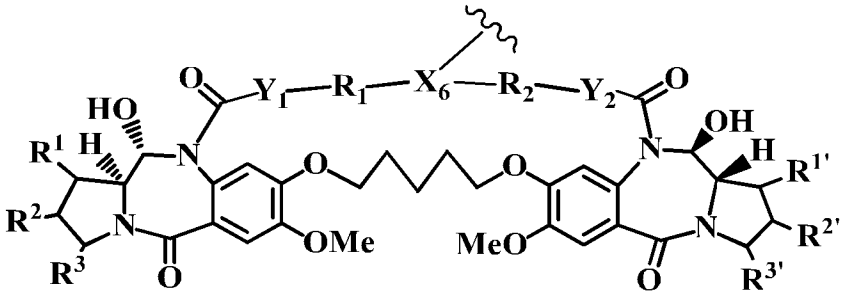
PB10,



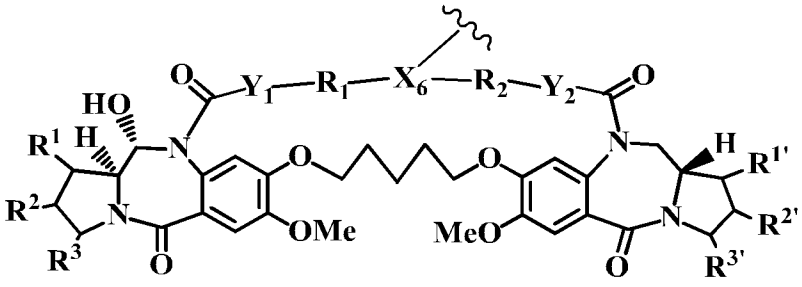
PB11,



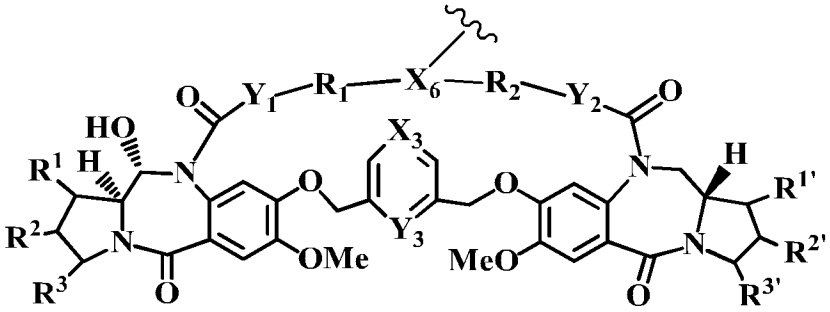
PB12,



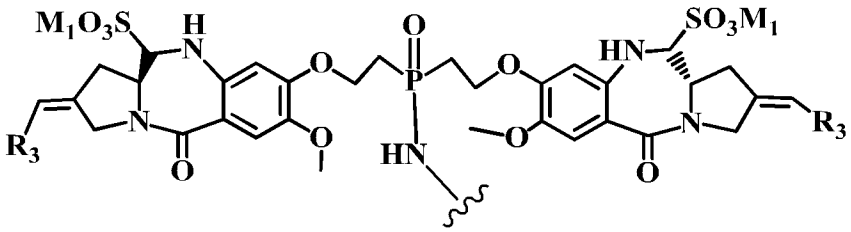
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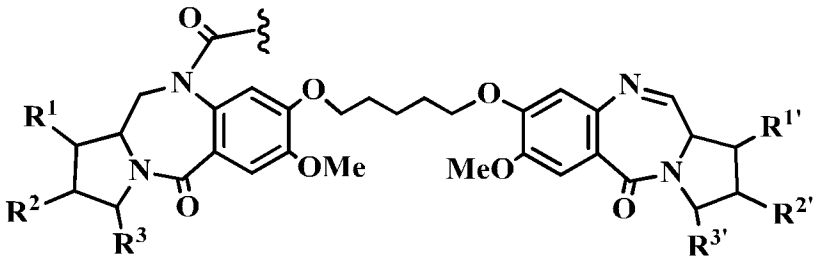
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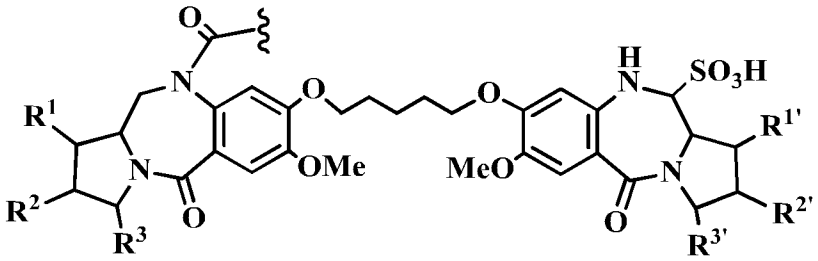
PB15,



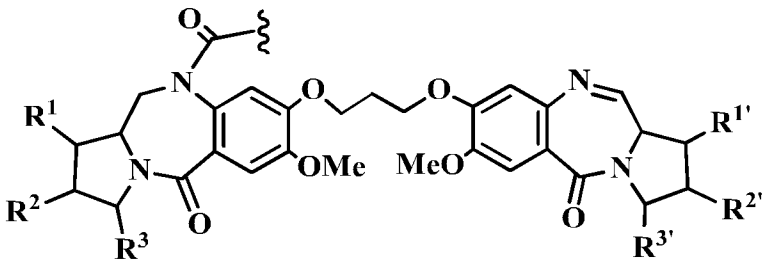
PB16,



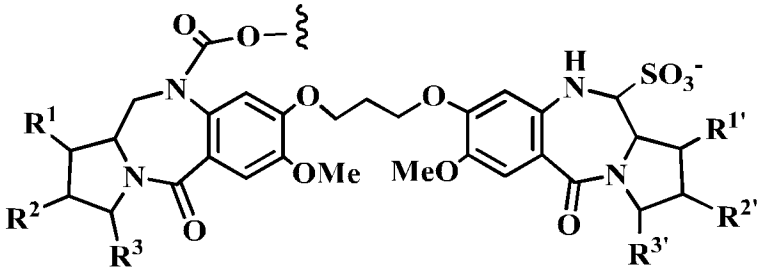
PB17,

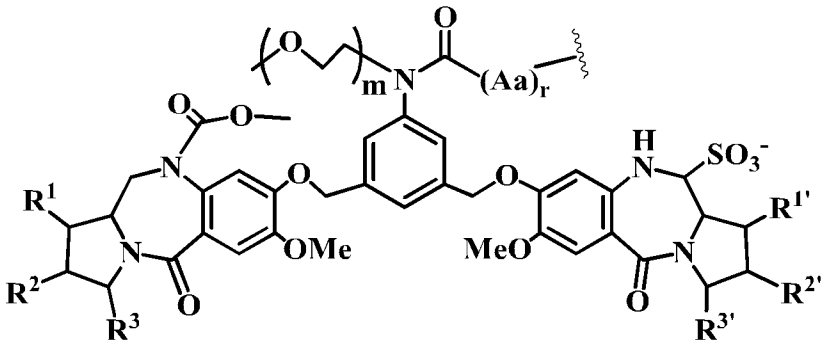


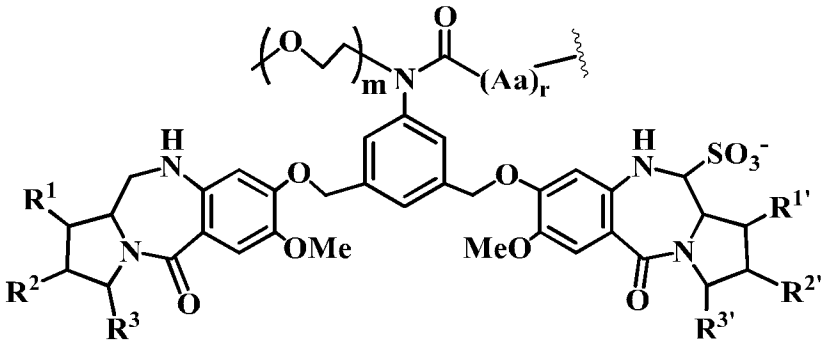
PB18,

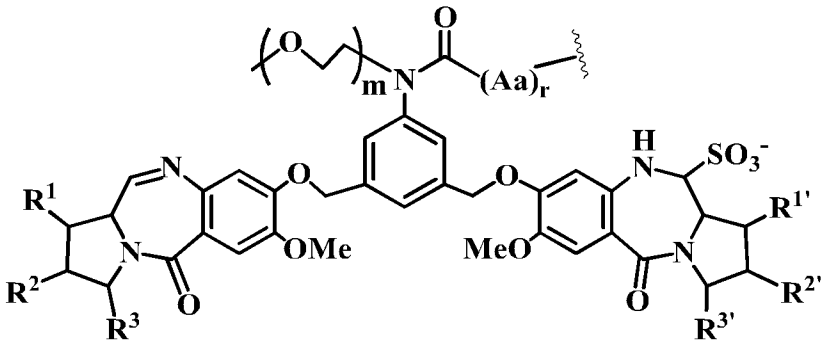


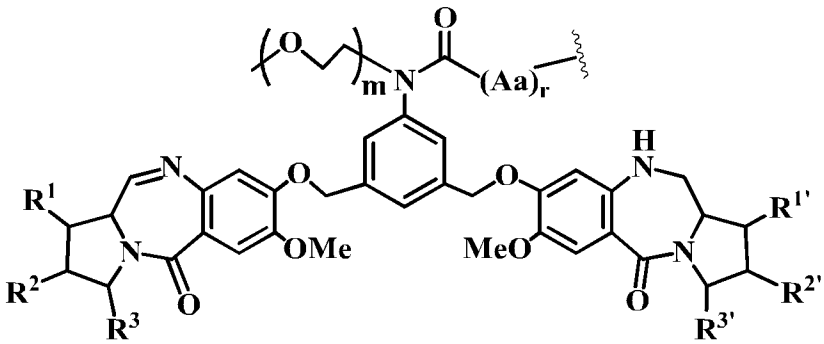
PB19,

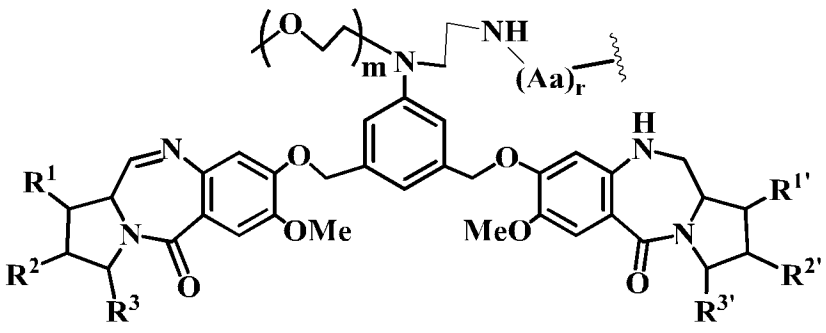

PB20,


PB21,

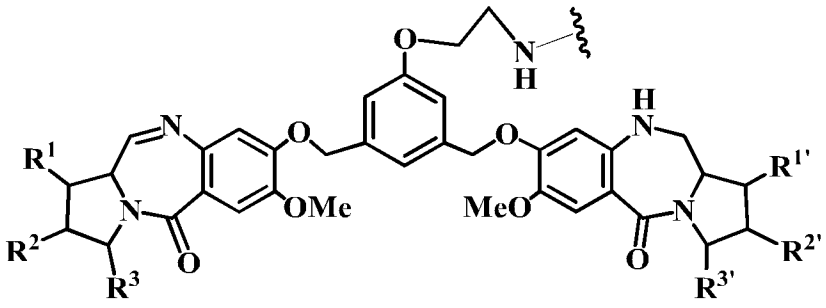

PB22,


PB23,

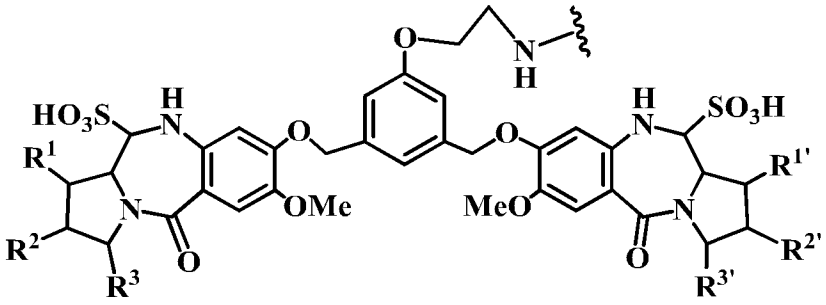

PB24,



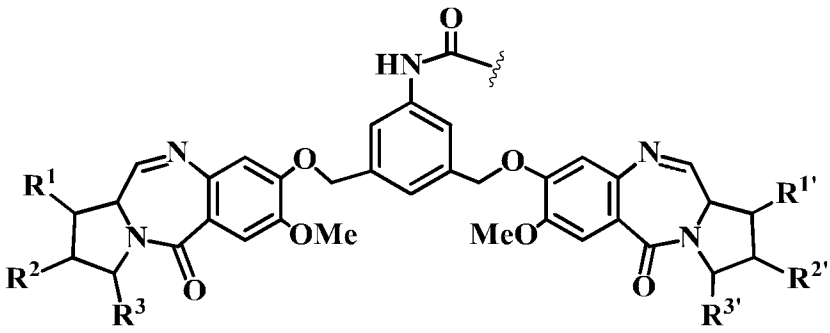
PB25,



PB26,



PB27,



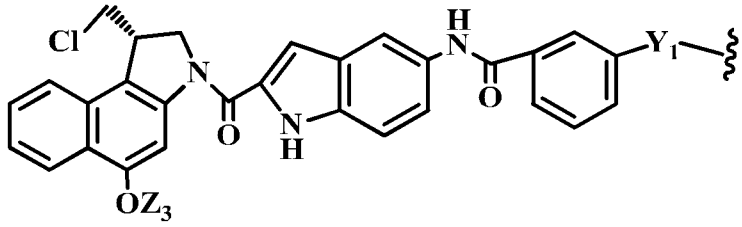
PB28,

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein X_1, X_2, Y_1, Y_2, Z_1, Z_2, and n are defined the same above; Preferably X_1, X_2, Y_1 and Y_2 are independently O, N, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, $OC(O)N(R_1)$, $N(R_1)C(O)N(R_1)$, CH, C(O)NHNHC(O) and $C(O)NR_1$; R^1, R^2, R^3, $R^{1'}$, $R^{2'}$, and $R^{3'}$ are independently H; F; Cl; =O; =S; OH; SH; C_1-C_8 linear or branched alkyl, aryl, alkenyl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester ($COOR_5$ or $-OC(O)R_5$), ether (OR_5), amide ($CONR_5$), carbamate ($OCONR_5$), amines (NHR_5, NR_5R_5'), heterocycloalkyl, or acyloxylamines (-C(O)NHOH, $-ONHC(O)R_5$); or peptides containing

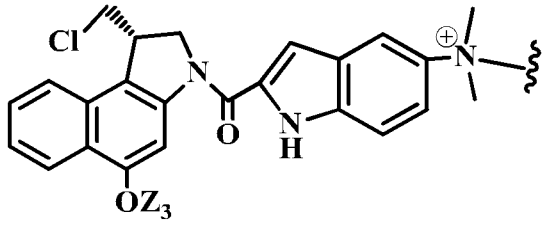
1-20 natural or unnatural aminoacids, or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 5000. The two Rs: R^1R^2, R^2R^3, R^1R^3, $R^{1'}R^{2'}$, $R^{2'}R^{3'}$, or $R^{1'}R^{3'}$ can independently form 3~8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group; X_3 and Y_3 are independently N, NH, CH_2 or CR_5, or one of X_3 and Y_3 can be absent; wherein R_1, and R_2 are C_1-C_8 linear or branched alkyl, heteroalkyl; C_3-C_8 aryl, heteroaryl, alkylcycloalkyl, acyloxyl, alkylaryl, alkylaryloxyl, alkylarylamino, alkylarylthiol; or 1-6 the same or different sequence of amino acid/peptides (Ar)r, r =1 -6; wherein R_4, R_5, R_5', R_6, R_{12} and R_{12}' are independently H, OH, NH_2, $NH(CH_3)$, $NHNH_2$, COOH, SH, OZ_3, SZ_3, F, Cl, or C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, acyloxylamines; Z_3 is H, $OP(O)(OM_1)(OM_2)$, $OCH_2OP(O)(OM_1)(OM_2)$, OSO_3M_1, or O-glycoside (glucoside, galactoside, mannoside, glucuronoside/glucuronide, alloside, fructoside, etc.), NH-glycoside, S-glycoside or CH_2-glycoside; M_1 and M_2 are independently H, Na, K, Ca, Mg, NH_4, $NR_1R_2R_3$; X_6 is CH, N, P(O)NH, $P(O)NR_1$, CHC(O)NH, C_3-C_8 aryl, heteroaryl, alkylcycloalkyl, acyloxyl, alkylaryl, alkylaryloxyl, alkylarylamino, or an Aa (amino acid, is preferably selected from Lys, Phe, Asp, Glu, Ser, Thr, His, Cys, Tyr, Trp, Gln, Asn, Arg); "⋯⋯" is either single bond or double bond; "〰" is defined the same above.

An CC-1065 analog and doucarmycin analogs are also preferred to be used for a conjugate of the present process invention. The examples of the CC-1065 analogues and doucarmycin analogs as well as their synthesis are described in: e.g. Warpehoski, et al, J. Med. Chem. 31:590-603 (1988); D. Boger et al., J. Org. Chem; 66; 6654-61, 2001; U. S. Patent Nos: 4169888, 4391904, 4671958, 4816567, 4912227, 4923990, 4952394, 4975278, 4978757, 4994578, 5037993, 5070092, 5084468, 5101038, 5117006, 5137877, 5138059, 5147786, 5187186, 5223409, 5225539, 5288514, 5324483, 5332740, 5332837, 5334528, 5403484, 5427908, 5475092, 5495009, 5530101, 5545806, 5547667, 5569825, 5571698, 5573922, 5580717, 5585089, 5585499, 5587161, 5595499, 5606017, 5622929, 5625126, 5629430, 5633425, 5641780, 5660829, 5661016, 5686237, 5693762, 5703080, 5712374, 5714586, 5739116, 5739350, 5770429, 5773001, 5773435, 5786377 5786486, 5789650, 5814318, 5846545, 5874299, 5877296, 5877397, 5885793, 5939598, 5962216, 5969108, 5985908, 6060608, 6066742, 6075181, 6103236, 6114598, 6130237, 6132722, 6143901, 6150584, 6162963, 6172197, 6180370, 6194612, 6214345, 6262271, 6281354, 6310209, 6329497, 6342480, 6486326, 6512101, 6521404, 6534660, 6544731, 6548530, 6555313, 6555693, 6566336, 6, 586, 618, 6593081, 6630579, 6, 756, 397, 6759509, 6762179, 6884869, 6897034, 6946455, 7, 049, 316, 7087600, 7091186, 7115573, 7129261, 7214663, 7223837, 7304032, 7329507, 7, 329, 760, 7, 388, 026, 7, 655, 660, 7, 655, 661, 7, 906, 545, and 8, 012, 978. Examples of the structures of the conjugate of the

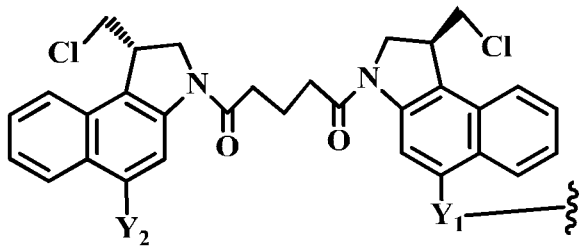
antibody-CC-1065 analogs via the linker of the patent are illustrated below CC01, CC02, CC03, CC04, CC05, CC06 and CC07:



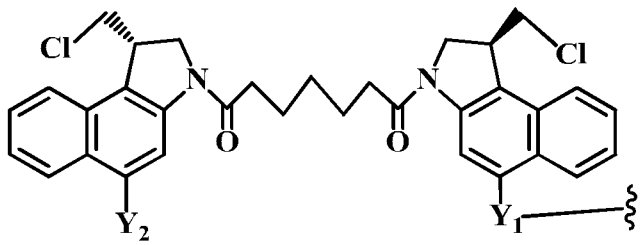
CC01,



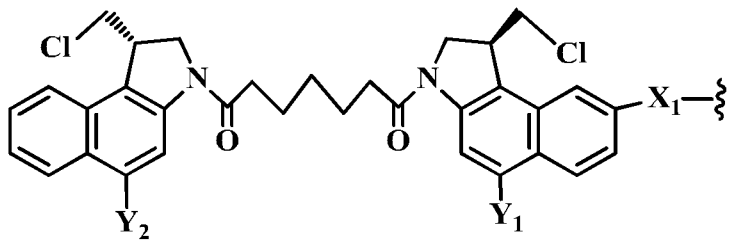
CC02,



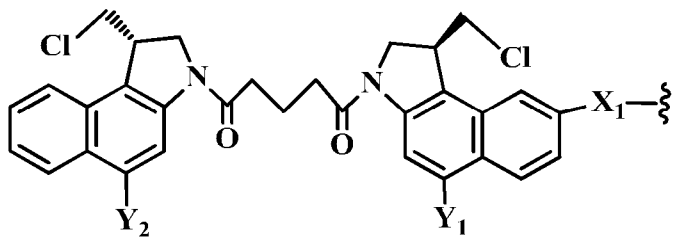
CC03,



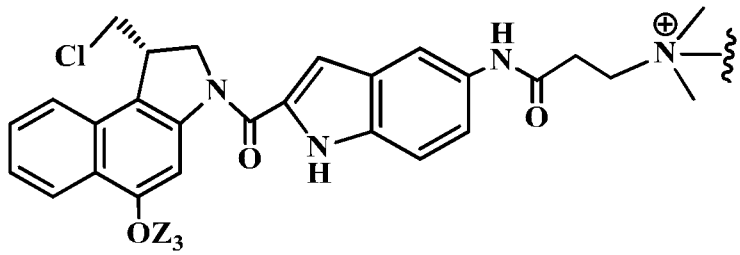
CC04,



CC05,



CC06,

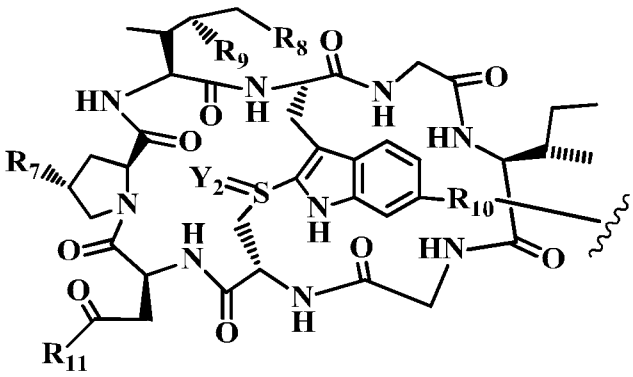


CC07,

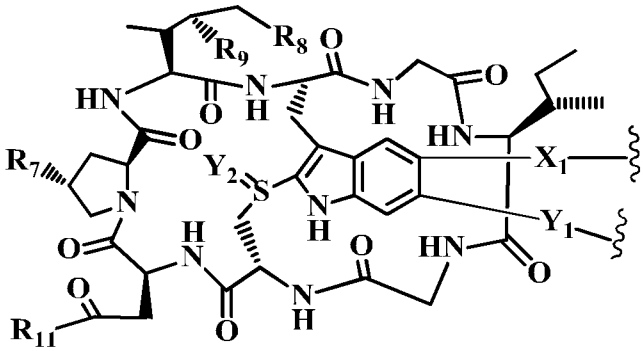
wherein X_1, X_2, Y_1 and Y_2 are independently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, $OC(O)N(R_1)$, $N(R_1)C(O)N(R_2)$, C(O)NHNHC(O) and $C(O)NR_1$ when linked to the connecting site " ∿∿ "; or OH, NH_2, $NHNH_2$, NHR_1, SH, C(O)OH, $C(O)NH_2$, $OC(O)NH_2$, OC(O)OH, $NHC(O)NH_2$, NHC(O)SH, $OC(O)NH(R_1)$, $N(R_1)C(O)NH(R_2)$, C(O)NHNHC(O)OH and $C(O)NHR_1$ when not linked to the connecting site " ∿∿ "; Z_3 is H, $PO(OM_1)(OM_2)$, SO_3M_1, $CH_2PO(OM_1)(OM_2)$, $CH_3N(CH_2CH_2)_2NC(O)$-, $O(CH_2CH_2)_2NC(O)$-, R_1, or glycoside; wherein R_1, R_2, R_3, M_1, M_2, and n are defined the same above;

An amatoxin and its analogs which are a subgroup of at least ten toxic compounds originally found in several genera of poisonous mushrooms, most notably Amanita phalloides and several other mushroom species, are also preferred for conjugation of the present patent. These ten amatoxins, named α-Amanitin, β-Amanitin, γ-Amanitin, ε-Amanitin, Amanullin, Amanullinic acid, Amaninamide, Amanin, Proamanullin, are rigid bicyclic peptides that are synthesized as 35-amino-acid proproteins, from which the final eight amino acids are cleaved by a prolyl oligopeptidase (Litten, W. 1975 Scientific American232 (3): 90–101; H. E. Hallen, et al 2007 Proc. Nat. Aca. Sci. USA 104, 19097–101; K. Baumann, et al, 1993 Biochemistry 32 (15): 4043–50; Karlson-Stiber C, Persson H. 2003, Toxicon 42 (4): 339–49; Horgen, P. A. et al. 1978 Arch. Microbio. 118 (3): 317–9). Amatoxins kill cells by inhibiting RNA polymerase II (Pol II), shutting down gene transcription and protein biosynthesis (Brodner, O. G. and Wieland, T. 1976 *Biochemistry*, 15(16): 3480–4; Fiume, L., Curr Probl Clin Biochem, 1977, 7: 23-8; Karlson-Stiber C, Persson H. 2003, Toxicon 42(4): 339–49; Chafin, D. R., Guo, H. & Price, D. H. 1995 J. Biol. Chem. 270 (32): 19114–19; Wieland (1983) Int. J. Pept. Protein Res. 22(3): 257-76.). Amatoxins can be produced from collected Amanita phalloides mushrooms (Yocum, R. R. 1978 Biochemistry 17(18): 3786-9; Zhang, P. et al, 2005, FEMS Microbiol. Lett.252(2), 223-8), or from fermentation using a basidiomycete (Muraoka, S. and Shinozawa T., 2000 J. Biosci. Bioeng. 89(1): 73-6) or from fermentation using A. fissa (Guo, X. W., et al, 2006 Wei Sheng Wu Xue Bao 46(3): 373-8), or from culturing Galerina fasciculata or Galerina helvoliceps, a strain belonging to the genus (WO/1990/009799, JP11137291). However, the yields from these isolation and fermentation were quite low (less than 5 mg/L culture). Several preparations of amatoxins and their analogs have been reported in the past three decades (W. E. Savige, A. Fontana, Chem. Commun. 1976, 600–1; Zanotti, G., et al, Int J Pept Protein Res, 1981.

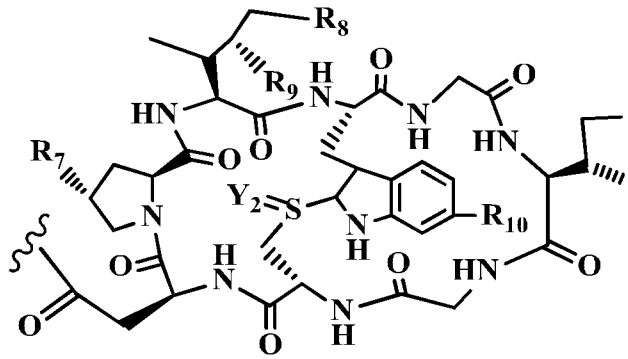
18(2): 162-8; Wieland, T., et al, Eur. J. Biochem. 1981, 117, 161–4; P. A. Bartlett, et al, Tetrahedron Lett. 1982, 23, 619–22; Zanotti, G., et al., Biochim Biophys Acta, 1986. 870(3): 454-62; Zanotti, G., et al., Int. J. Peptide Protein Res. 1987, 30, 323–9; Zanotti, G., et al., Int. J. Peptide Protein Res. 1987, 30, 450–9; Zanotti, G., et al., Int J Pept Protein Res, 1988. 32(1): 9-20; G. Zanotti, T. et al, Int. J. Peptide Protein Res. 1989, 34, 222–8; Zanotti, G., et al., Int J Pept Protein Res, 1990. 35(3): 263-70; Mullersman, J. E. and J. F. Preston, 3rd, Int J Pept Protein Res, 1991. 37(6): 544-51; Mullersman, J.E., et al, Int J Pept Protein Res, 1991. 38(5): 409-16; Zanotti, G., et al, Int J Pept Protein Res, 1992. 40(6): 551-8; Schmitt, W. et al, J. Am. Chem. Soc. 1996, 118, 4380–7; Anderson, M.O., et al, J. Org. Chem., 2005, 70(12): 4578-84; J. P. May, et al, J. Org. Chem. 2005, 70, 8424–30; F. Brueckner, P. Cramer, Nat. Struct. Mol. Biol. 2008, 15, 811–8; J. P. May, D. M. Perrin, Chem. Eur. J. 2008, 14, 3404–9; J. P. May, et al, Chem. Eur. J. 2008, 14, 3410–17; Q. Wang, et al, Eur. J. Org. Chem. 2002, 834–9; May, J. P. and D. M. Perrin, Biopolymers, 2007. 88(5): 714-24; May, J. P., et al., Chemistry, 2008. 14(11): 3410-7; S. De Lamo Marin, et al, Eur. J. Org. Chem. 2010, 3985–9; Pousse, G., et al., Org Lett, 2010. 12(16): 3582-5; Luo, H., et al., Chem Biol, 2014. 21(12): 1610-7; Zhao, L., et al., Chembiochem, 2015. 16(10): 1420-5) and most of these preparations were by partial synthesis. Because of their extreme potency and unique mechanism of cytotoxicity, amatoxins have been used as payloads for conjugations (Fiume, L., Lancet, 1969. 2 (7625): 853-4; Barbanti-Brodano, G. and L. Fiume, Nat New Biol, 1973. 243(130): 281-3; Bonetti, E., M. et al, Arch Toxicol, 1976. 35(1): p. 69-73; Davis, M. T., Preston, J. F. Science 1981, 213, 1385–1388; Preston, J.F., et al, Arch Biochem Biophys, 1981. 209(1): 63-71; H. Faulstich, et al, Biochemistry 1981, 20, 6498–504; Barak, L.S., et al., Proc Natl Acad Sci U S A, 1981. 78(5): 3034-8; Faulstich, H. and L. Fiume, Methods Enzymol, 1985. 112: 225-37; Zhelev, Z., A. et al, Toxicon, 1987. 25(9): 981-7; Khalacheva, K., et al, Eksp Med Morfol, 1990. 29(3): 26-30; U. Bermbach, H. Faulstich, Biochemistry 1990, 29, 6839–45; Mullersman, J. E. and J. F. Preston, Int. J. Peptide Protein Res. 1991, 37, 544–51; Mullersman, J.E. and J.F. Preston, Biochem Cell Biol, 1991. 69(7): 418-27; J. Anderl, H. Echner, H. Faulstich, Beilstein J. Org. Chem. 2012, 8, 2072–84; Moldenhauer, G., et al, J. Natl. Cancer Inst. 2012, 104, 622–34; A. Moshnikova, et al; Biochemistry 2013, 52, 1171–8; Zhao, L., et al., Chembiochem, 2015. 16(10): 1420-5; Zhou, B., et al., Biosens Bioelectron, 2015. 68: 189-96; WO2014/043403, US20150218220, EP 1661584). We have been working on the conjugation of amatoxins for a while. Examples of the structures of the amatoxins used for the present application are preferred the following structures of Am01, Am02, Am03 and Am04:



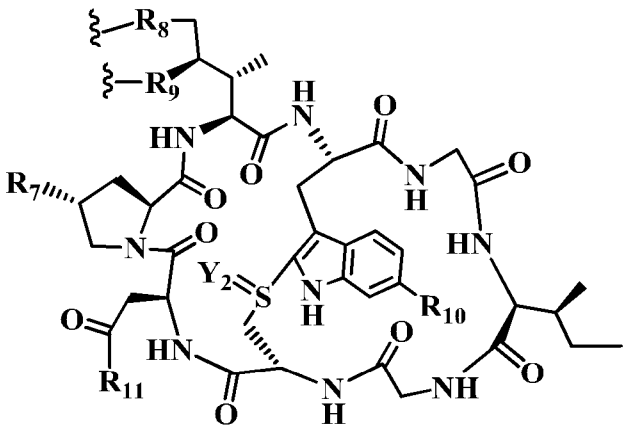
Am01,



Am02,



Am03,

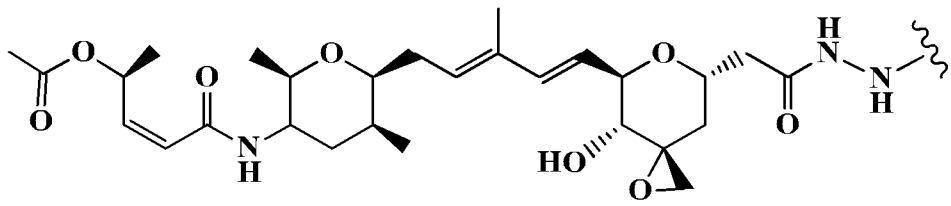


Am04

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein X_1, and Y_1 are independently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH_2, CHNH, CH_2O, C(O)NHNHC(O) and C(O)NR_1; R_7, R_8, and R_9 are

independently H, OH, OR_1, NH_2, NHR_1, C_1-C_6 alkyl, or absent; Y_2 is O, O_2, NR_1, NH, or absent; R_{10} is CH_2, O, NH, NR_1, NHC(O), NHC(O)NH, NHC(O)O, OC(O)O, C(O), OC(O), OC(O)(NR_1), (NR_1)C(O)(NR_1), C(O)R_1 or absent; R_{11} is OH, NH_2, NHR_1, $NHNH_2$, NHNHCOOH, O-R_1-COOH, NH-R_1-COOH, NH-$(Aa)_r$COOH, $O(CH_2CH_2O)_pCH_2CH_2OH$, $O(CH_2CH_2O)_pCH_2CH_2NH_2$, $NH(CH_2CH_2O)_pCH_2CH_2NH_2$, NR_1R_2, $O(CH_2CH_2O)_pCH_2CH_2$-COOH, $NH(CH_2CH_2O)_pCH_2CH_2COOH$, NH-Ar-COOH, NH-Ar-$NH_2$, $O(CH_2CH_2O)_pCH_2CH_2$-$NHSO_3H$, $NH(CH_2CH_2O)_pCH_2CH_2NHSO_3H$, R_1-$NHSO_3H$, NH-R_1-$NHSO_3H$, $O(CH_2CH_2O)_p$-$CH_2CH_2NHPO_3H_2$, $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, OR_1, R_1-$NHPO_3H_2$, R_1-OPO_3H_2, $O(CH_2CH_2O)_pCH_2CH_2OPO_3H_2$, OR_1-$NHPO_3H_2$, NH-R_1-$NHPO_3H_2$, or $NH(CH_2CH_2O)_pCH_2$-$CH_2NHPO_3H_2$, wherein $(Aa)_r$ is 1-8 aminoacids; n and m_1 are independently 1-20; p is 1 -5000; R_1, R_2 and Ar, are the same defined through out the application; " ∿∿ " is defined the same above.

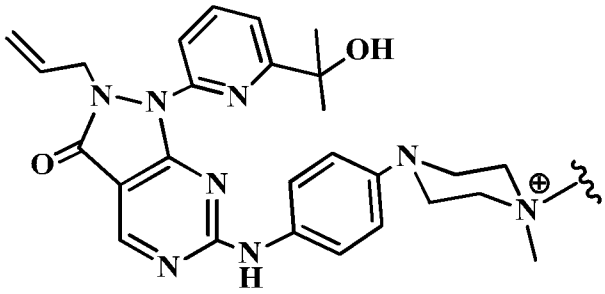
Spliceostatins and pladienolides are anti-tumor compounds which inhibit splicing and interacts with spliceosome, SF3b. Examples of spliceostatins include, but are not limited to, spliceostatin A, FR901464, and (2S, 3Z)-5-{[(2R, 3R, 5S, 6S)-6-{(2E, 4E)-5-[(3R, 4R, 5R, 7S)-7-(2-hydrazinyl-2-oxoethyl)-4-hydroxy-1, 6-dioxaspiro[2.5]oct-5-yl]-3-methylpenta-2, 4-dien-1-y-l}-2, 5-dimethyltetrahydro-2H-pyran-3-yl]amino}-5-oxopent-3-en-2-yl acetate having the core structure:



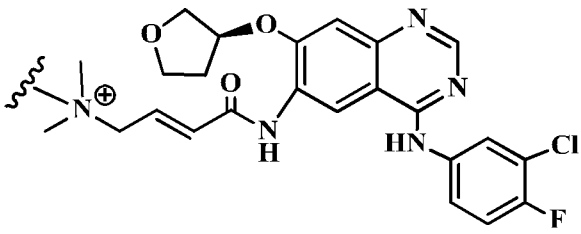
(Sp-01),

Examples of pladienolides include, but are not limited to, Pladienolide B, Pladienolide D, and E7107.

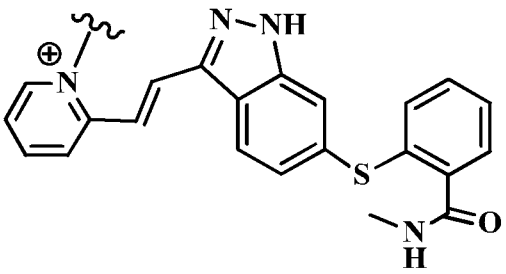
Protein kinase inhibitors that block the action of an enzyme to add a phosphate (PO_4) group to serine, threonine, or tyrosine amino acids on an antibody-like protein, and can modulate the protein function. The protein kinase inhibitors can be used to treat diseases due to hyperactive protein kinases (including mutant or overexpressed kinases) in cancer or to modulate cell functions to overcome other disease drivers. The structures of protein kinase inhibitors are preferred to selected from Adavosertib, Afatinib, Axitinib, Bafetinib, Bosutinib, Cobimetinib, Crizotinib, Cabozantinib, Dasatinib, Entrectinib, Erdafitinib, Erlotinib, Erlotinib, Fostamatinib, Gefitinib, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Pazopanib, Pegaptanib, Ponatinib, Rebastinib, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, SU6656, Tofacitinib, Vandetanib, Vemurafenib, Entrectinib, Palbociclib, Ribociclib, Abemaciclib, Dacomitinib, Neratinib, Rociletinib (CO-1686), Osimertinib, AZD3759, Nazartinib (EGF816), having the following formula, PK01 ~ PK40:



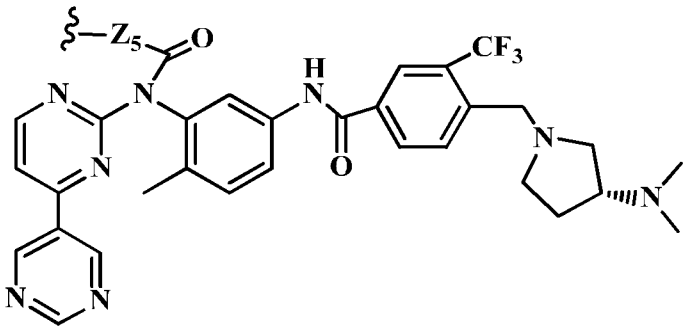
PK01, Adavosertib,



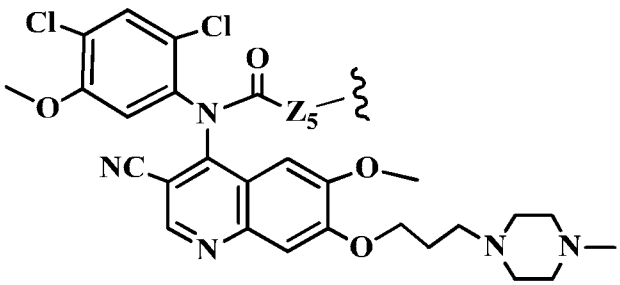
PK02, Afatinib,



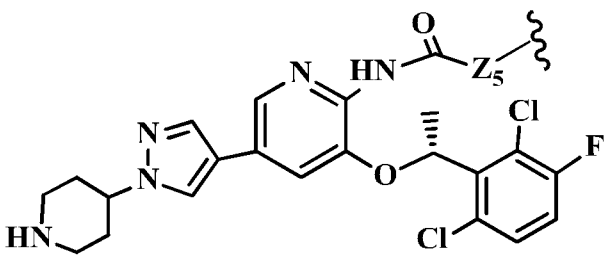
PK03, Axitinib,



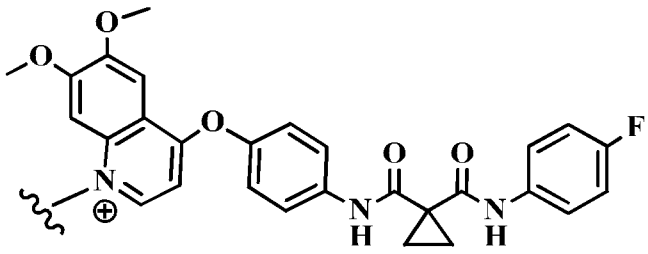
PK04, Bafetinib



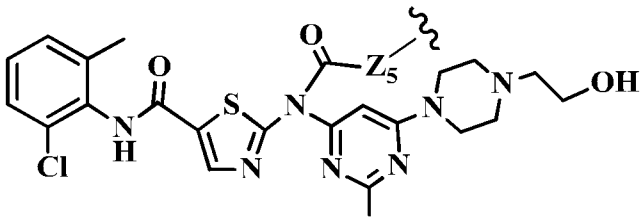
PK05, Bosutinib,



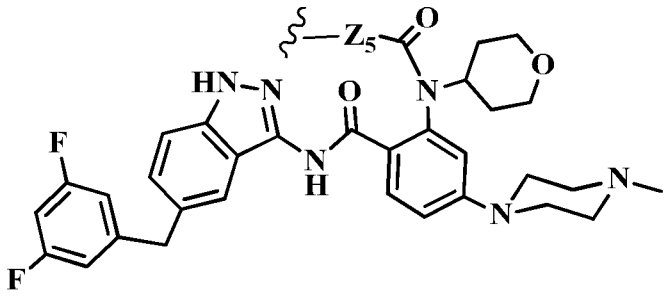
PK06, Crizotinib,



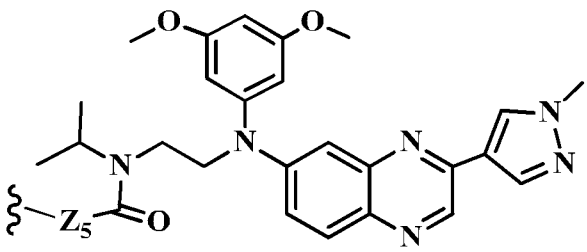
PK07, Cabozantinib,



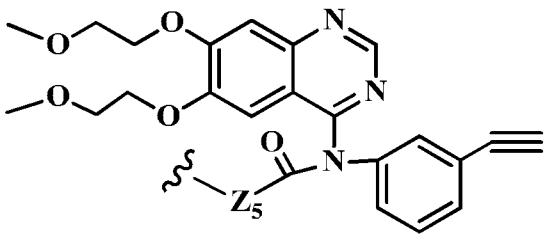
PK08, Dasatinib,



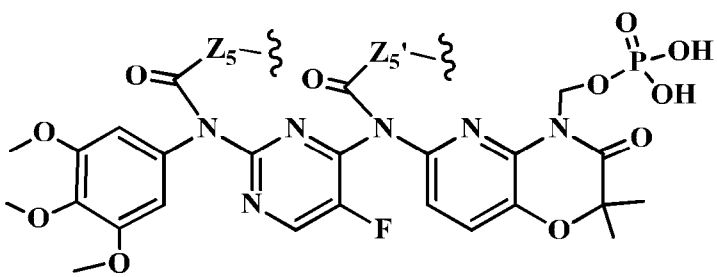
PK09, Entrectinib,



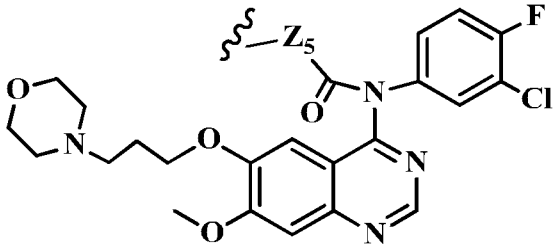
PK10, Erdafitinib,



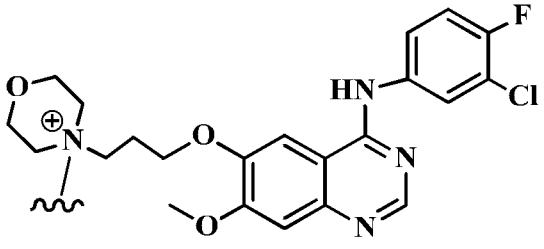
PK11, Erlotinib,



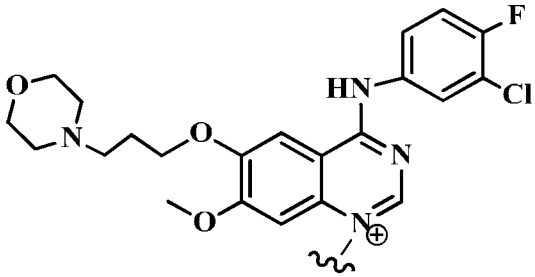
PK12, Fostamatinib,



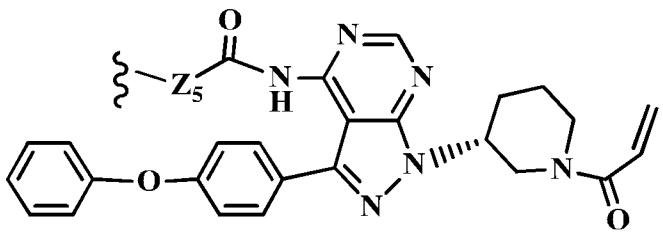
PK13, Gefitinib,



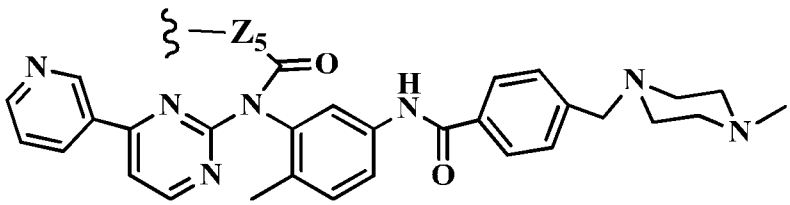
PK14, Gefitinib,



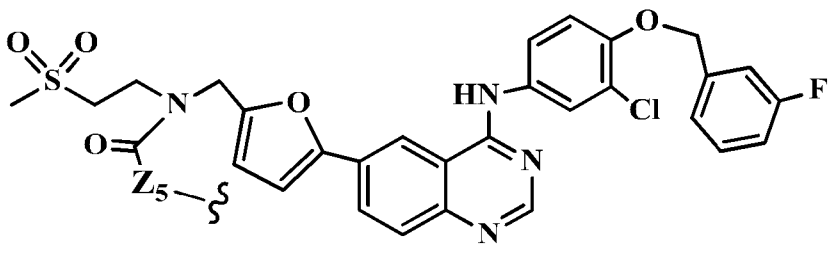
PK15, Gefitinib,



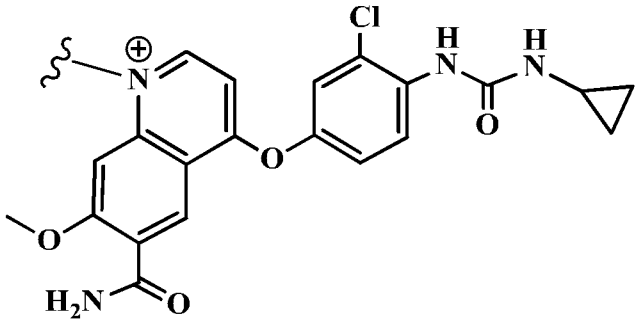
PK16, Ibrutinib,



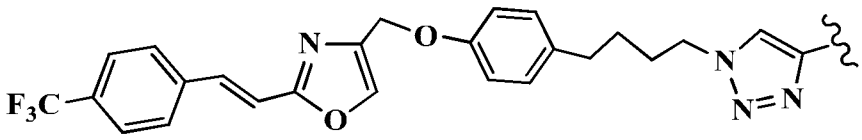
PK17, Imatinib,



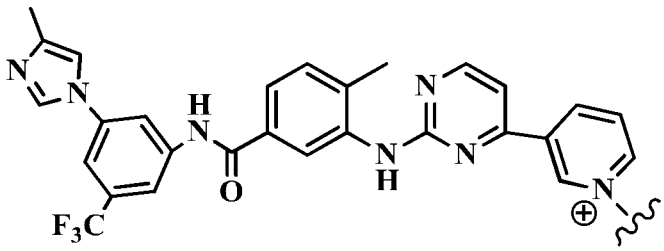
PK18, Lapatinib,



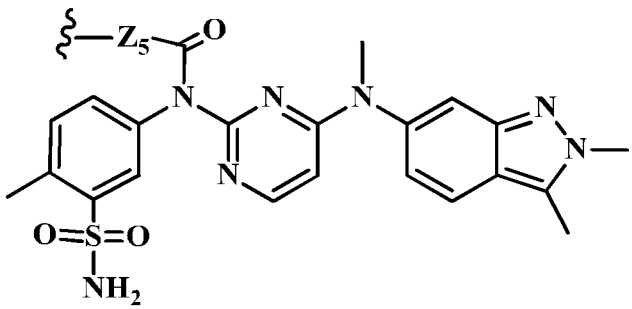
PK19, Lenvatinib,



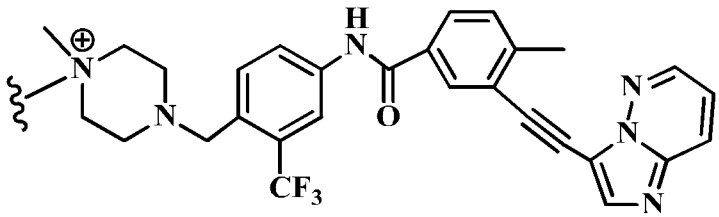
PK20, Mubritinib,



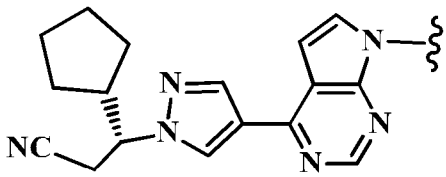
PK21, Nilotinib,



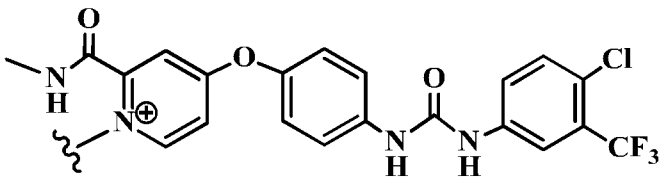
PK22, Pazopanib,



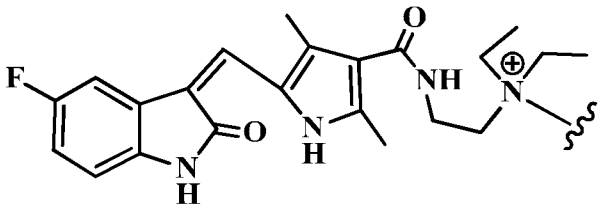
PK23, Ponatinib,



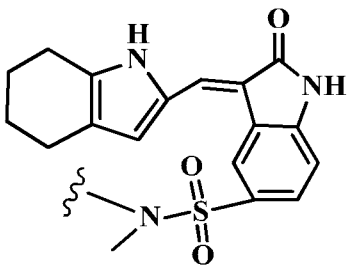
PK24, Ruxolitinib,



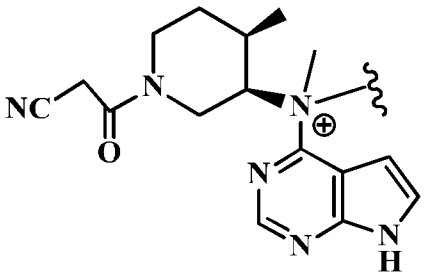
PK25, Sorafenib,



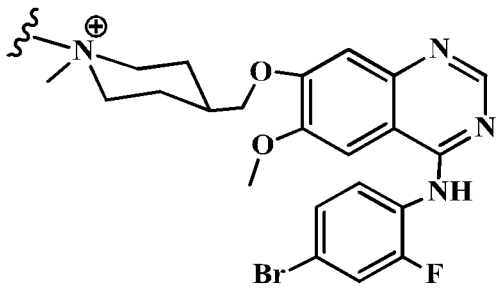
PK26, Sunitinib,



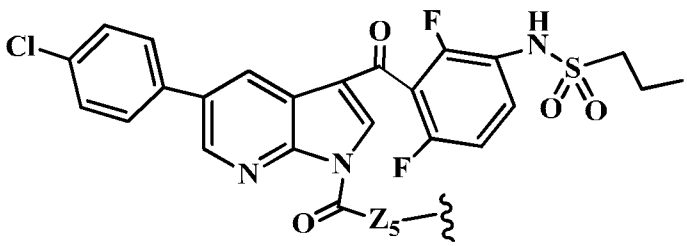
PK27, SU6656,



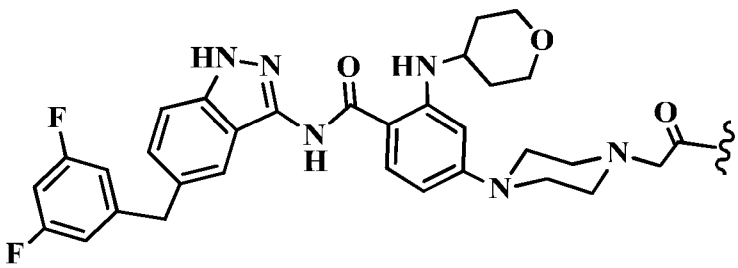
PK28, Tofacitinib,



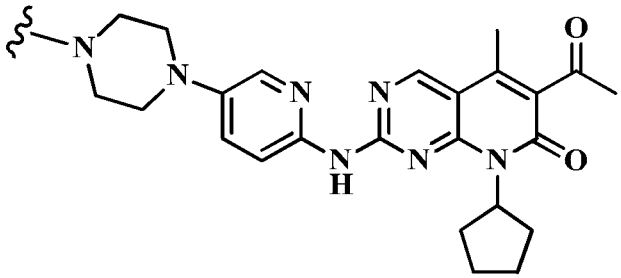
PK29, Vandetanib,



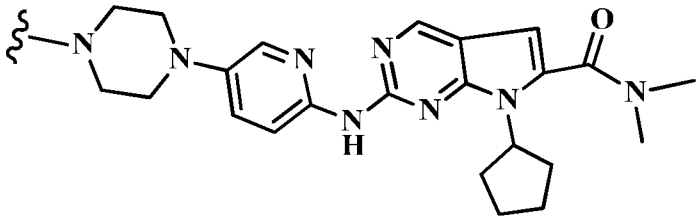
PK30, Vemurafenib;



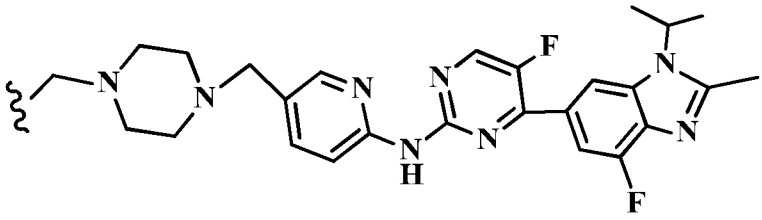
PK 31, Entrectinib;



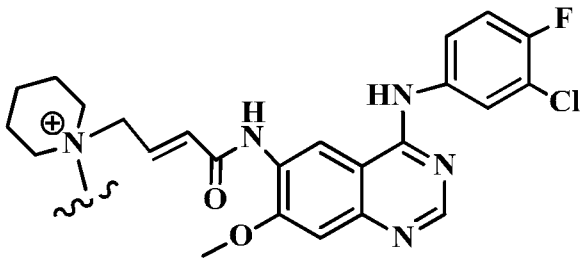
PK-32, Palbociclib analog,



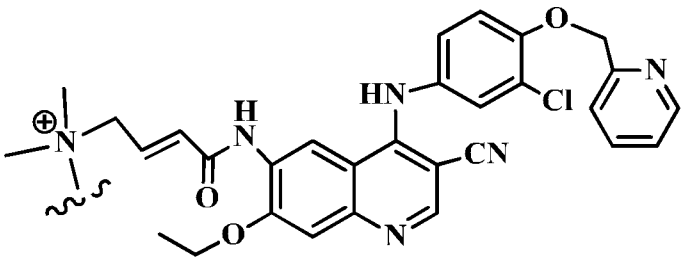
PK-33, Ribociclib,



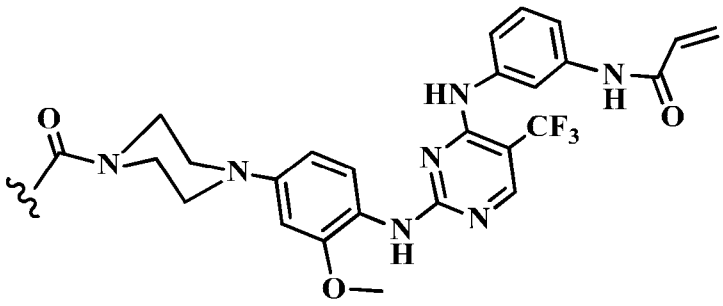
PK-34, Abemaciclib,



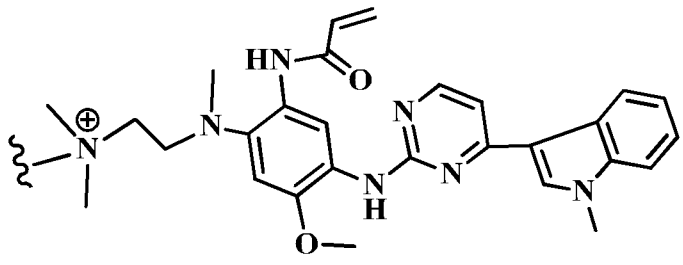
PK-35, Dacomitinib,



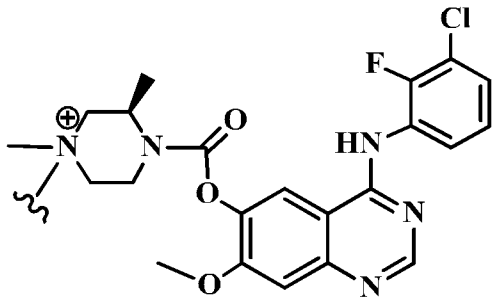
PK-36, Neratinib,



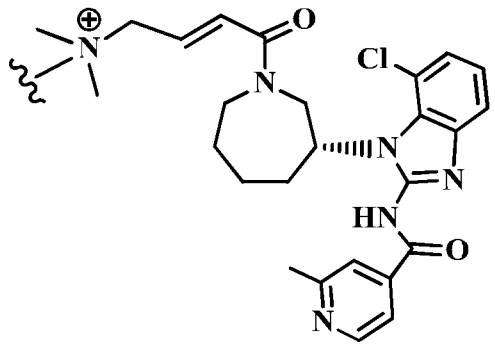
PK-37, Rociletinib (CO-1686),



PK-38, Osimertinib,



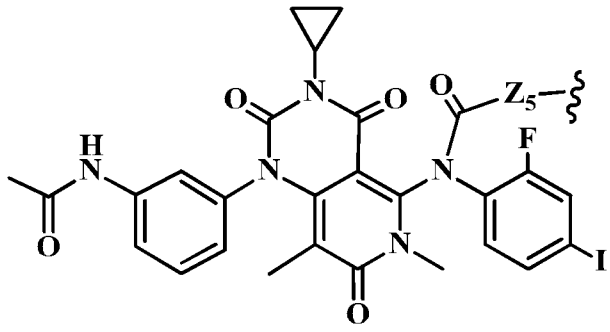
PK-39, AZD3759,



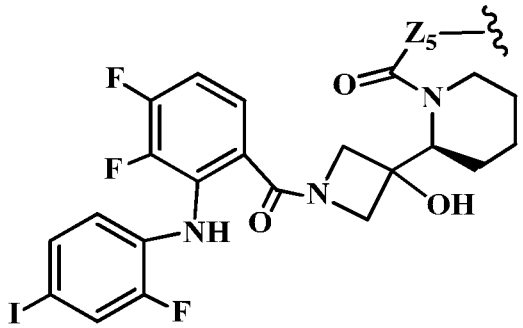
PK-40, Nazartinib (EGF816),

wherein Z_5 and Z_5' are independently selected from O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_2), C(O)NHNHC(O) and C(O)NR_1; " ∿∿∿ " is defined the same above.

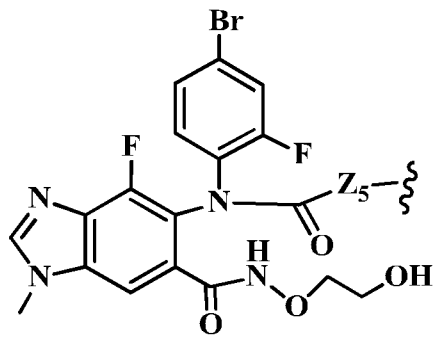
A MEK inhibitor inhibits the mitogen-activated protein kinases MEK1 and/or MEK2 which is often overactive in some cancers. MEK inhibitors are especially used for treatment of BRAF-mutated melanoma, and KRAS/BRAF mutated colorectal cancer, breast cancer, and non-small cell lung cancer (NSCLC). MEK inhibitors are selected from PD0325901, selumetinib (AZD6244), cobimetinib (XL518), refametinib, trametinib (GSK1120212), pimasertib, Binimetinib (MEK162), AZD8330, RO4987655, RO5126766, WX-554, E6201, GDC-0623, PD-325901 and TAK-733. The preferred MEK inhibitors are selected from Trametinib (GSK1120212), Cobimetinib (XL518), Binimetinib (MEK162), selumetinib having the following formula:



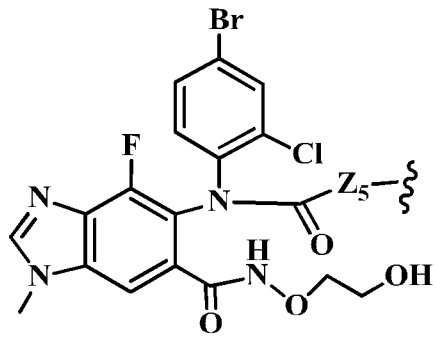
MEK01, Trametinib,



MEK02, Cobimetinib,



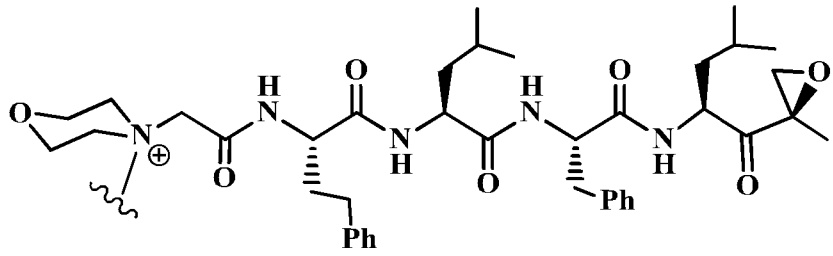
MEK03, Binimetinib,



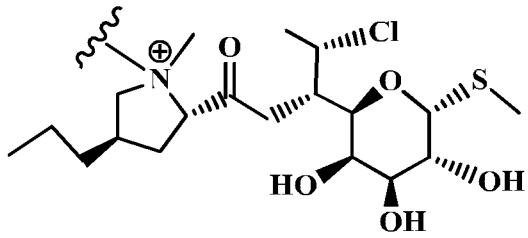
MEK04, selumetinib,

wherein Z_5 is selected from O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_2), C(O)NHNHC(O) and C(O)NR_1; R_1 and R_2 are the same as defined above.

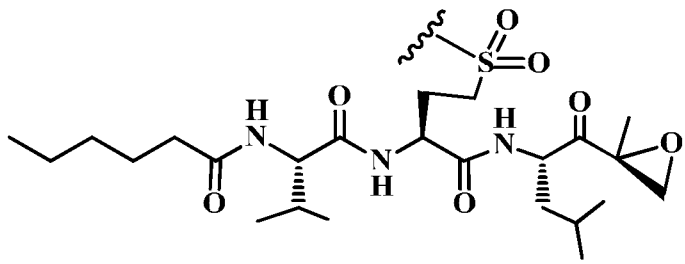
A proteinase inhibitor that are used as a payload is preferably selected from: Carfilzomib, Clindamycin, Retapamulin, Indibulin, as shown in the following structures:



PI01, Carfilzomib,



PI02, Clindamycin,



PI03, Carmaphycin analog,

An immunotoxin herein is a macromolecular drug which is usually a cytotoxic protein derived from a bacterial or plant protein, such as Diphtheria toxin (DT), Cholera toxin (CT), Trichosanthin (TCS), Dianthin, Pseudomonas exotoxin A (ETA′), Erythrogenic toxins, Diphtheria toxin, AB toxins, Type III exotoxins, etc. It also can be a highly toxic bacterial pore-forming protoxin that requires proteolytic processing for activation. An example of this protoxin is proaerolysin and its genetically modified form, topsalysin. Topsalysin is a modified recombinant protein that has been engineered to be selectively activated by an enzyme in the prostate, leading to localized cell death and tissue disruption without damaging neighboring tissue and nerves; An immunotoxin herein is preferably conjugated via the process of the application through an amino acid having free amino, thiol or carboxyl acid group; and more preferably through N-terminal amino acid.

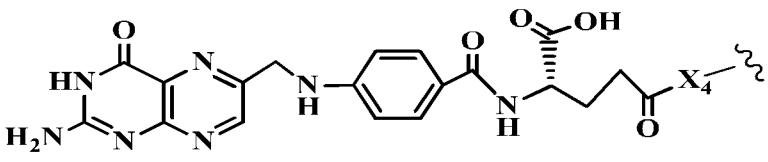
In addition, a certain cell-binding ligand or cell receptor agonist, a cell stimulating molecule or intracellular signalling molecule can be as a drug D conjugated via the process of the invention.

A cell-binding ligand or receptor agonist selected from: Folate derivatives; Glutamic acid urea derivatives; Somatostatin and its analogs (selected from the group consisting of octreotide (Sandostatin) and lanreotide (Somatuline)); Aromatic sulfonamides; Pituitary adenylate cyclase activating peptides (PACAP) (PAC1); Vasoactive intestinal peptides (VIP/PACAP) (VPAC1, VPAC2); Melanocyte-stimulating hormones (α-MSH); Cholecystokinins (CCK) /gastrin receptor agonists; Bombesins (selected from the group consisting of Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2)/gastrin-releasing peptide (GRP); Neurotensin receptor ligands (NTR1,

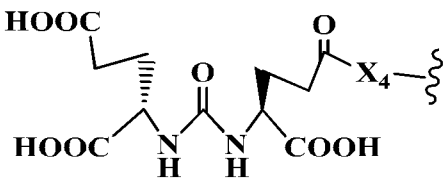
NTR2, NTR3); Substance P (NK1 receptor) ligands; Neuropeptide Y (Y1–Y6); Homing Peptides include RGD (Arg-Gly-Asp), NGR (Asn-Gly-Arg), the dimeric and multimeric cyclic RGD peptides (selected from cRGDfV), TAASGVRSMH and LTLRWVGLMS (Chondroitin sulfate proteoglycan NG2 receptor ligands) and F3 peptides; Cell Penetrating Peptides (CPPs); Peptide Hormones, selected from the group consisting of luteinizing hormone-releasing hormone (LHRH) agonists and antagonists, and gonadotropin-releasing hormone (GnRH) agonist, acts by targeting follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as testosterone production, selected from the group consisting of buserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(OtBu)-Leu-Arg-Pro-NHEt), Gonadorelin (Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), Goserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(OtBu)-Leu-Arg-Pro-AzGly-NH_2), Histrelin (Pyr-His-Trp-Ser-Tyr-D-His(N-benzyl)-Leu-Arg-Pro-NHEt), leuprolide (Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt), Nafarelin (Pyr-His-Trp-Ser-Tyr-2Nal-Leu-Arg-Pro-Gly-NH_2), Triptorelin (Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), Nafarelin, Deslorelin, Abarelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-(N-Me)Tyr-D-Asn-Leu-isopropylLys-Pro-DAla-NH_2), Cetrorelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2), Degarelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-4-aminoPhe(L-hydroorotyl)-D-4-aminoPhe(carba-moyl)-Leu-isopropylLys-Pro-D-Ala-NH_2), and Ganirelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-Tyr-D-(N9, N10-diethyl)-homoArg-Leu-(N9, N10-diethyl)-homoArg-Pro-D-Ala-NH_2); Pattern Recognition Receptor (PRRs), selected from the group consisting of Toll-like receptors' (TLRs) ligands, C-type lectins and Nodlike Receptors' (NLRs) ligands; Calcitonin receptor agonists; integrin receptors' and their receptor subtypes' (selected from the group consisting of$\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$, $\alpha_6\beta_4$, $\alpha_7\beta_1$, $\alpha_L\beta_2$, $\alpha_{IIb}\beta_3$) agonists (selected from the group consisting of GRGDSPK, cyclo(RGDfV) (L1) and its derives [cyclo(-N(Me)R-GDfV), cyclo(R-Sar-DfV), cyclo(RG-N(Me)D-fV), cyclo(RGD-N(Me)f-V), cyclo(RGDf-N(Me)V-)(Cilengitide)]; Anticalin (a derivative of Lipocalins); Adnectins (10th FN3 (Fibronectin)); Designed Ankyrin Repeat Proteins (DARPins); Avimers; EGF receptors, or VEGF receptors' agonists;

A cell-binding molecule/ligand or a cell receptor agonist selected from the following: LB01 (Folate), LB02 (PMSA ligand), LB03 (PMSA ligand), LB04 (PMSA ligand), LB05 (Somatostatin), LB06 (Somatostatin), LB07 (Octreotide, a Somatostatin analog), LB08 (Lanreotide, a Somatostatin analog), LB09 (Vapreotide (Sanvar) , a Somatostatin analog), LB10 (CAIX ligand), LB11 (CAIX ligand), LB12 (Gastrin releasing peptide receptor (GRPr), MBA), LB13 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH), LB14 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand), LB15 (GnRH antagonist, Abarelix), LB16 (cobalamin, vitamin B12 analog), LB17 (cobalamin, vitamin B12 analog), LB18 (for $\alpha_v\beta_3$ integrin receptor, cyclic RGD

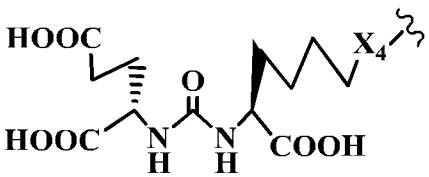
pentapeptide), LB19 (hetero-bivalent peptide ligand for VEGF receptor), LB20 (Neuromedin B), LB21 (bombesin for a G-protein coupled receptor), LB22 (TLR_2 for a Toll-like receptor,), LB23 (for an androgen receptor), LB24 (Cilengitide/cyclo(-RGDfV-) for an α_v integrin receptor, LB23 (Fludrocortisone), LB25 (Rifabutin analog), LB26 (Rifabutin analog), LB27 (Rifabutin analog), LB28 (Fludrocortisone), LB29 (Dexamethasone), LB30 (fluticasone propionate), LB31 (Beclometasone dipropionate), LB32 (Triamcinolone acetonide), LB33 (Prednisone), LB34 (Prednisolone), LB35 (Methylprednisolone), LB36 (Betamethasone), LB37 (Irinotecan analog), LB38 (Crizotinib analog), LB39 (Bortezomib analog), LB40 (Carfilzomib analog), LB41 (Carfilzomib analog), LB42 (Leuprolide analog), LB43 (Triptorelin analog), LB44 (Clindamycin), LB45 (Liraglutide analog), LB46 (Semaglutide analog), LB47 (Retapamulin analog), LB48 (Indibulin analog), LB49 (Vinblastine analog), LB50 (Lixisenatide analog), LB51 (Osimertinib analog), LB52 (a nucleoside analog), LB53 (Erlotinib analog) or LB54 (Lapatinib analog) which are shown in the following structures:



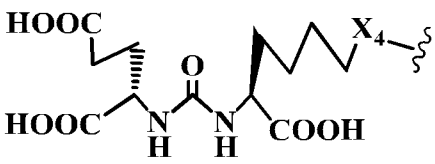
LB01 (Folate),



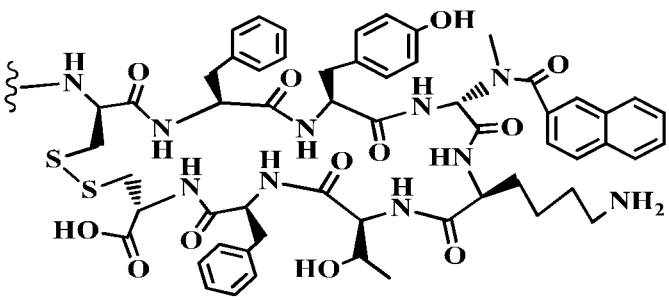
LB02 (PMSA ligand conjugate),



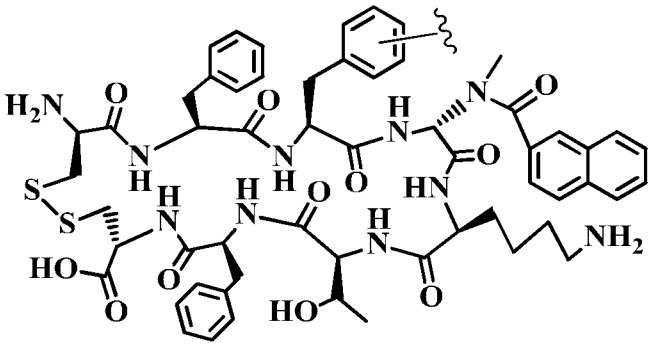
LB03 (PMSA ligand),



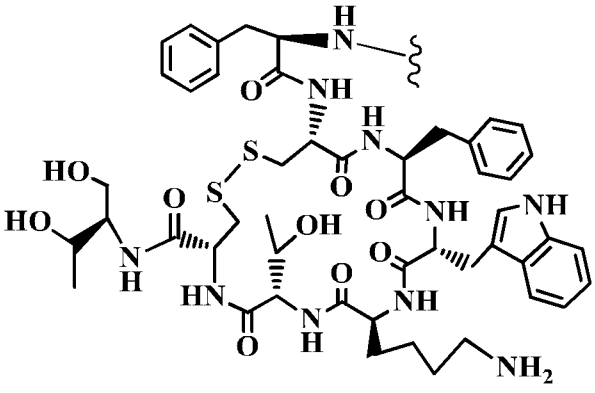
LB04 (PMSA ligand),



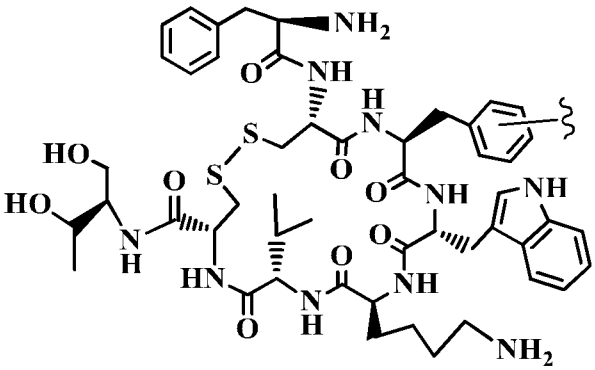
LB05 (Somatostatin),



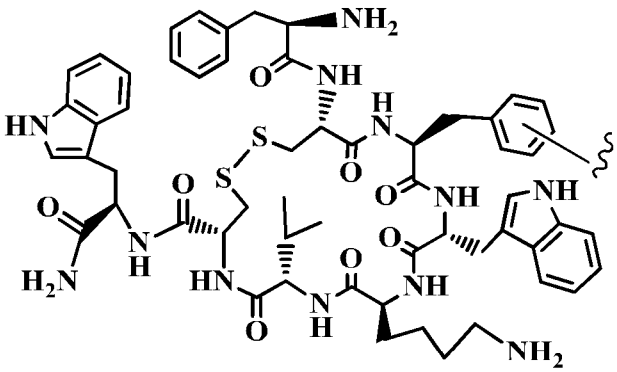
LB06 (Somatostatin),



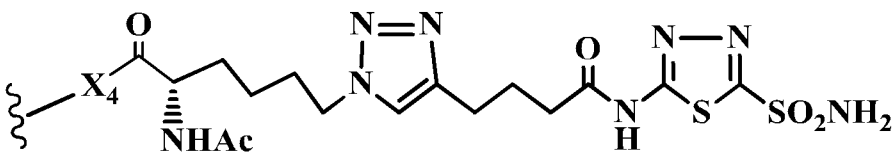
LB07 (Octreotide, a Somatostatin analog),



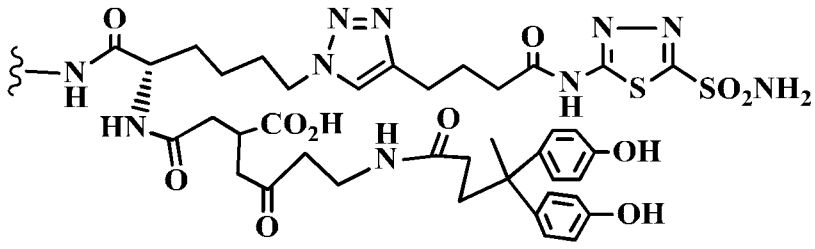
LB08 (Lanreotide, a Somatostatin analog),



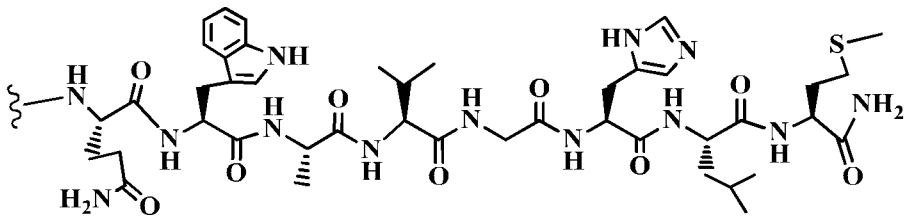
LB09 (Vapreotide (Sanvar), a Somatostatin analog),



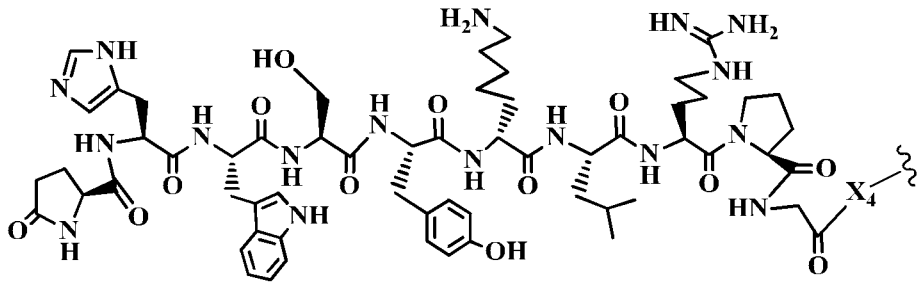
LB10 (CAIX ligand),



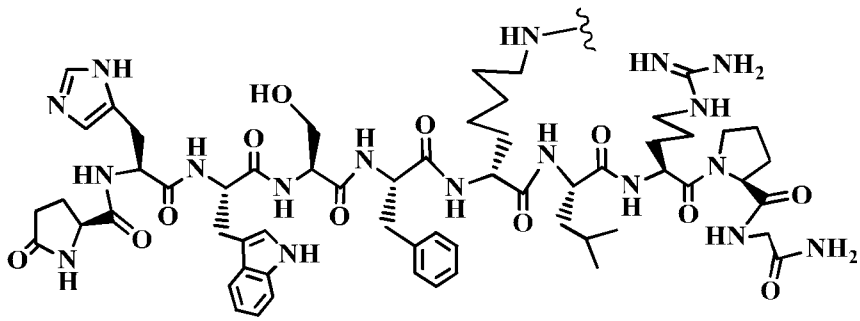
LB11 (CAIX ligand),



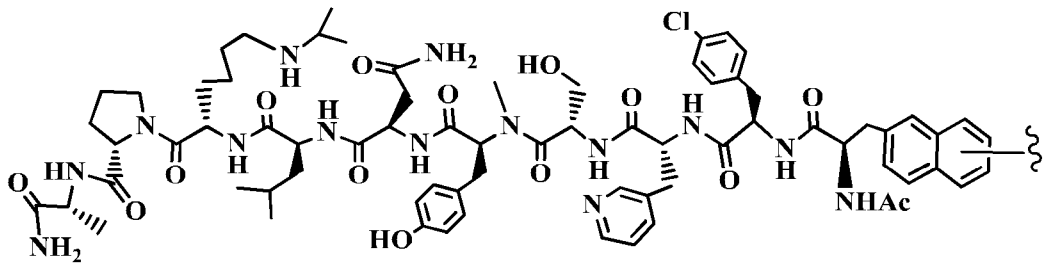
LB12 (Gastrin releasing peptide receptor (GRPr), MBA),



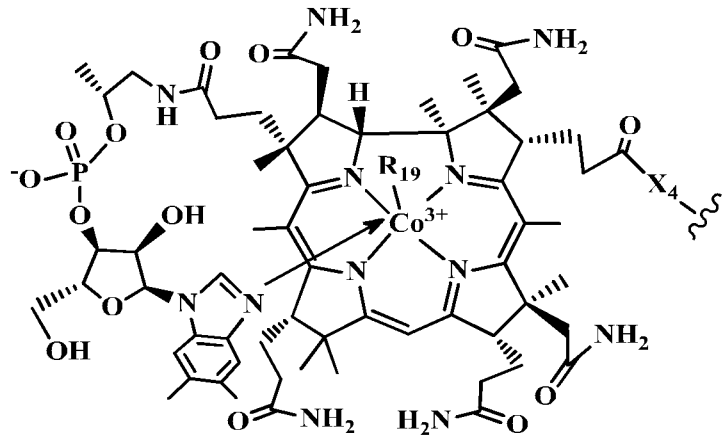
LB13 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH),



LB14 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand),

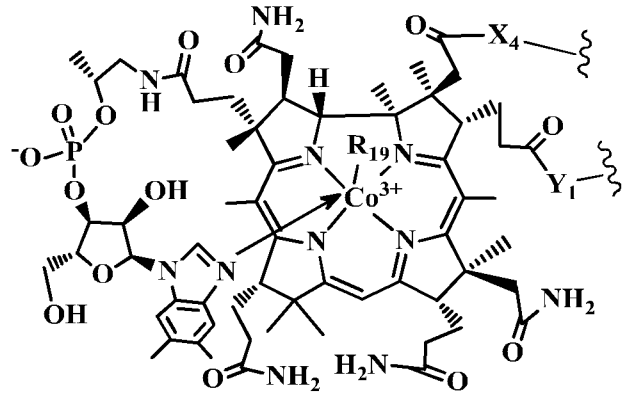


LB15 (GnRH antagonist, Abarelix),

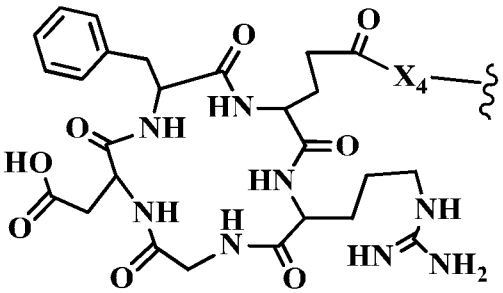


R_{19} is 5'deoxyadenosyl, Me, OH, CN;

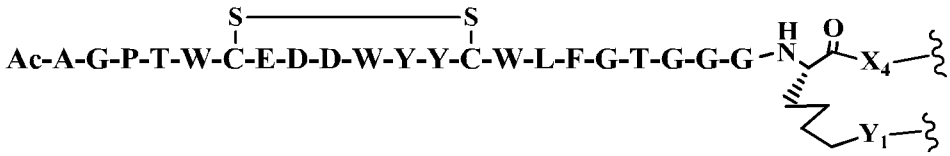
LB16 (cobalamin, vitamin B12 analog),



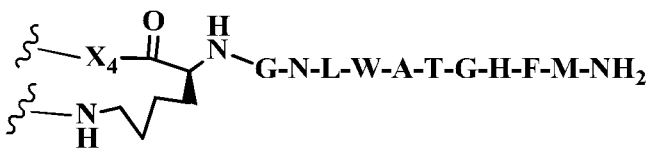
R_{19} is 5'deoxyadenosyl, Me, OH, CN; LB17 (cobalamin, vitamin B12 analog),



LB18 (for $\alpha_v\beta_3$ integrin receptor, cyclic RGD pentapeptide),



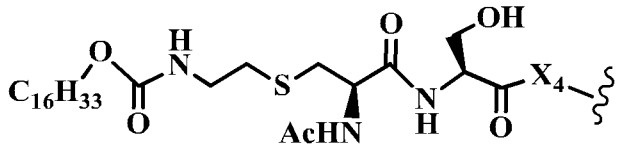
LB19 (hetero-bivalent peptide ligand conjugate for VEGF receptor),



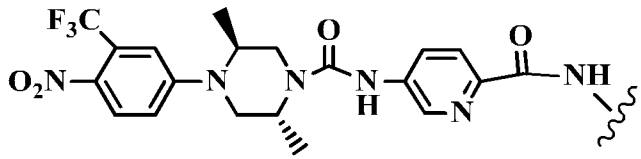
LB20 (Neuromedin B),



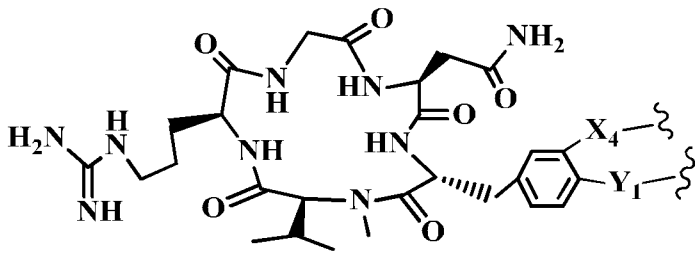
LB21 (bombesin conjugate for a G-protein coupled receptor),



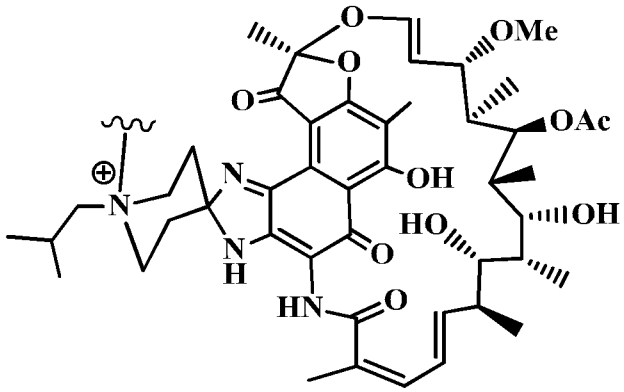
LB22 (TLR_2 conjugate for a Toll-like receptor),



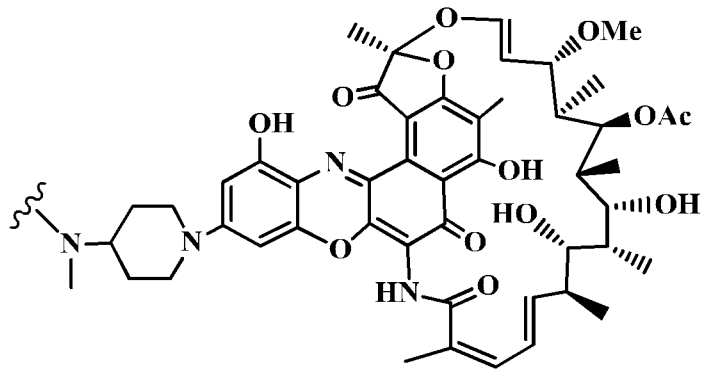
LB23 (an androgen receptor),



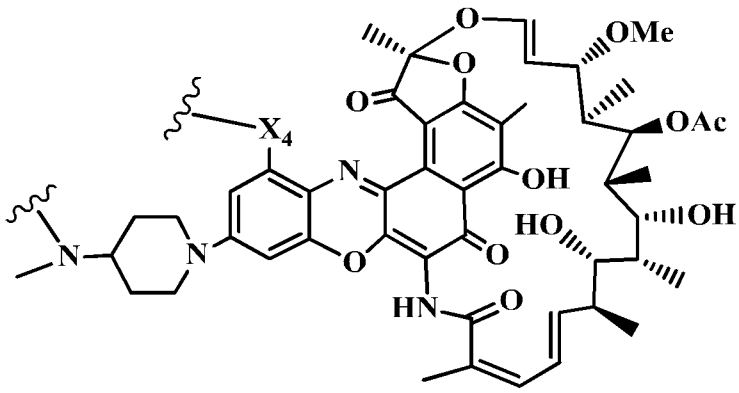
LB24 (Cilengitide/cyclo(-RGDfV-) conjugate for an α_v integrin receptor)



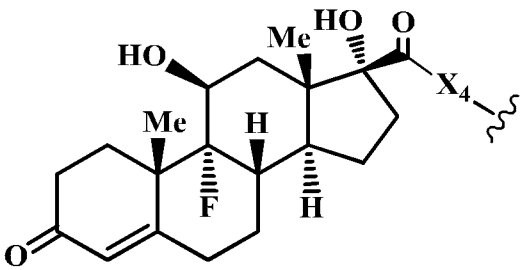
LB25 (Rifabutin analog),



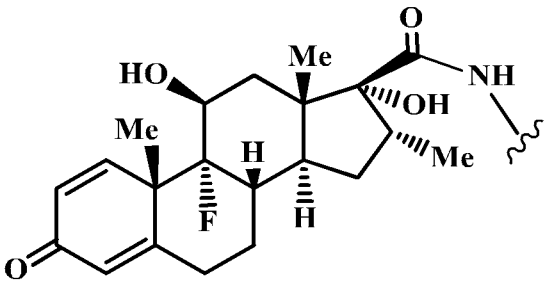
LB26 (Rifabutin analog),



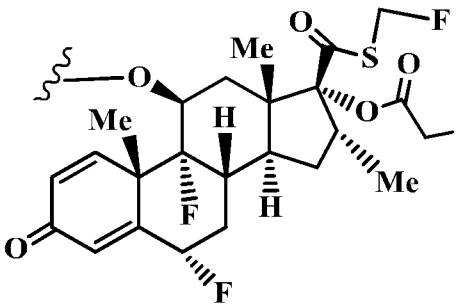
LB27 (Rifabutin analog),



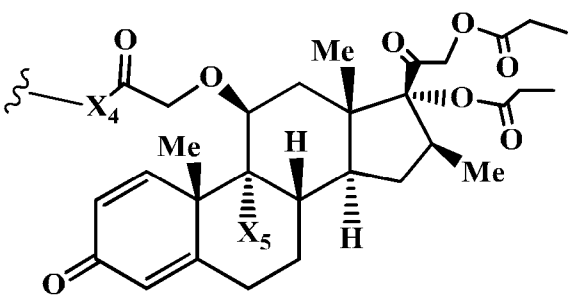
LB28 (Fludrocortisone),



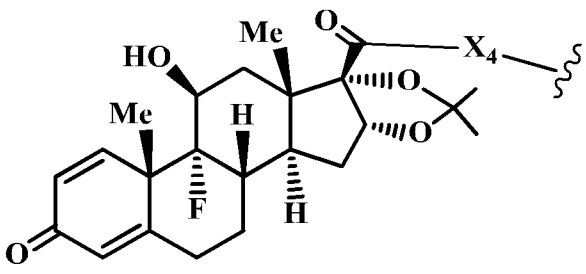
LB29 (Dexamethasone),



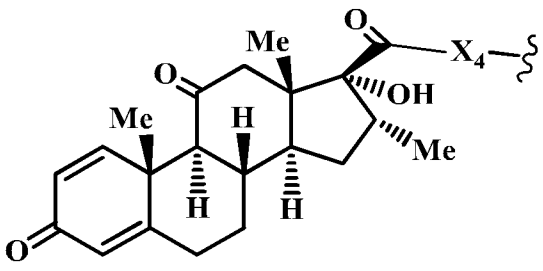
LB30 (fluticasone propionate),



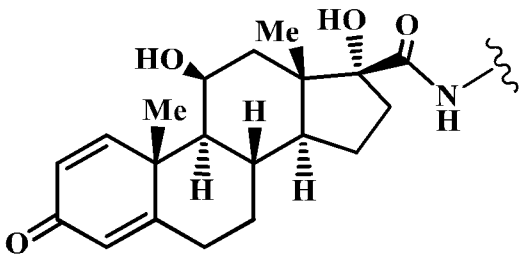
LB31 (Beclometasone dipropionate),



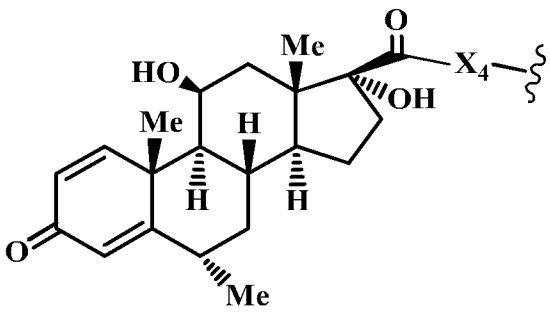
LB32 (Triamcinolone acetonide),



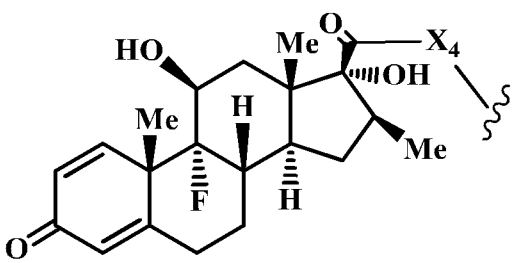
LB33 (Prednisone),



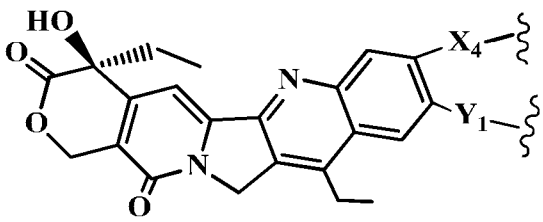
LB34 (Prednisolone),



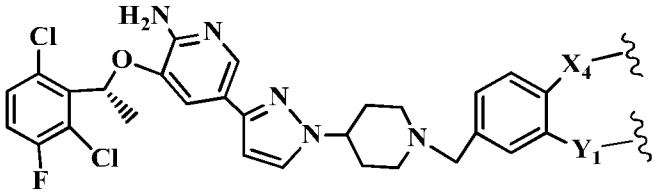
LB35 (Methylprednisolone),



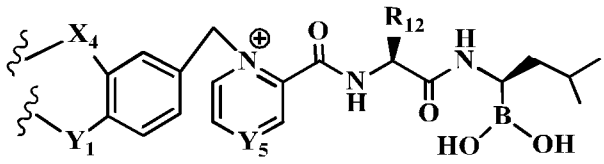
LB36 (Betamethasone),



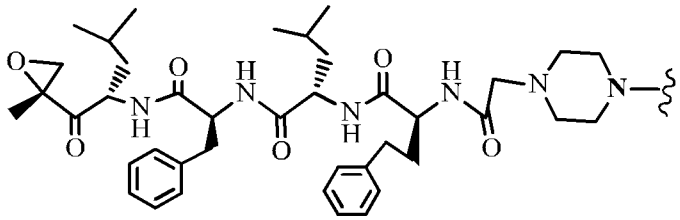
LB37 (Irinotecan analog),



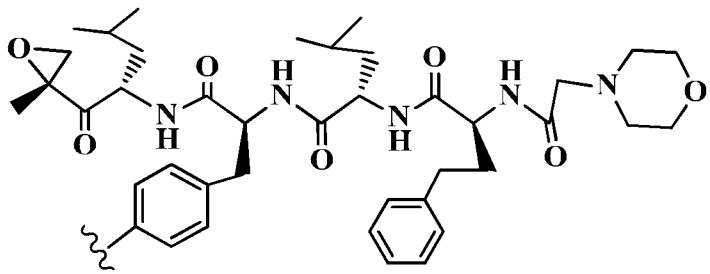
LB38 (Crizotinib analog),



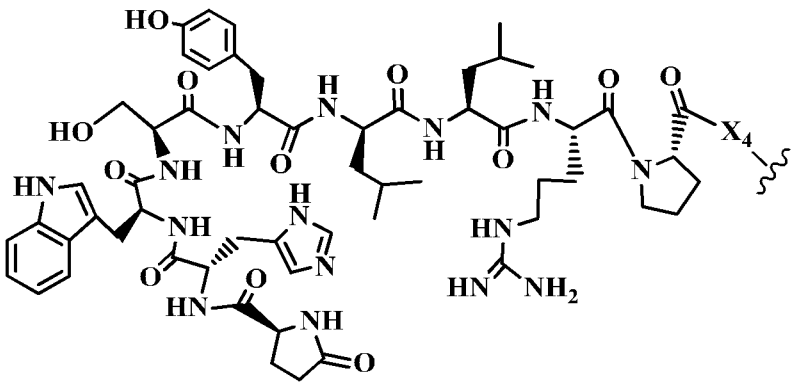
LB39 (Bortezomib analog), wherein Y_5, is N, CH, C(Cl), $C(CH_3)$, or $C(COOR_1)$; R_{12} is H, C_1-C_6 Alkyl, C_3-C_8 Ar;



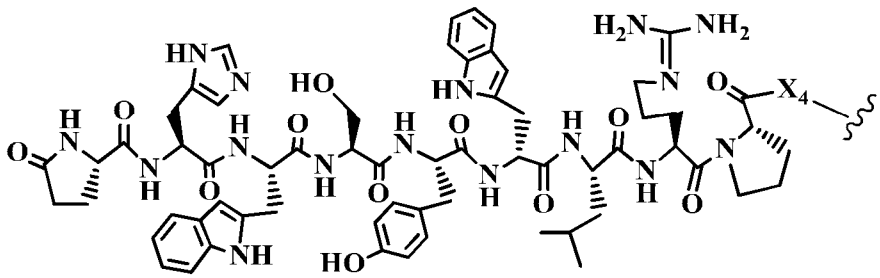
LB40 (Carfilzomib analog),



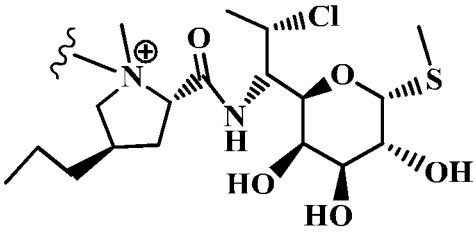
LB41 (Carfilzomib analog),



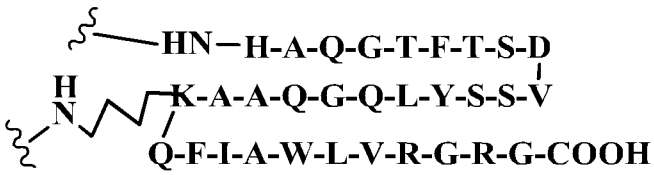
LB42 (Leuprolide analog),



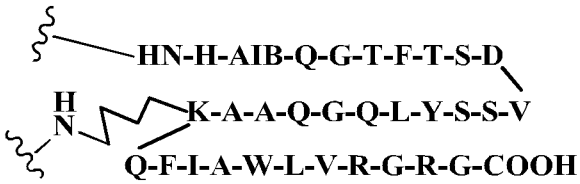
LB43 (Triptorelin analog),



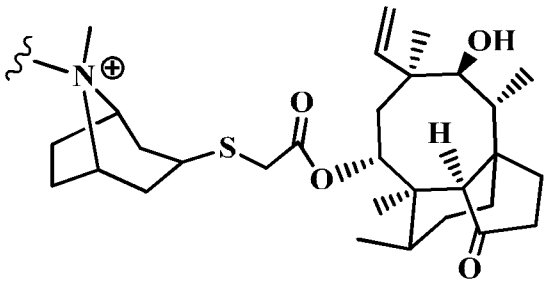
LB44 (Clindamycin),



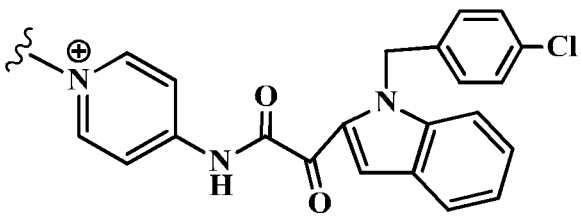
LB45 (Liraglutide analog),



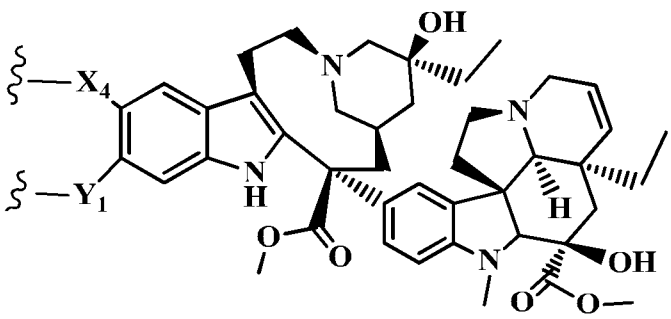
LB46 (Semaglutide analog),



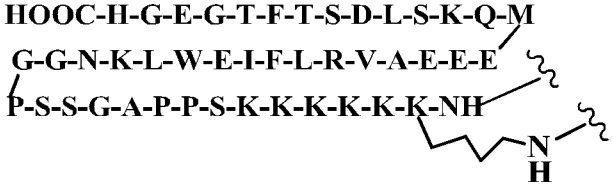
LB47 (Retapamulin analog),



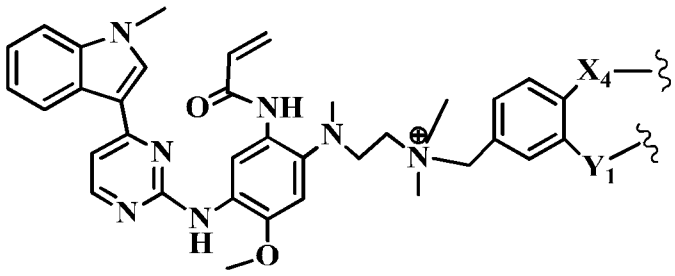
LB48 (Indibulin analog),



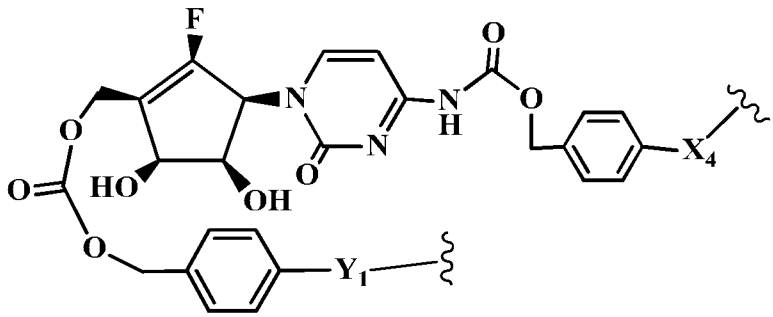
LB49 (Vinblastine analog),



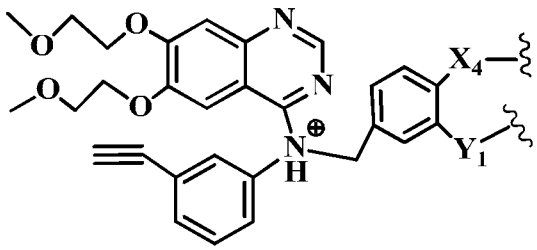
LB50 (Lixisenatide analog),



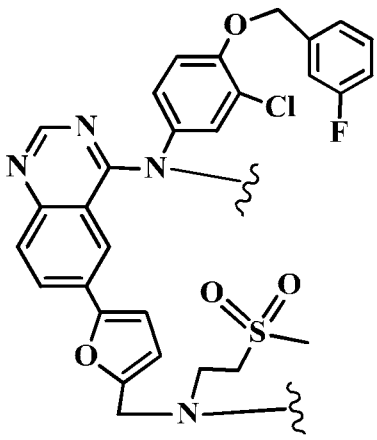
LB51 (Osimertinib analog),



LB52 (a nucleoside analog),



LB53 (Erlotinib analog),



LB54 (Lapatinib analog);

Wherein X_4, and Y_1 are independently O, NH, NHNH, NR_1, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH_2, C(O)NHNHC(O) and C(O)NR_1; R_1, R_2 and Ar, are defined the same above.

In certain embodiments, one, two or more DNA, RNA, mRNA, small interfering RNA (siRNA), microRNA (miRNA), and PIWI interacting RNAs (piRNA) can be as a drug D conjugated via the process of the invention:



, SI01,



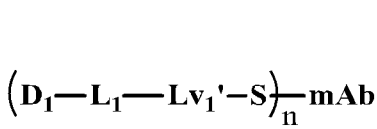
, SI02;

wherein " ~~~ " is the site to link the side chain linker of the present patent;  is single or double strands of DNA, RNA, mRNA, siRNA, miRNA, or piRNA; X_1,and Y are independently O, NH, NHNH, NR_1, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH_2, C(O)NHNHC(O) and C(O)NR_1; R_1, and R_2 are defined the same above.

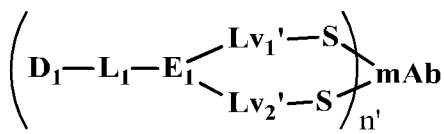
In certain embodiments, the oxidant which can be added in step (c) (after the step of the conjugation reaction) in the process of invention is preferably selected from dehydroascorbic acid (DHAA) to re-oxidize unreacted thiol groups, thus leading to restore the disulfide linkage in the antibody or antibody-like protein for having longer half-life. The concentration of the oxidant in the reaction solution may be 0.01 mM - 1.0 mM.

Instead of addition of DHAA in the process of invention, an excess amount of disulfide compound, such as cystine can be added in the step (c) to replace DHAA. Thus the disulfide compound can be reduced by the excess reductant, such as TCEP in step (b), to form a thiol compound, which simultaneously reacts to the excessive conjugation linker or linker/payload complex containing thiol reactive groups (e. g. maleimide), and following by removing of the generated thiol-succinimide linker/payload complex by chromatography.

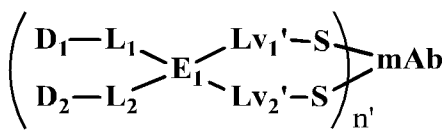
In some embodiments, under conjugation process of the present invention to introduce specific thiols in an antibody or antibody-like protein (preferably mainly at the sites of disulfide bonds between heavy-light chain), then the thiols simultaneously or sequentially in conjugation process react to formula (I), (II), (III) or (IV) independently to the antibody or antibody-like protein to form a conjugate of formula (VI), (VII), (VIII) or (IX) accordingly as represented below:



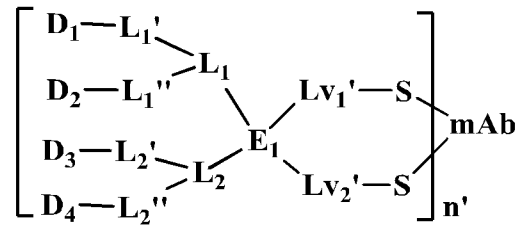
(VI),



(VII),



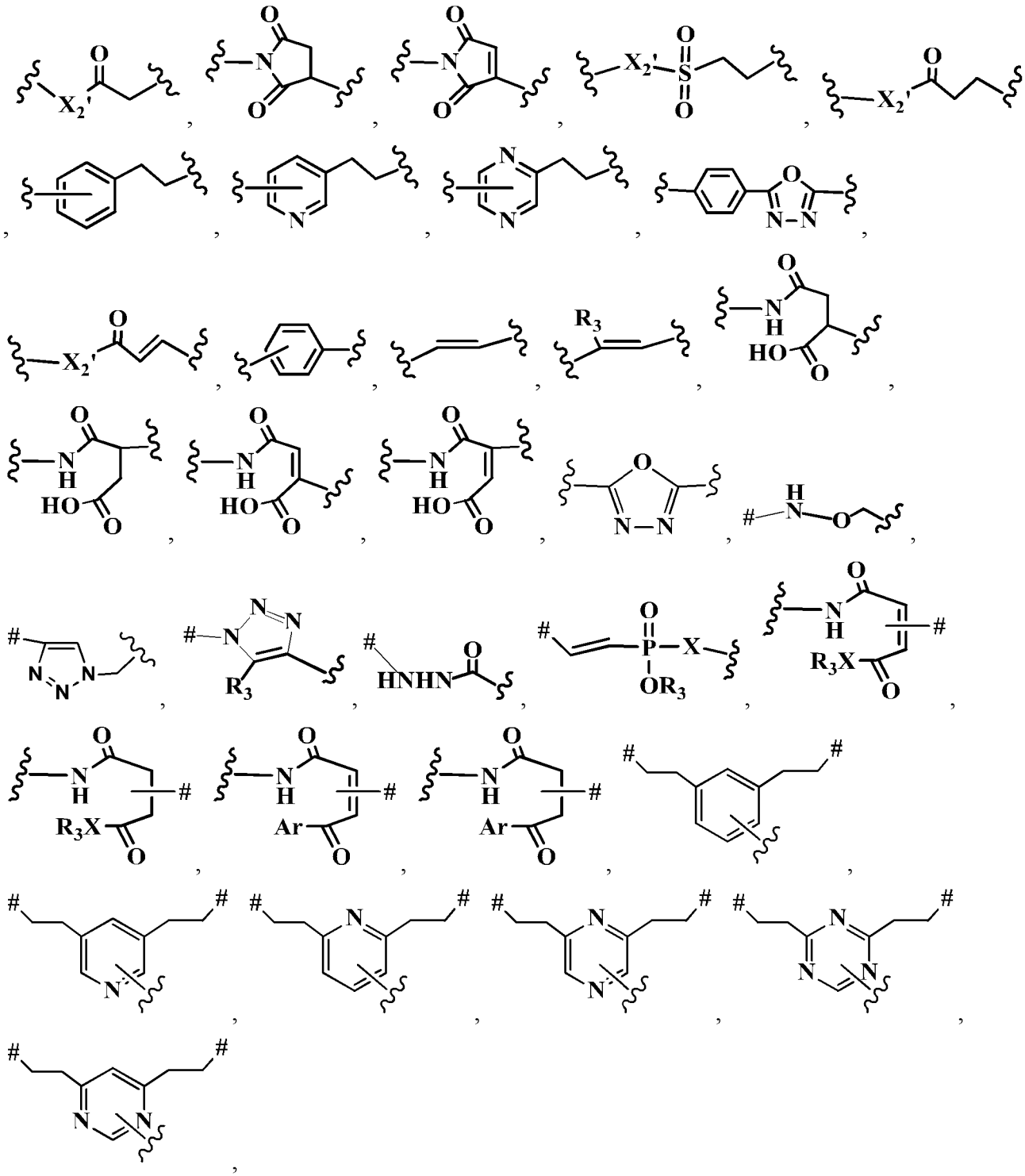
(VIII),



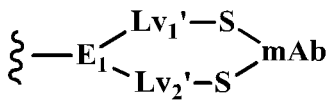
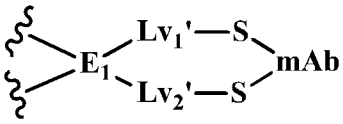
(IX),

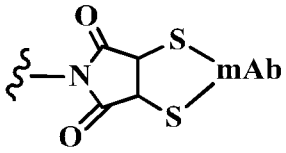
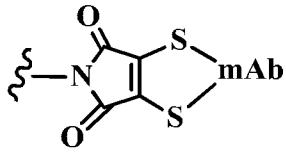
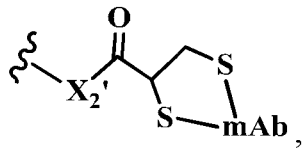
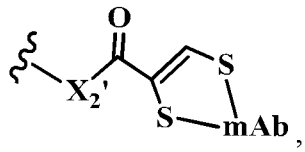
wherein n is 1 – 20; n' is 1-10; preferably n is 1 -8 and n' is 1 - 4; more preferably n is 2 -4 and n' is 1 - 2; D_1, D_2, L_1, L_2, and E_1 are described the same above; S (sulfur) is generated from the reduction of disulfide bonds in the antibody-like protein (e.g. antibody) under process of the

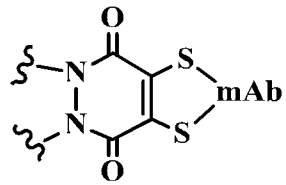
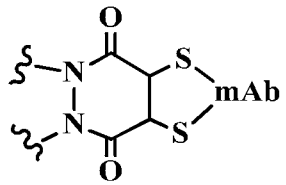
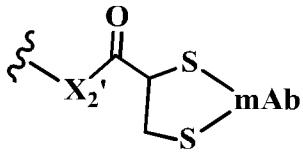
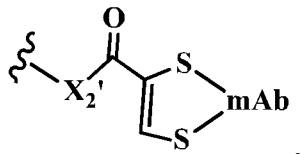
invention; mAb is an antibody-like protein; Wherein Lv_1' and Lv_2' are independently the resulting groups that a thiol in mAb reacted with Lv_1 and Lv_2, whose structures described above. Wherein Lv_1' and Lv_2' are independently having the following structures:

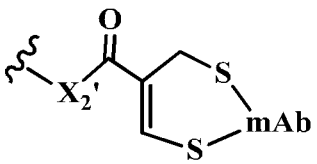
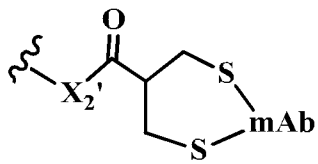
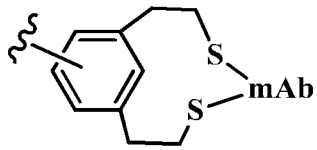


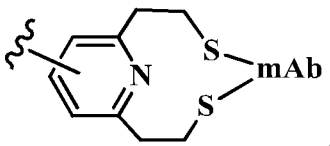
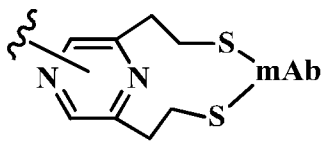
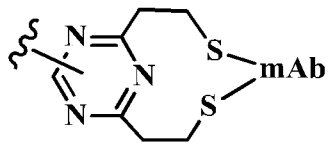
wherein Ar, X_2' and R_3 are defined the same previously.

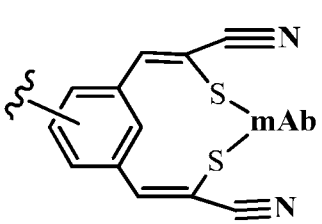
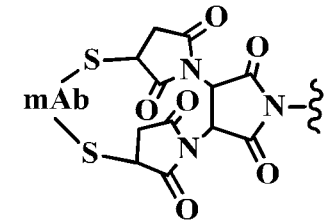
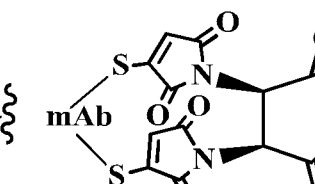
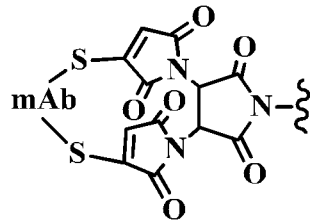
In the formula (VII), (VII) and (IX) wherein the fragments of,  and  can independently be selected from:

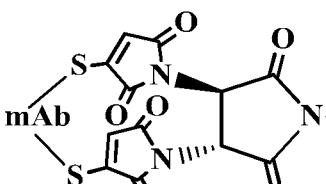
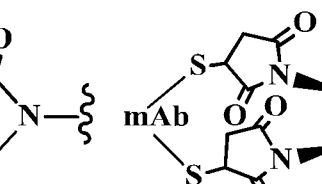
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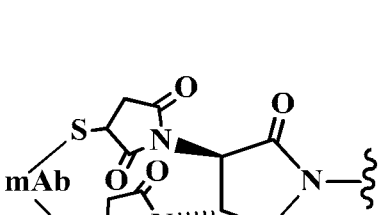
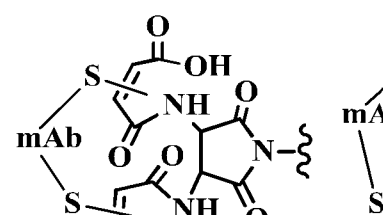
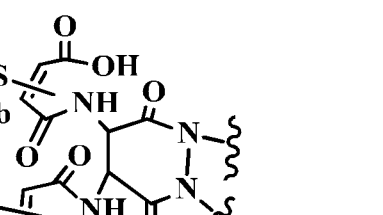
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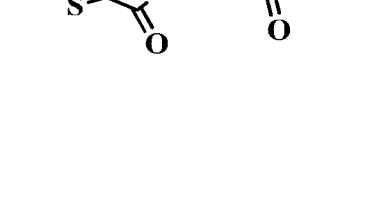
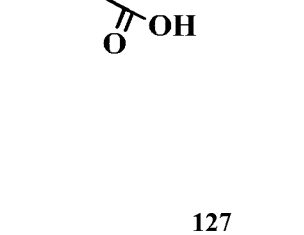
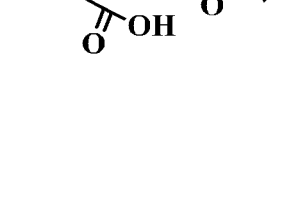
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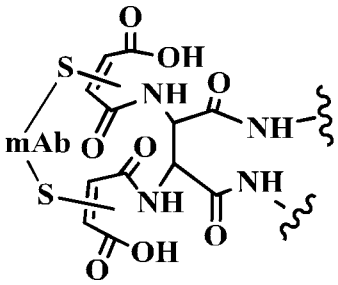
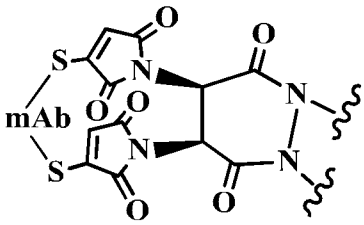
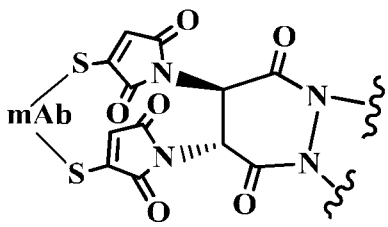
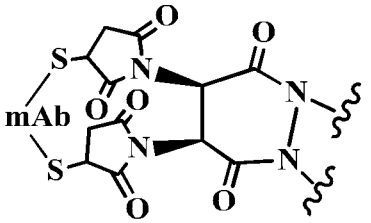
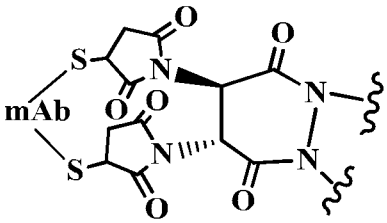
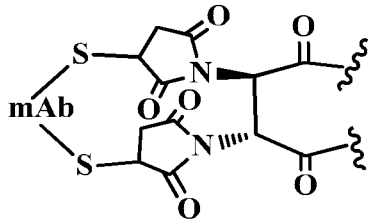
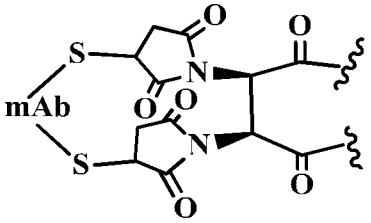
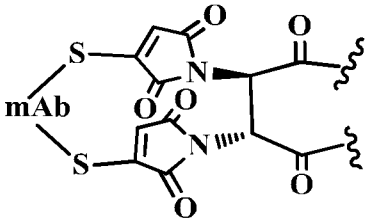
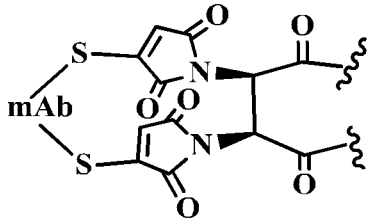
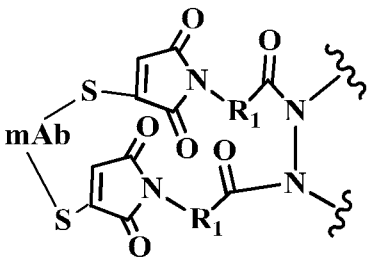
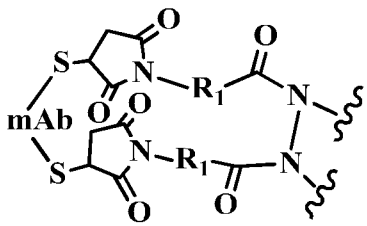
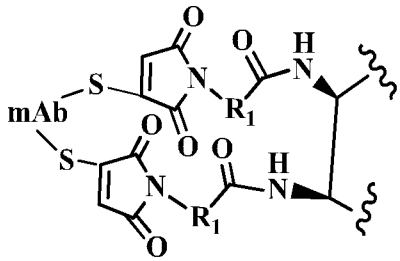
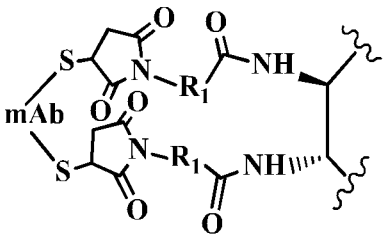
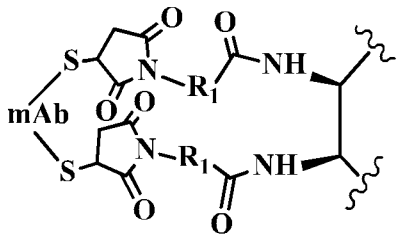
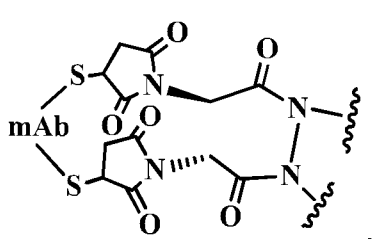
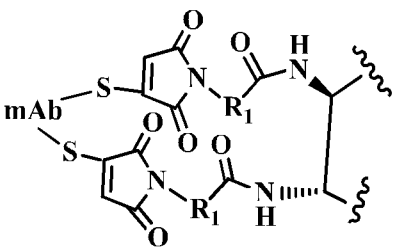
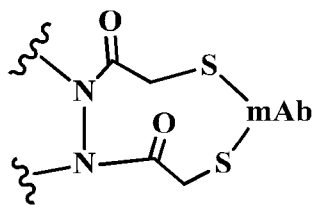
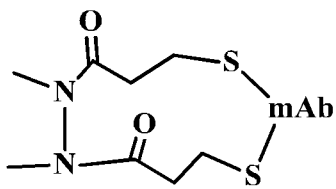
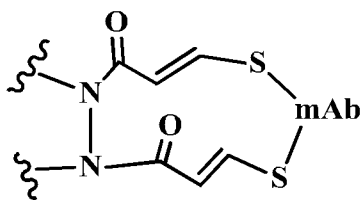
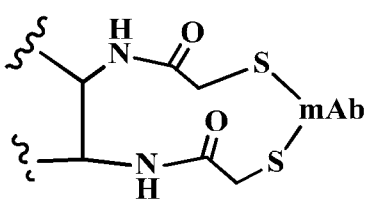
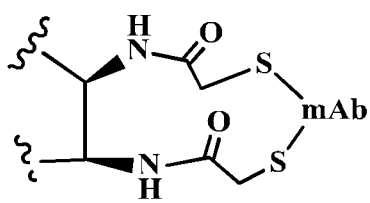
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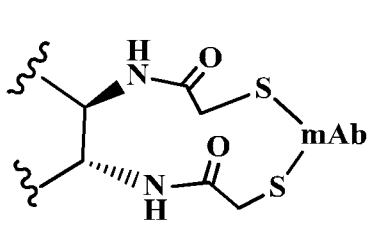
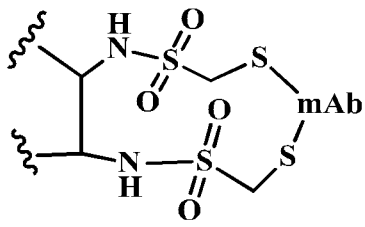
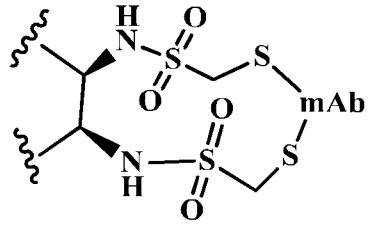
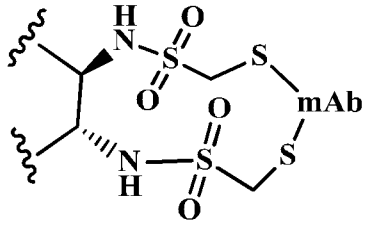
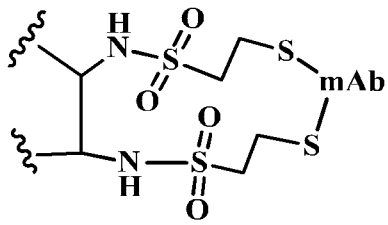
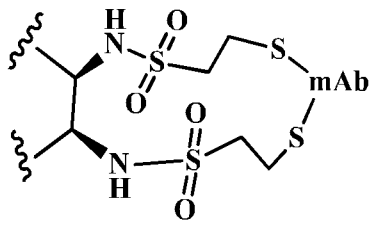
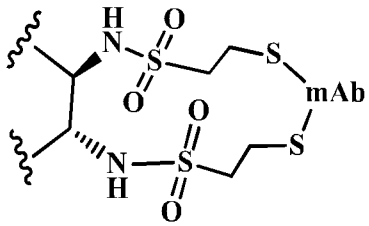
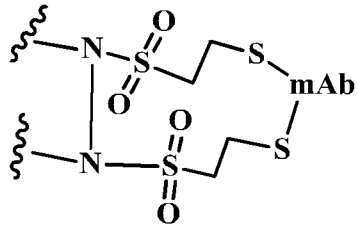
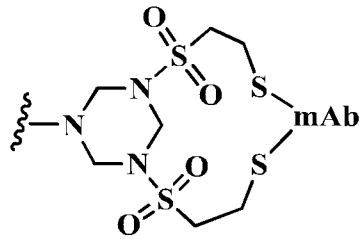
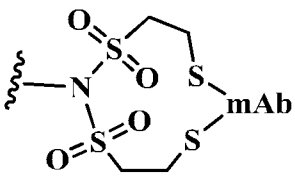
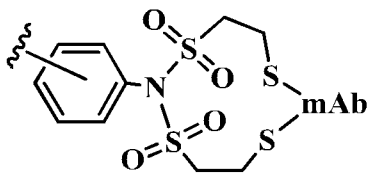
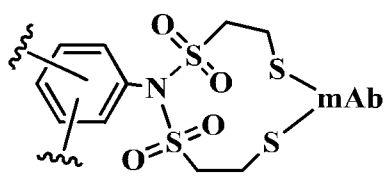
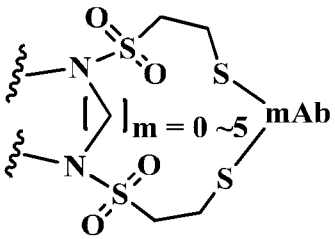
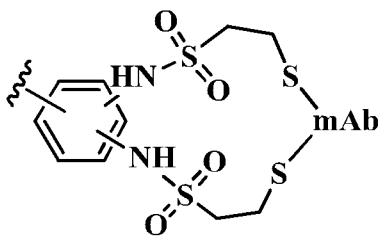
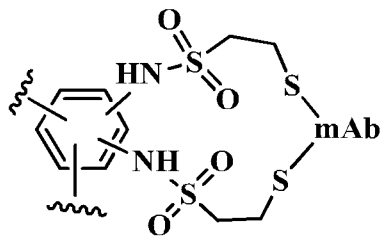
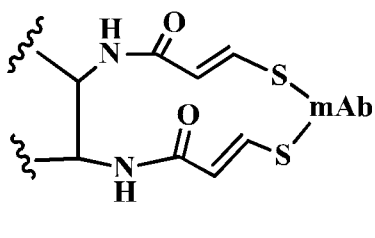
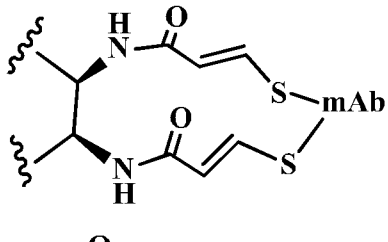
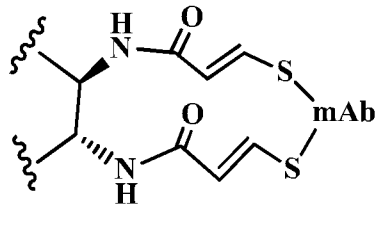
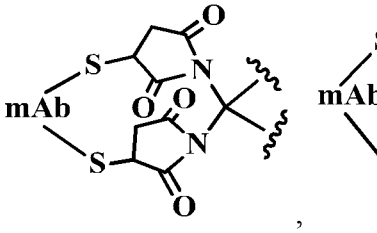
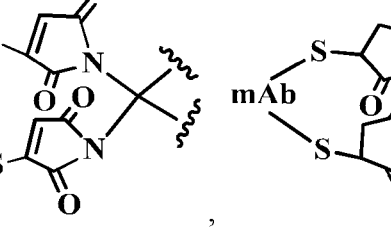
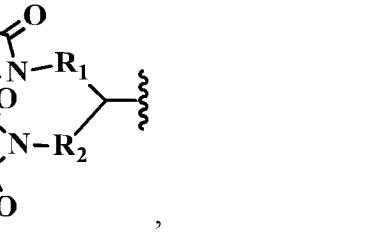
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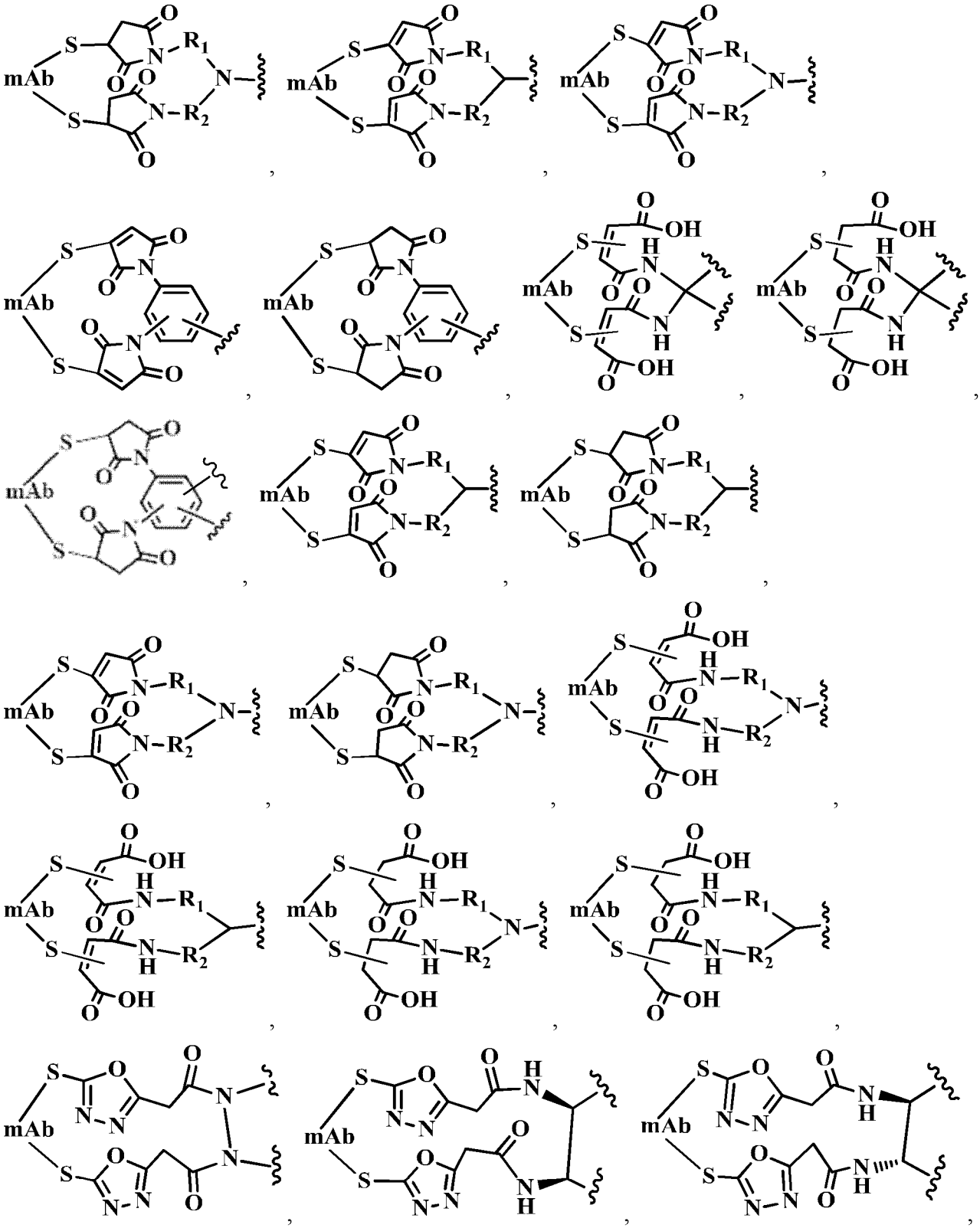
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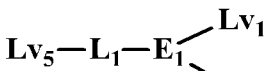


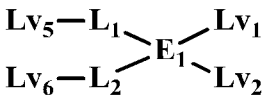
wherein R_1, R_2, X_2 are defined the same above; mAb is an antibody-like protein, preferably an antibody. Preferably, the conjugates are specifically linked to the tiols between heavy-light chains of the antibody when an antibody-like protein is specifically an antibody.

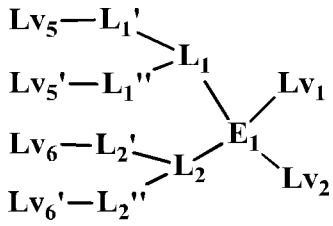
In other embodiments, under process of the present patent invention, wherein a linker having formula (X), (XI), (XII) or (XIII) illustrated below can react first to the selectively reduced thiols in

the antibody or antibody-like protein (e. g. typically thiols between heavy-light chain when the antibody or antibody-like protein is IgG antibody) independently, followed by condensation with a cytotoxic drug or cytotoxic drug/linker complex to form the conjugates of formula (VI), (VII), (VIII) or (IX) as shown above:

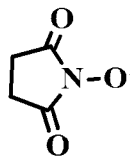
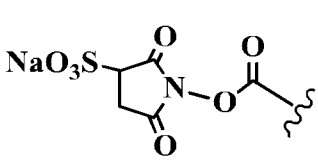
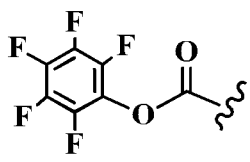
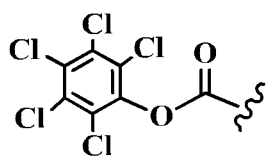
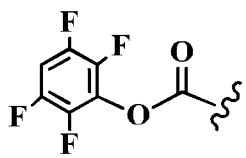
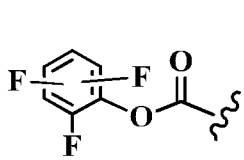
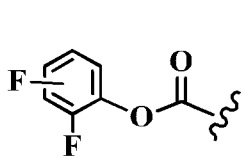
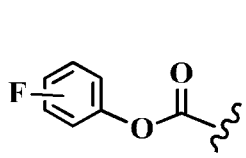
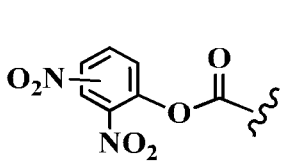
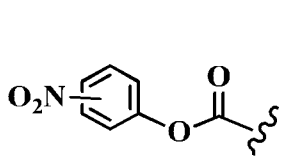
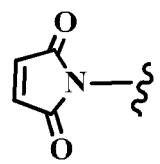
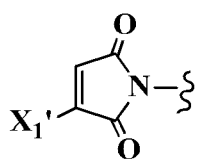
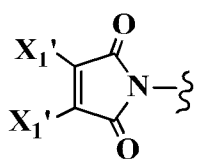
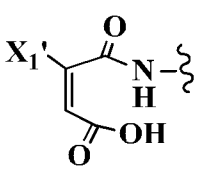
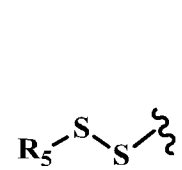
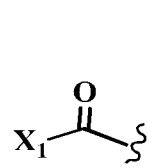
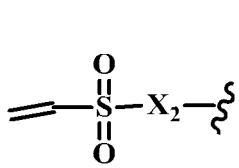
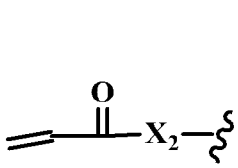
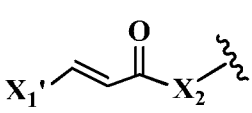
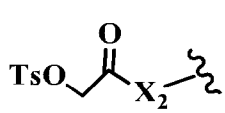
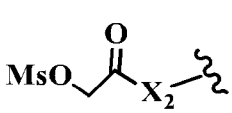
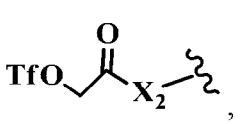
(X),

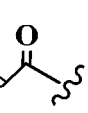
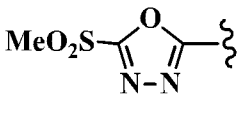
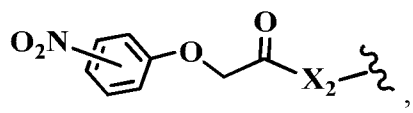
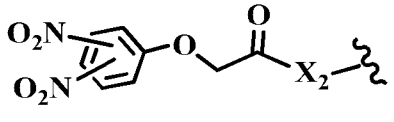
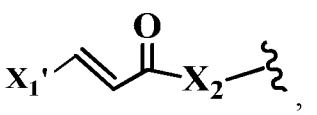
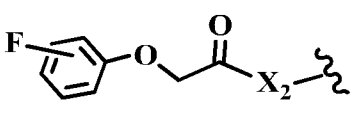
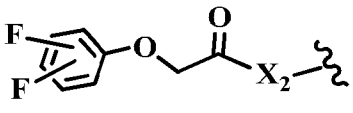
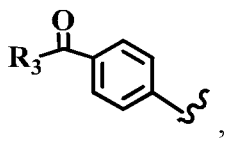

(XI),

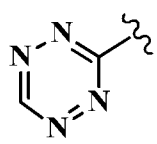
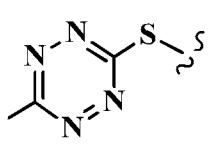
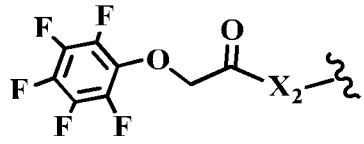

(XII),

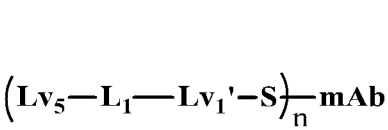

(XIII),

Wherein L_1, L_1', L_2, L_2', E_1, Lv_1, and Lv_2 are defined the same above for Formula (VI), (VII) (VIII) and (IX); wherein Lv_5 and Lv_6 are independently selected from

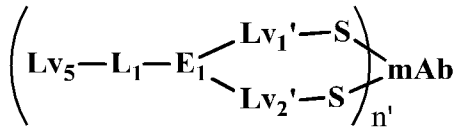

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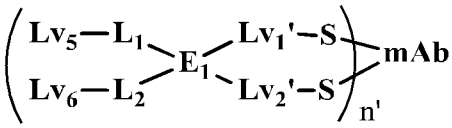






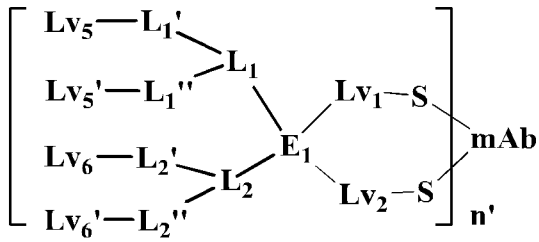
(XIV),



(XV),



(XVI),



(XVII),

wherein Lv_5, Lv_6, L_1, L_2, L_1', L_2', E_1, Lv_1' Lv_2', mAb, n and n' are described the same above.

In further embodiments, under process of the present patent invention, wherein the linker of formula (X), (XI), (XII) or (XIII) illustrated above can react first with a cytotoxic drug to form the cytotoxic drug/linker complex molecule of formula (I), (II), (III) or (IV), follow by reaction with the reduced thiols in the antibody or antibody-like protein independently to form the conjugate of formula (VI), (VII), (VIII) or (IX) under process of this invention. The first step condensation reaction of the formula (X), (XI), (XII) or (XIII) to a cytotoxic drug can be in a separated pot, and the resulted cytotoxic drug/linker complex molecules of formula (I), (II), (III) or (IV) can be optionally purified by a chromatography, extraction or precipitation before for conjugation to the reduced thiols in the antibody-like protein. The first step of specific reduction of disulfide bonds in an antibody-like protein and conjugation reaction with formula (I), (II), (III) or (IV) are preferred in the same pot without separation of intermediates.

To distinguish the reactions between Lv_5 and/or Lv_6 to a cytotoxic drug, and Lv_1 and/or Lv_2 to a thiol in an antibody-like protein, each step of the reactions for the linker of formula (X), (XI), (XII) or (XIII) can be conducted at different conditions in the same or different reaction pots. For instance, a drug containing an amino group can undergo condensation with a carboxylic acid group in the linker in the present of a condensation regent, e. g. EDC, TBTU or BroP, to give a modified drug/linker complex of Formula (I), (II), (III) or (IV) bearing amide bonds. This condensation reaction can be performed at physiological buffer solution wherein the carboxylic acid group at one terminal of the linker of formula (X), (XI), (XII) or (XIII) is activated to be N-hydroxysuccinimidyl (NHS), pentfluorophenyl, dinitrophenyl ester, or carboxylic acid chloride group, etc, which can react to a drug bearing an amino group to provide drug/linker complex of Formula (I), (II), (III) or (IV), then subsequently or simultaneously undergo the conjugation to thiols of an antibody-like protein according to the process of the present application to form the conjugate of formula (VI), (VII), (VIII) or (IX). In another practice, the linker of formula (X), (XI), (XII) or (XIII) bearing both a thiol reactive group (e. g. maleimido, vinylsulfonyl, haloacetyl, acrylic, substituted propiolic) at one terminal and a drug reactive group (e. g. hydoxylsuccinimidyl (NHS), pentfluorophenyl, dinitrophenyl ester, amino, alkyloxylamino or clickable chemistry group (e. g. azide, alkyne,

dibenzocyclooctyne, BCN ((1*R*, 8*S*, 9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol)) at the other terminal can undergo undergo the conjugation to thiols of an antibody-like protein (preferably an antibody) according to the process of the present application in a buffer solution at pH 4.5 -7.5, 2 °C – 40 °C (preferably 2 °C -8 °C) with or without addition of 0~30% of water mixable (miscible) organic solvents to form the antibody-linker conjugate of formula (XIV), (XV), (XVI) or (XVII) independently. Then a drug bearing a reactive group which are matched to the reactive group in the antibody-linker conjugate of formula (XIV), (XV), (XVI) or (XVII) accordingly can be subsequently or simultaneously added to the reaction solution to provide the conjugate of formula (VI), (VII), (VIII) or (IX). In the second step reaction, the antibody-linker conjugate of formula (XIV), (XV), (XVI) or (XVII) can be optionally purified before proceeding the condensation with a drug, and the condensation condition of the second step can be adjusted, e. g. the pH is adjusted to 6.5 – 8.0, and/or temperature is adjusted to 20 -45 °C.

In other embodiments, during the process of the conjugation of this invention, prior to conjugating with a drug, the antibody can be modified through attachment of a heterobifunctional cross linker of formula (XIV), (XV), (XVI) or (XVII), such as with linkers of Amine-to-Sulfhydryl (succinimidyl (NHS) ester/maleimide, NHS ester/ pyridyldithiol, NHS esters/ haloacetyl), diazirine (SDA)–to-Sulfhydryl, Azide-to-Sulfhydryl, Alkyne-to-Sulfhydryl, Sulfhydryl-to-Carbohydrate (Maleimide/Hydrazide, Pyridyldithiol /Hydrazide, haloacetyl /Hydrazide), Hydroxyl-to-Sulfhydryl (Isocyanate/Maleimide), Sulfhydryl-to-DNA (Maleimide/ Psoralen, Pyridyldithiol / Psoralen, haloacetyl/Psoralen), Sulfhydryl-to-Carboxyl (Carbodiimide).

The reactive group of a drug/cytotoxic agent that reacting to a modified an antibody-linker conjugate of formula (XIV), (XV), (XVI) or (XVII) to give the final conjugate can be in different ways accordingly. For example, the conjugate linked via disulfide bonds is achieved via the first step, a linker of formula (X), (XI), (XII) or (XIII) is conjugated to the antibody at 2 °C - 8 °C, pH 4.5 – 6.0, according to the present invention of reduction and conjugation of an antibody, following by a disulfide exchange between a drug containing a free thiol group and the disulfide bond ((e. g. pyridyldithio moiety) in the linker attached to the modified antibody at pH 6.5 – 8.0, at 20 °C - 40 °C. Before the addition of the drug containing a free thiol for conjugation, the excess reduction agent (e. g. TCEP, or tri(3-hydroxylpropyl)phosphine) is preferably removed from the reaction pot. Synthesis of the conjugates linked via thioether is achieved by first reaction of a linker containing both thiol reactive terminals of maleimido or haloacetyl or ethylsulfonyl or substituted propiolic group to the thiols in an antibody which are reduced by the process of the present patent application at 2 °C - 8 °C, pH 4.5 – 6.0 to give the antibody-linker conjugate of formula (XIV), (XV), (XVI) or (XVII), following by reaction of a drug containing a thiol at pH 6.5 – 8.0, at 20 °C - 40 °C to provide the conjugate of formula (VI), (VII), (VIII) or (IX). If the same pH and/or temeperature conditions are

chosen for the two step reactions for thioether linked conjugates, the over four times equivalents of the linker containing dual terminal thiol reactive are used for the conjugation. It should be noted that the preferred methods of synthesis of the disulfide or thiol-ether linked conjugates are through the first chemical synthesis the drug-linker complex having disulfide or thiol-ether bonds of the formula (I), (II), (III) or (IV), following by reaction with the thiols in the protein (antibody) according the process of the invention. Synthesis of conjugates bearing an acid labile hydrazone linkage can be achieved by reaction of a carbonyl group with the hydrazide moiety in the linker, by methods known in the art (see, for example, P. Hamann et al., Cancer Res. 53, 3336-34, 1993; B. Laguzza et al., J. Med. Chem., 32; 548-55, 1959; P. Trail et al., Cancer Res., 57; 100-5, 1997). Synthesis of conjugates bearing triazole linkage can be achieved by reaction of a 1-yne group of the drug with the azido moiety in the linker, through the click chemistry (Huisgen cycloaddition) (Lutz, J-F. et al, 2008, Adv. Drug Del. Rev. 60, 958–70; Sletten, E. M. et al, 2011, AccChem. Research 44, 666–76). Synthesis of the conjugates linked via oxime is achieved by reaction of a modified antibody-like protein containing a ketone or aldehyde and a drug containing oxyamine group. A drug bearing a hydroxyl group or a thiol group can be reacted with a modified linker of Formula (XIV), (XV), (XVI) or (XVII), bearing a halogen, particularly the alpha halide of carboxylates, in the presence of a mild base, e.g. pH 8.0 ~ 9.5, to give a modified drug/linker complex bearing an ether or thiol ether linkage of Formula (I), (II), (III) or (IV). A drug containing a hydroxyl group can be condensed with a linker of Formula (XIV), (XV), (XVI) or (XVII) bearing a carboxyl group, in the presence of a dehydrating agent, such as EDC or DCC, to give ester linkage, then the subject drug/linker complex undergoes the conjugation with an antibody-like protein under the process of the present invention. A drug containing an amino group can condensate with a carboxyl ester of NHS, imidazole, nitrophenoxyl; N-hydroxysuccinimide (NHS); methylsufonylphenoxyl; dinitrophenoxyl; pentafluorophenoxyl; tetrafluorophenoxyl; difluorophenoxyl; monofluorophenoxyl; pentachlorophenoxyl; triflate; imidazole; dichlorophenoxyl;tetrachlorophenoxyl;1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3′-sulfonate on the antibody-linker of Formula (XIV), (XV), (XVI) or (XVII) to give a conjugate via amide bond linkage of Formula (VI), (VII), (VII) or (IX). For a linker of formula (X), (XI), (XII) or (XIII) which contains both different thiol reactive terminals (e. g. one end is maleimido group(s) and the other end is vinyl sulfonyl group(s)), then the conjugation can be proceeded at different pHs or temperatures or with a catalyst to differentiate the one terminal reaction with a thiol group of a drug or of an antibody from another end of terminal reaction. In the case of both one end is maleimido group(s) and the other end is vinyl sulfonyl group(s)), the reaction (Michael addition) of a thiol group of a drug or of an antibody with a maleimido group can be proceeded at pH below 5.5 (normally at pH 4.5 ~ 7.0) of a buffer solution,

at low temperature (2 ~ 15 °C), and in a short time (30 min ~ 2 hrs.), but the reaction (Michael addition) of a thiol group of a drug or of an antibody with a vinyl sulfonyl group has to be proceeded at pH over 7.0 (normally at pH 7.0 ~ 8.5) of a buffer solution, at high temperature (20 ~ 37 °C), and for at least 8 hrs. Thus, a thiol group of a drug or of an antibody can first react to a maleimido group in a linker of formula (X), (XI), (XII) or (XIII), at low pH (e. g. below 6) for less than 2 hrs., following by addition of a thiol reduced antibody (containing a thiol) or a drug containing a thiol to react a vinyl sulfonyl group at the other end of the linker complex at higher pH (e. g. 7.5).

In further embodiments, under process of the present patent invention, the resulted conjugates of formula (VI), (VI)), (VIII) or (IX) are over 75% linked to the cysteine sites between heavy-light chains of an antibody, and are less than 15% linked to the cysteine sites between heavy-heavy chains (hinge region) of an antibody. Typically, for formula (VI) or (VII), when drug/antibody ratio (DAR) is set to be 4, the distributions in percentage of the numbers of drugs in the antibody are: D0 <1%, D2<10%, D4>75%, D6<10%, D8<10%; for formula (VIII) or (IX), when drug/antibody ratio (DAR) is set to be 8, the distributions in percentage of the numbers of drugs in the antibody are: D0 <1%, D1<10%, D2>75%, D3<10%, D4<10%.

The resulted conjugate may be purified by standard biochemical means, such as gel filtration on a Sephadex G25 or Sephacryl S300 column, adsorption chromatography, ion (cation or anion) exchange chromatography or by dialysis (ultrafiltration or hyperfiltration (UF) and diafiltration (DF)). In some cases, a small size molecule of antibody-like protein (e.g. < 10 KD) conjugated with a small molecular drugs can be purified by chromatography such as by HPLC, medium pressure column chromatography or ion exchange chromatography.

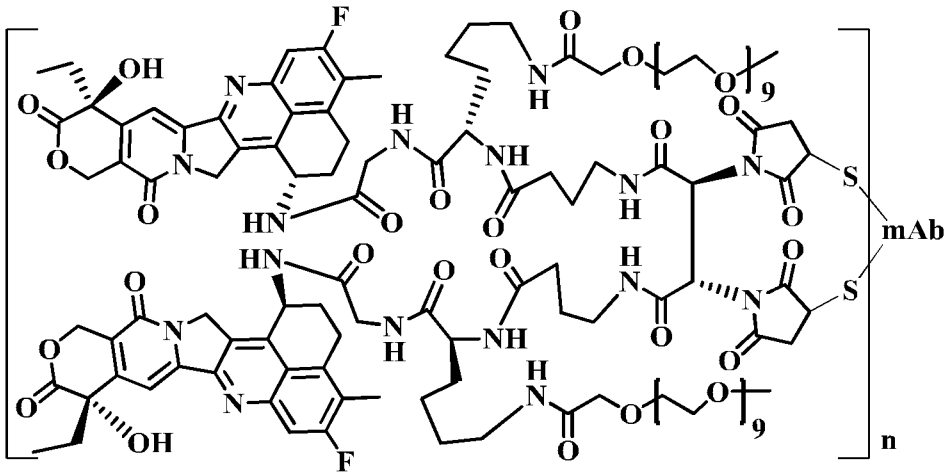
In general, the conjugate of Formula (VI), (VII), (VIII) or (IX) is preferably generated from a drug/linker complex of Formula (I), (II), (III) or (VI), as in a one pot reaction. When a thiol reduced from an antibody protein reacts a thiol reactive group in the terminal of drug/linker complex of Formula (I), (II), (III) or (IV), the Ellman reagent can be optionally used to monitor the efficient reduction of the disulfide bonds and conjugation of the tiols through measurement of the numbers of the free thiols during the reactions. A UV spectrometry at wavelength of range 190-390 nm, preferably at 240-380 nm, more preferably at 240-330 nm is preferred to be used in assisting the reaction (via monitoring the conjugation). The conjugation reaction can be thus measured or conducted in a quartz cell or Pyrex flask in temperature control environment. The drug/protein (antibody) ratios (DAR) of the conjugates can also be measured by UV at wavelength of range 240-380 nm via calculation of the concentrations of the drug and the protein, by Hydrophobic Interaction Chromatography (HIC-HPLC) via measurement of the integration areas of each drug/protein fragment, by Capilary electrophoresis (CE), and/or by LC-MS or LC-MS/MS or CE-MS (the

combination of liquid chromatography (LC) or CE with mass spectrometry (MS) via measurement of both the integration areas of LC or CE and Peak intensity of MS for each drug/protein fragment). It is also noted in the conjugation process of the present invention, when a drug or a drug/linker complex is not well soluble in a water based buffer solution, up to 30% of water mixable (miscible) organic solvents, such as DMA, DMF, ethanol, methanol, acetone, acetonitrile, THF, isopropanol, dioxane, propylene glycol, or ethylene diol can be added as the co-solvent in water based buffer solution.

The aqueous solutions for the modification of the antibody-like protein are buffered between pH 4 and 9, preferably between 6.0 and 7.5 and can contain any non-nucleophilic buffer salts useful for these pH ranges. Typical buffers include phosphate, acetate, triethanolamine HCl, HEPES, and MOPS buffers, which can contain additional components, such as cyclodextrins, sucrose and salts, for examples, NaCl and KCl. Other biological buffers that are used for the conjugation process are listed in the definition section. The progress of the reaction can be monitored by measuring the decrease in the absorption at a certain UV wavelength, such as at 254 nm, or increase in the absorption at a certain UV wavelength, such as 280 nm, or the other appropriate wavelength. After the reaction is complete, isolation of the modified cell-binding antibody-like protein agent can be performed in a routine way, using for example gel filtration chromatography, or adsorptive chromatography.

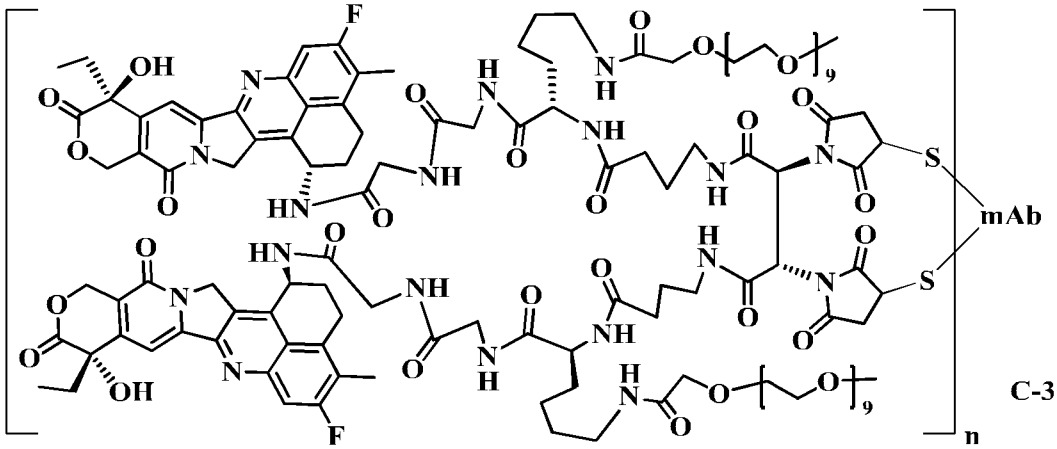
When disulfide exchange reaction is used for modification of an antibody-like protein, the extent of the modification can be assessed by measuring the absorbance of the nitropyridine thione, dinitropyridine dithione, pyridine thione, carboxylamidopyridine dithione and dicarboxyl-amidopyridine dithione group released via UV spectra. For the conjugation without a chromophore group, the modification or conjugation reaction can be monitored by LC-MS, preferably by UPLC-QTOF mass spectrometry, or Capilary electrophoresis–mass spectrometry(CE-MS). The linker compounds have diverse functional groups that can react with drugs, preferably cytotoxic agents that possess a suitable substituent. For examples, the modified antibody-like protein bearing an amino or hydroxyl substituent can react with drugs bearing an N-hydroxysuccinimide (NHS) ester, the modified antibody-like protein bearing a thiol substituent can react with drugs bearing a maleimido or haloacetyl group. Additionally, the modified antibody-like protein bearing a carbonyl (ketone or aldehyde) substituent can react with drugs bearing a hydrazide or an alkoxyamine. One skilled in the art can readily determine which linker to use based on the known reactivity of the available functional group on the linkers.

In certain embodiments, examples of preferred conjugates of formula (VI), (VII), (VIII) and (IX) which can be constructed under the process of the invention are illustrated below:



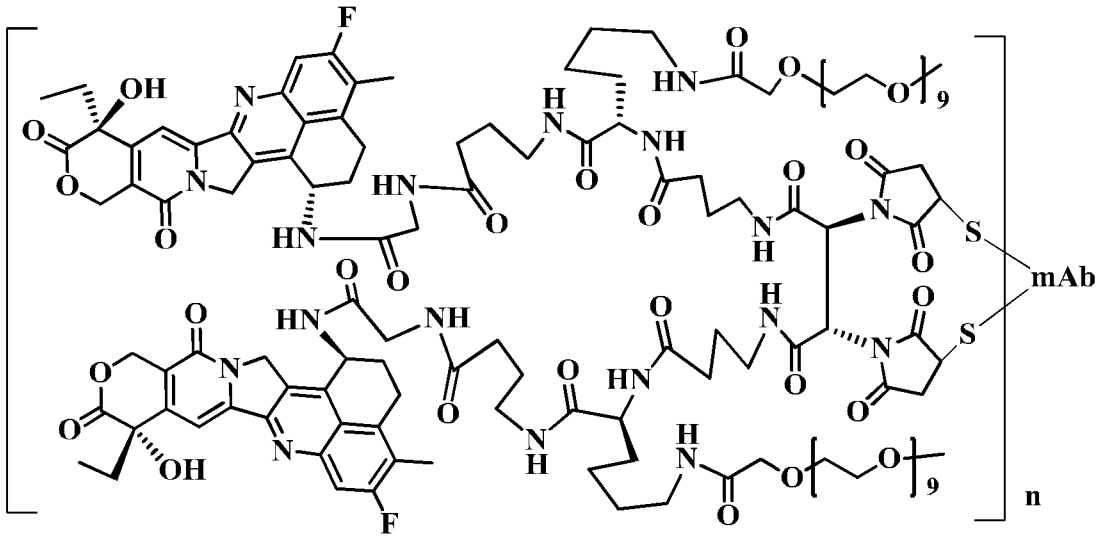
C-25

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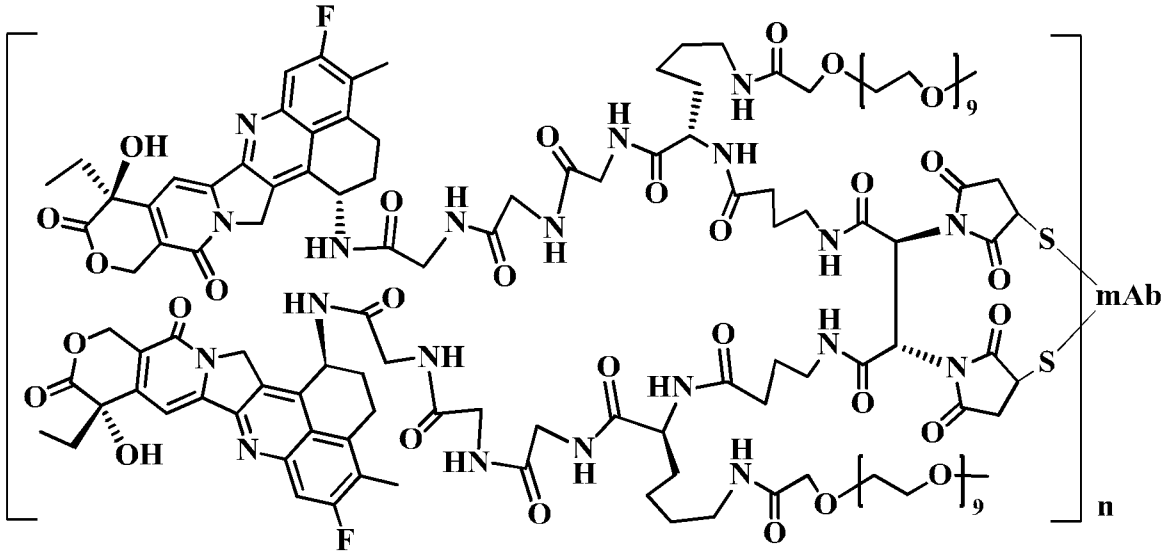
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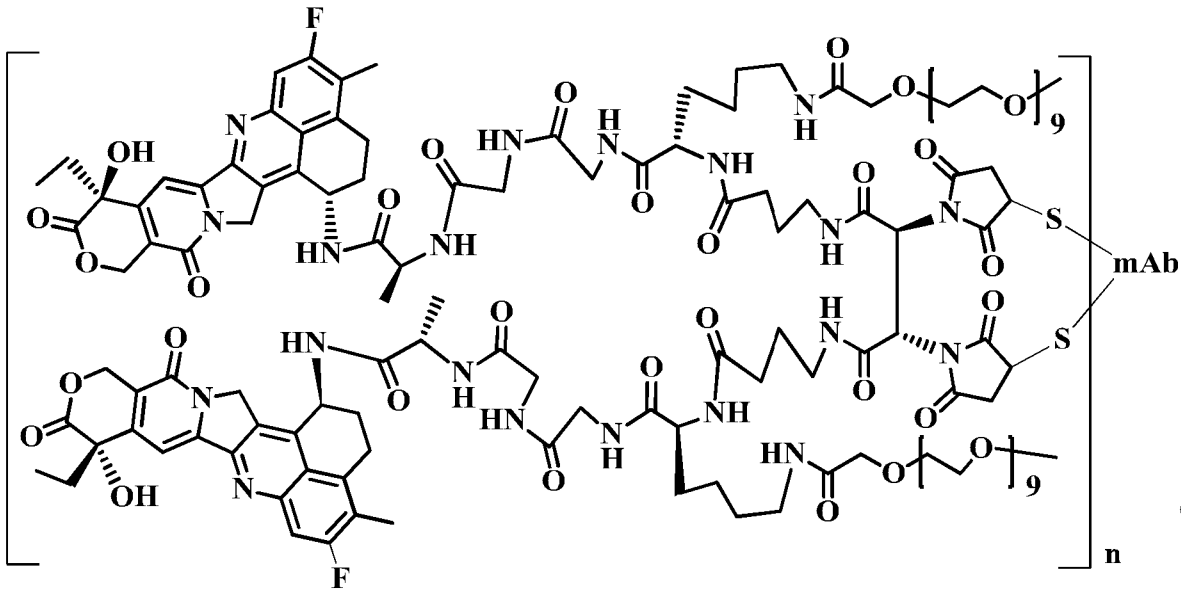


C-36

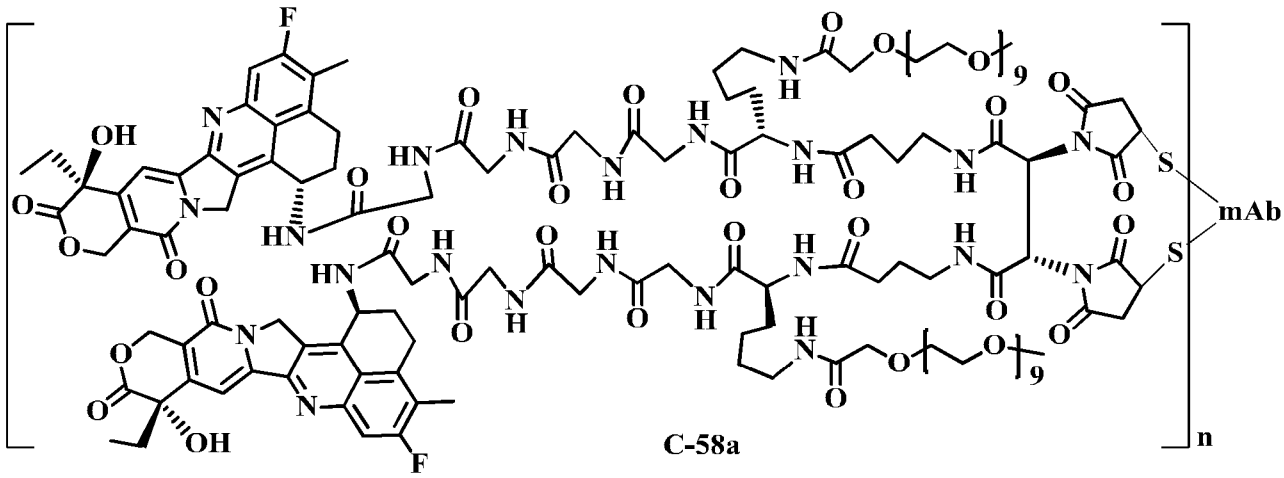
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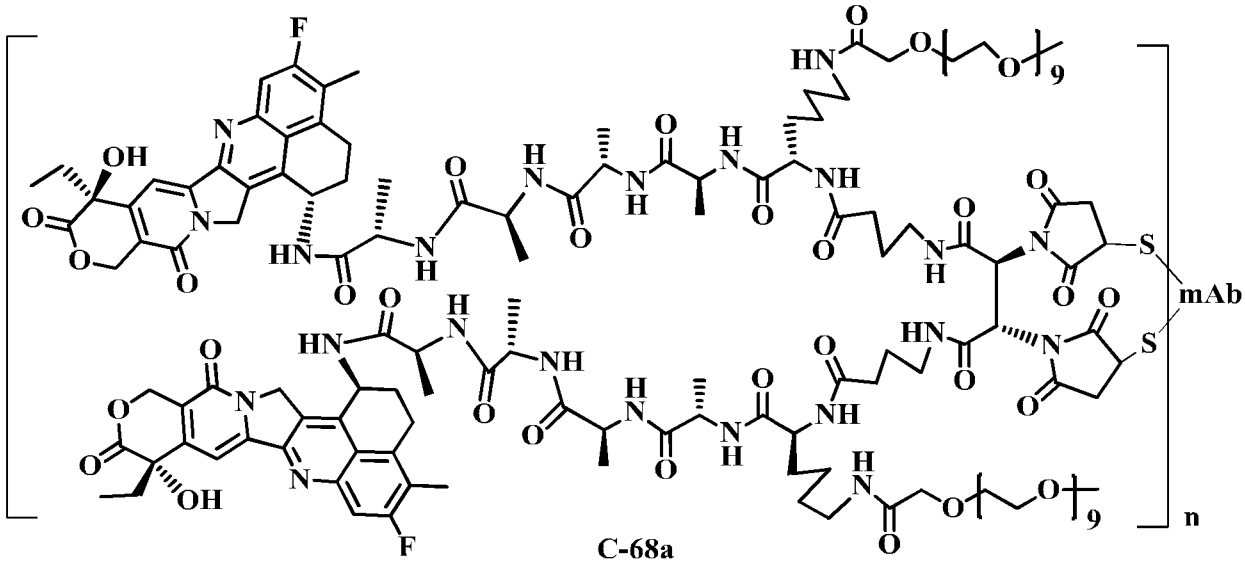
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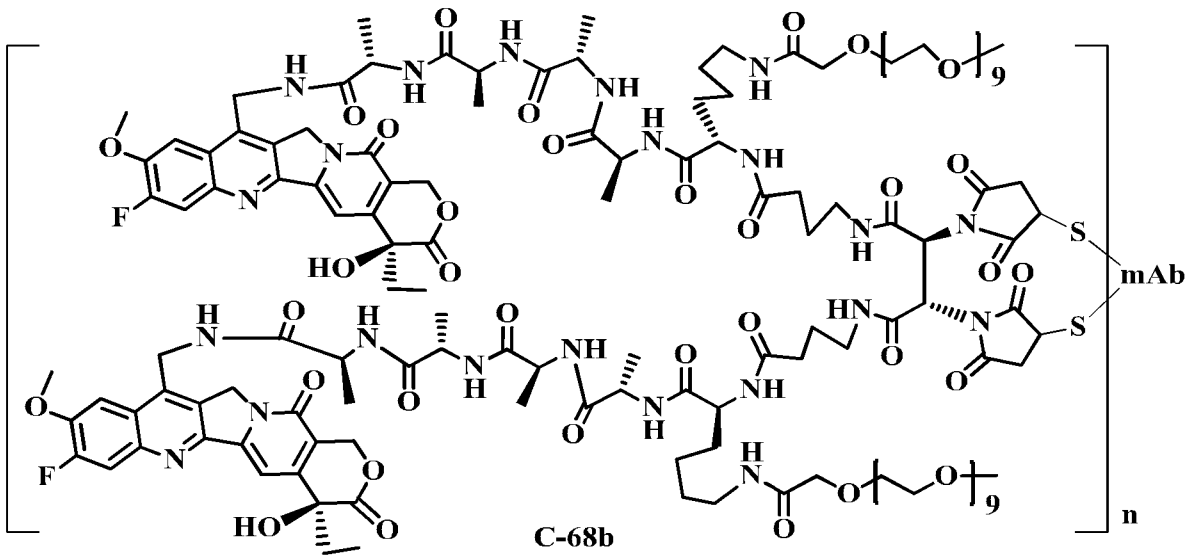
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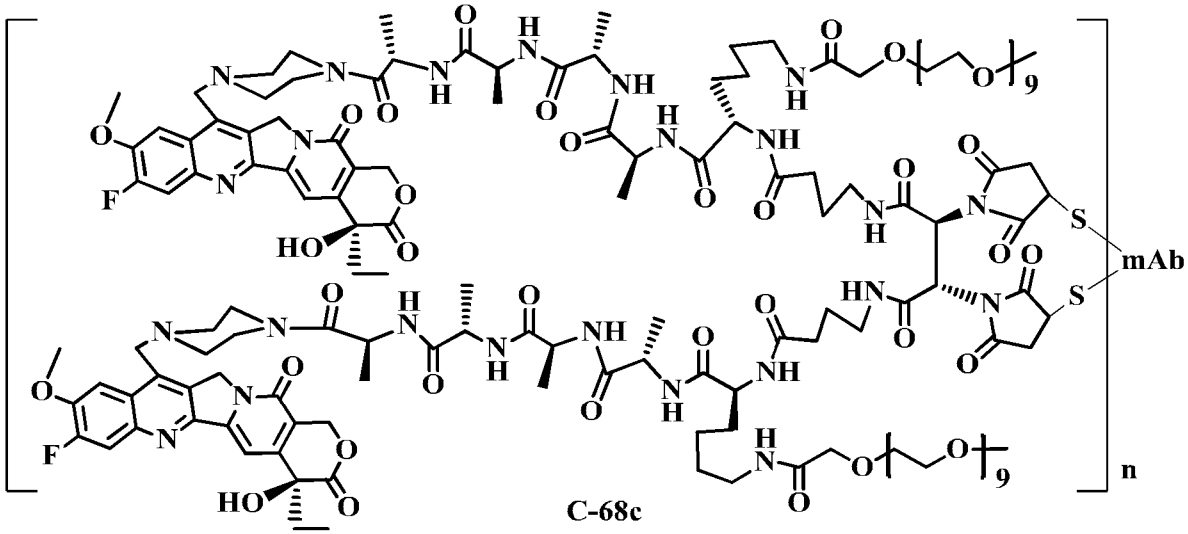
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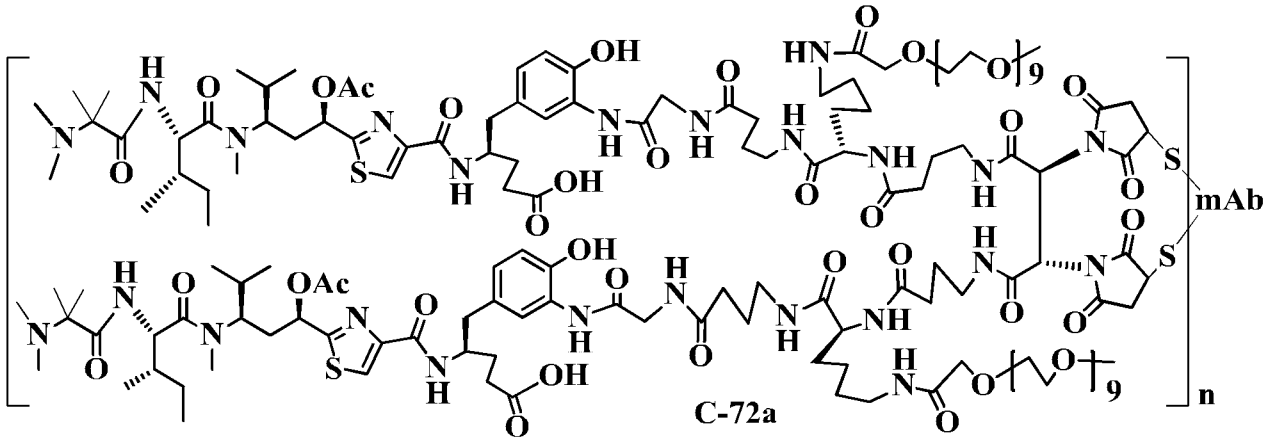
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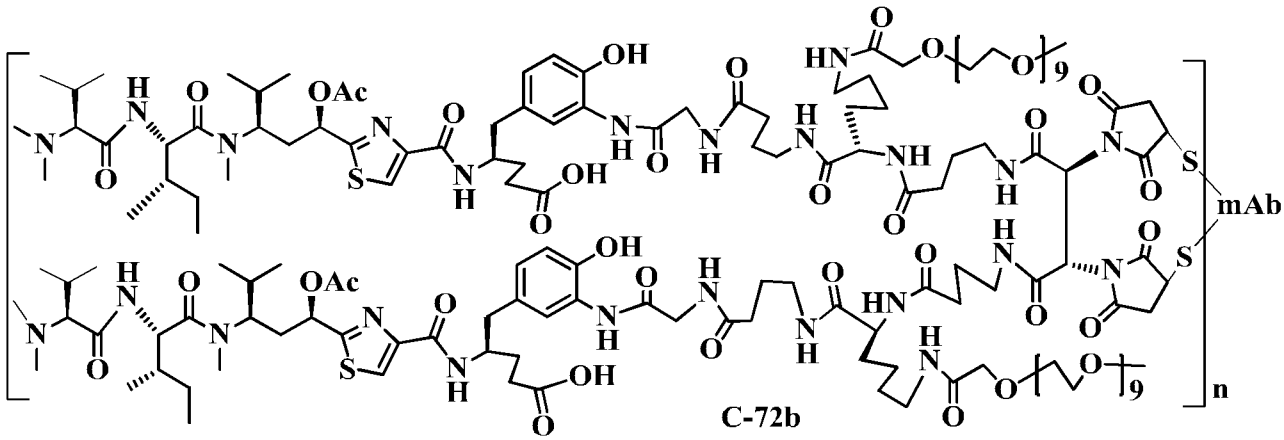
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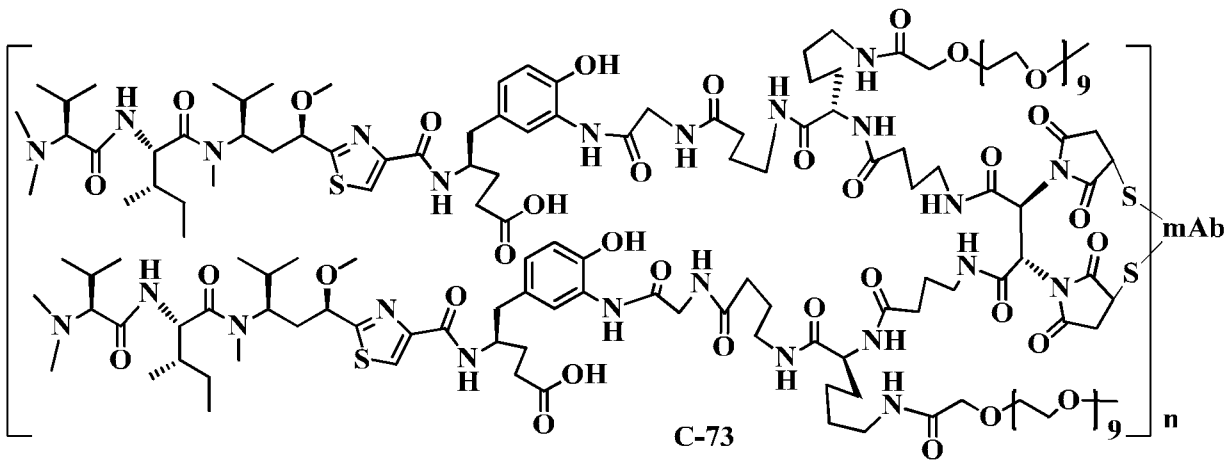
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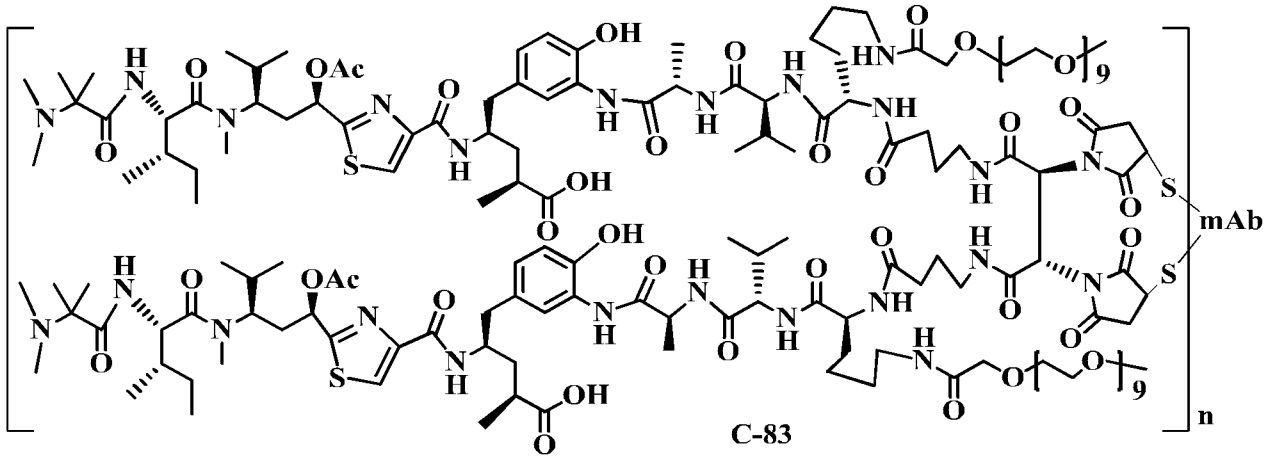
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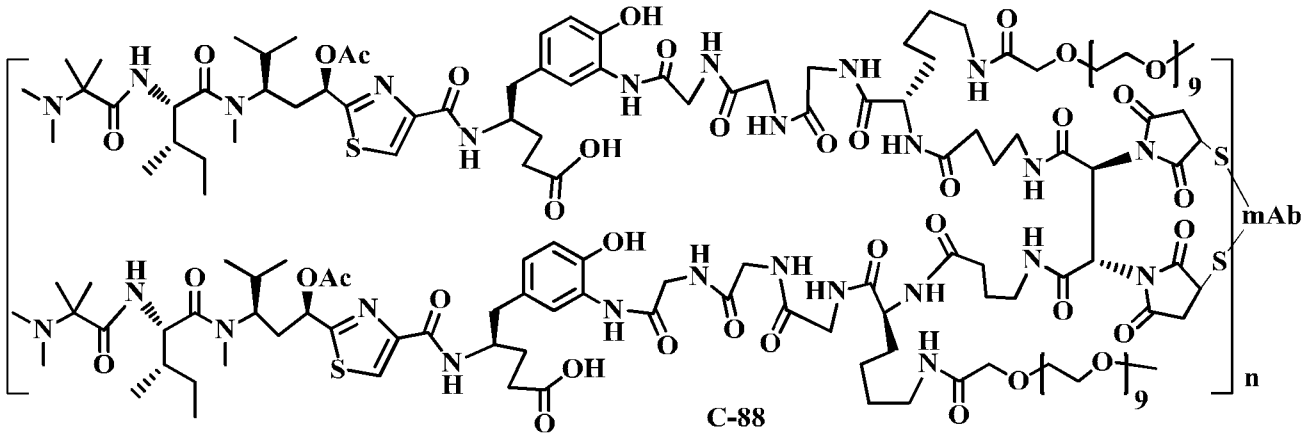
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C-73

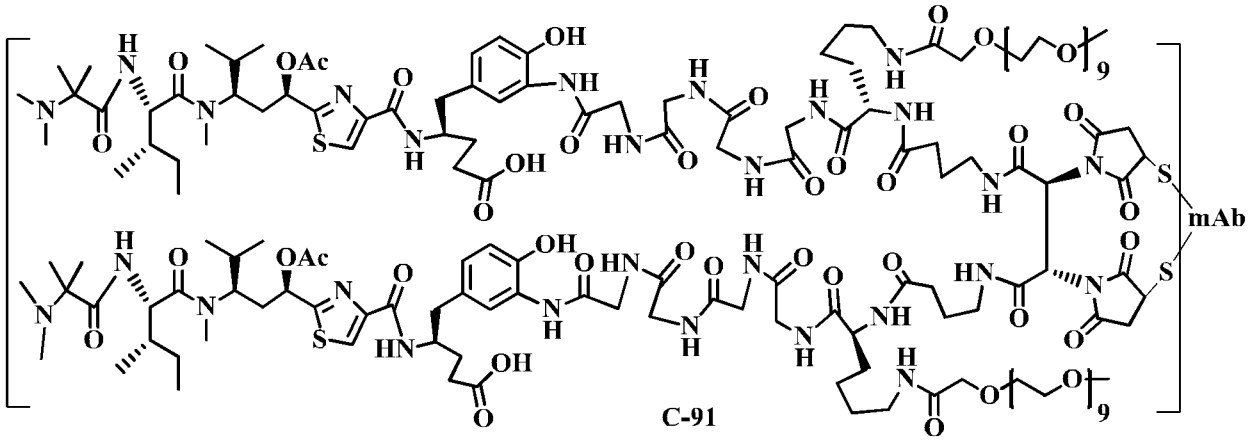


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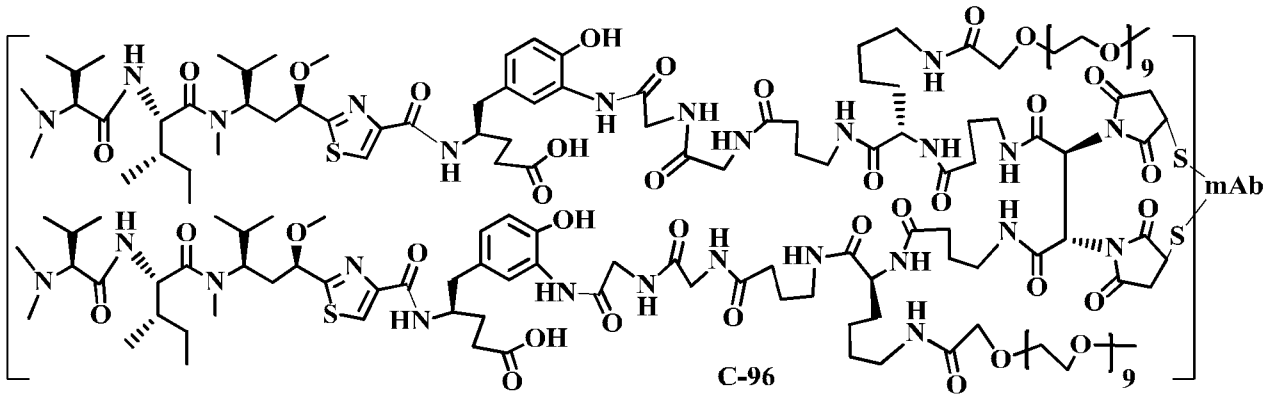
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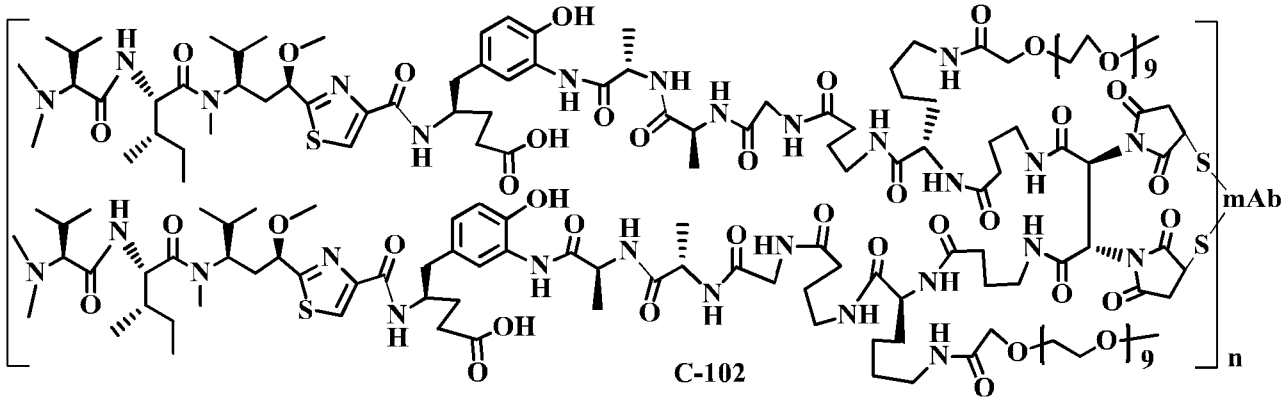
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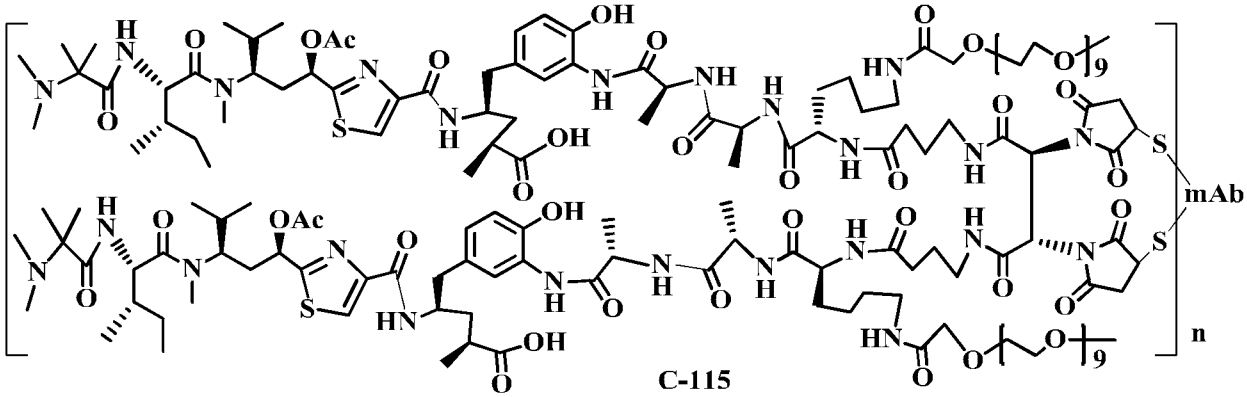
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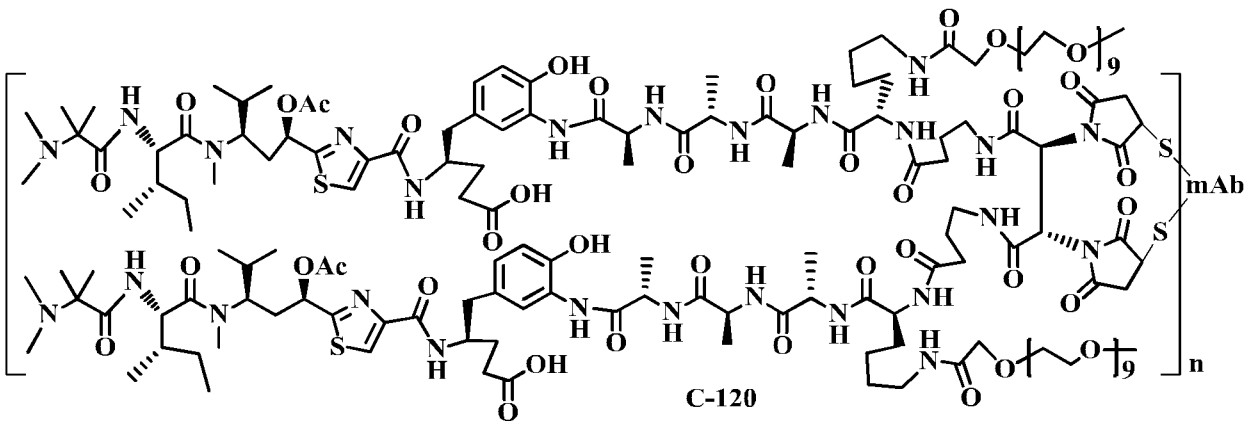


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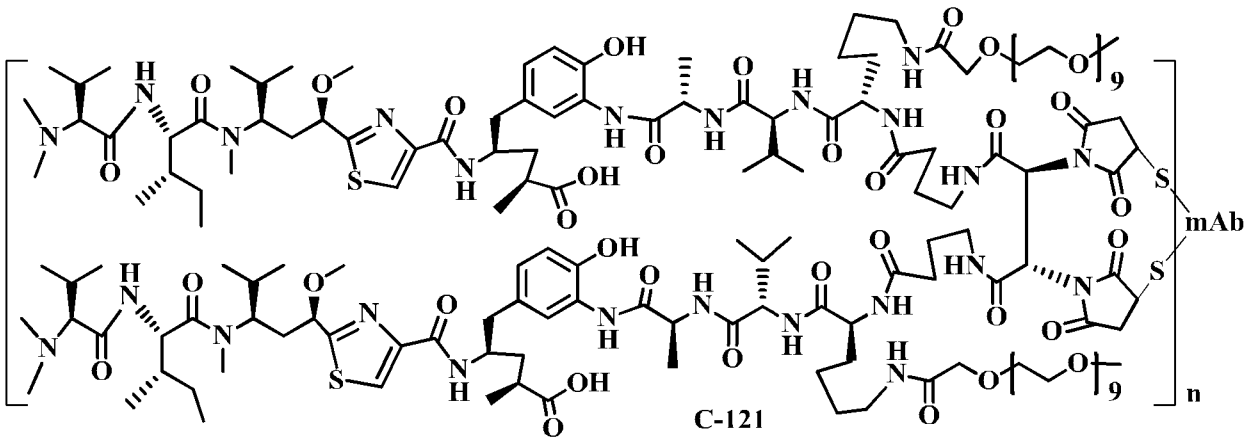
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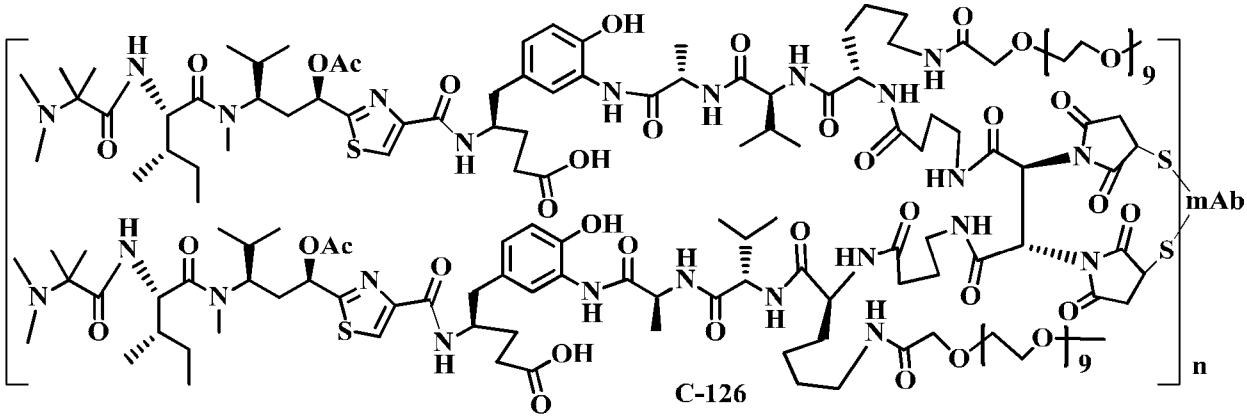
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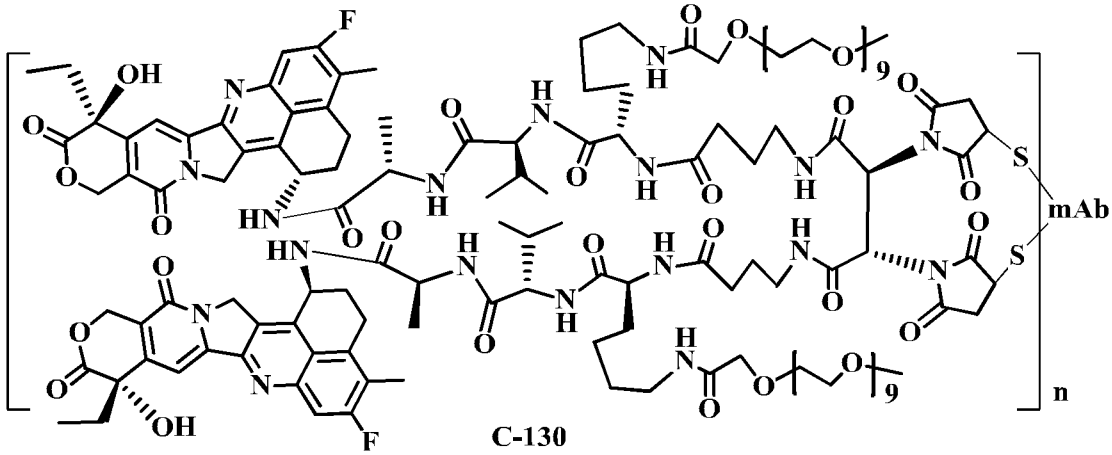
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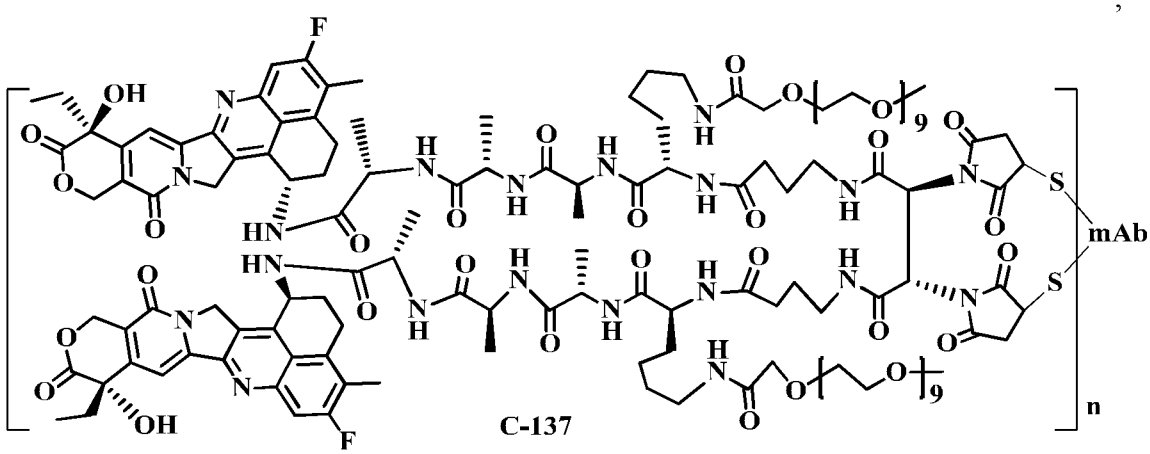
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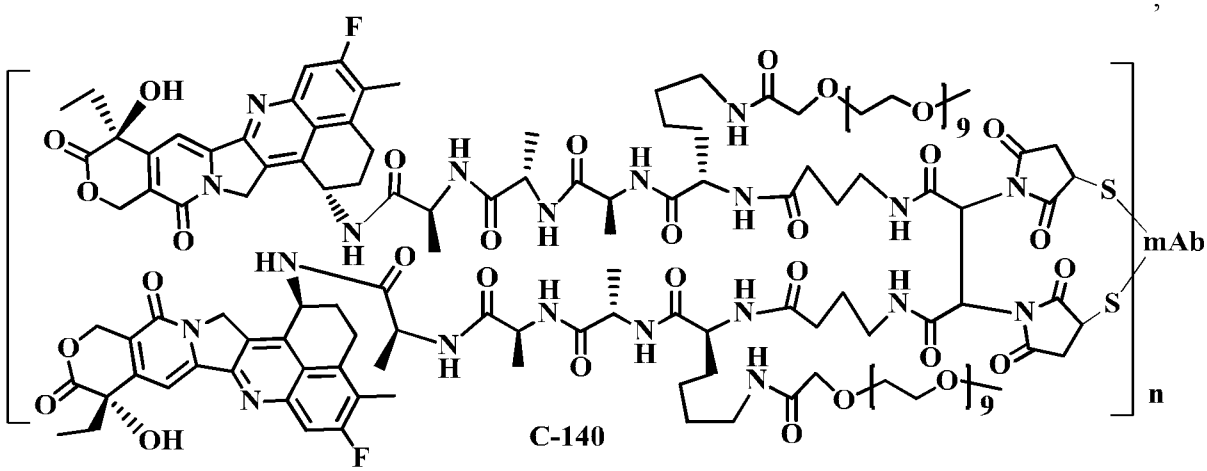
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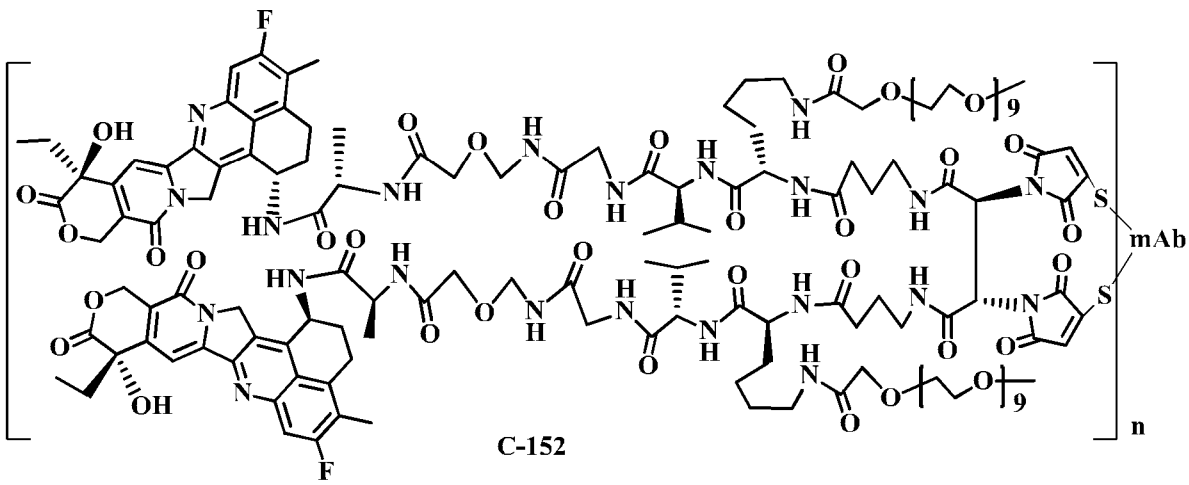
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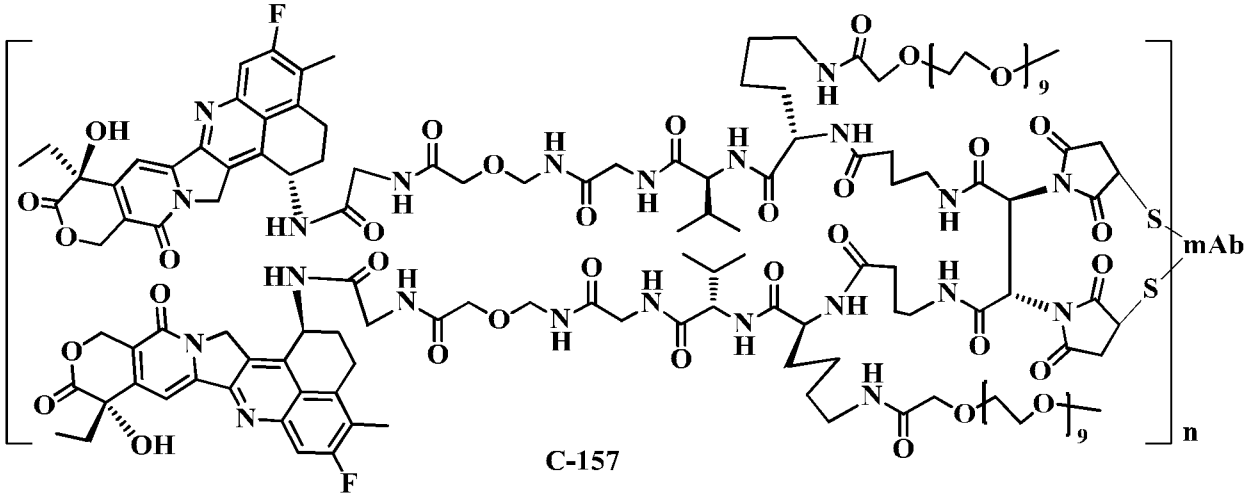
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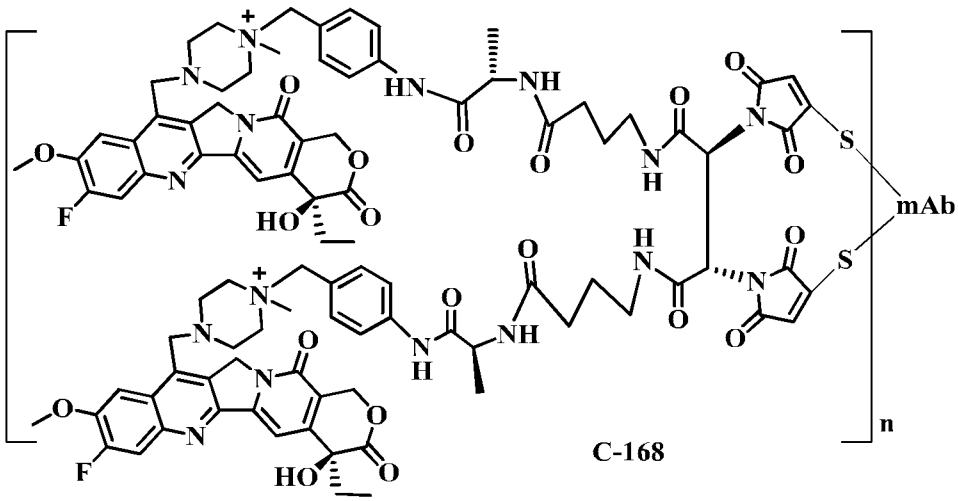
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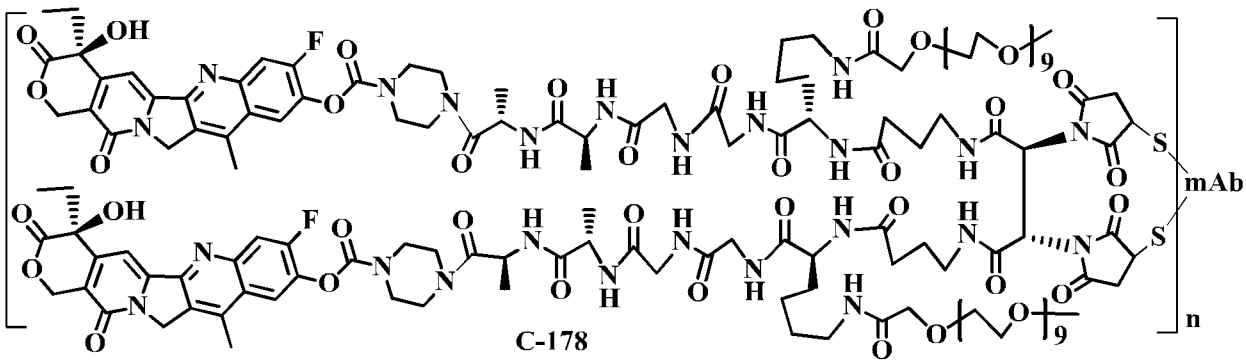
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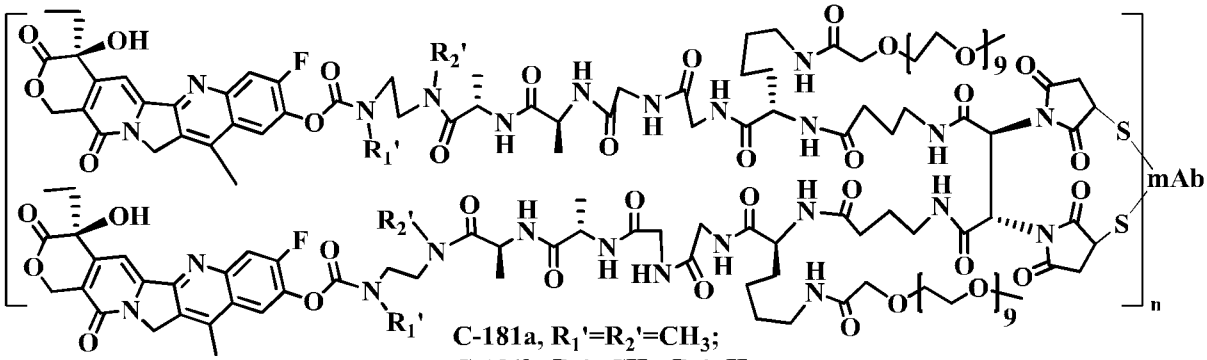
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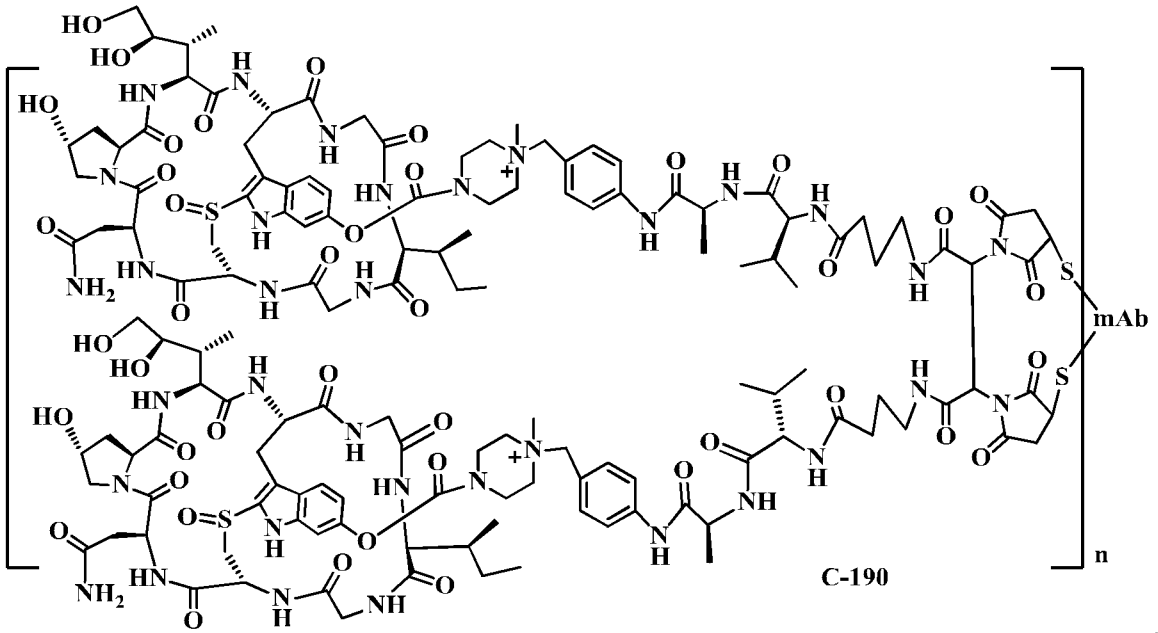
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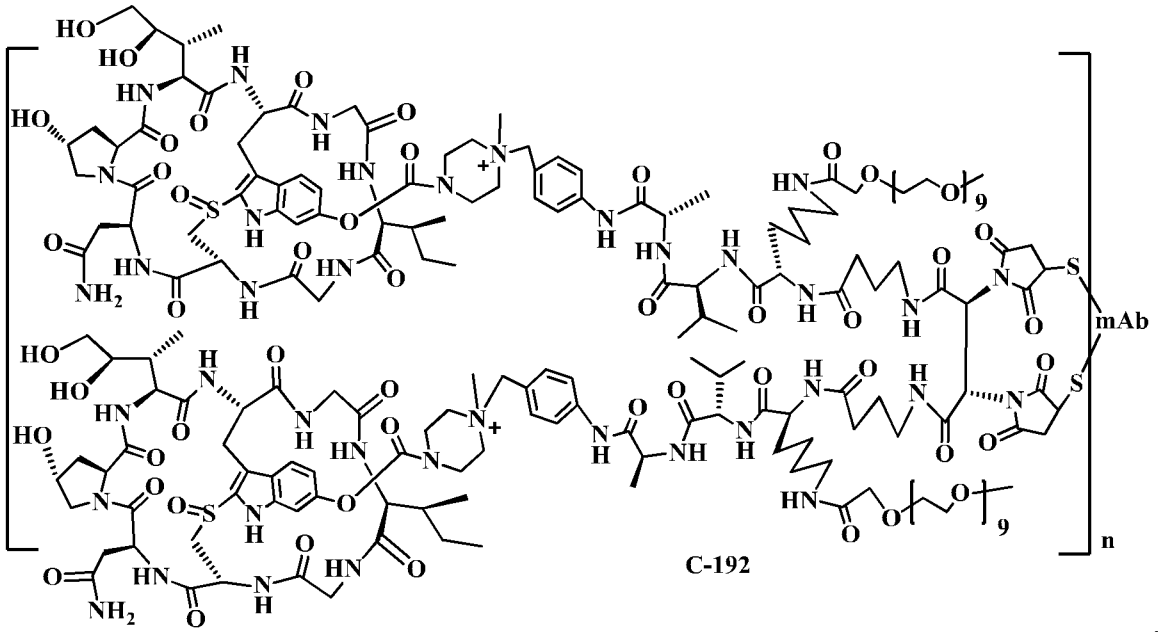
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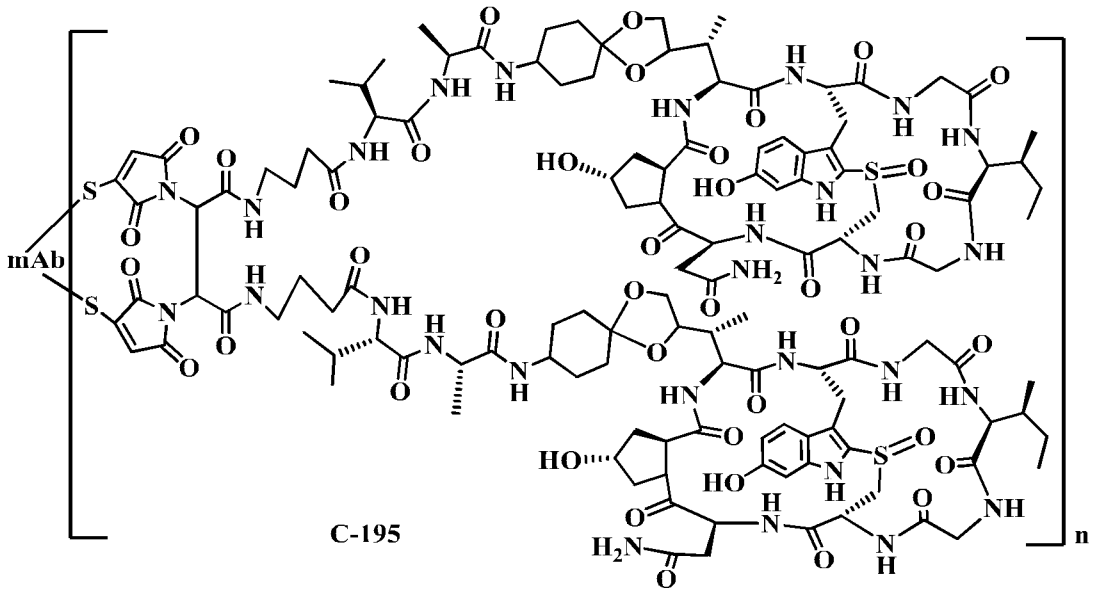
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C-181b, $R_1'=CH_3$, $R_2'=H$;
C-181c, $R_1'=H$, $R_2'=CH_3$;
C-181d, $R_1'=R_2'=H$;



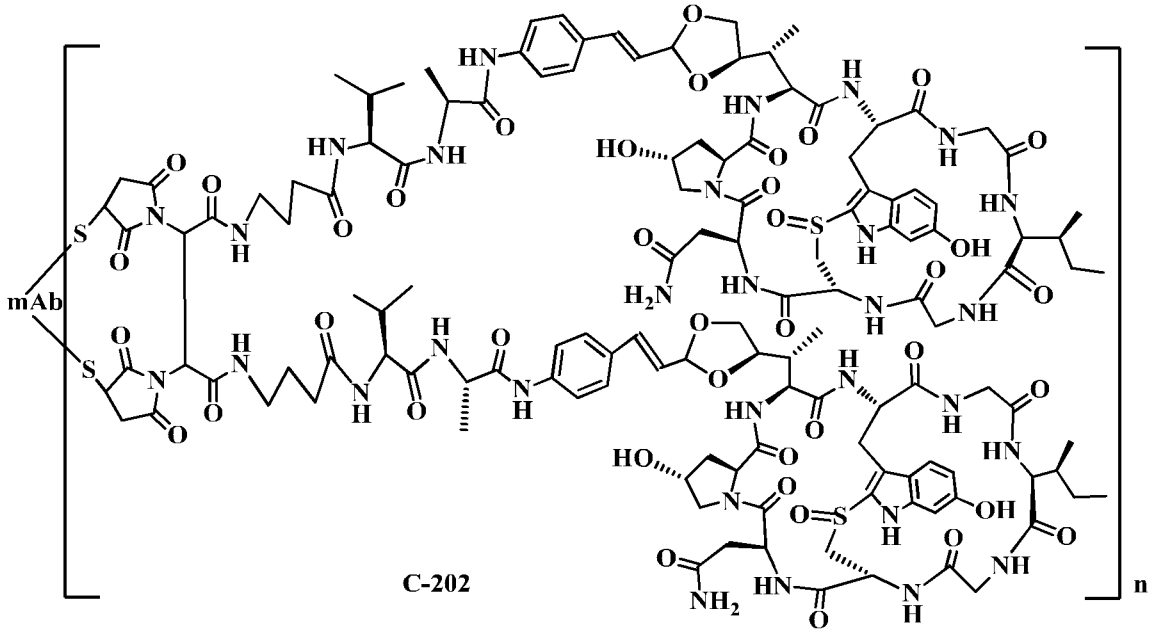
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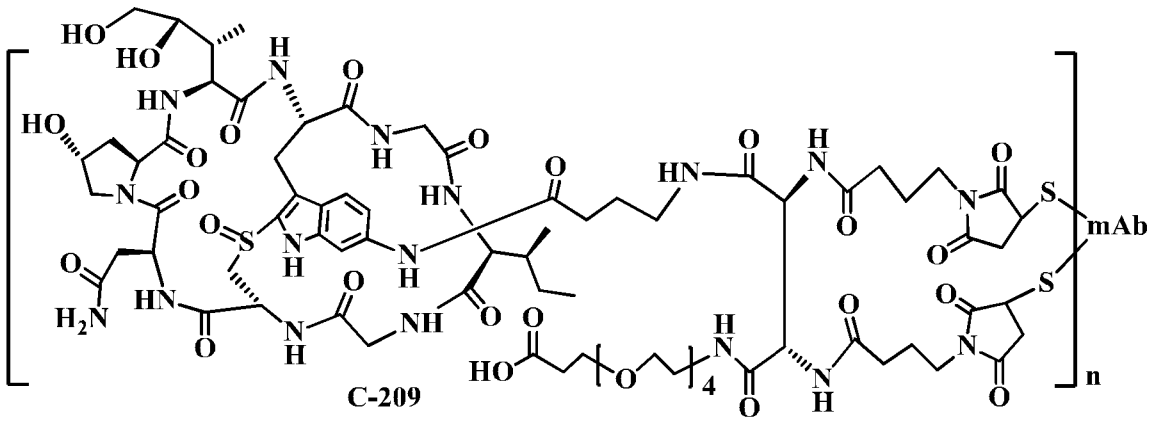
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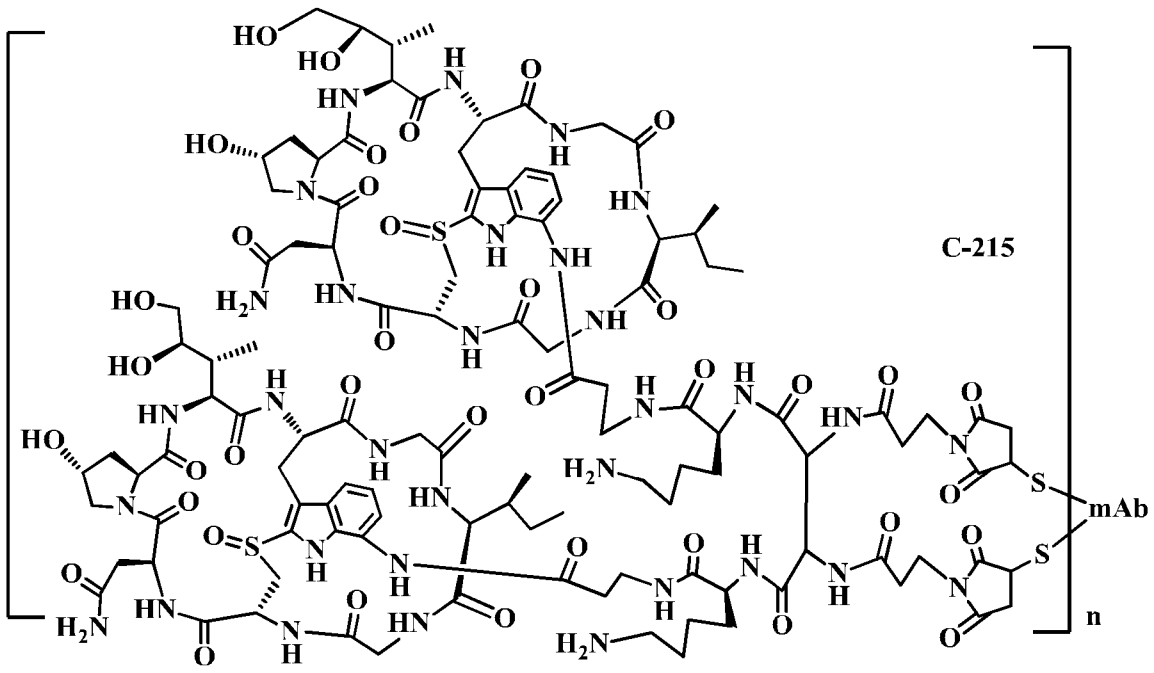
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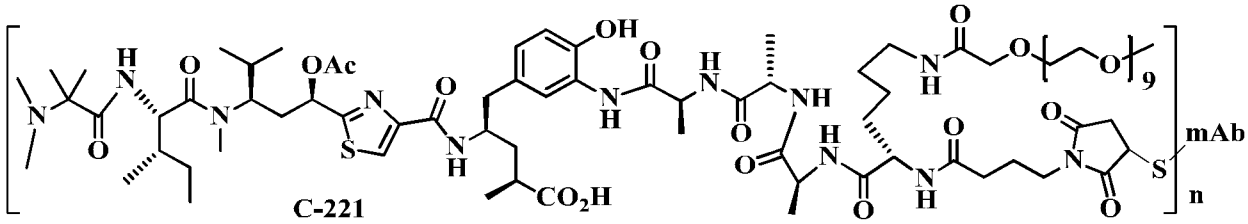
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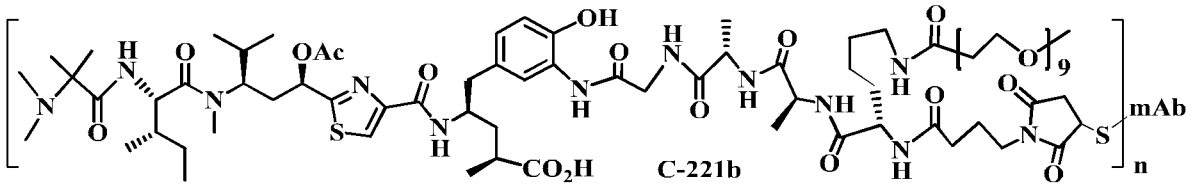
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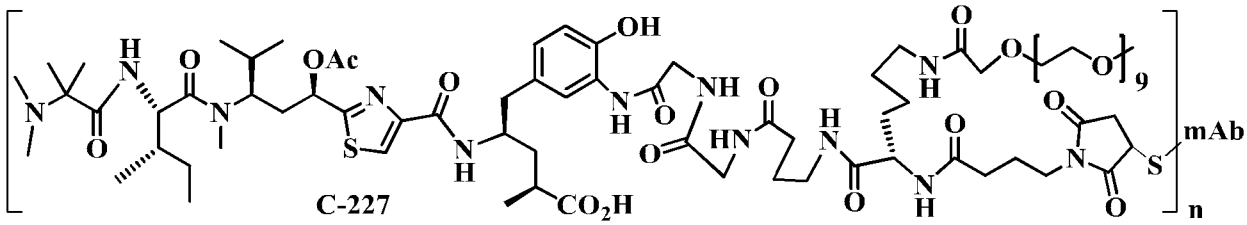
C-215 ,


C-221
mAb
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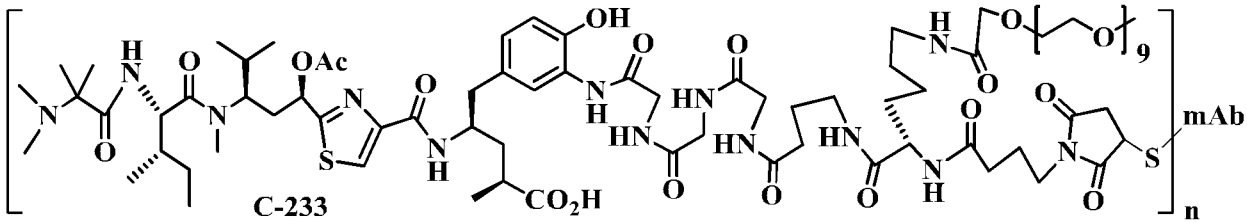
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C-221b
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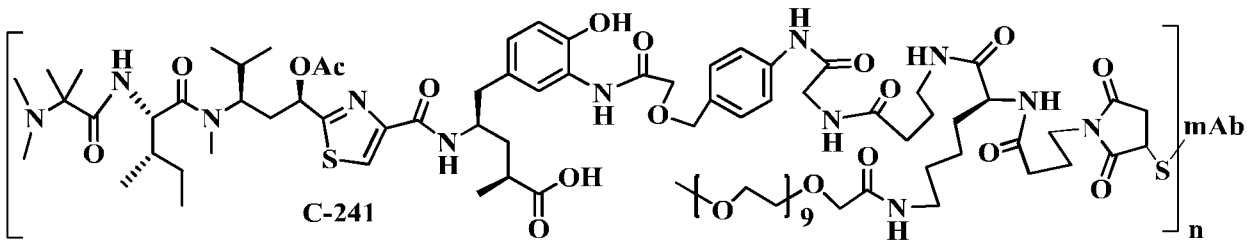
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C-227
mAb
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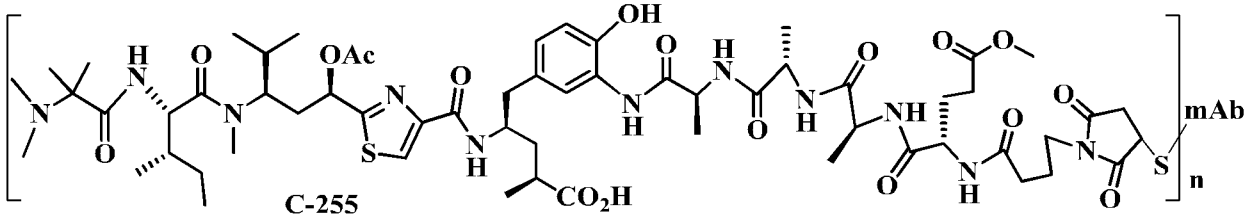
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C-233
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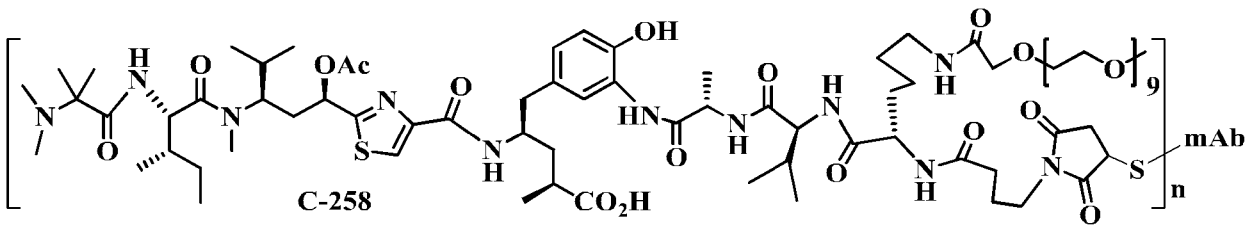
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C-241
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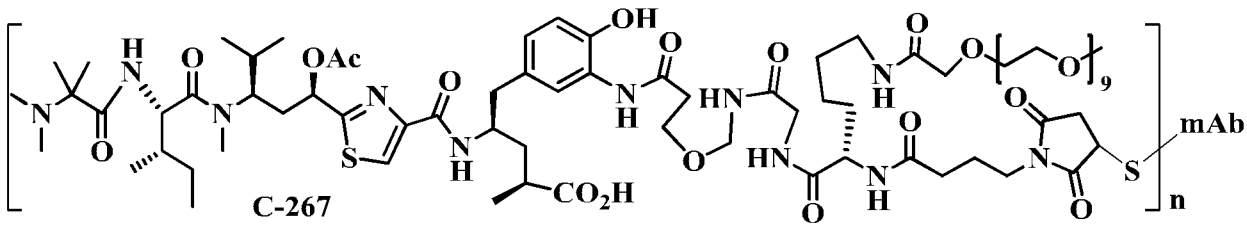
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C-255
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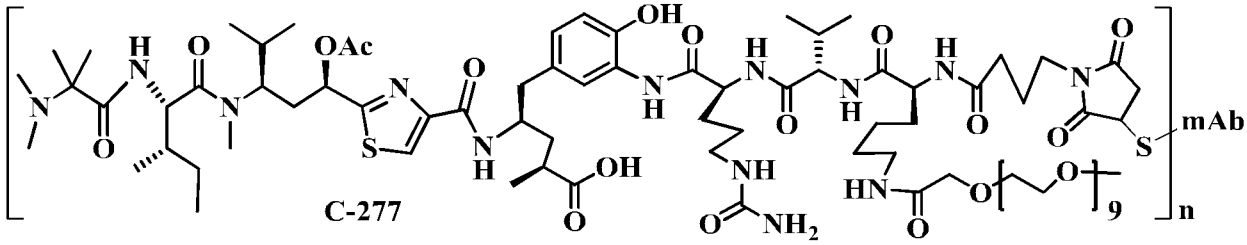
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C-258
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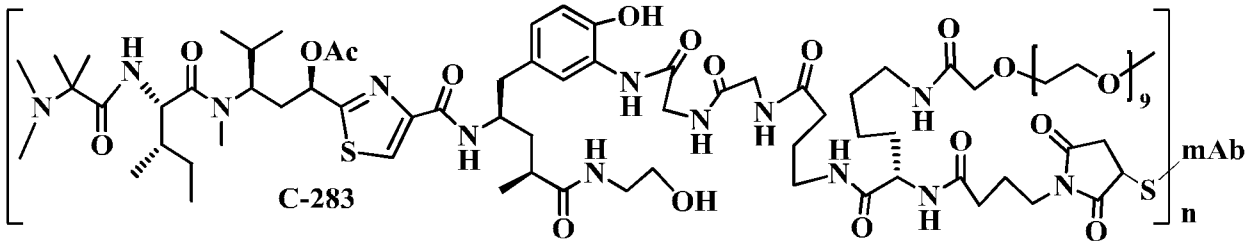
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C-267
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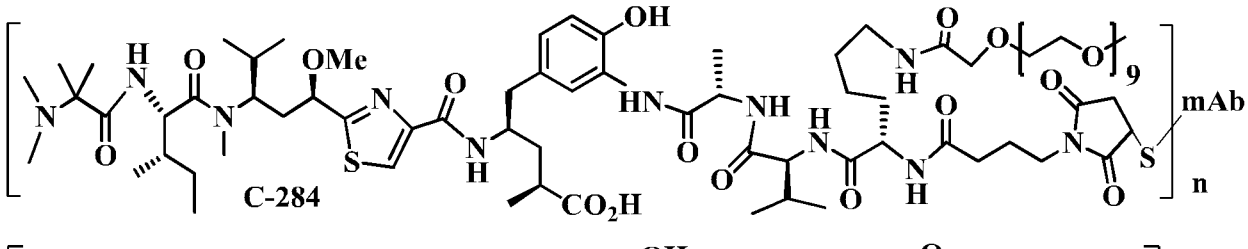
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C-277
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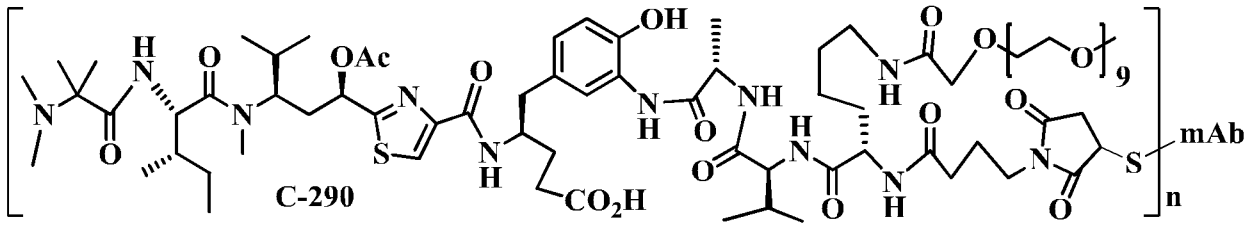
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C-283
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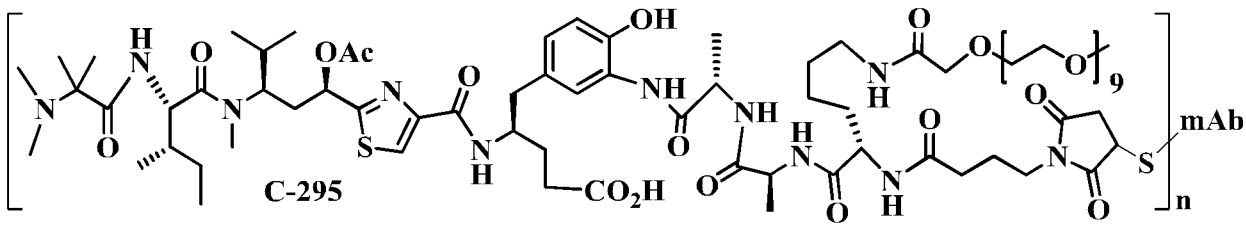
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C-284
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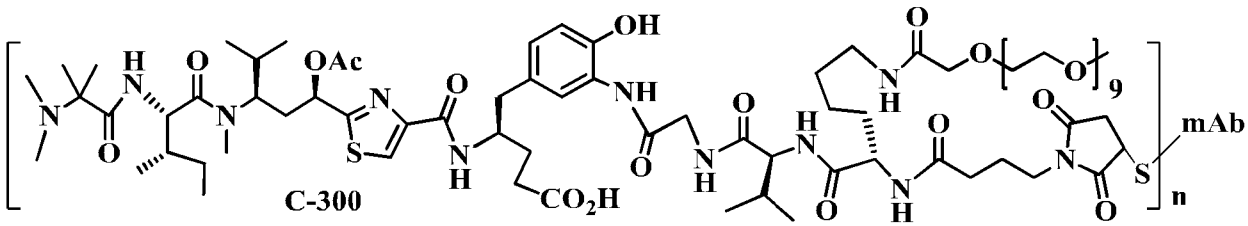
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C-290
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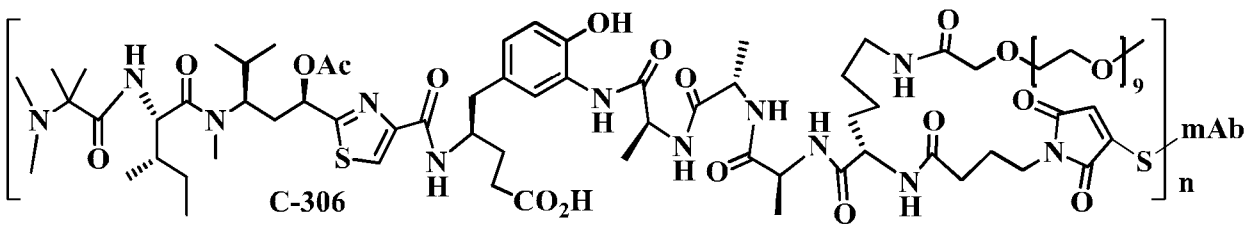
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C-295
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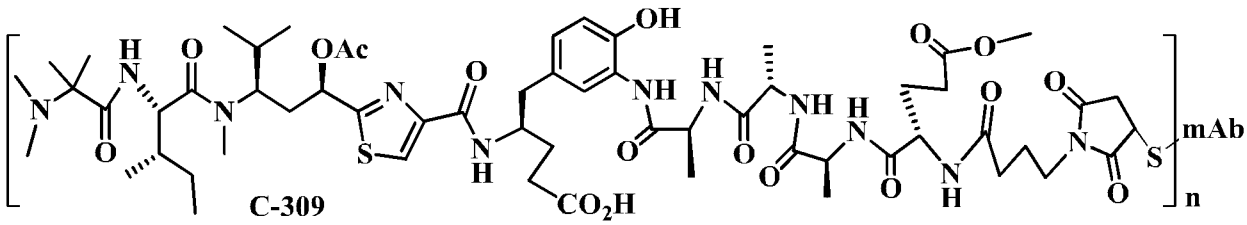
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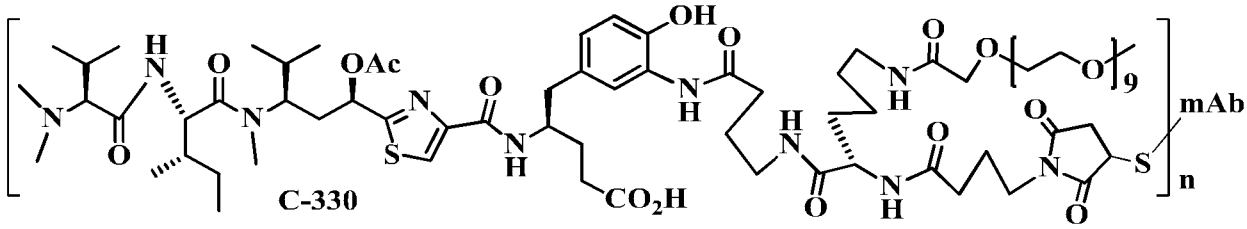

C-300
mAb
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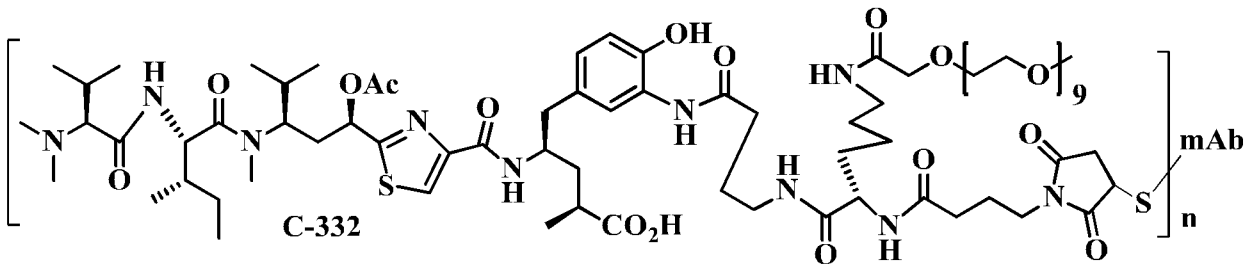

C-306
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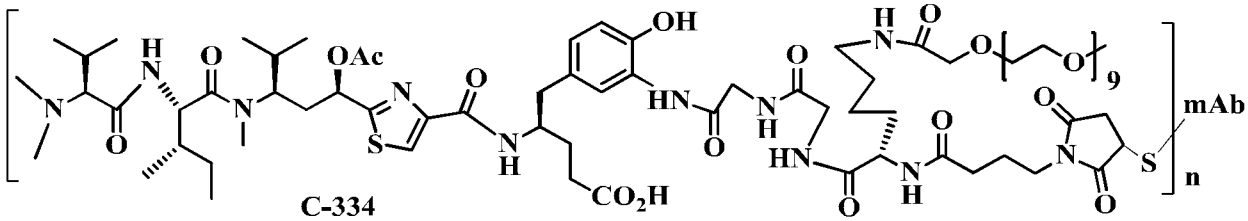

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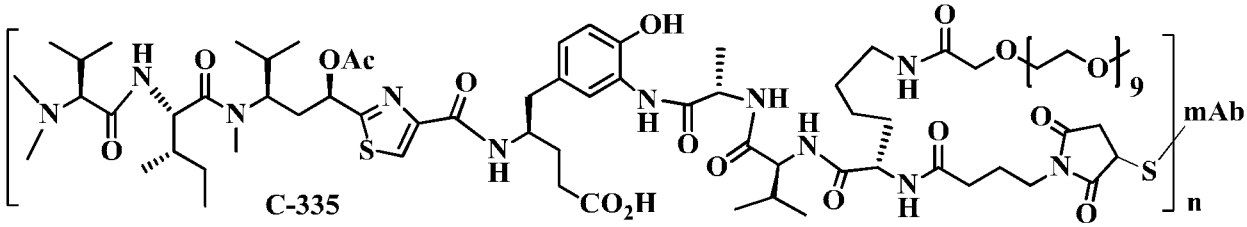
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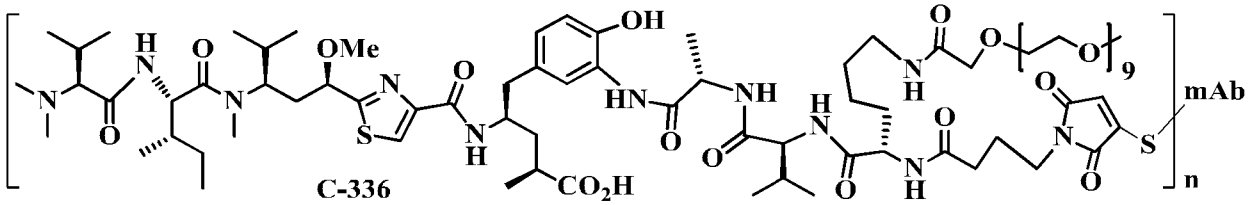
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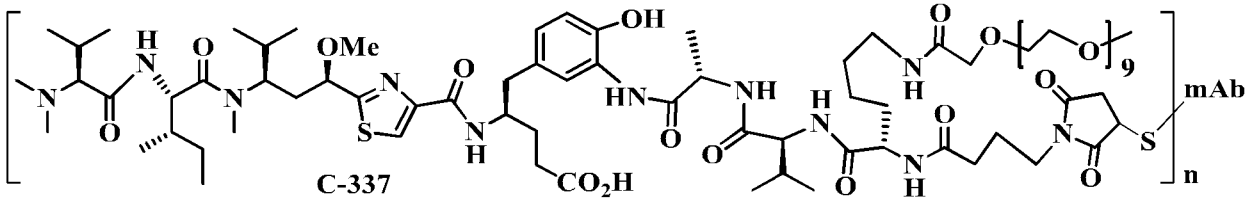
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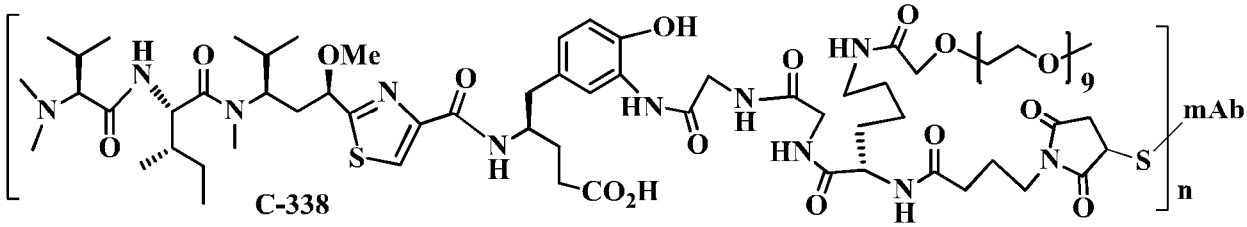
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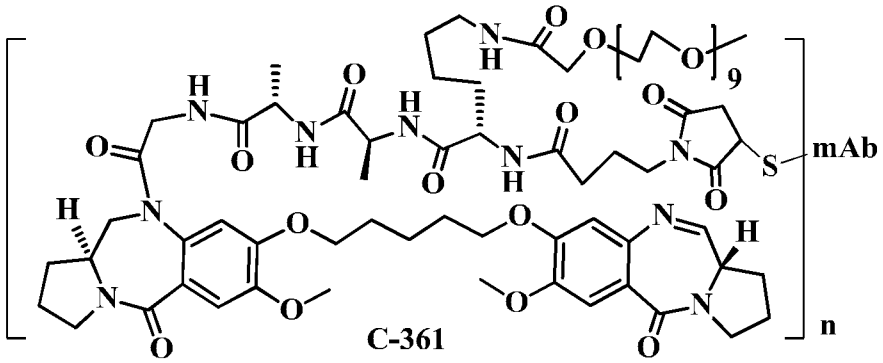
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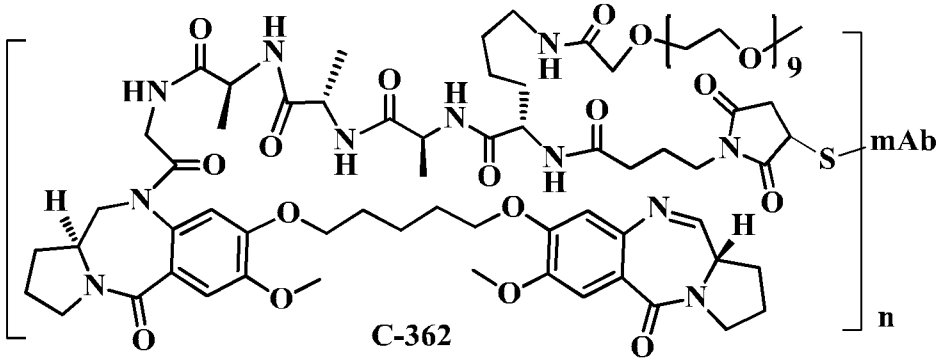
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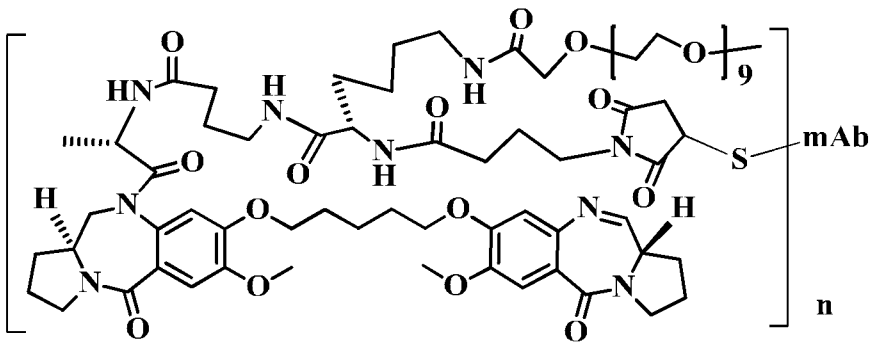
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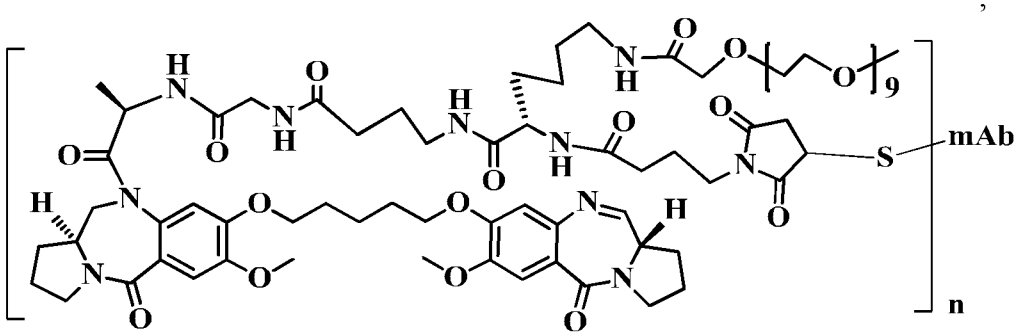
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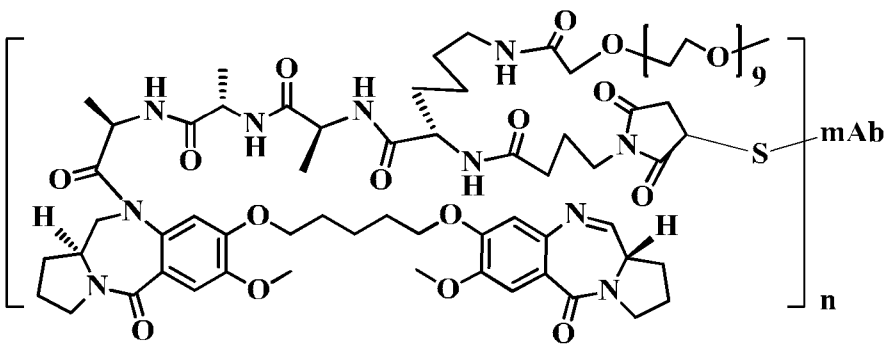
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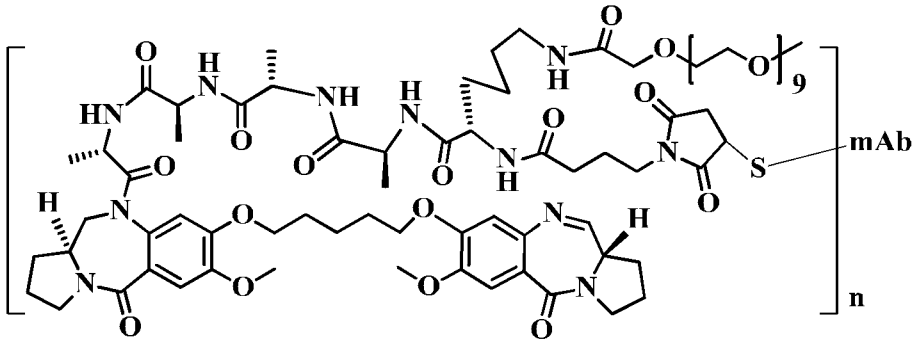
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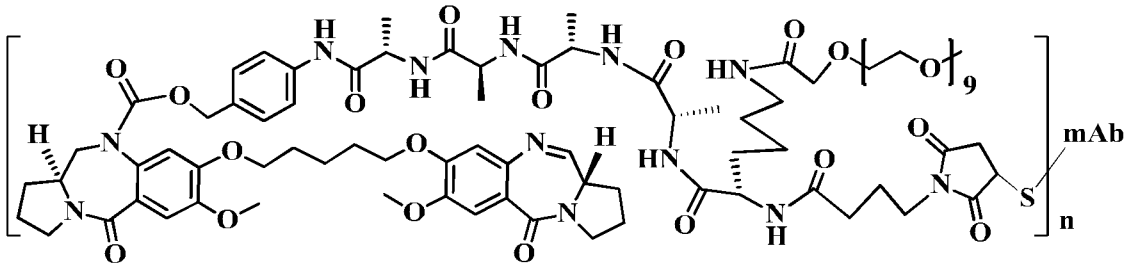
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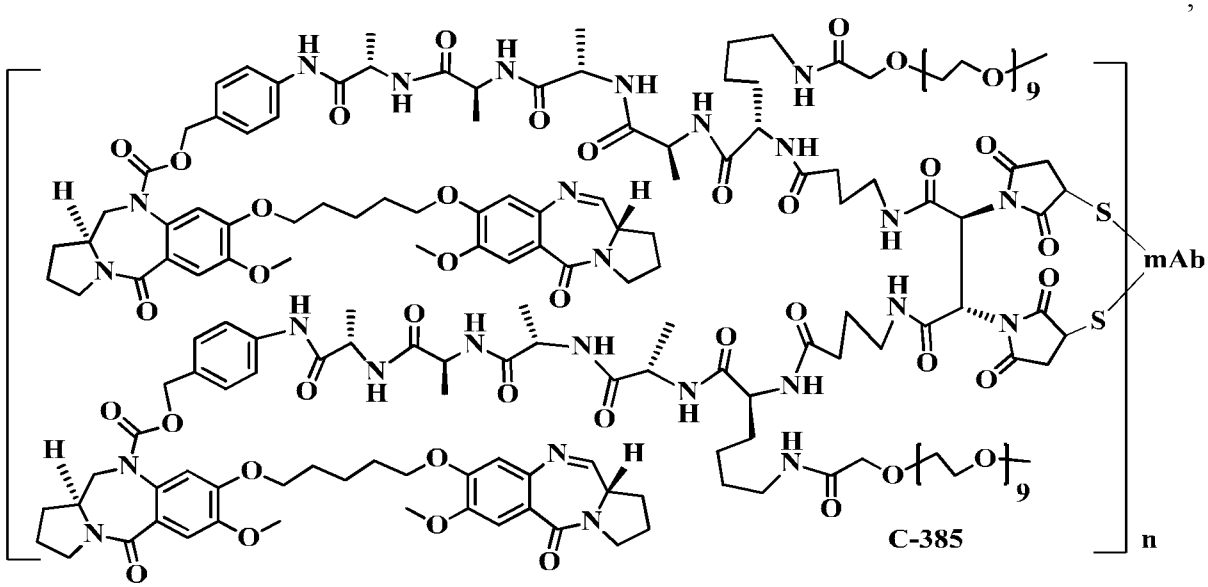
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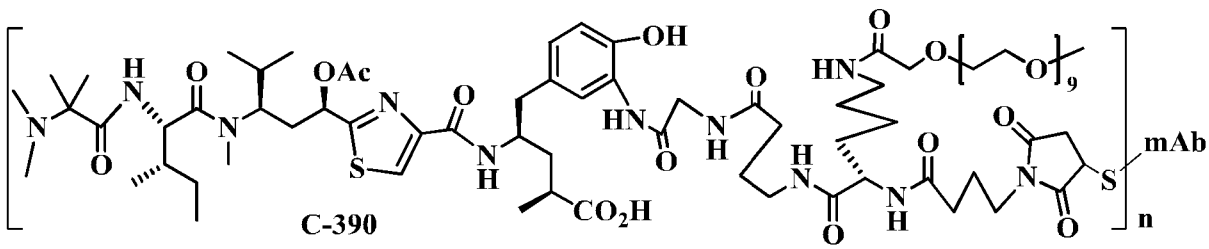
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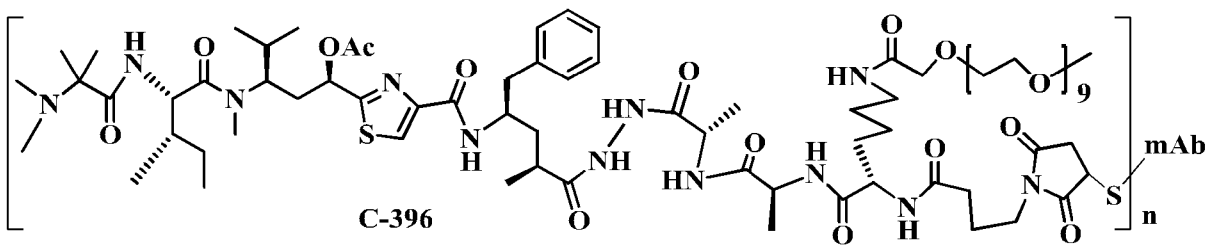
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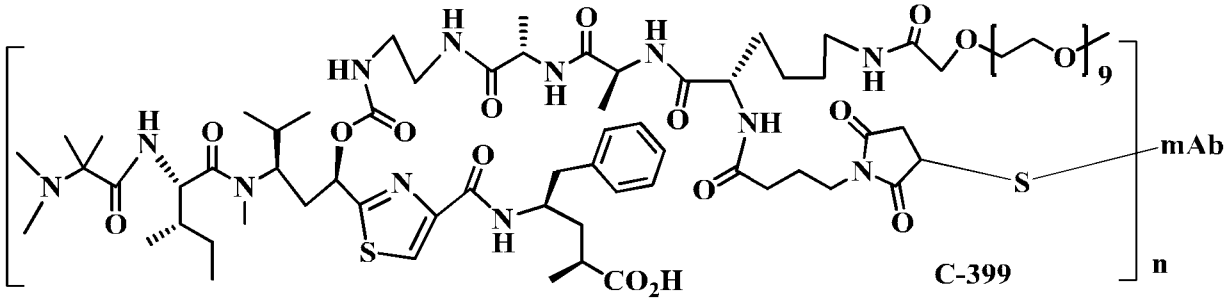
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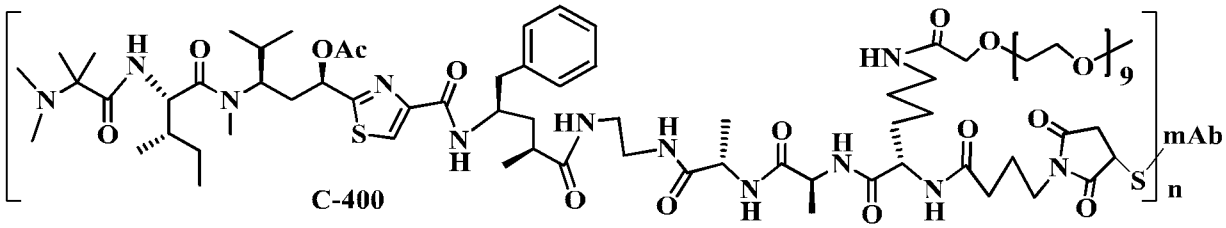
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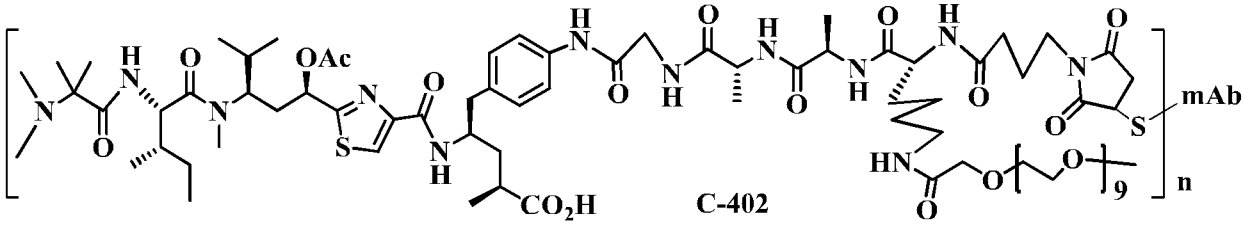
C-396 ,


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C-399

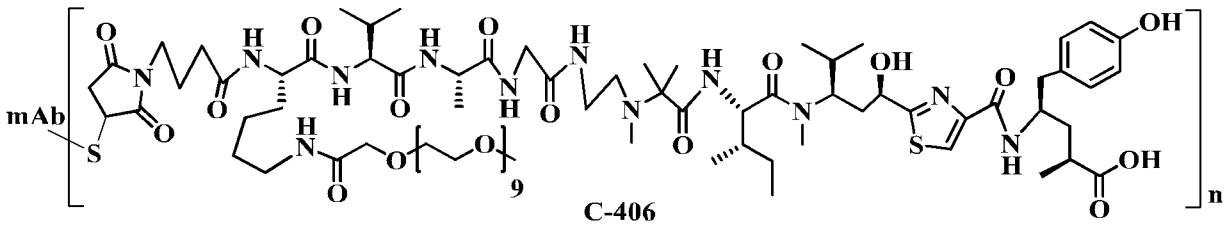
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C-400

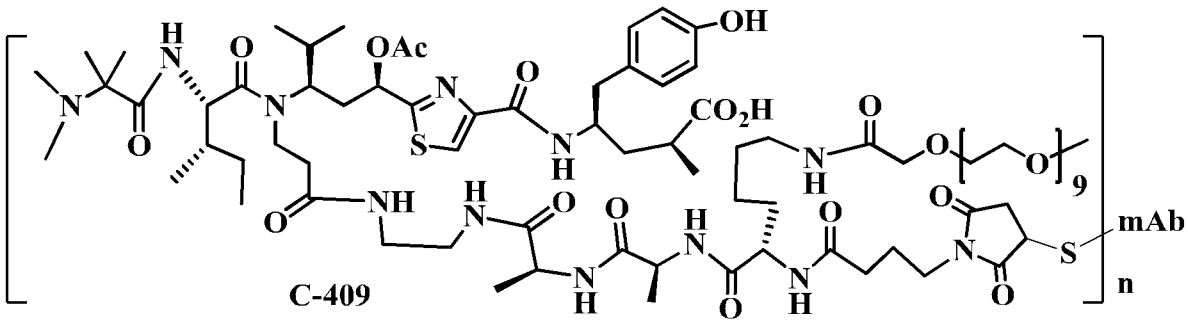
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C-402

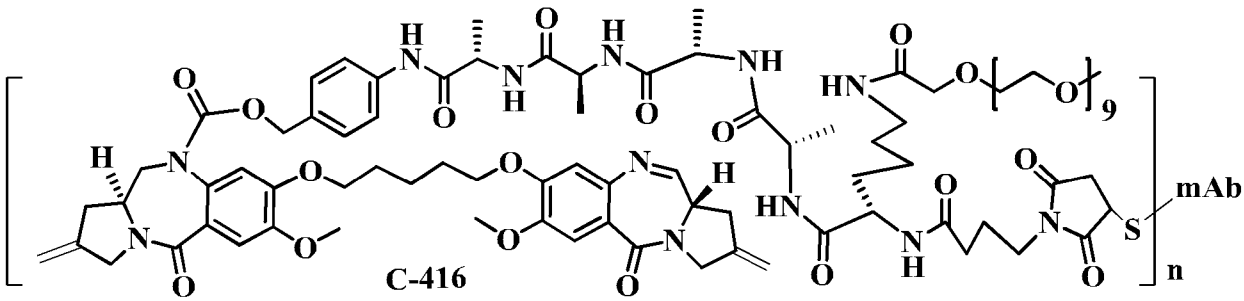
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mAb
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C-406

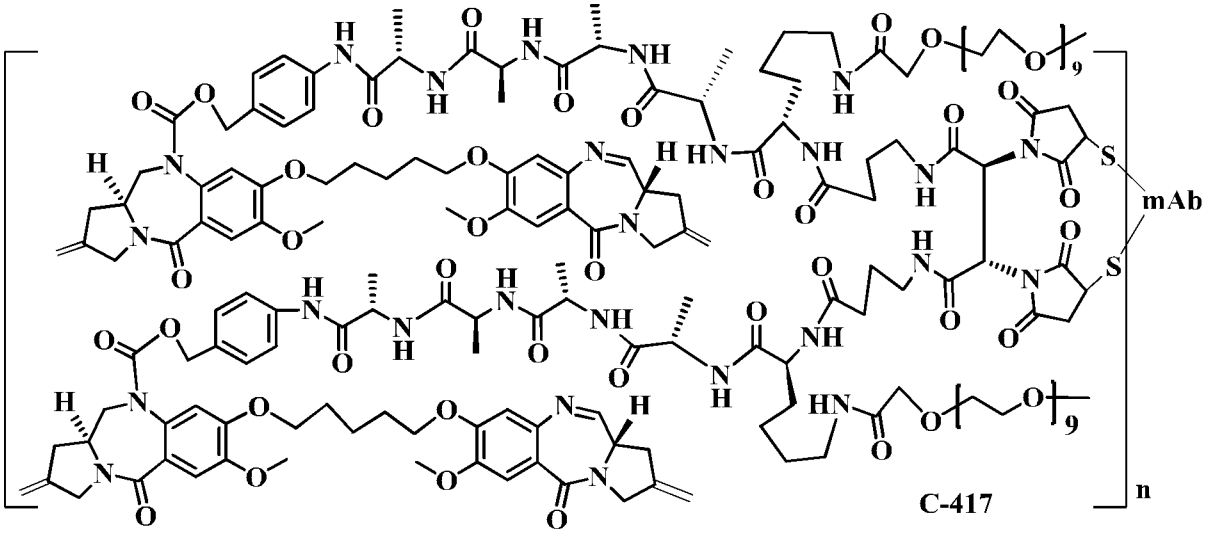
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mAb
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C-409

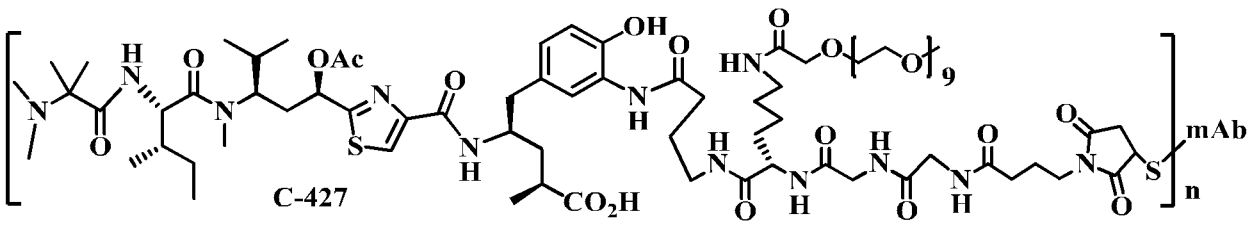
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mAb
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C-416

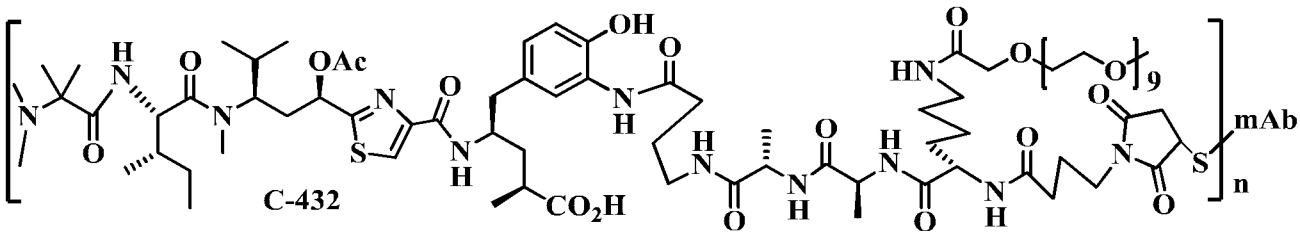
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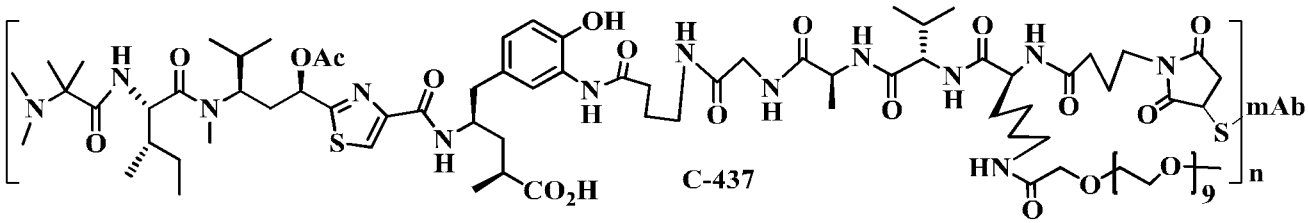

C-417
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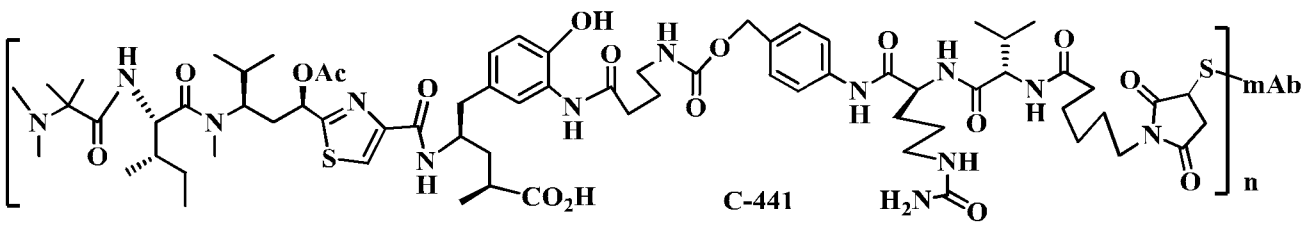

C-427
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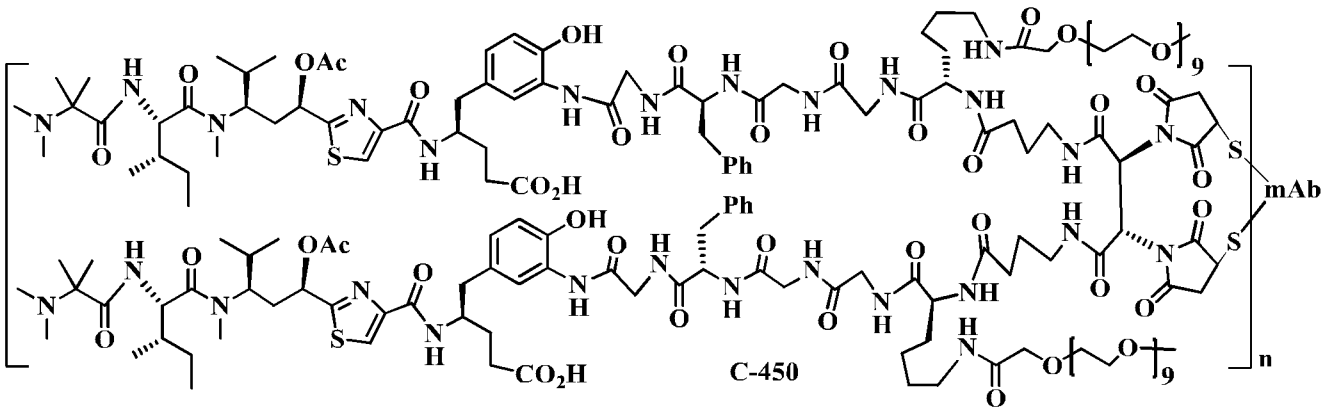

C-432
mAb
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C-437
mAb
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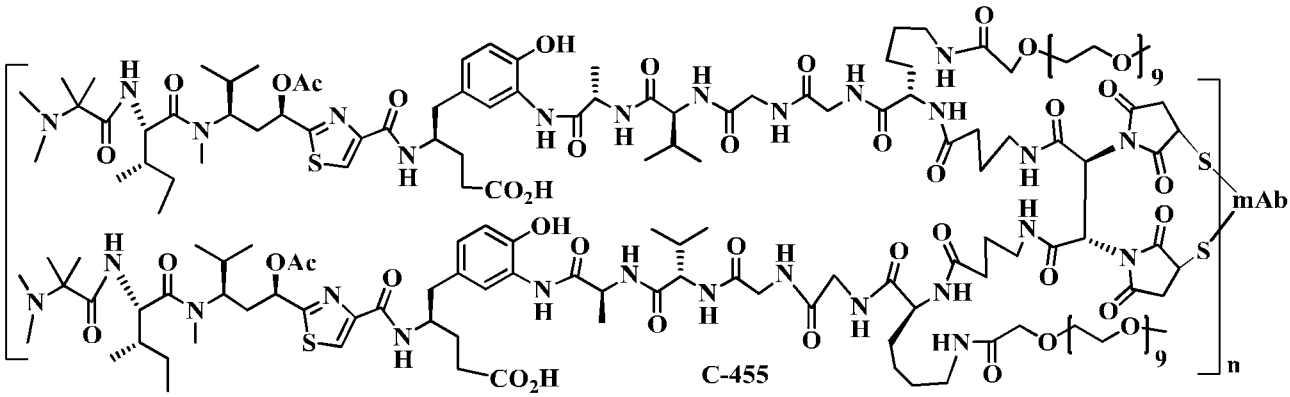
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C-441
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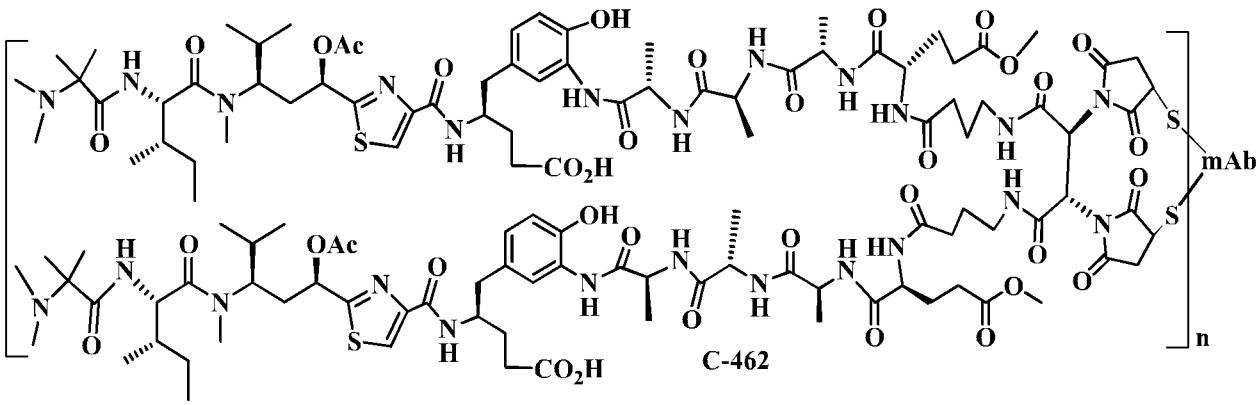
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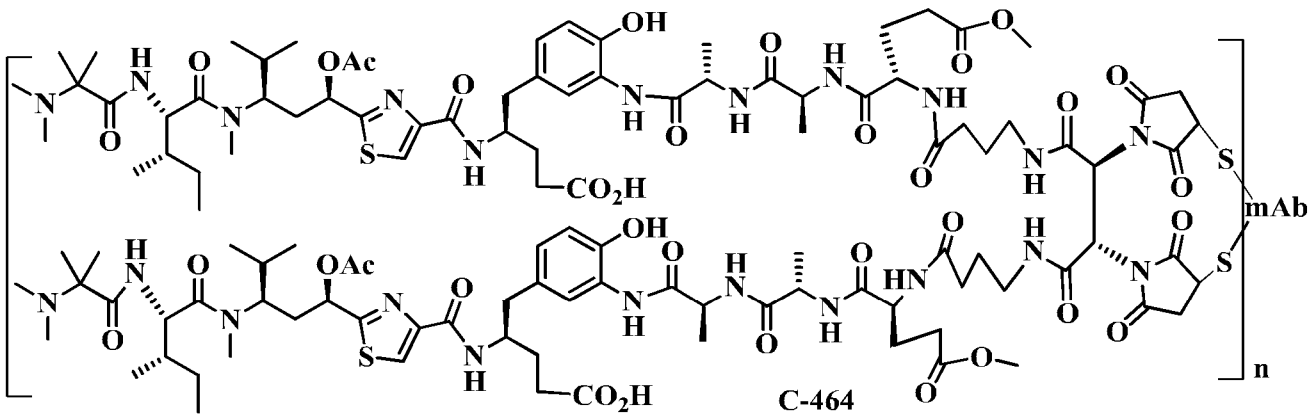
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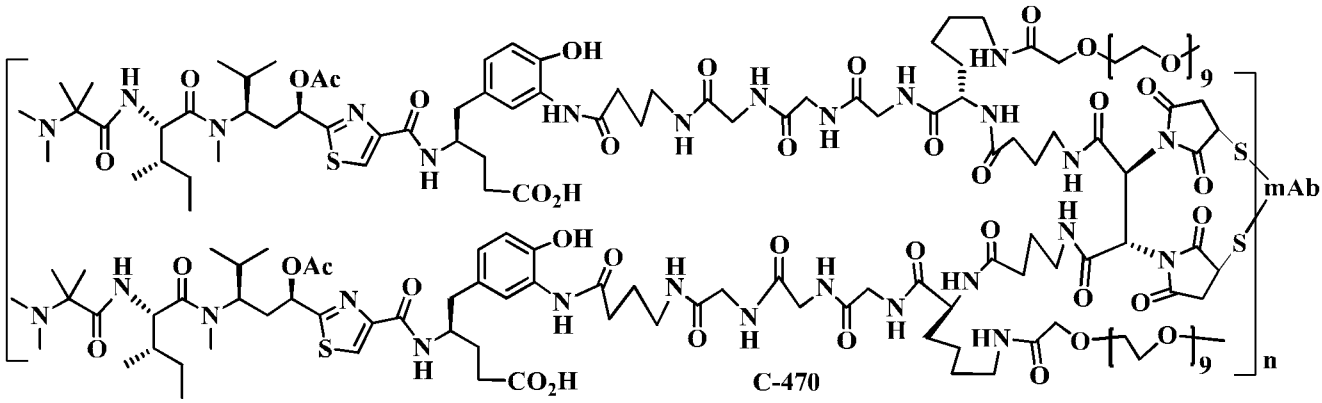
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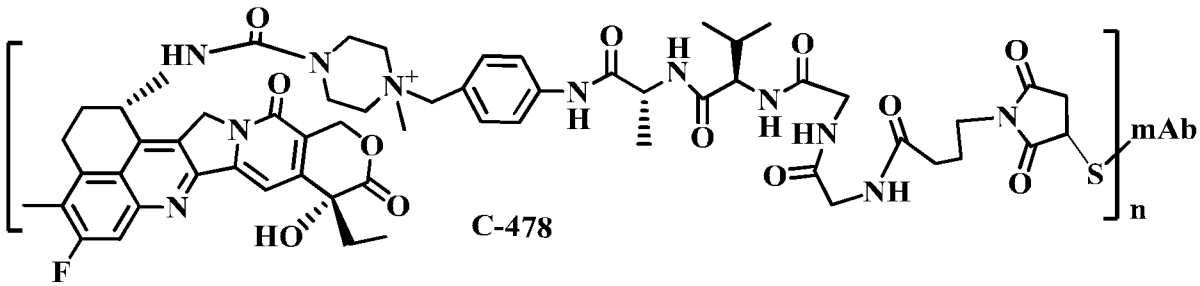
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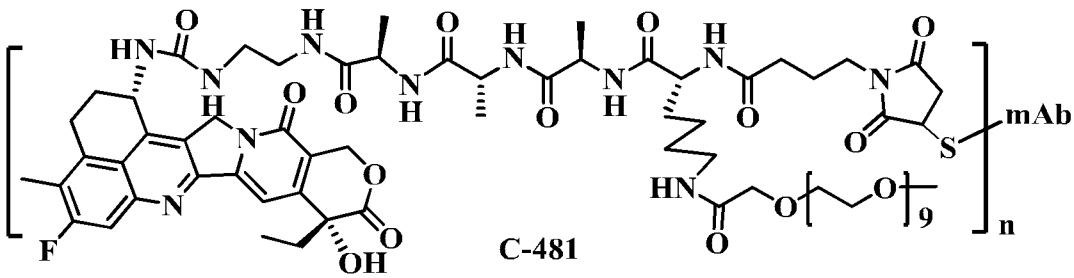
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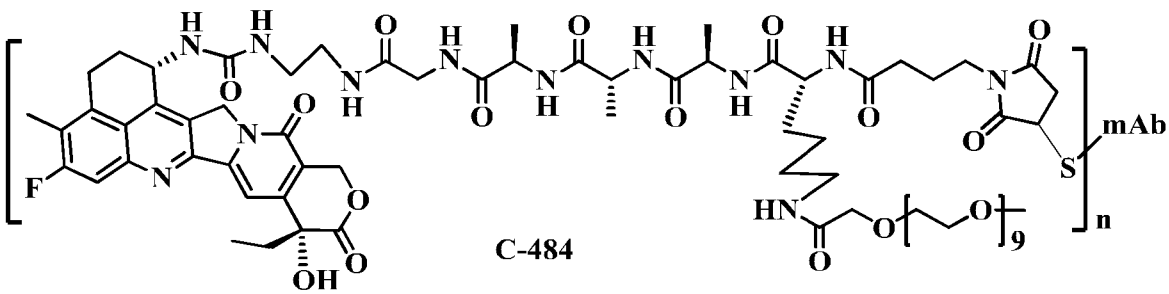
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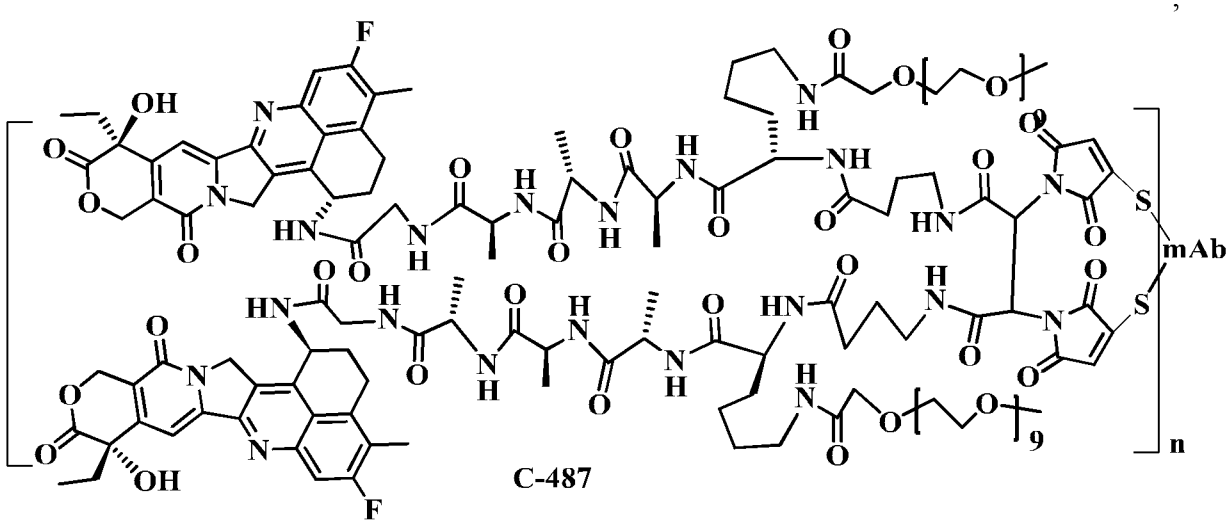
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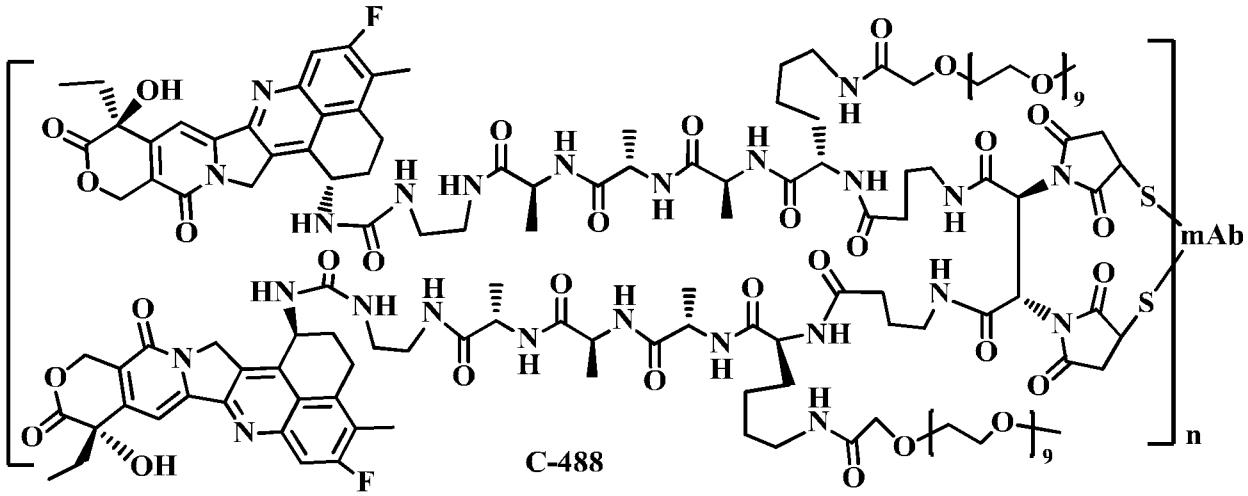
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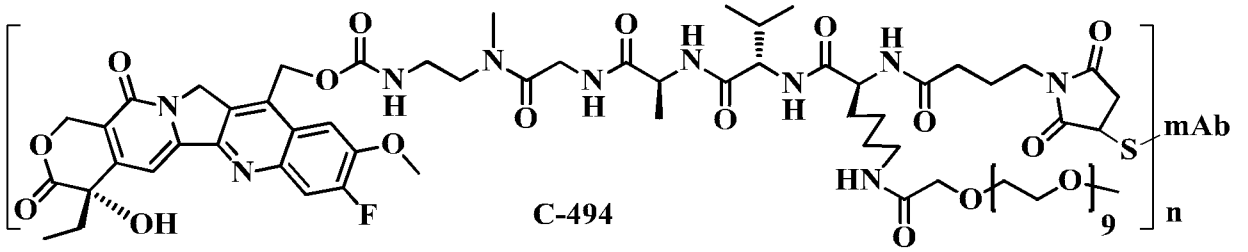
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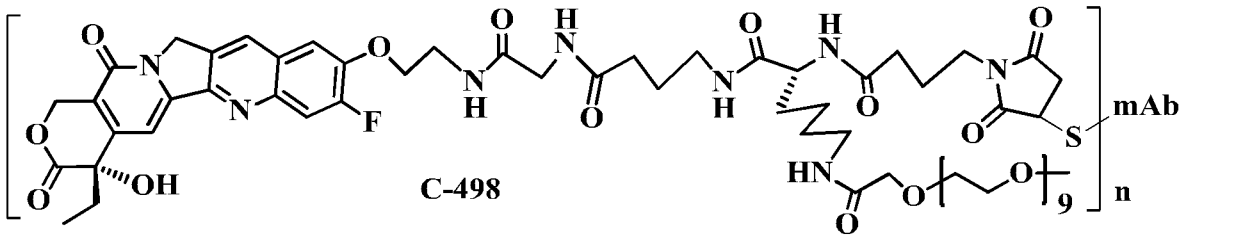
C-487


C-488
mAb
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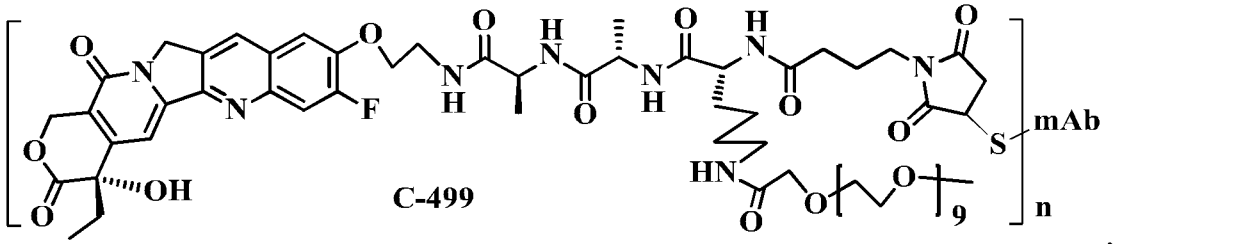
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C-494
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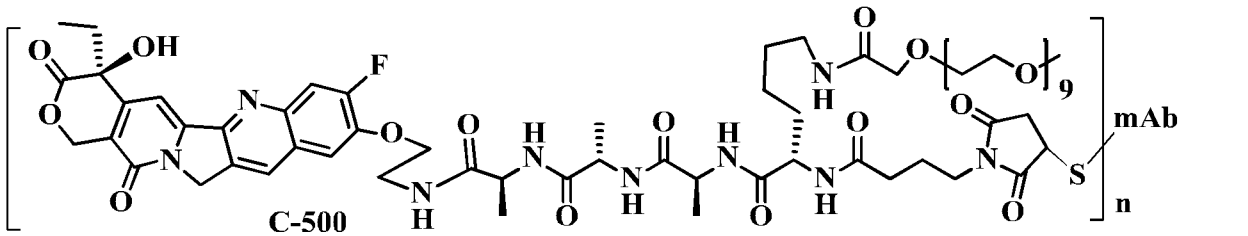
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C-498
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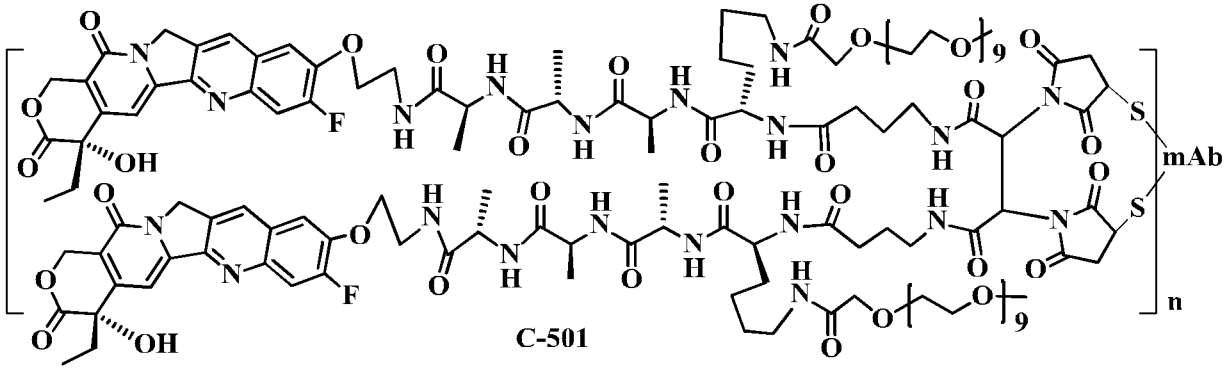
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C-499
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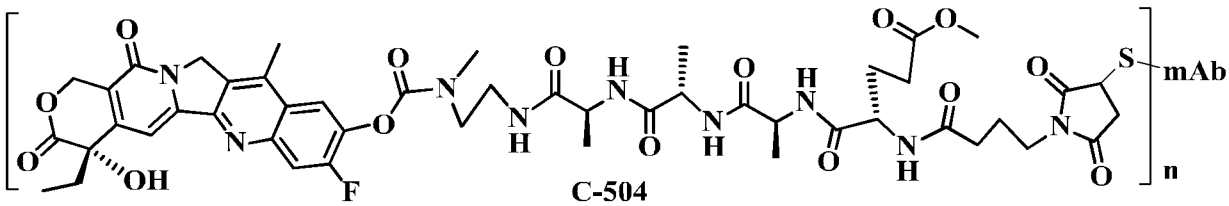
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C-500
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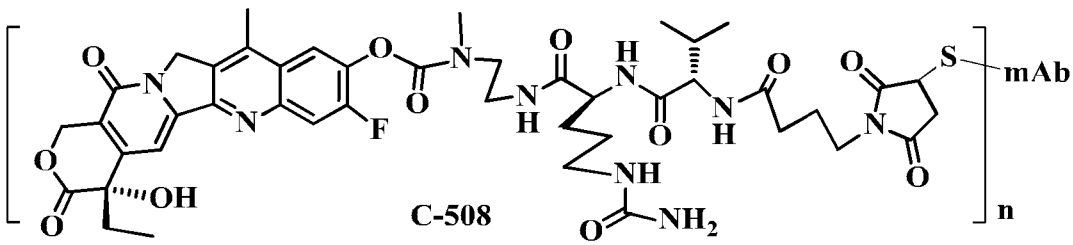
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C-501
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C-504
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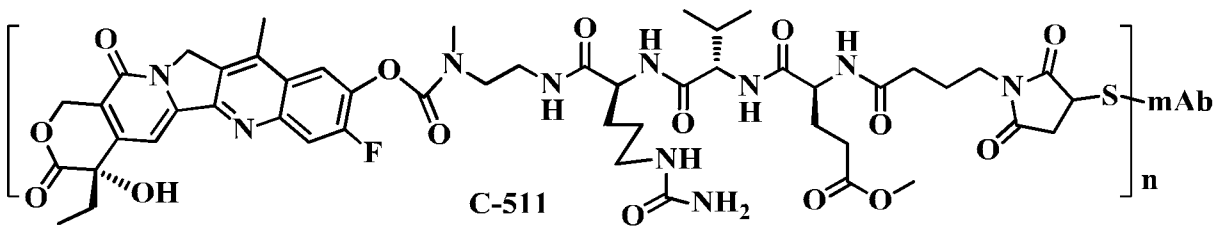
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C-508
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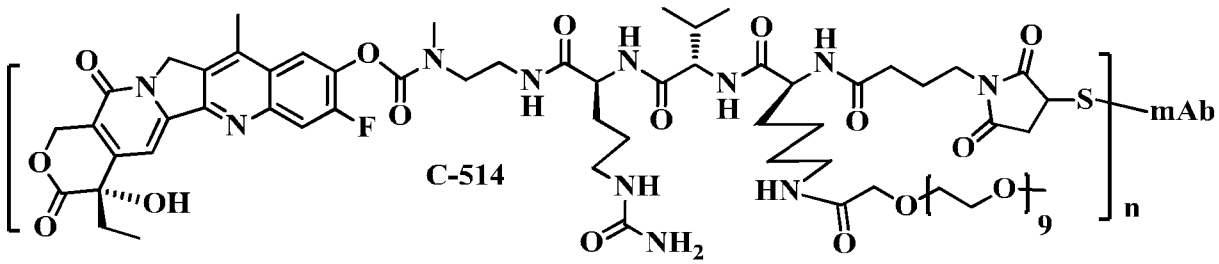
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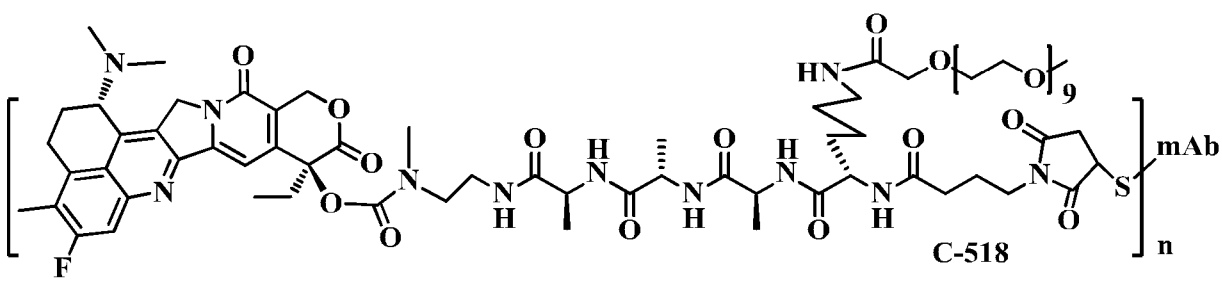
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C-511
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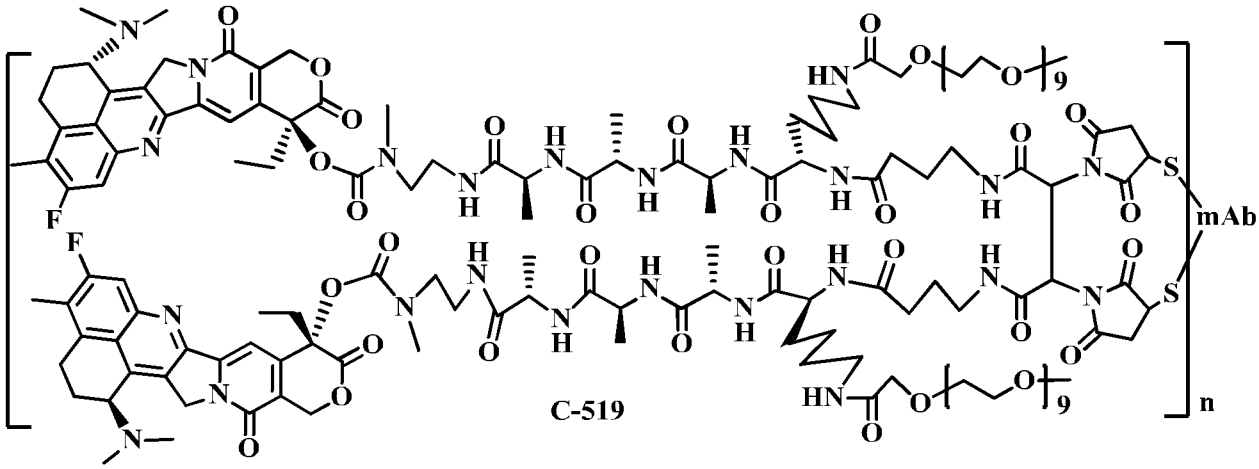
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C-514
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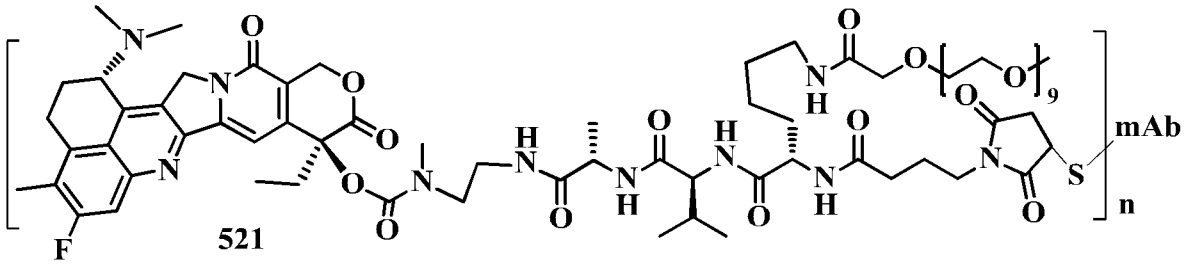
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C-518
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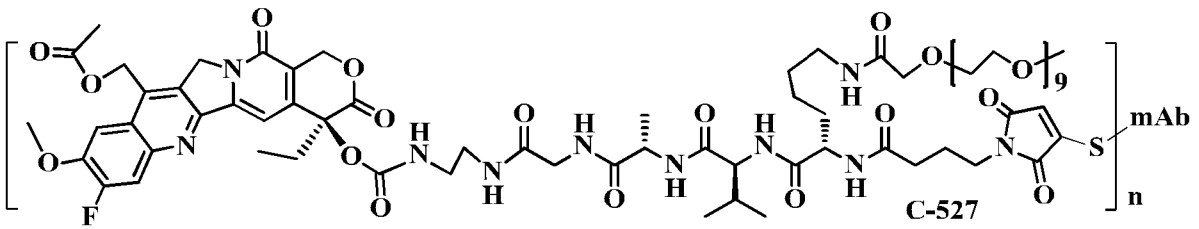
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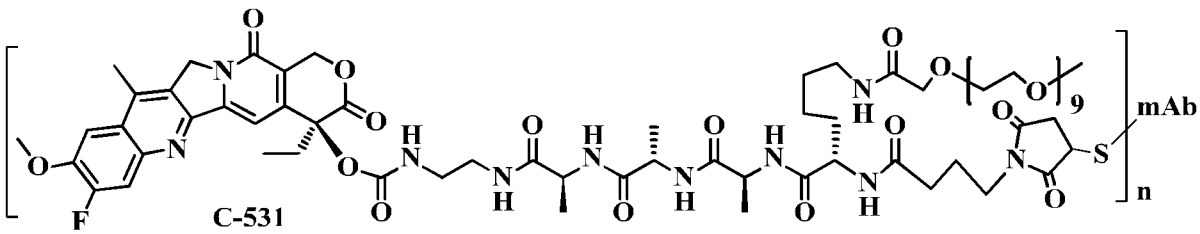
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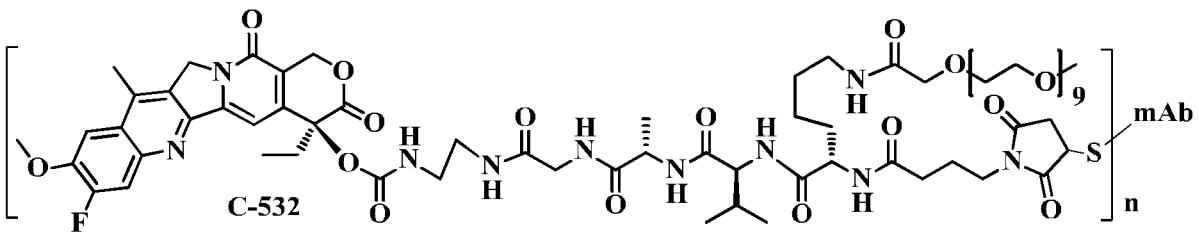
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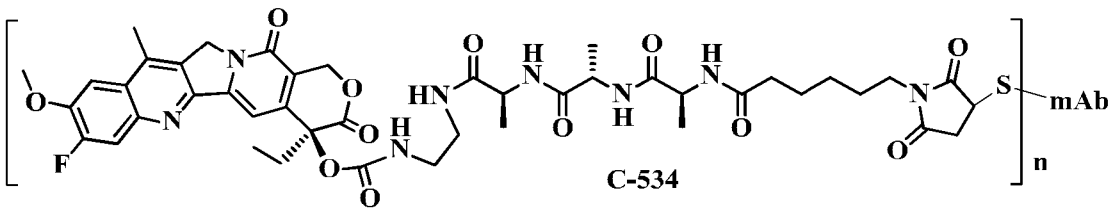
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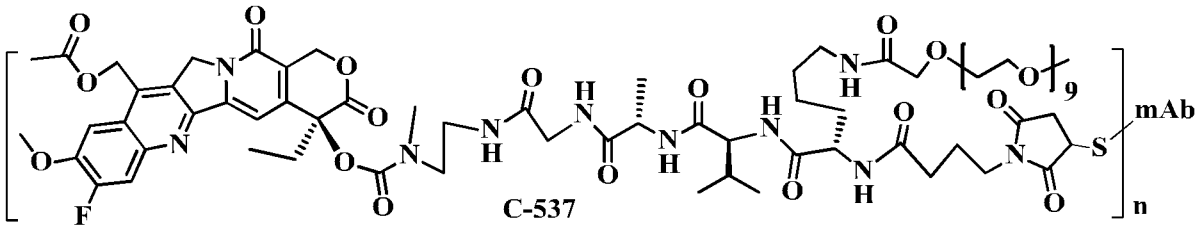
C-531 ,



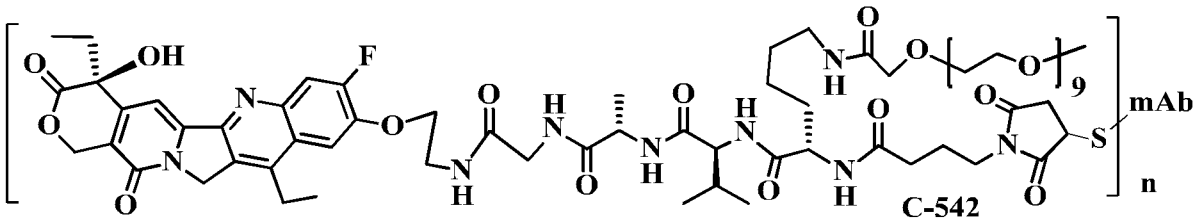
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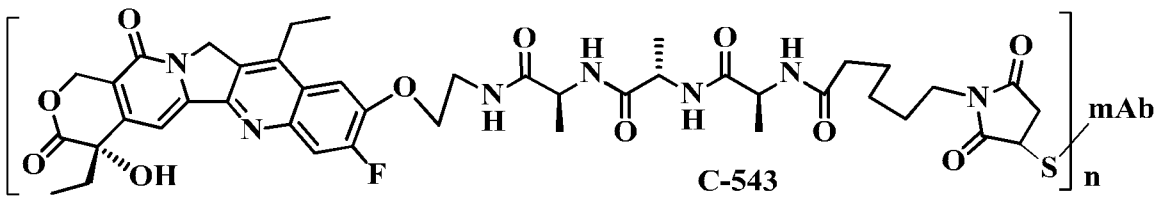
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C-537
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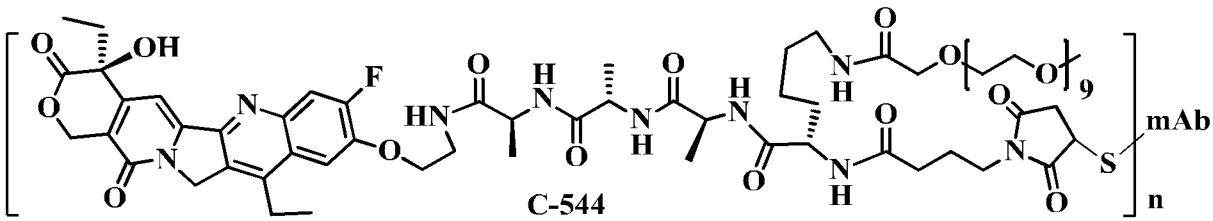
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C-542
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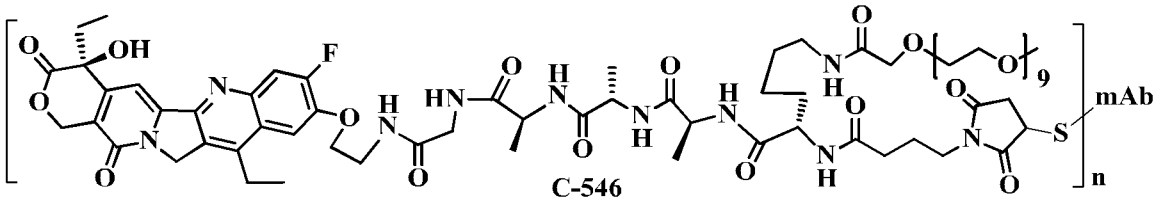
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C-543
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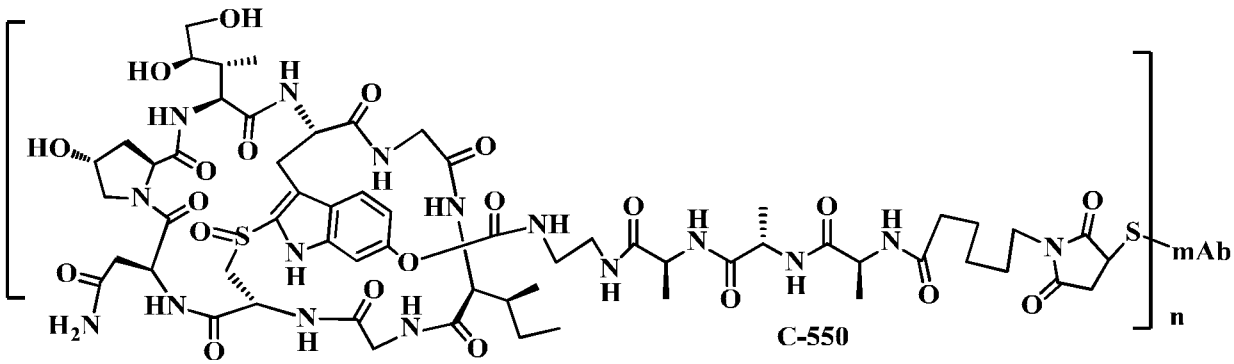
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C-544
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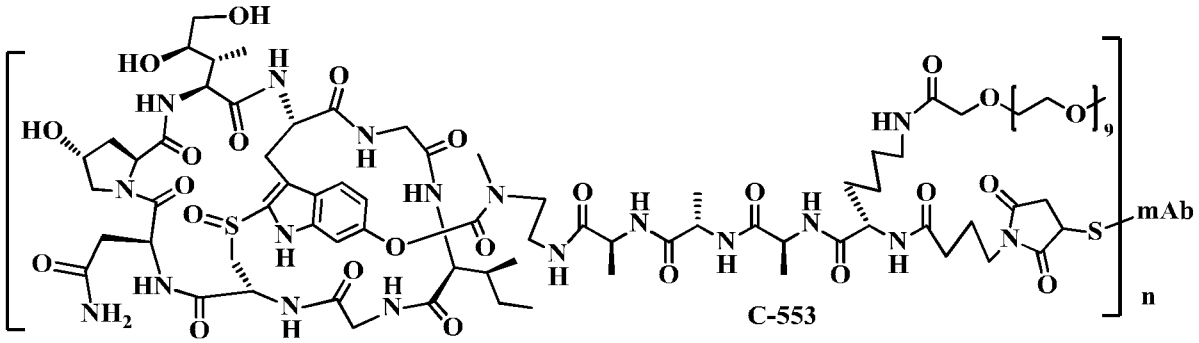
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C-546
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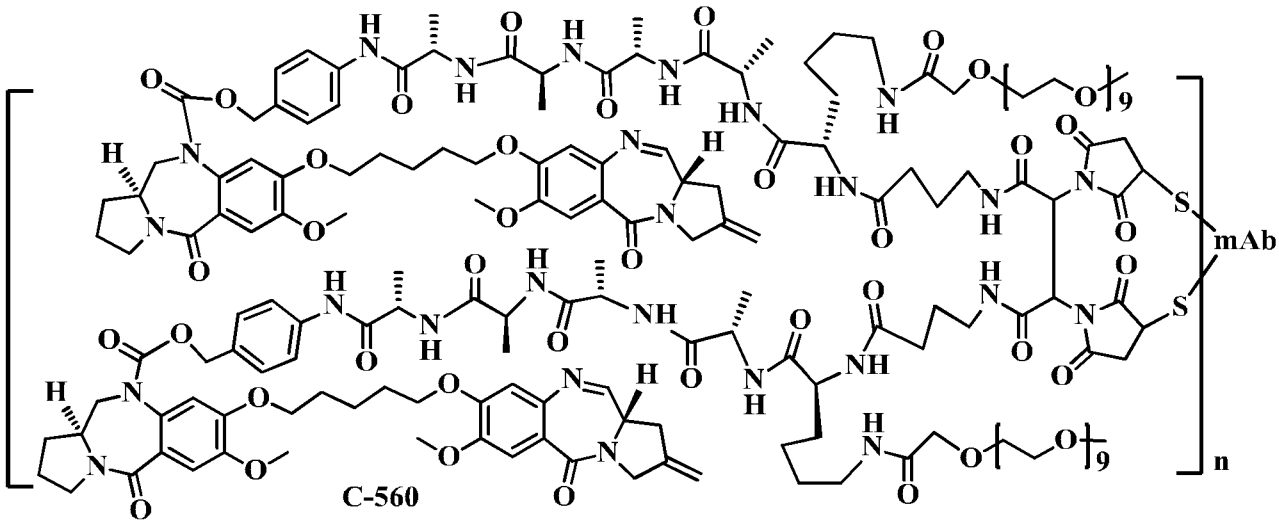
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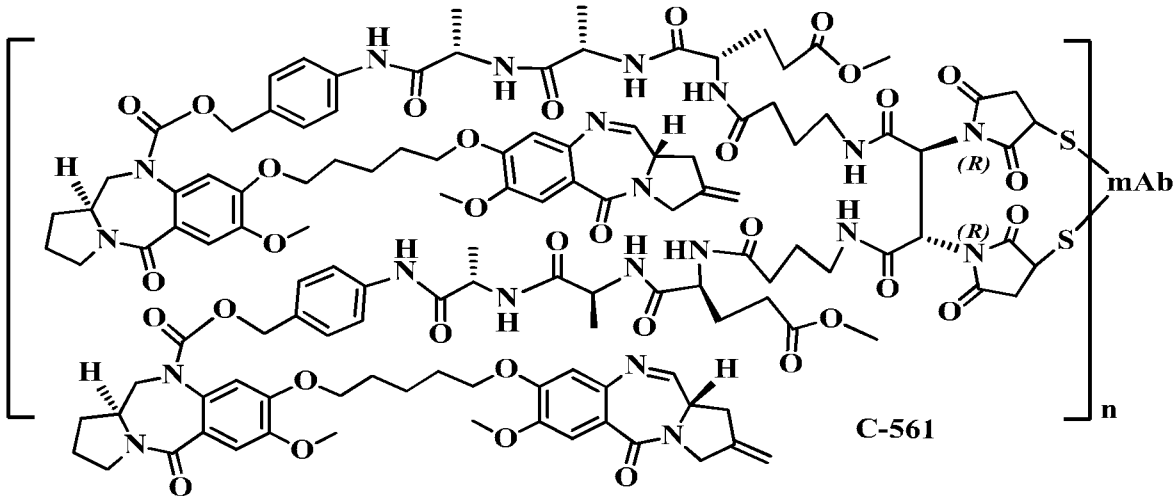

C-553
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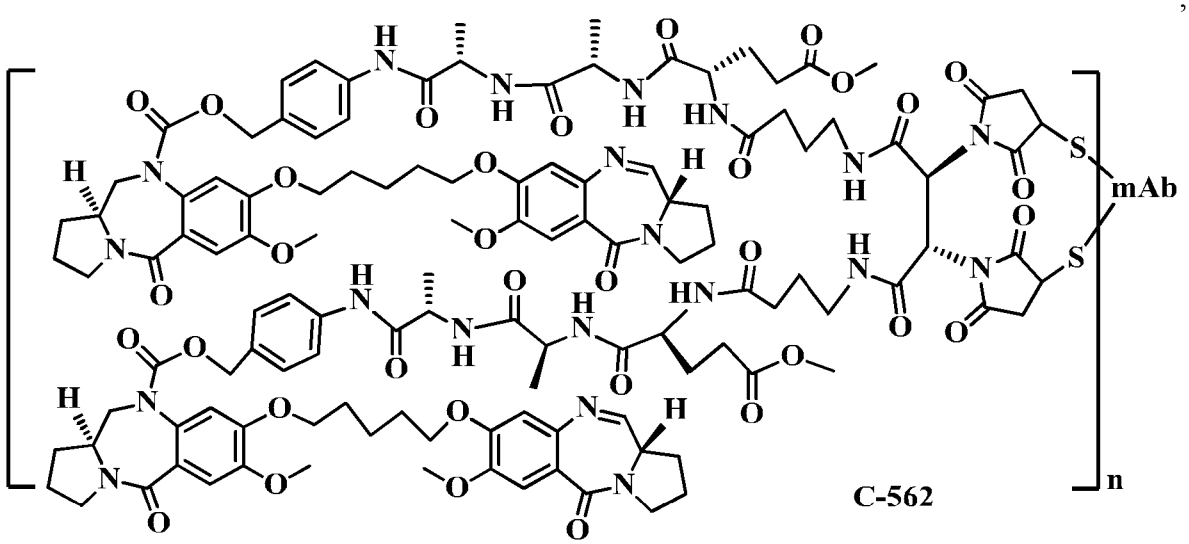
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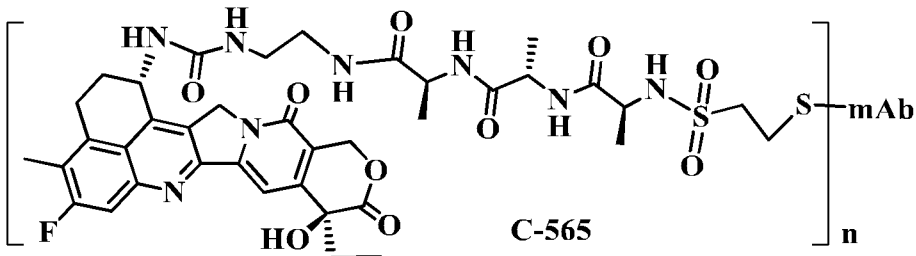
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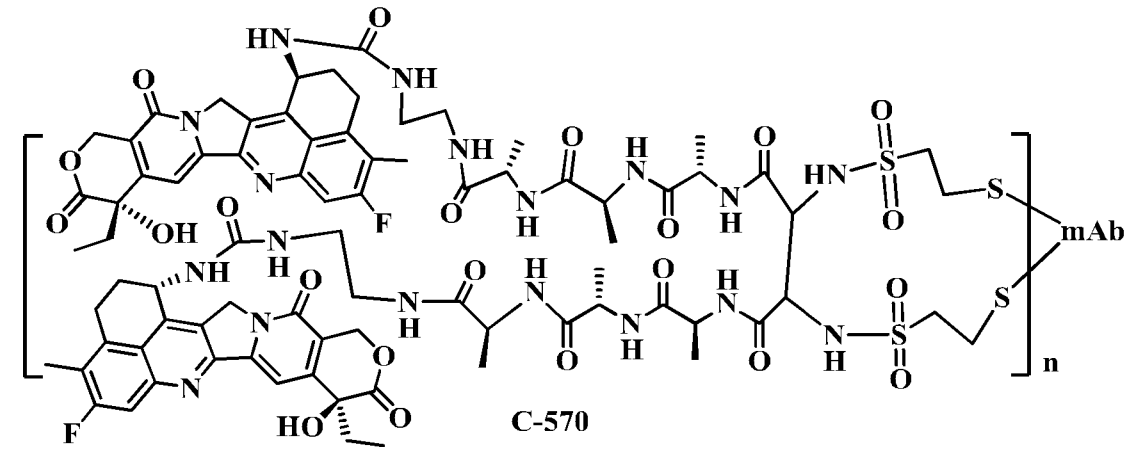


C-562

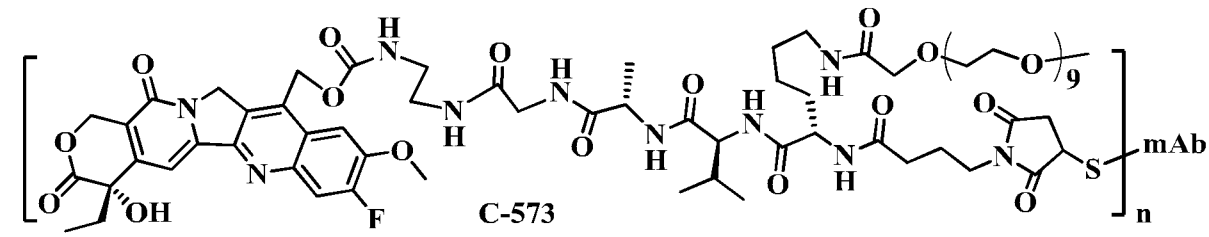
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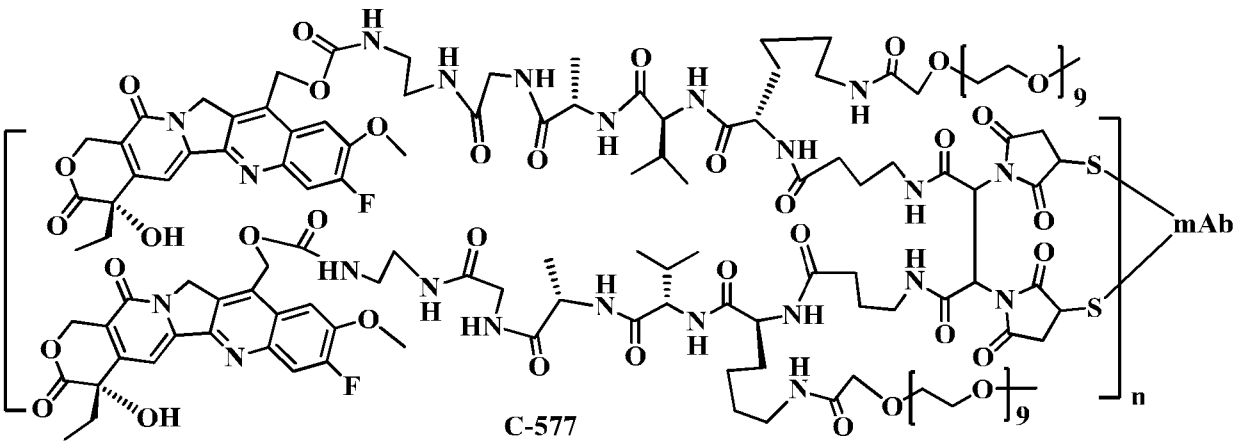
C-565



C-570

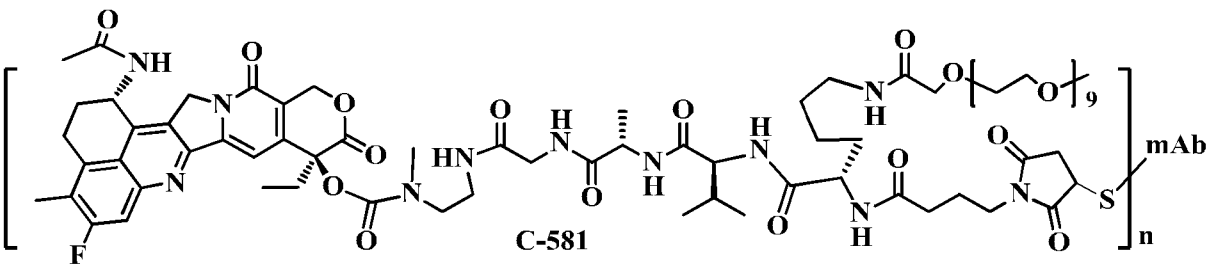


C-573



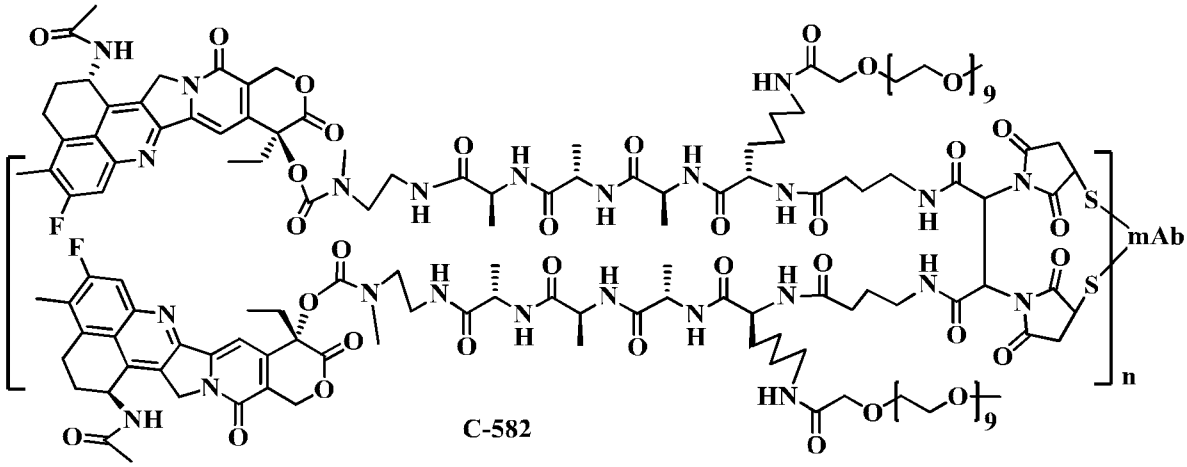
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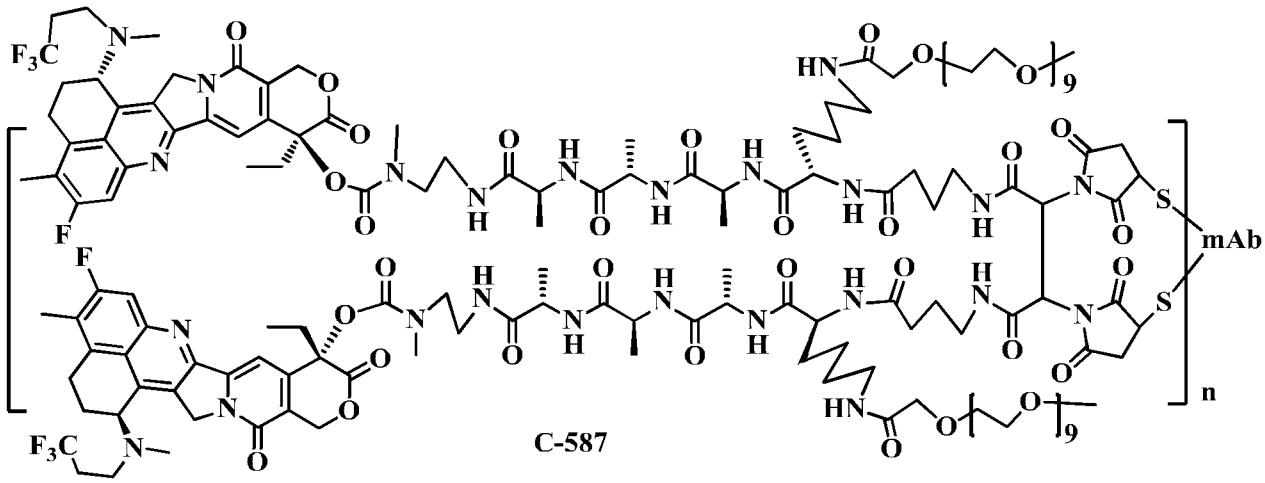


C-581

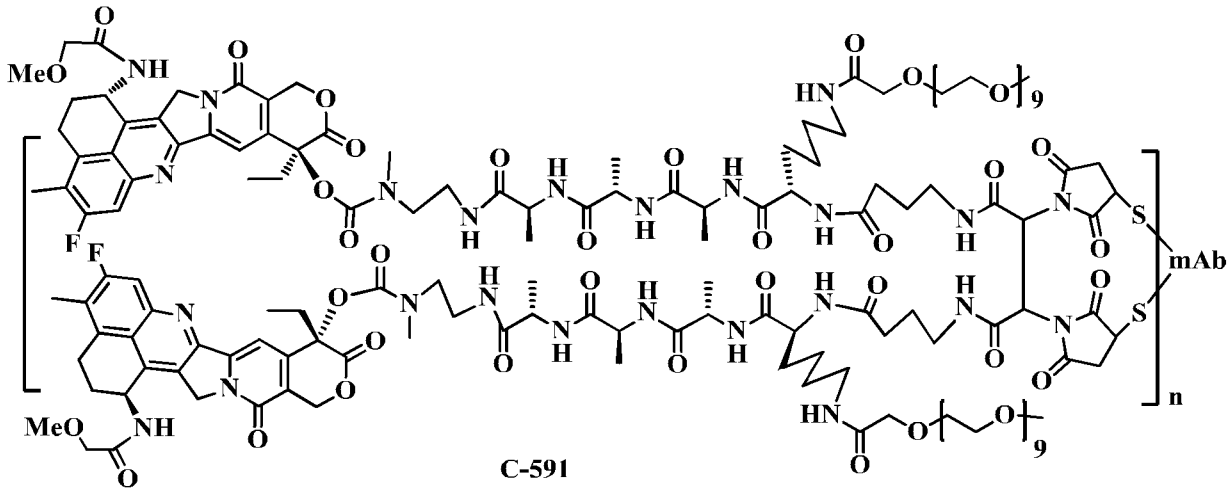
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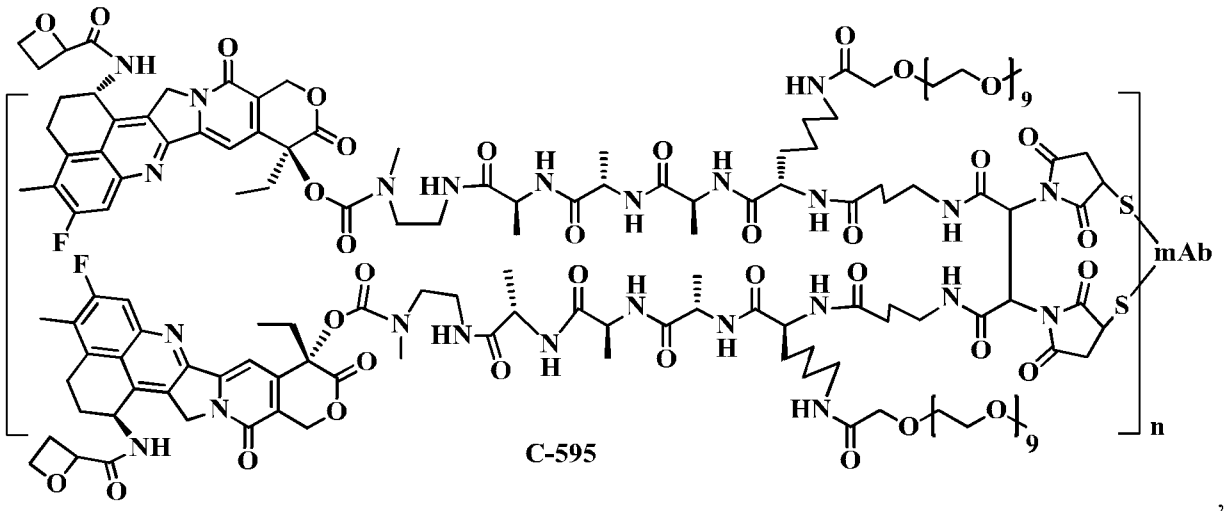
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C-587 ,

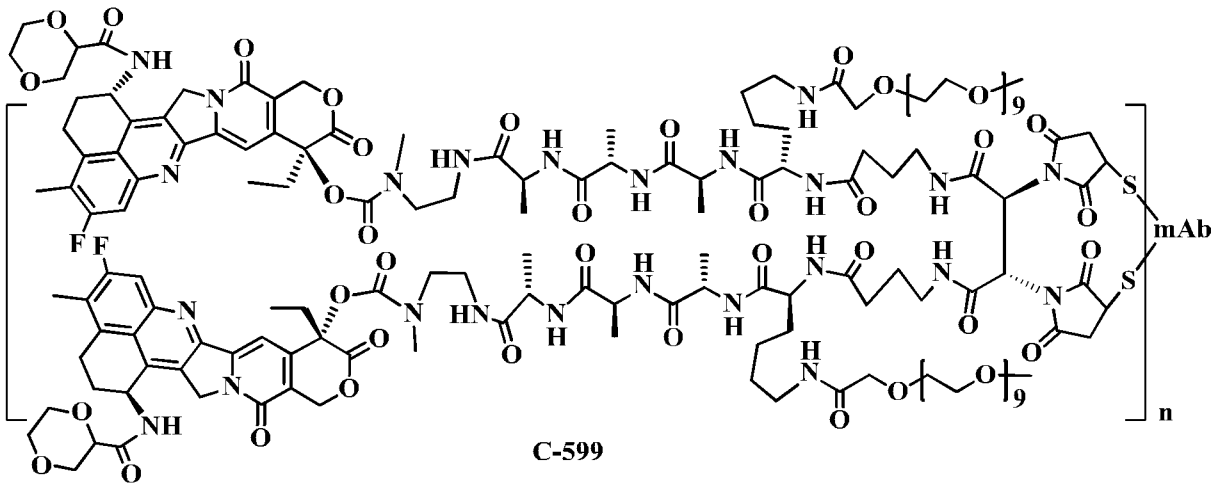


C-591 ,



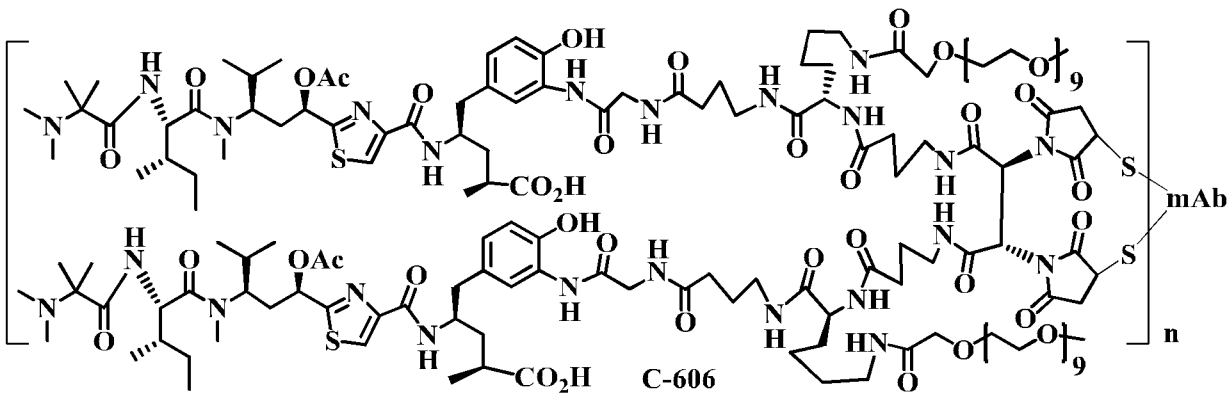
C-595

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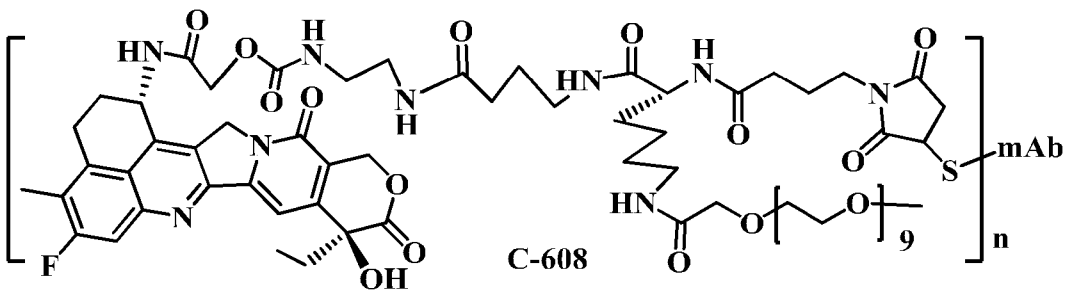
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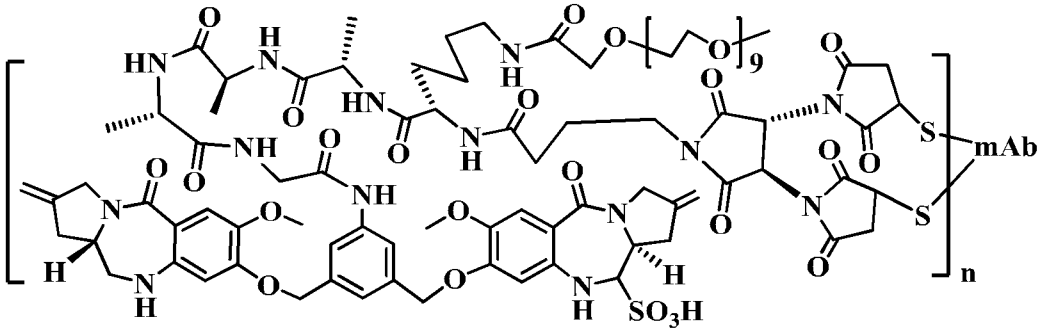
C-606

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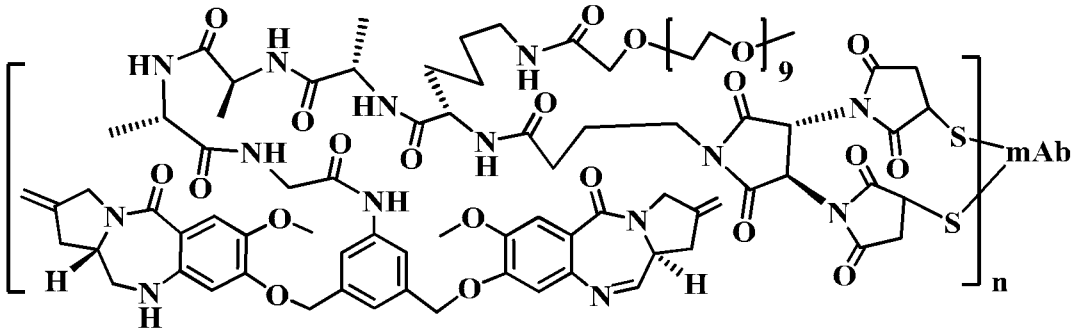


C-608

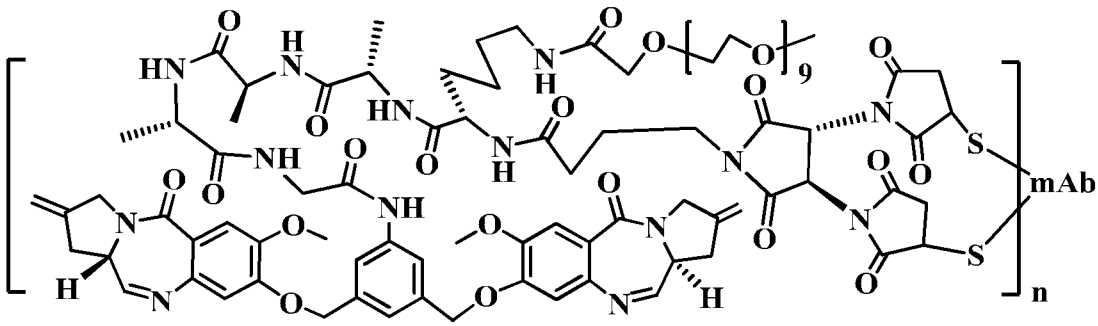
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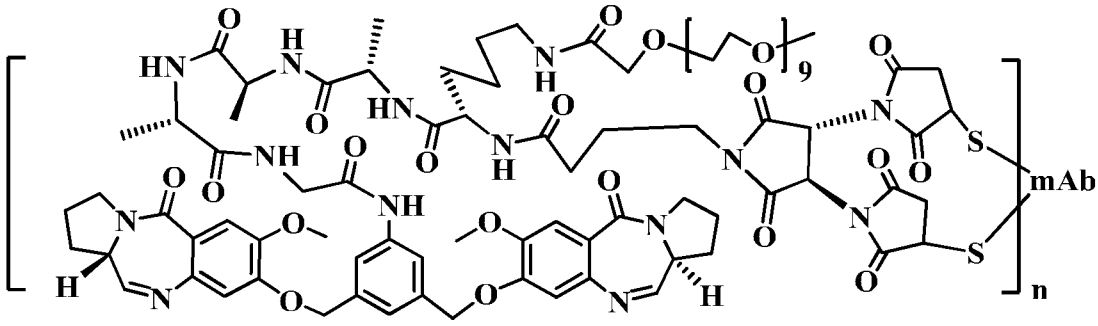
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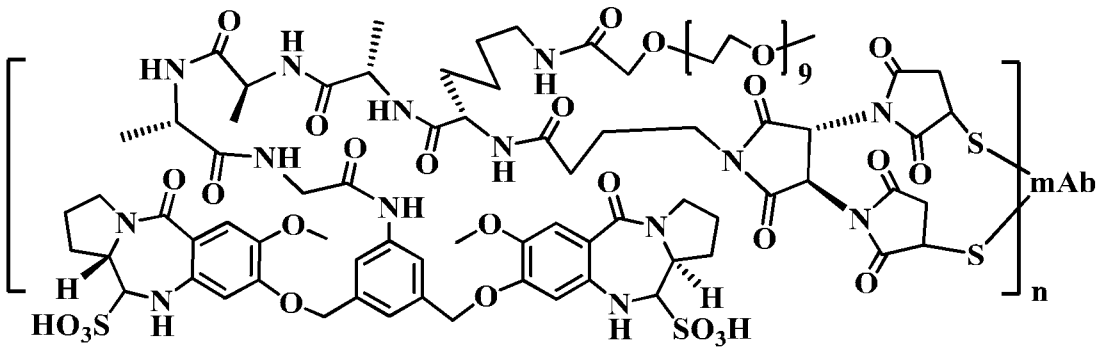
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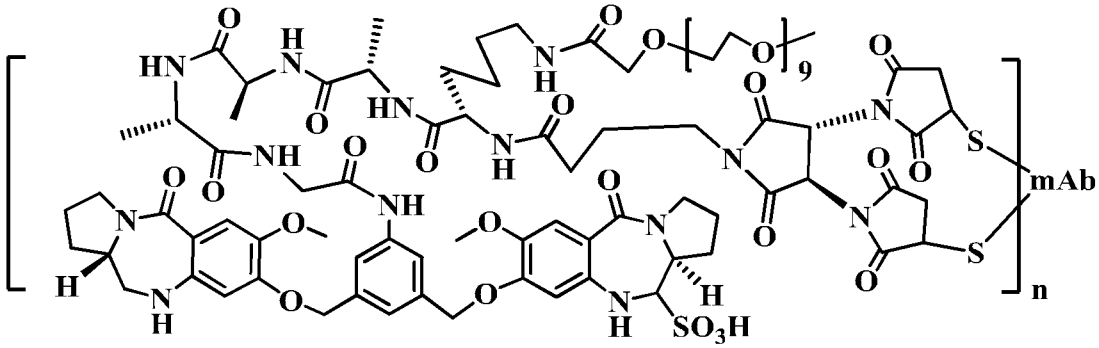
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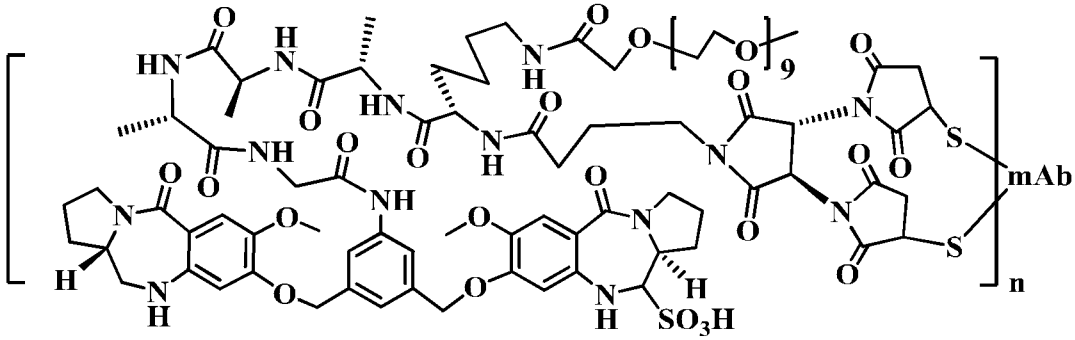
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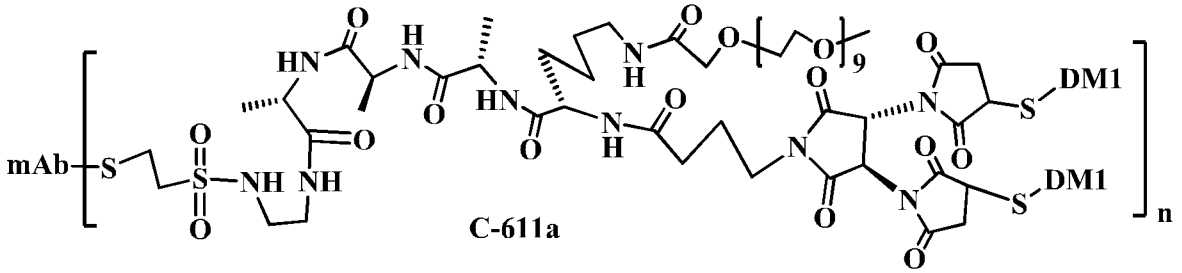
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C-610c,

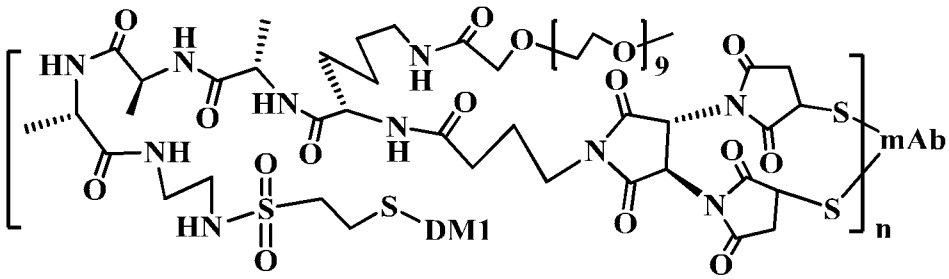


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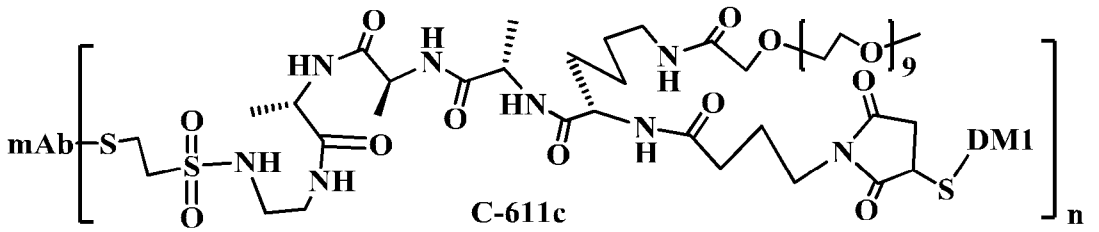
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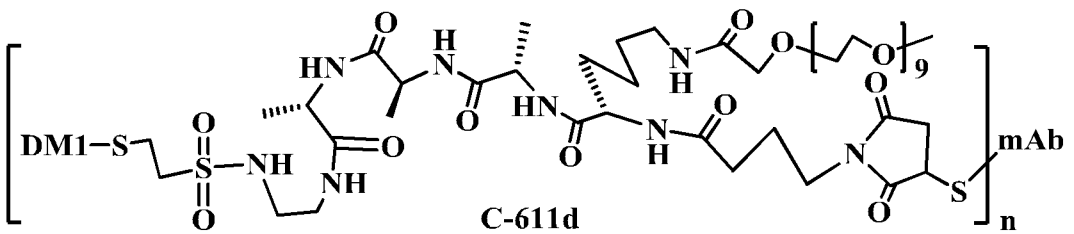
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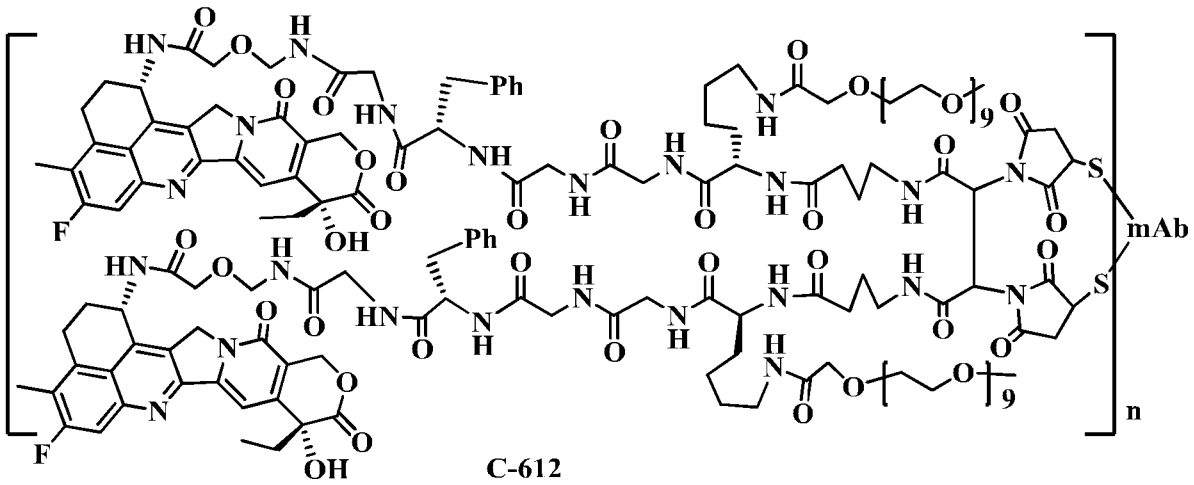
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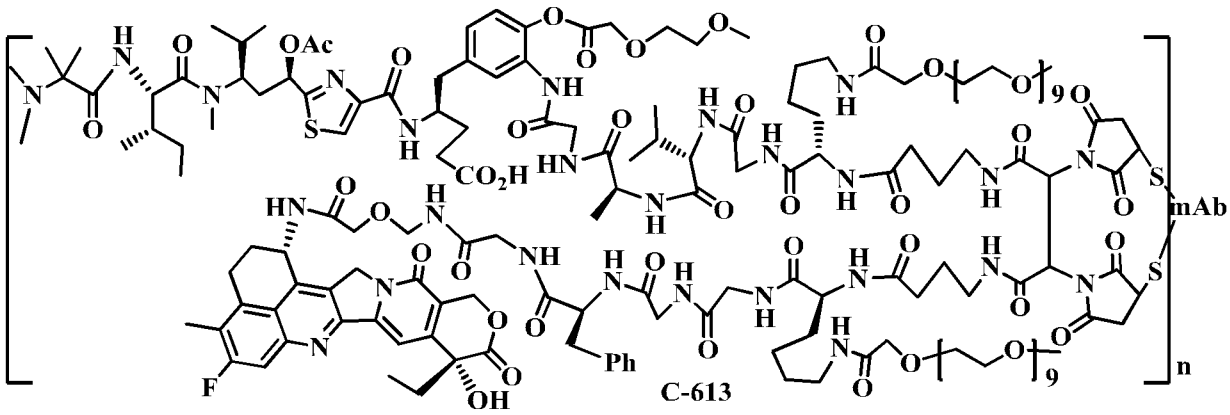


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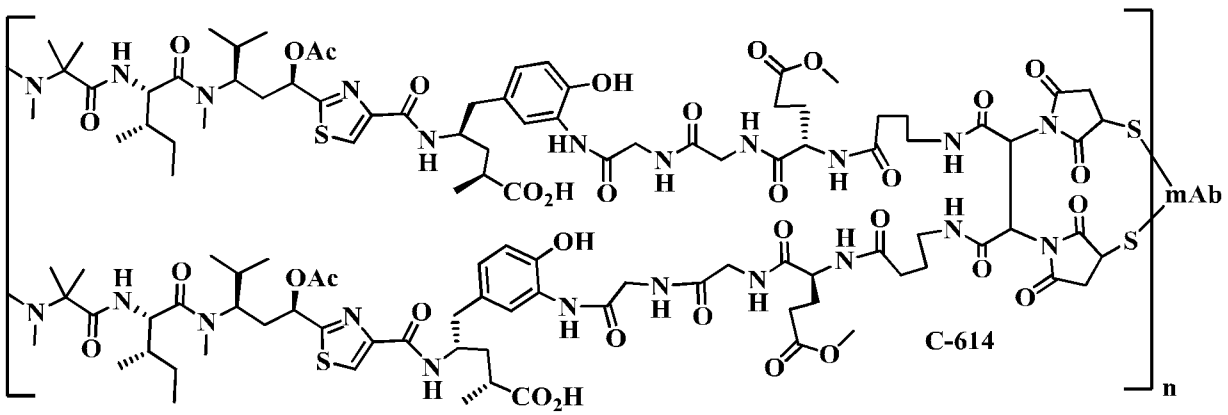
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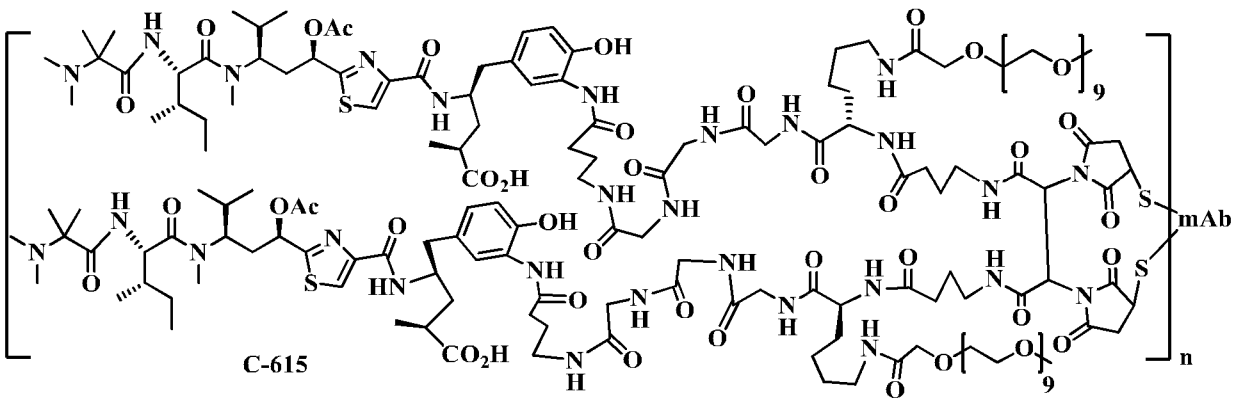
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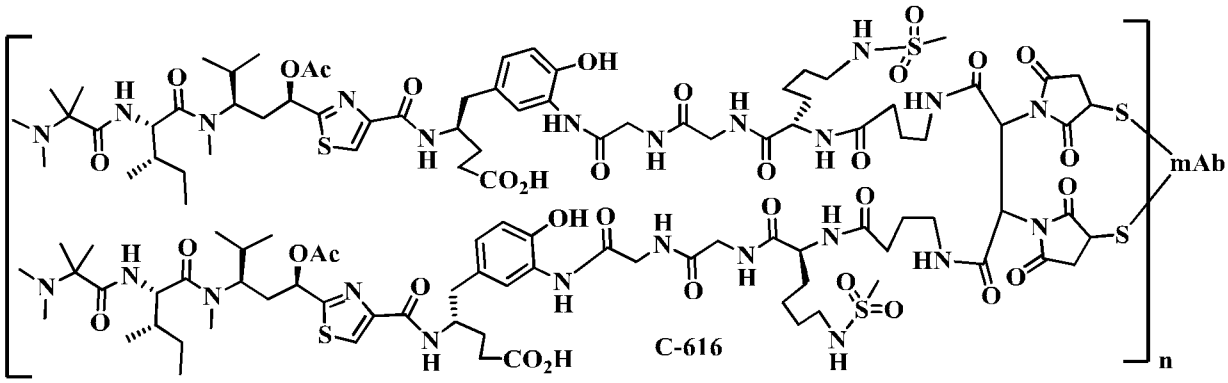
C-613



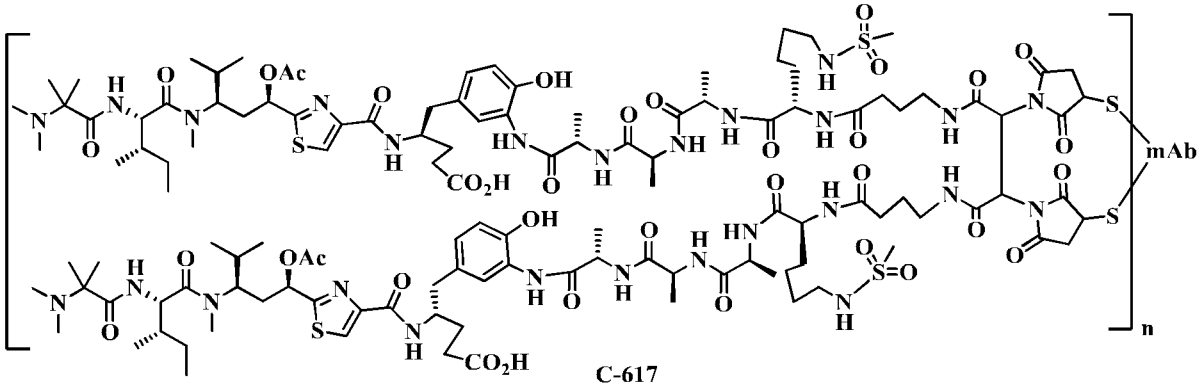
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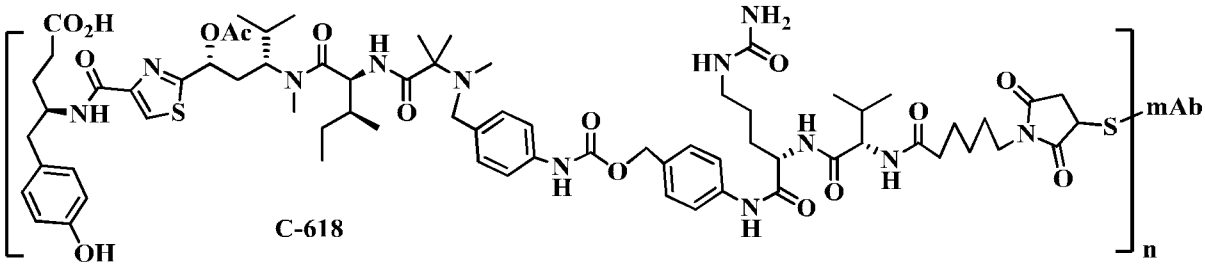
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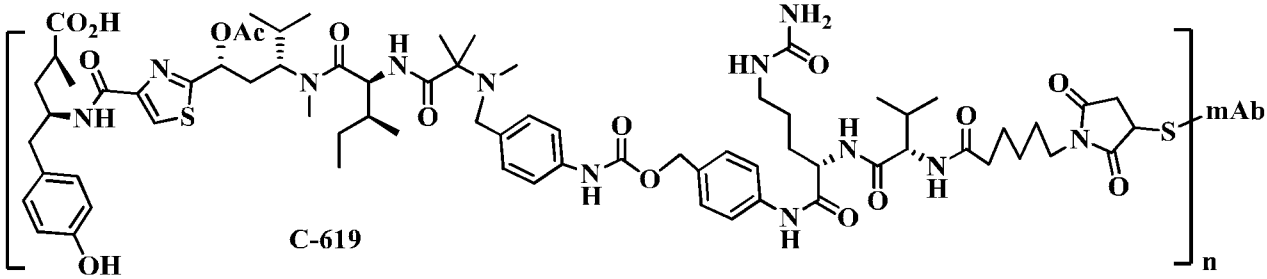
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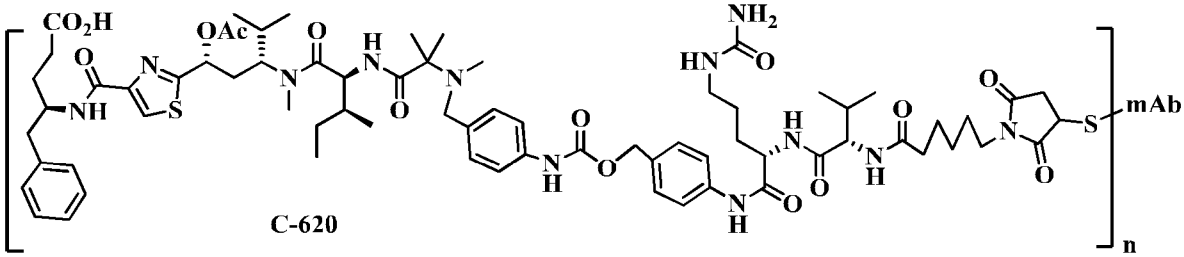
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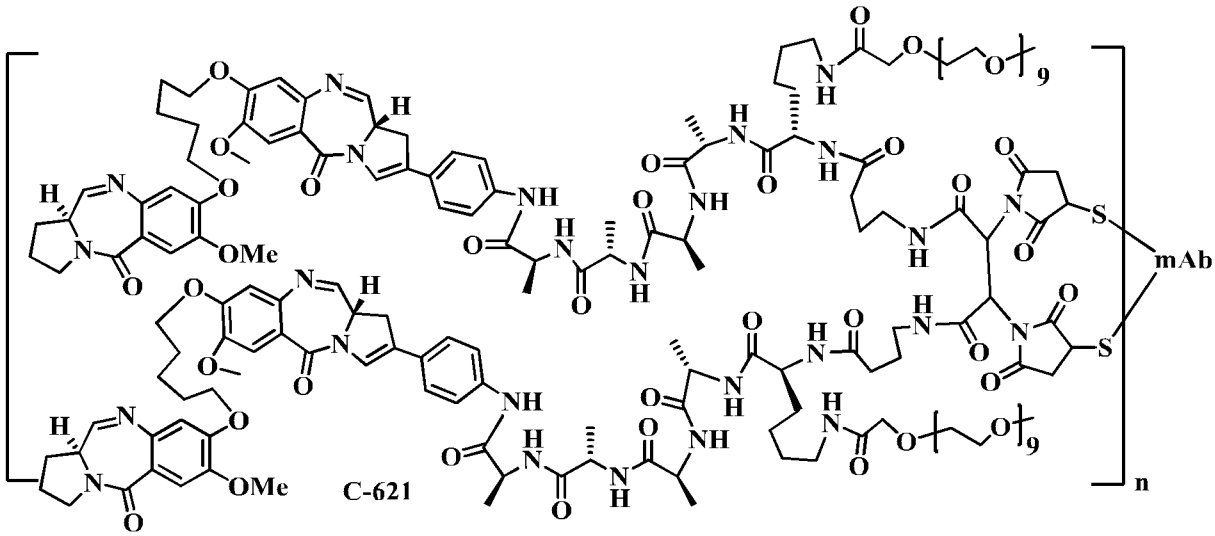
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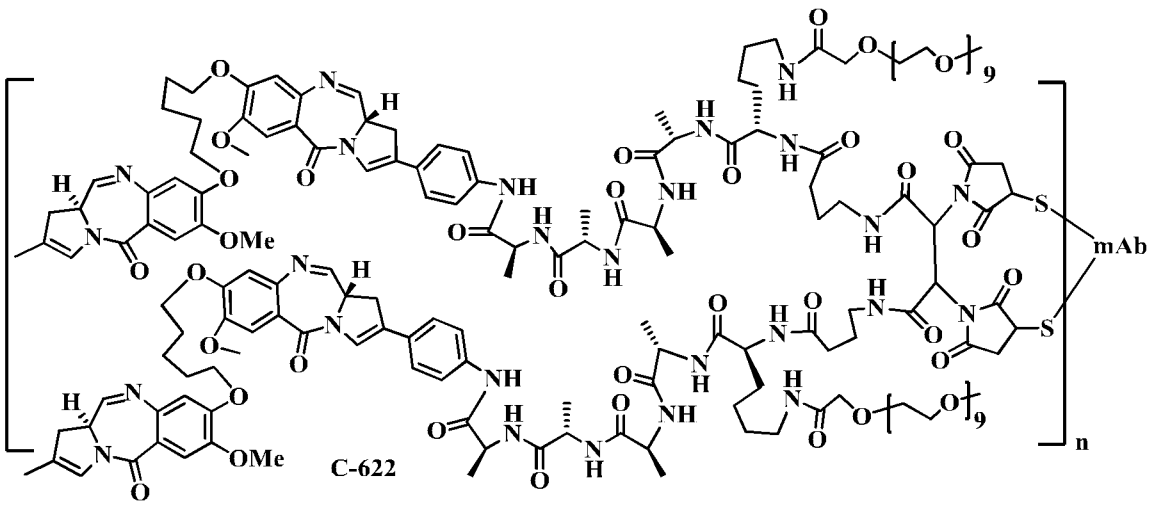
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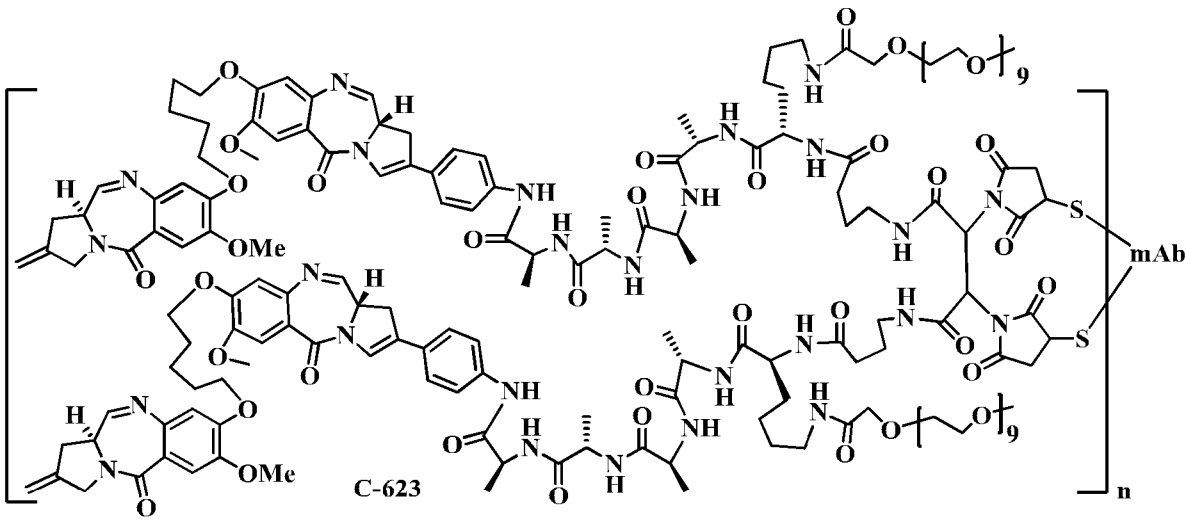
C-620



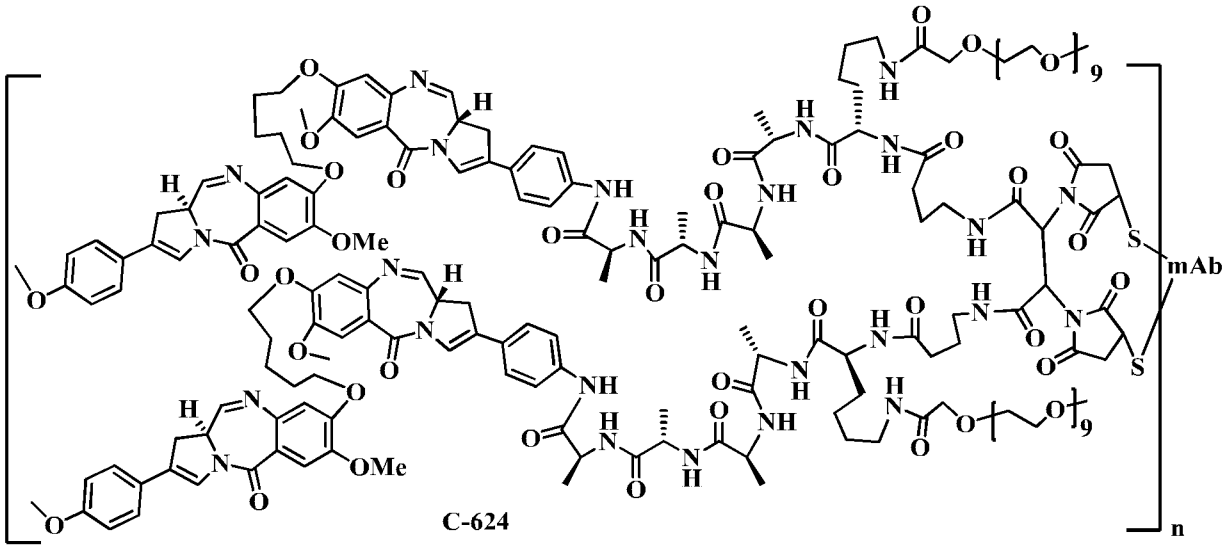
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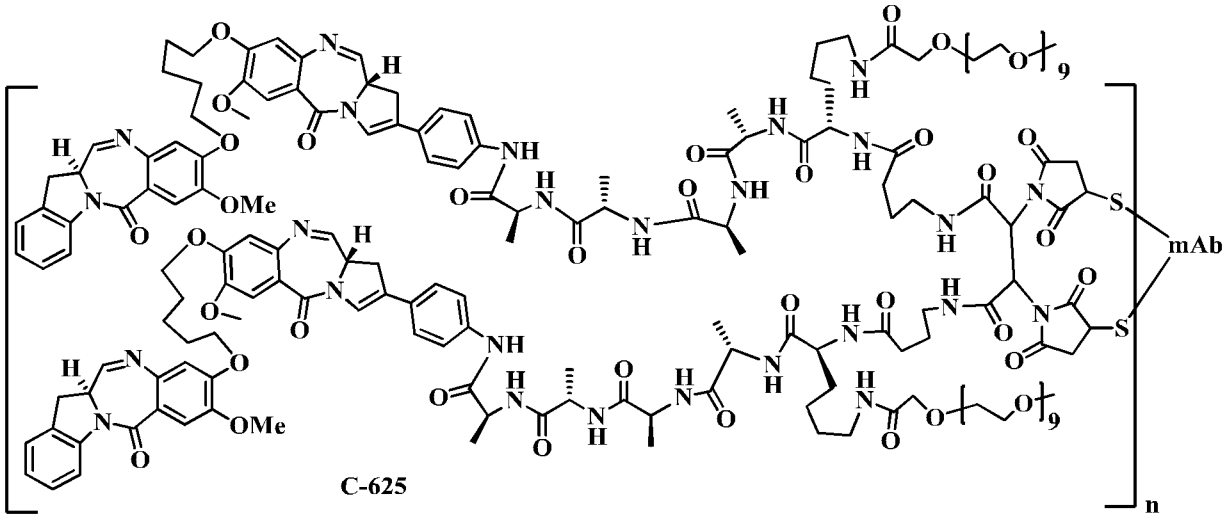
C-622



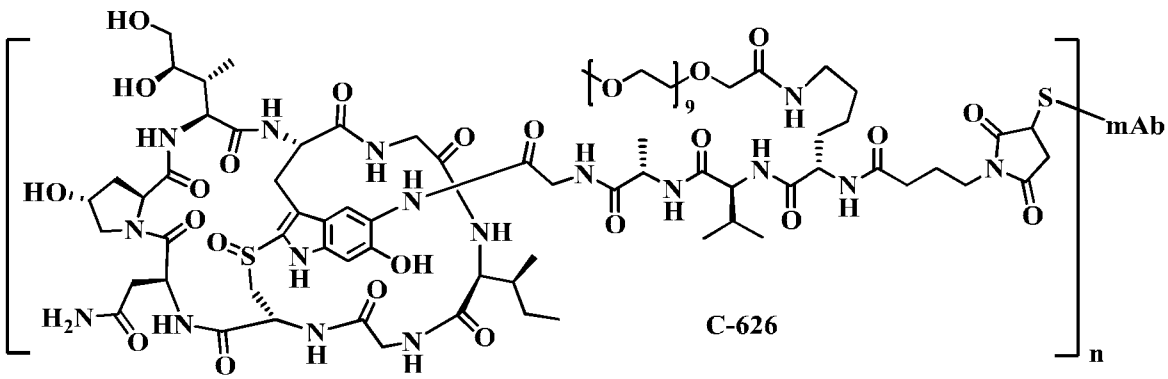
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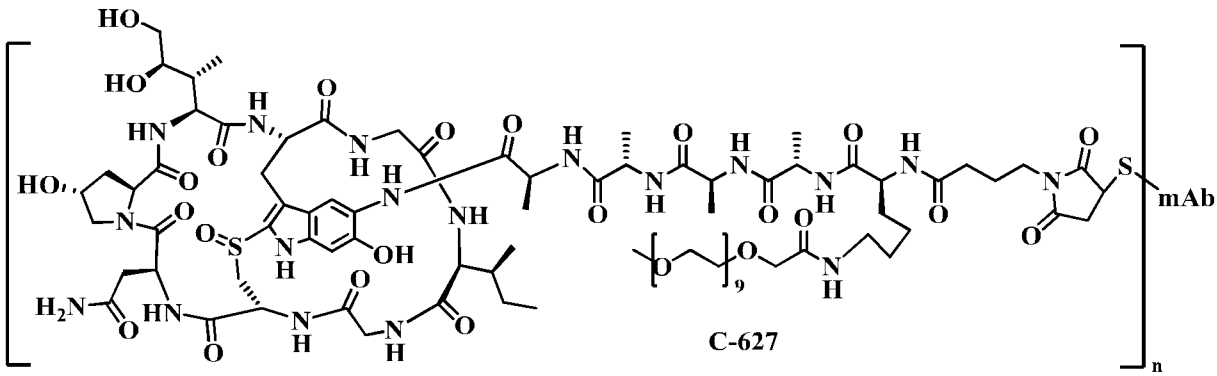
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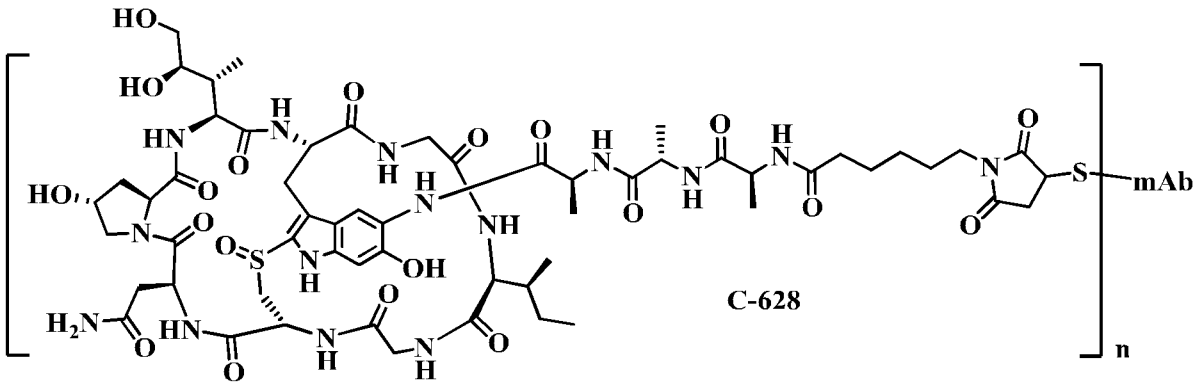
C-625



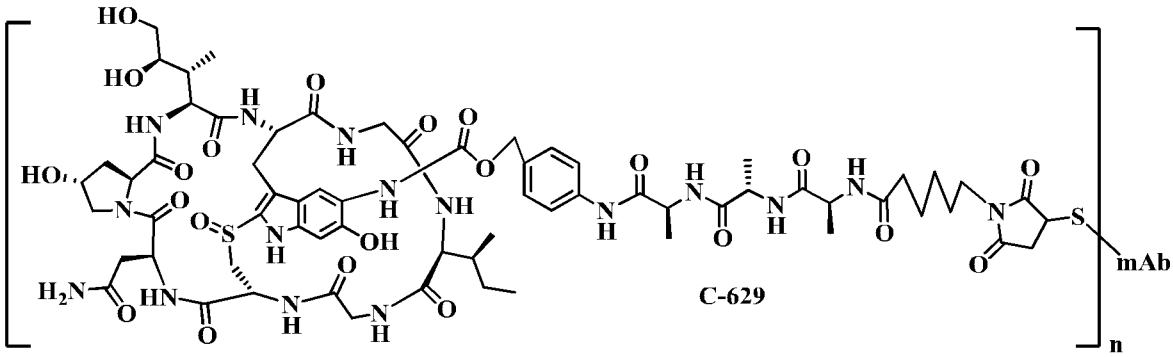
C-626



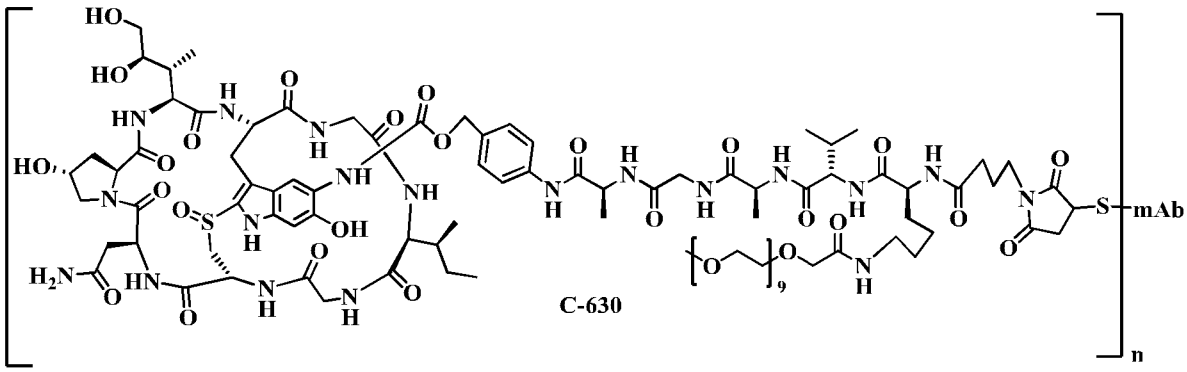
C-627



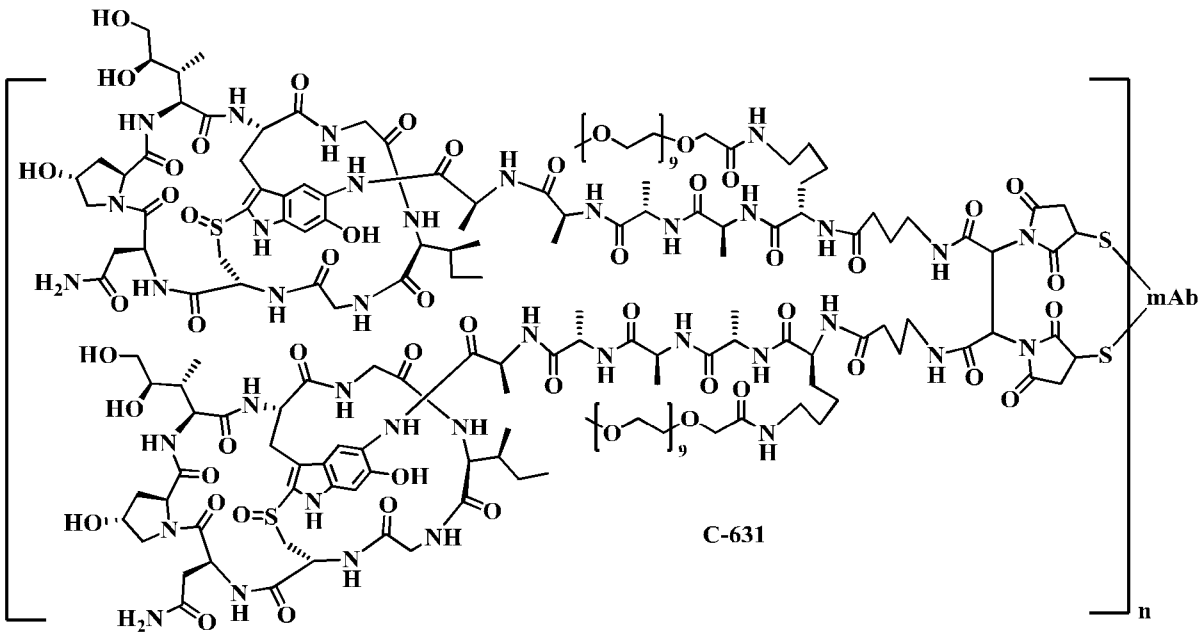
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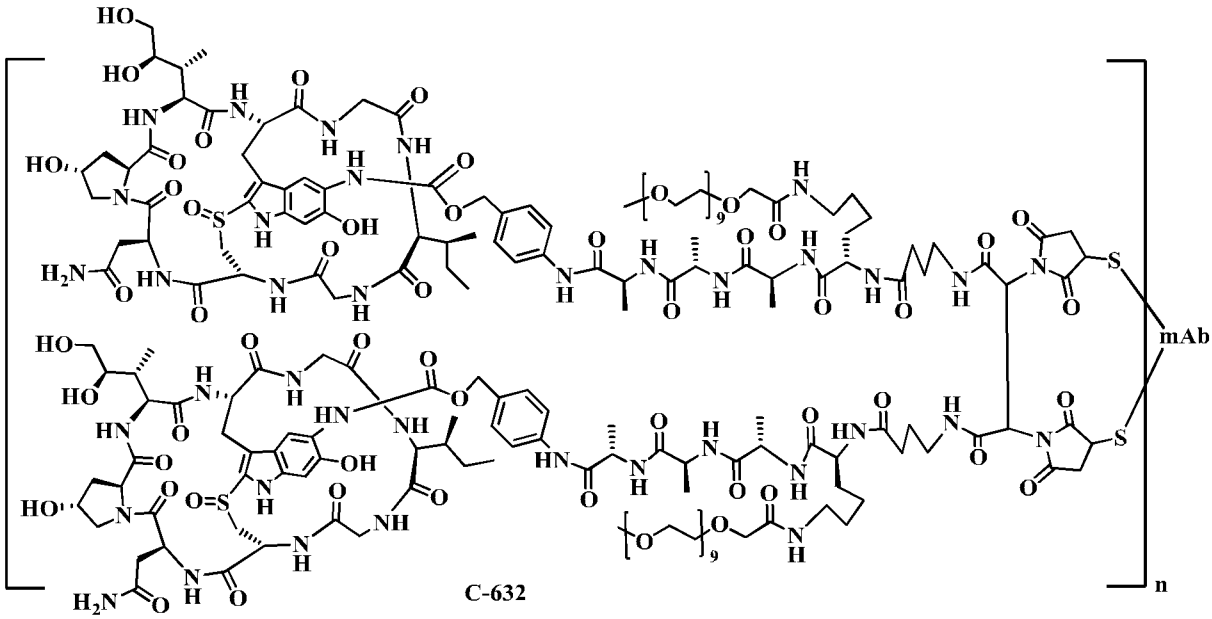
C-629



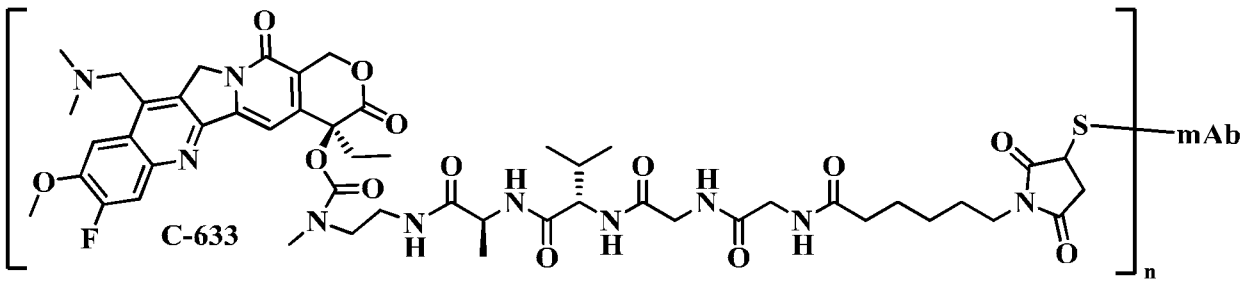
C-630



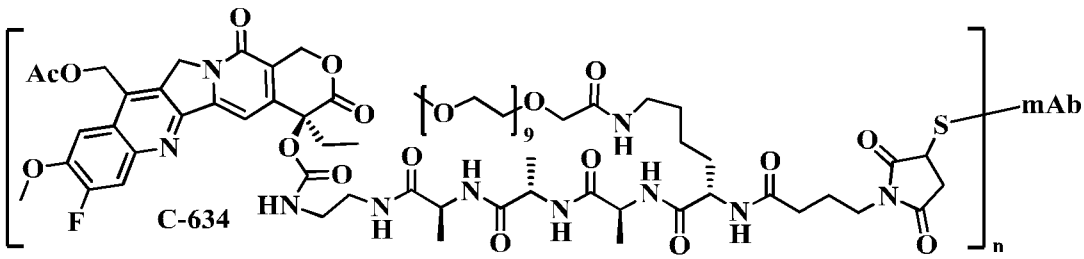
C-631



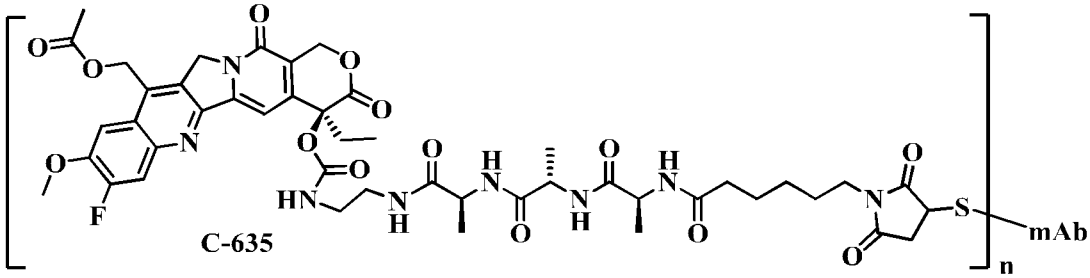
C-632



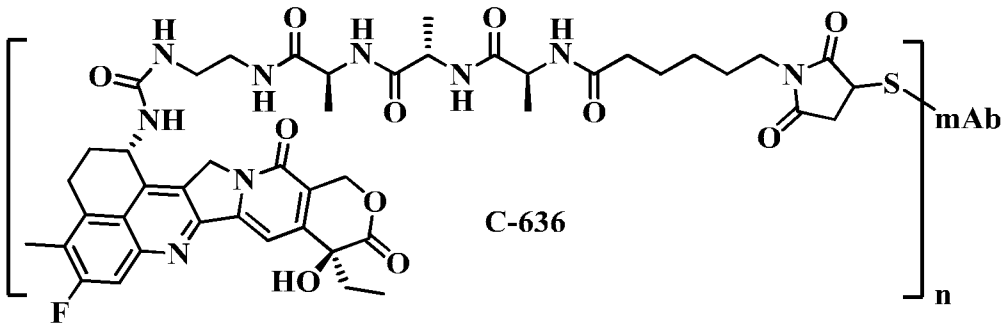
C-633



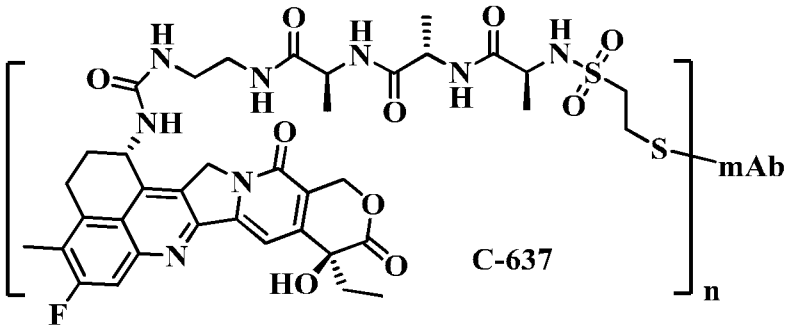
C-634



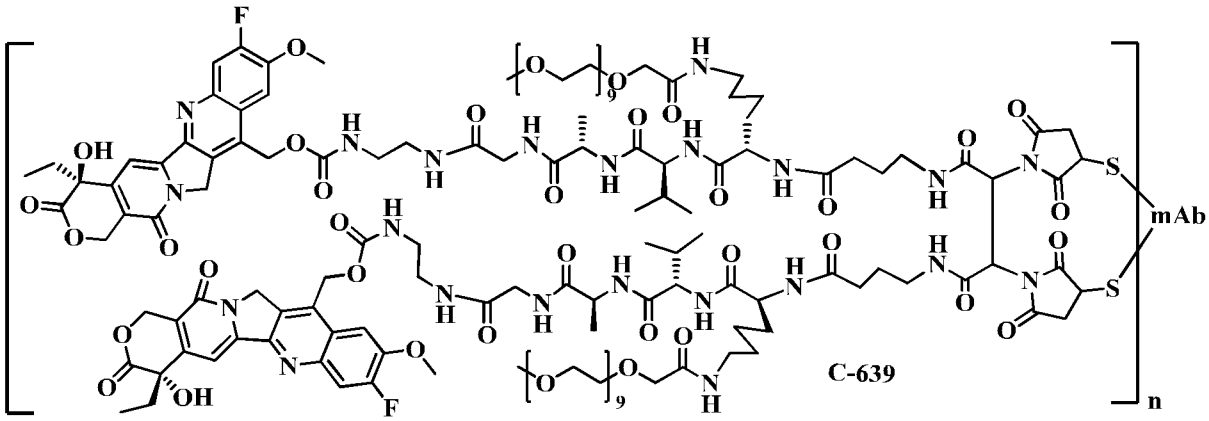
C-635



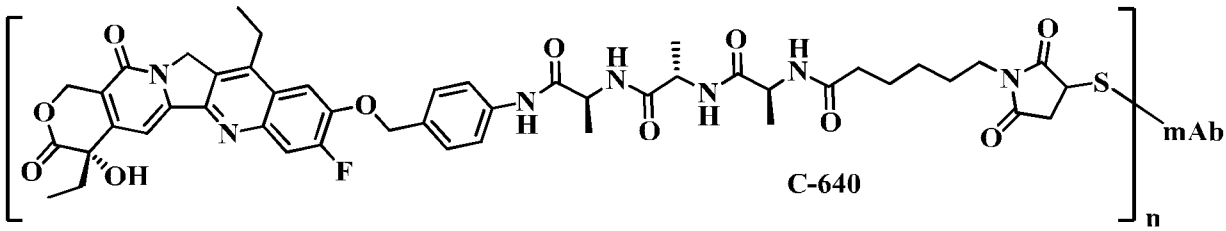
C-636



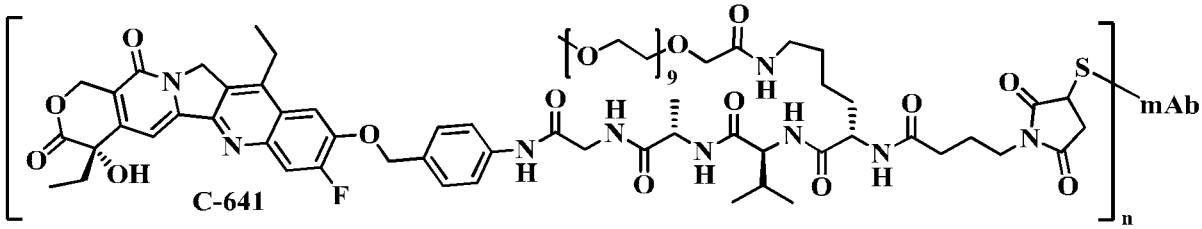
C-637



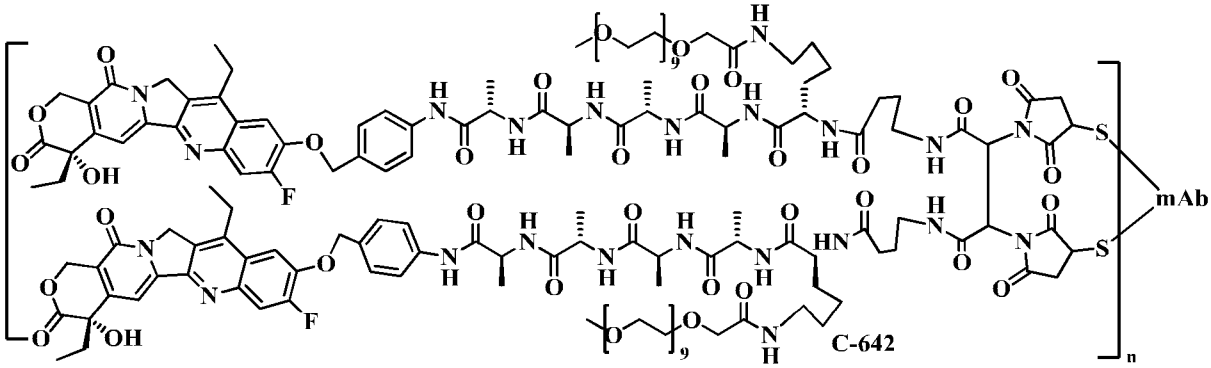
C-639



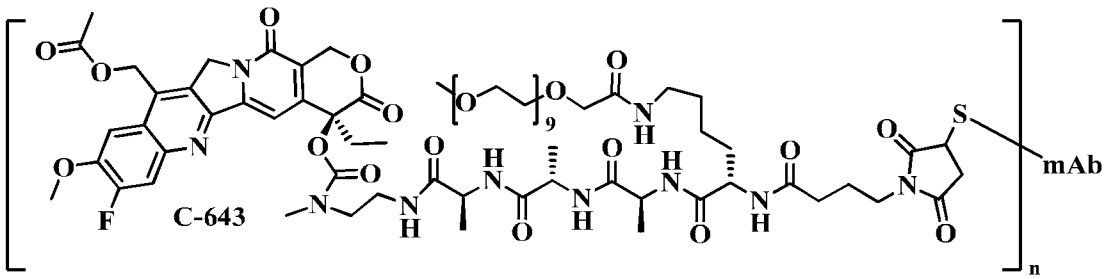
C-640



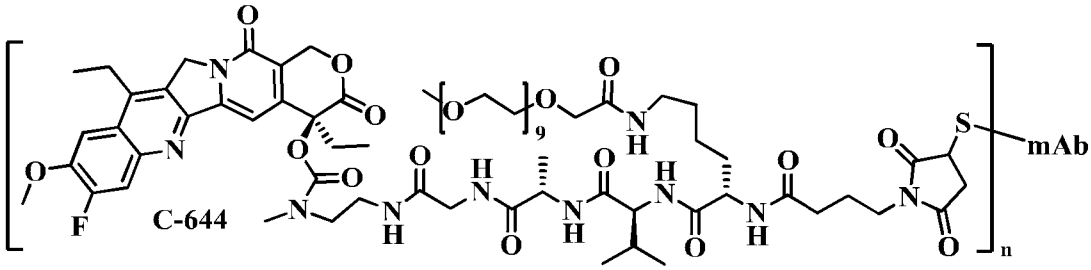
C-641

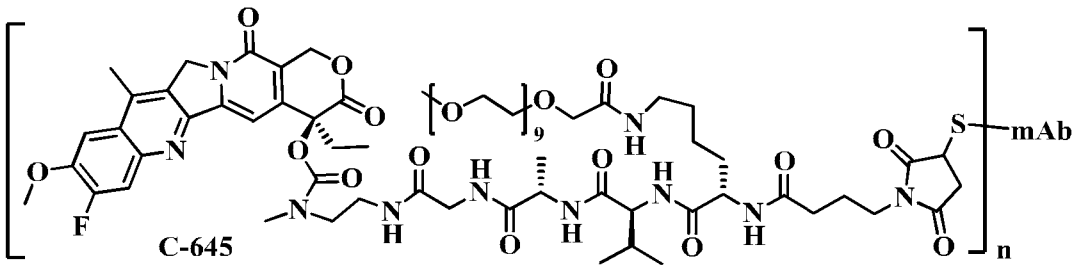


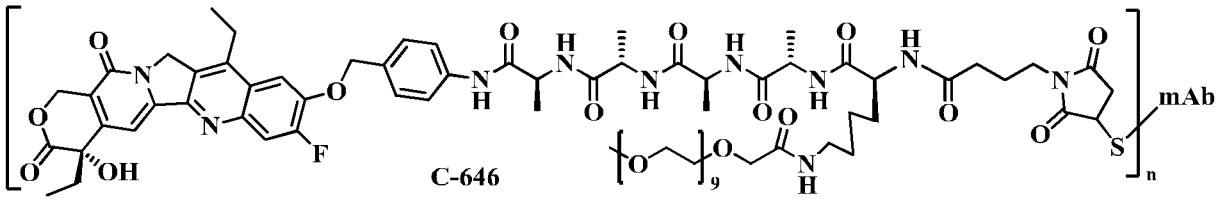
C-642

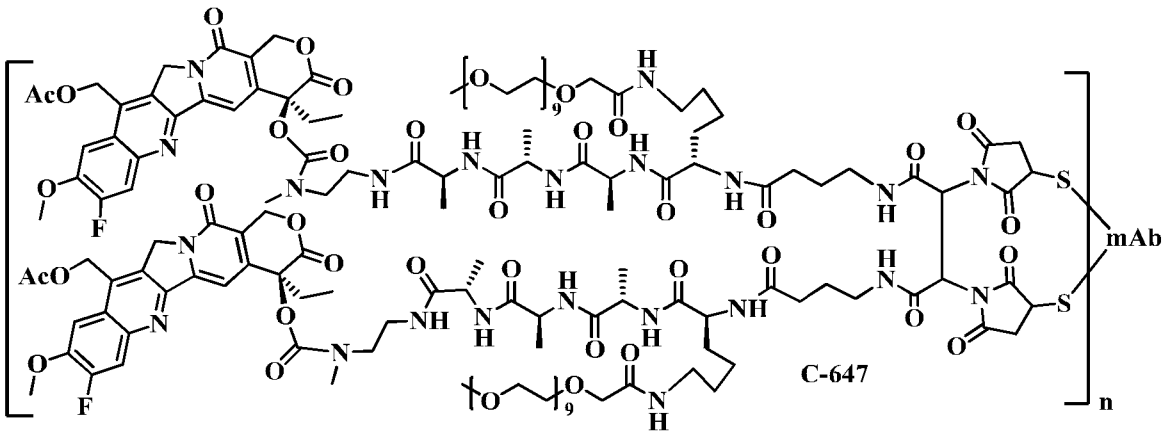


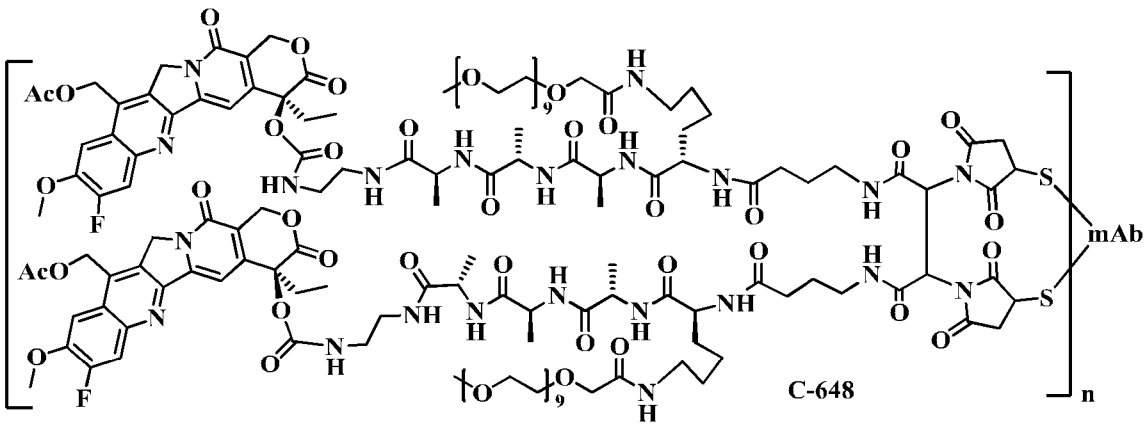
C-643

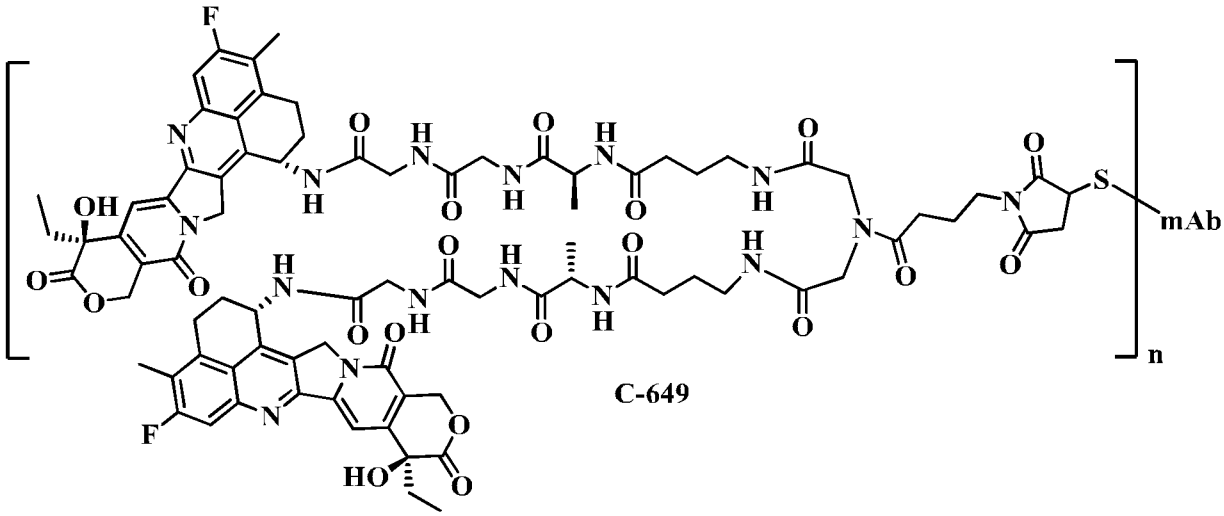

C-644
mAb
n


C-645
mAb
n

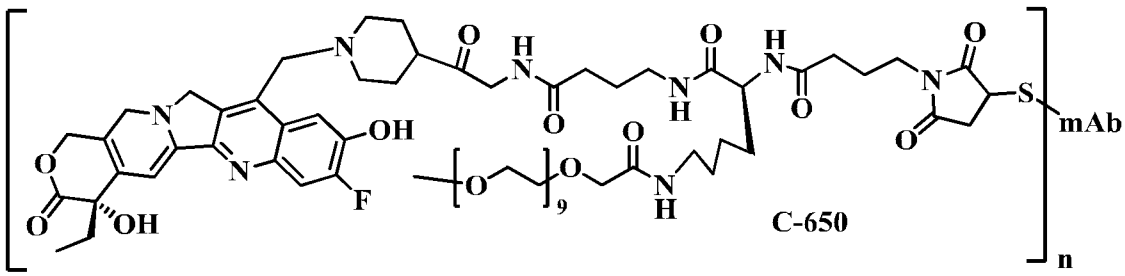

C-646
mAb
n


C-647
mAb
n

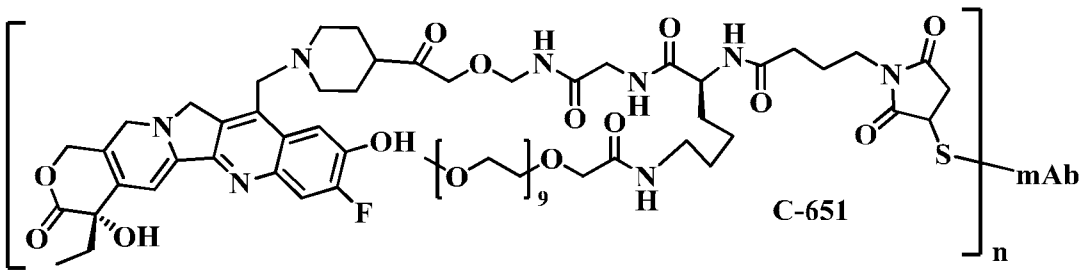

C-648
mAb
n



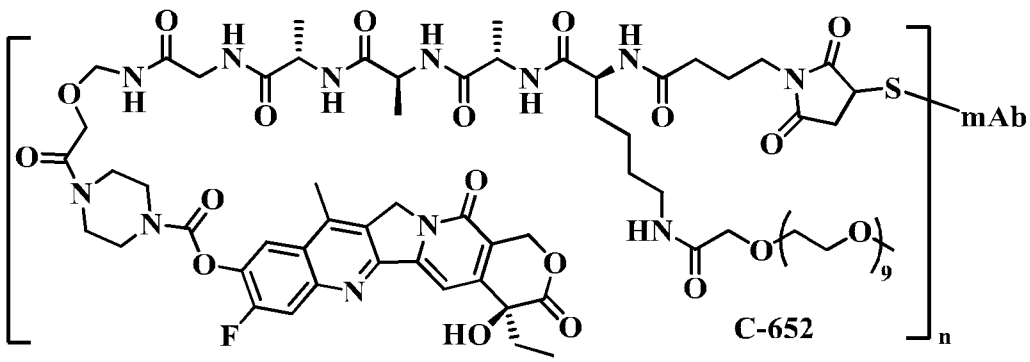
C-649



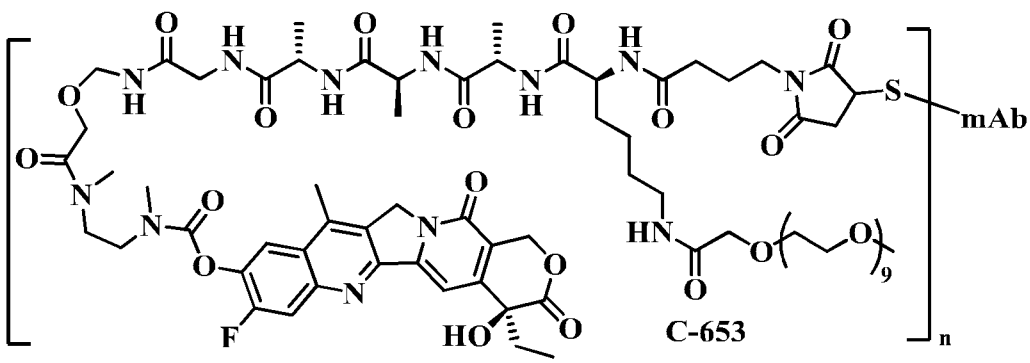
C-650



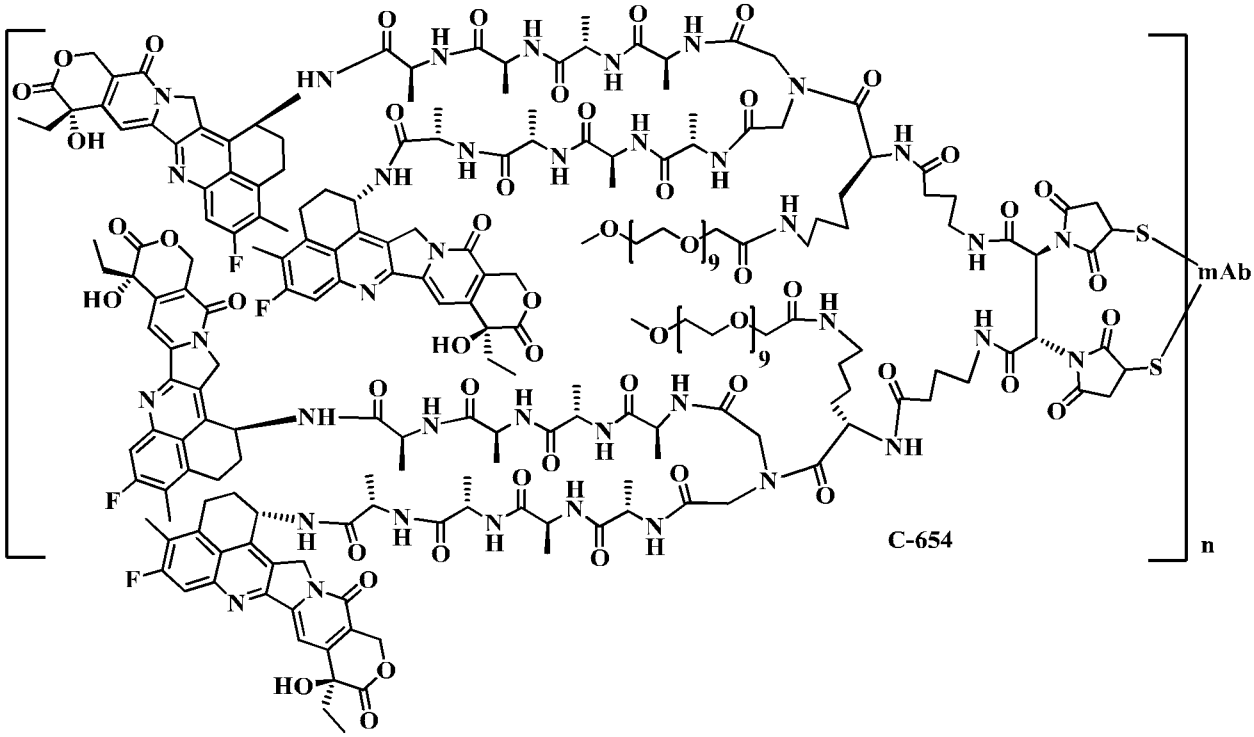
C-651



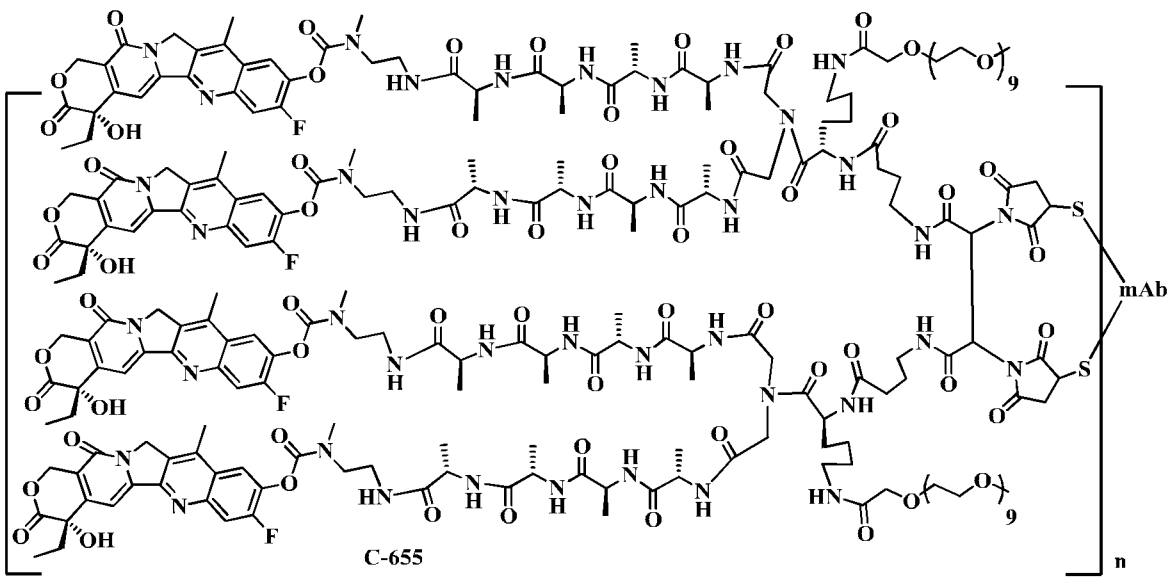
C-652



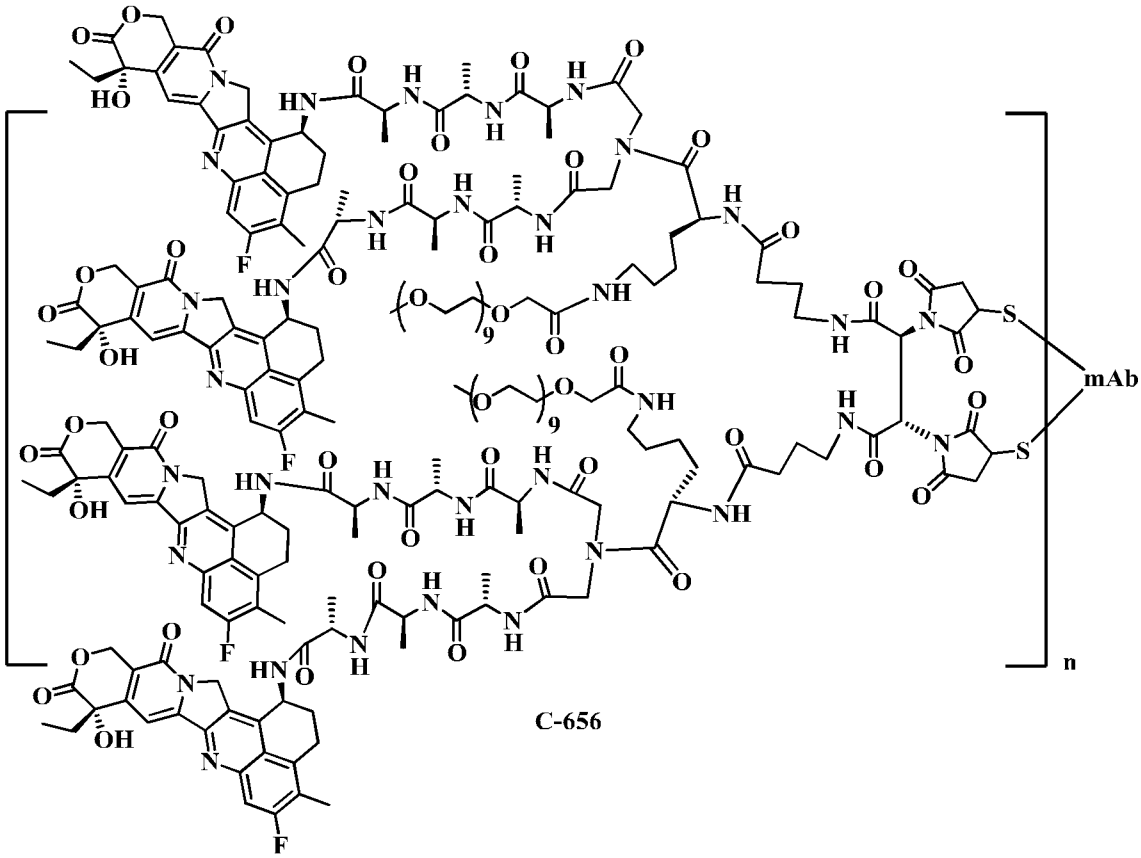
C-653



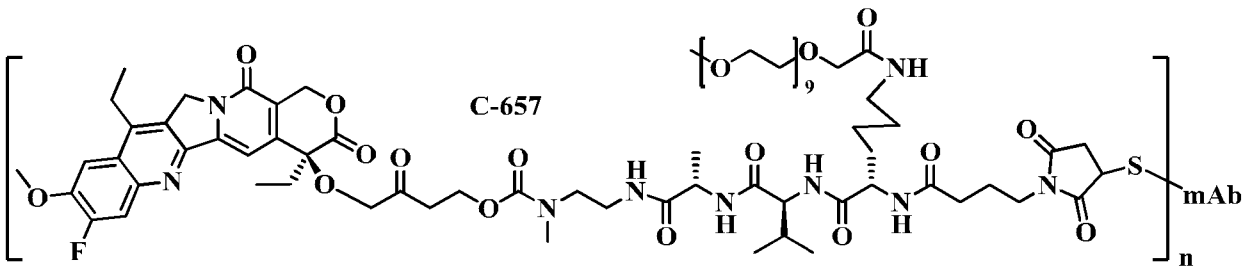
C-654



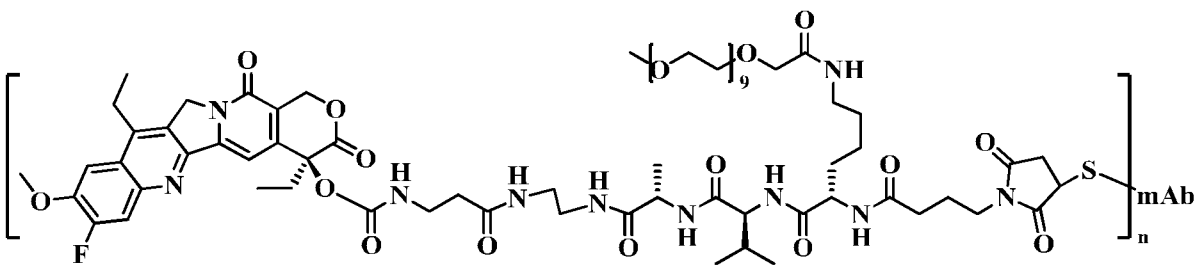
C-655



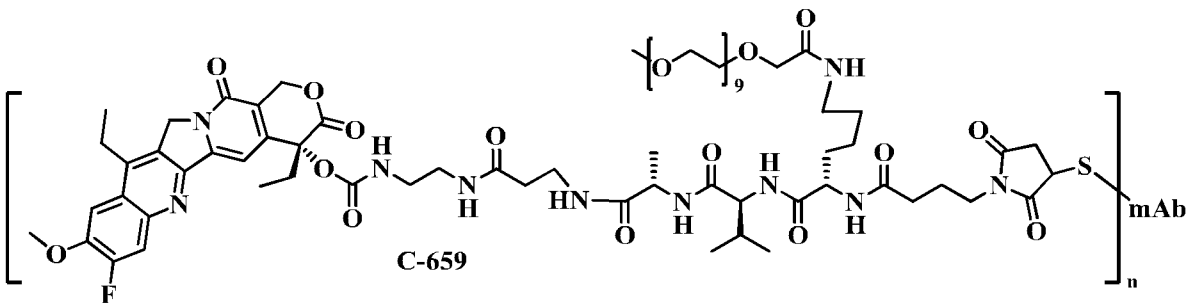
C-656



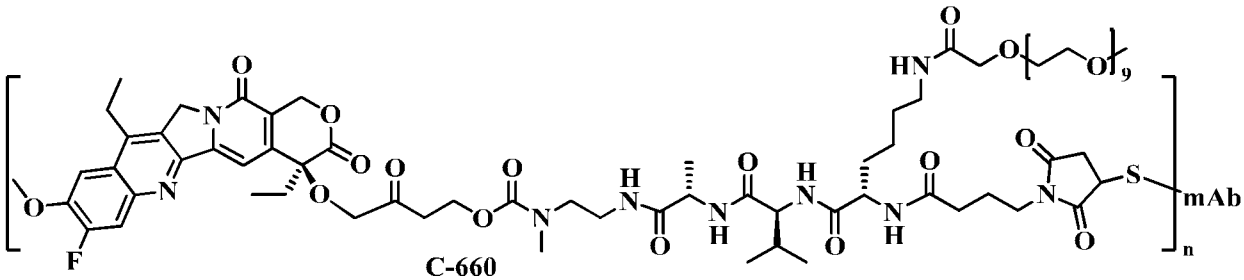
C-657



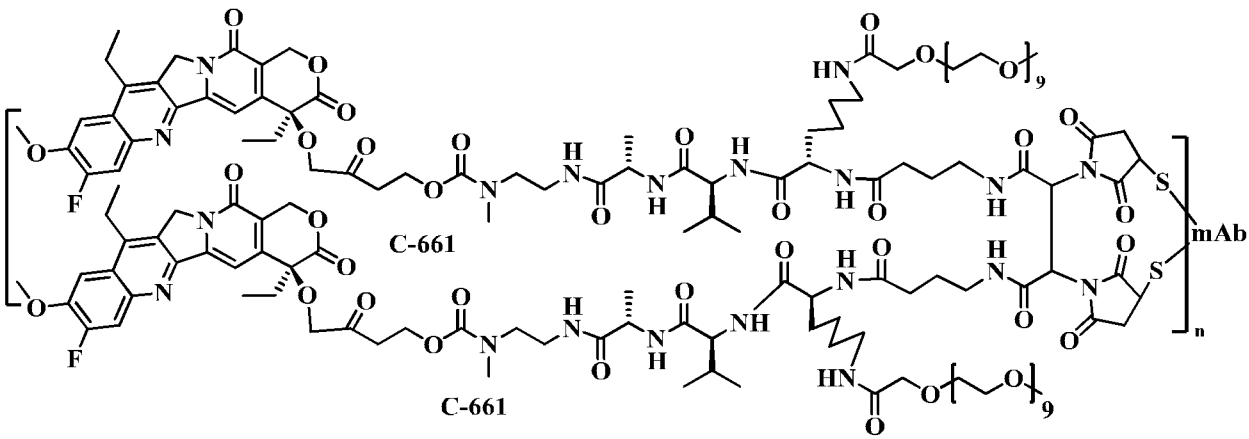
C-658



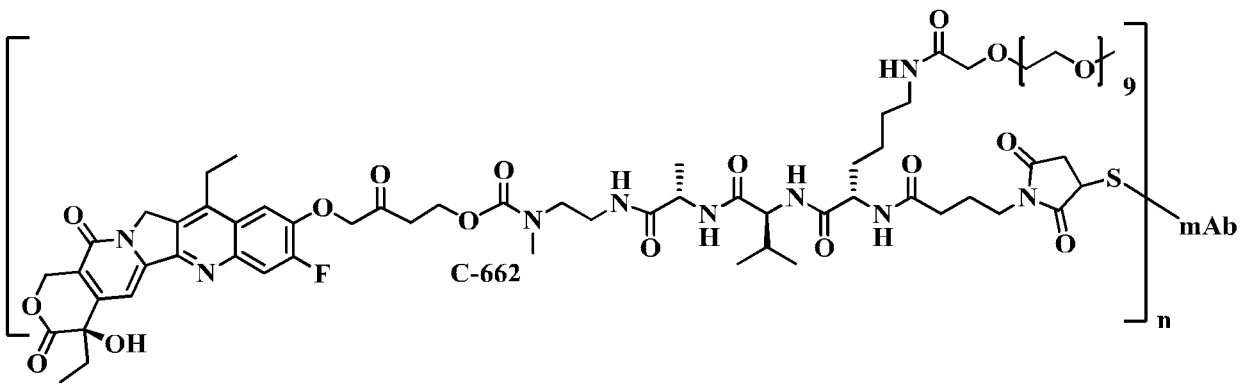
C-659



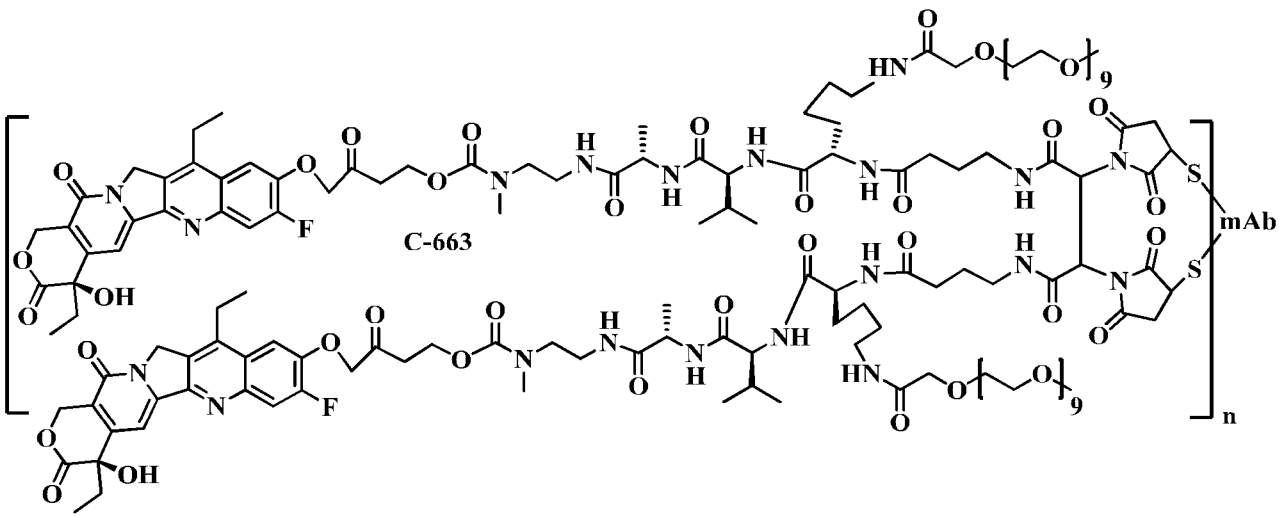
C-660



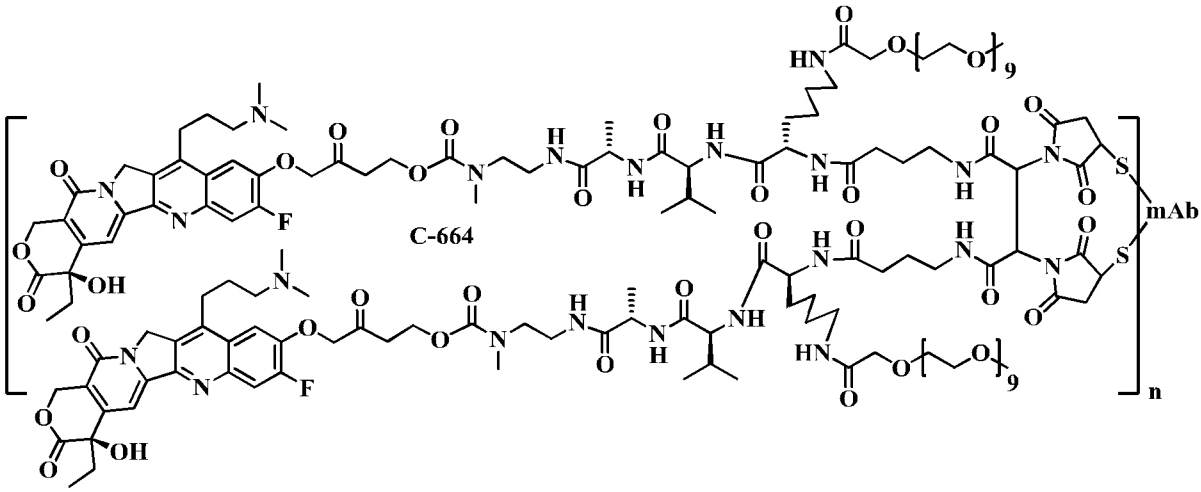
C-661



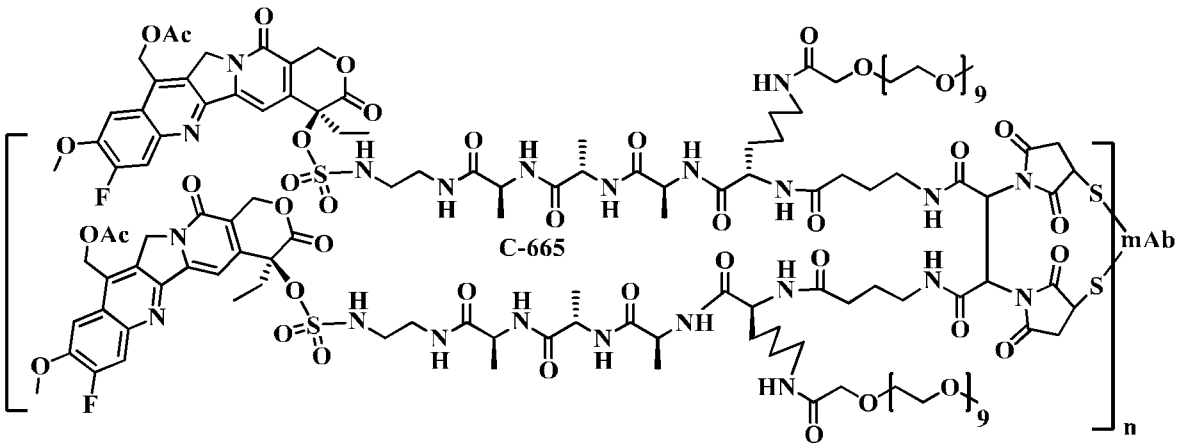
C-662



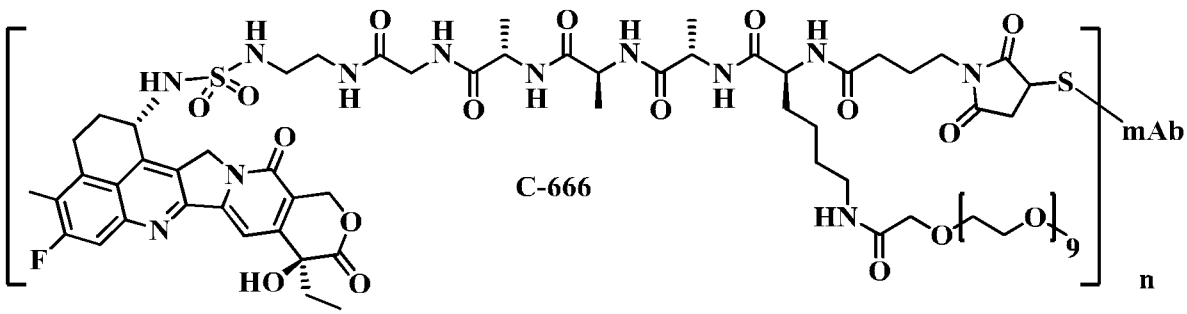
C-663



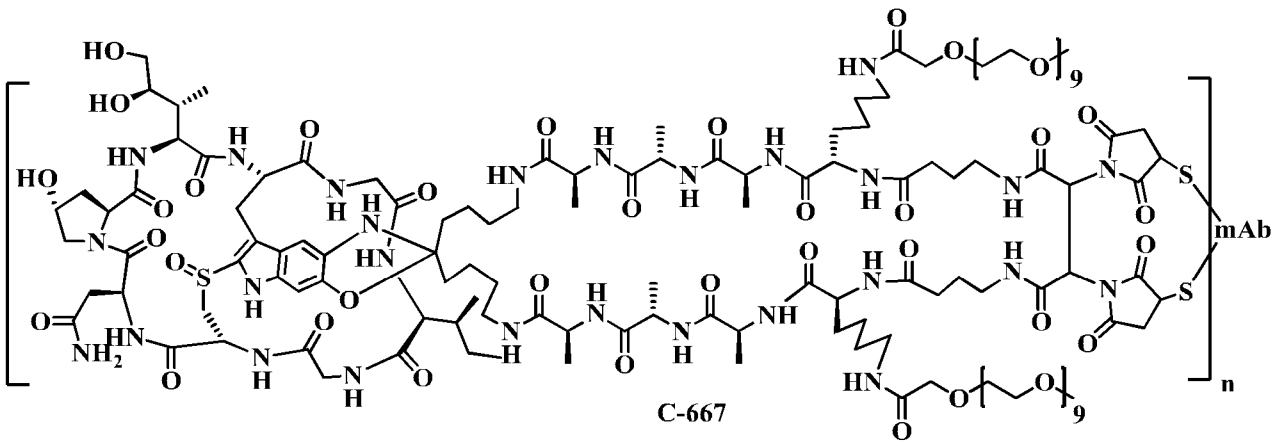
C-664



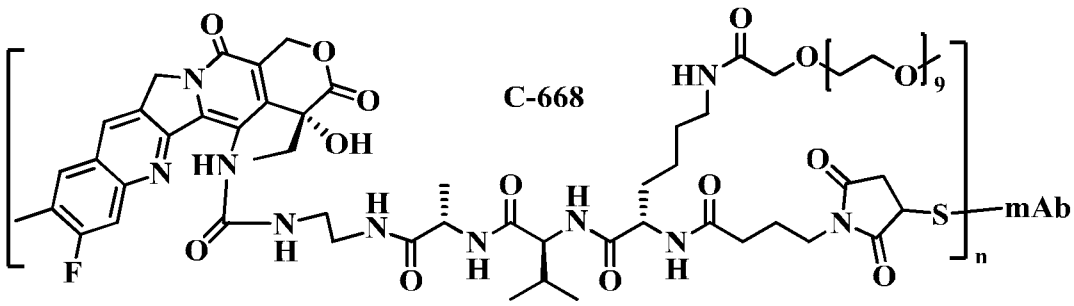
C-665



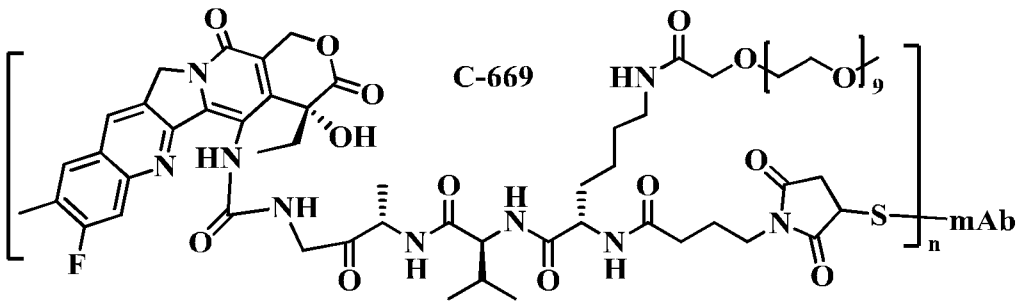
C-666



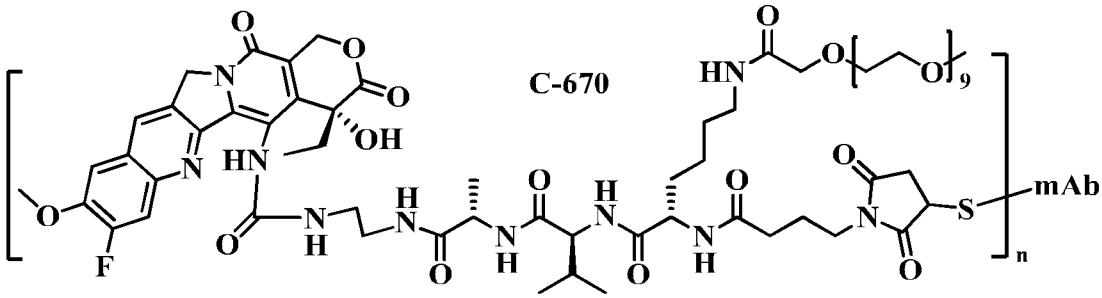
C-667



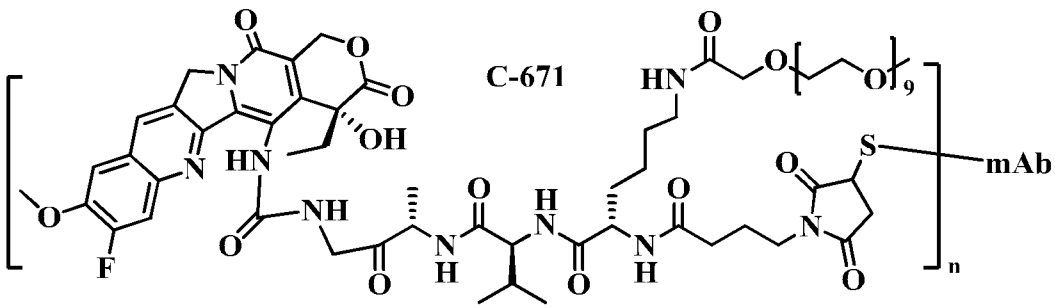
C-668



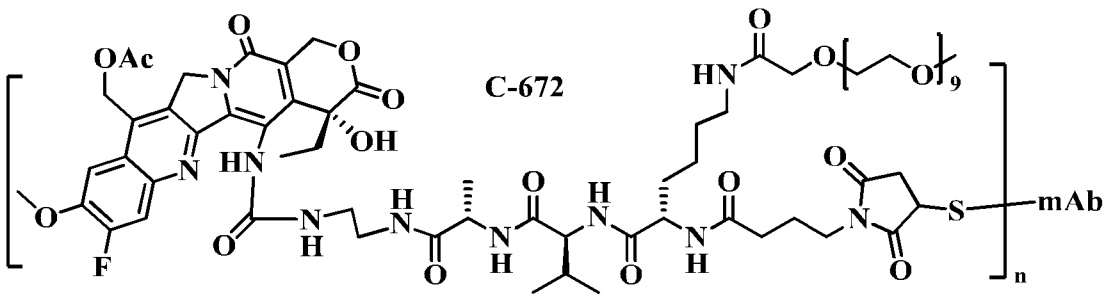
C-669



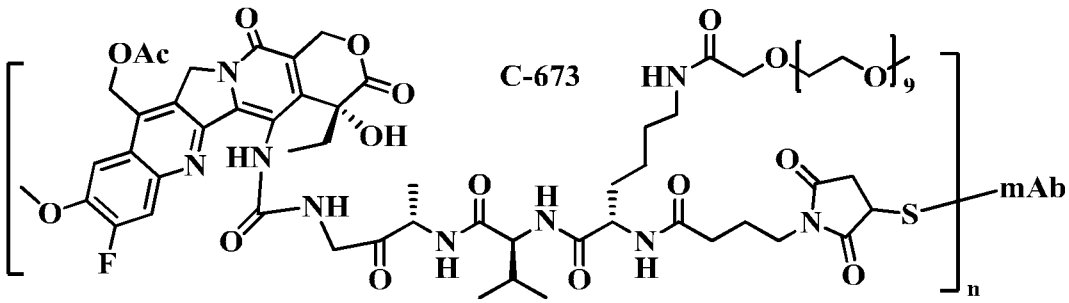
C-670

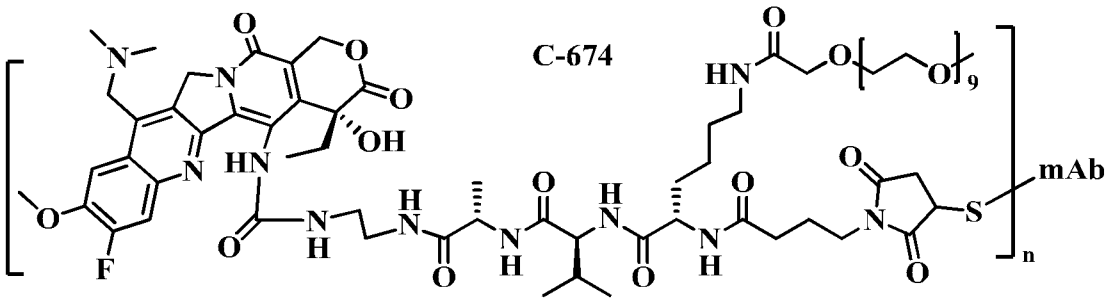


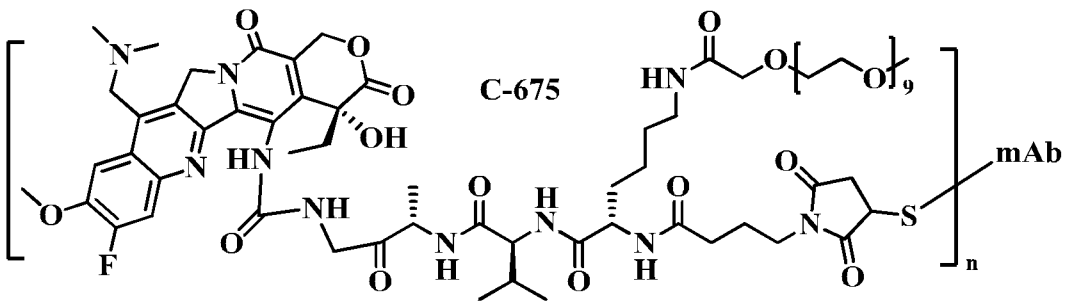
C-671

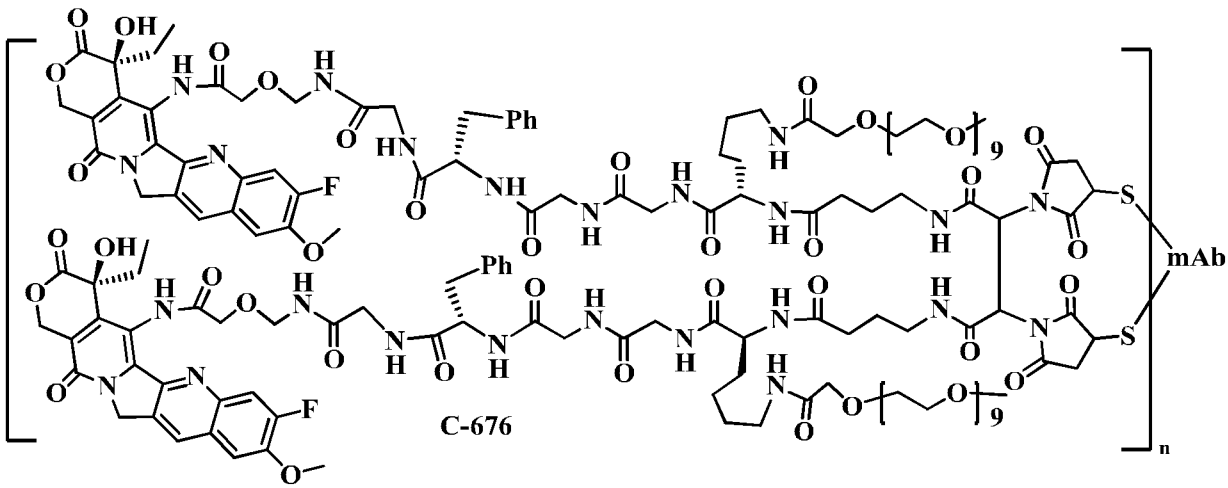


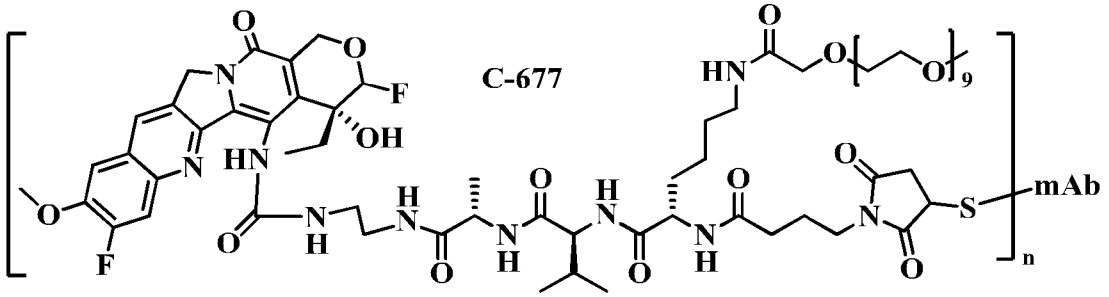
C-672

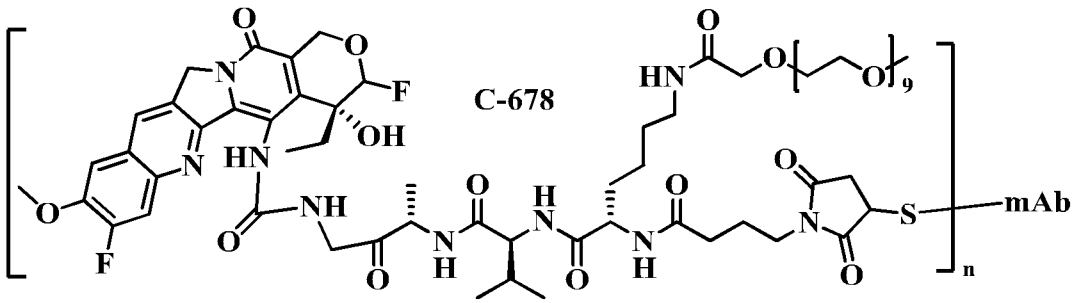

C-673
OAc
OH
HN
NH
HN
O
F
mAb
n


C-674
OH
HN
HN
O
F
mAb
n

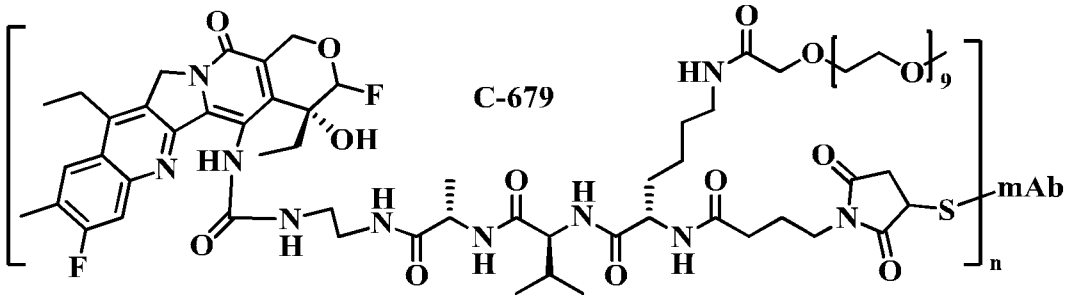

C-675
OH
HN
HN
O
F
mAb
n


C-676
OH
Ph
HN
NH
F
mAb
n

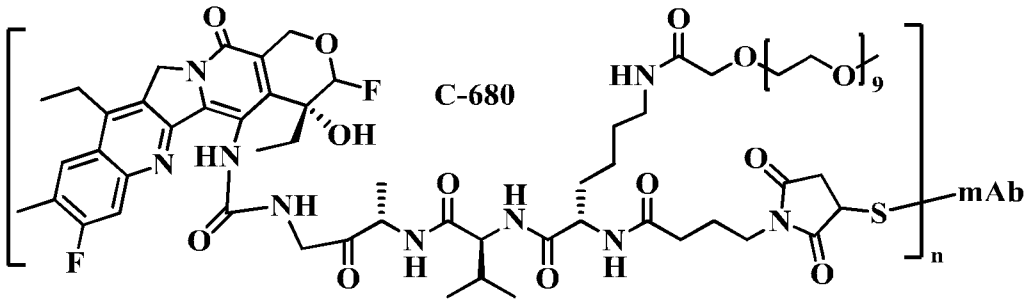

C-677
OH
HN
HN
O
F
mAb
n



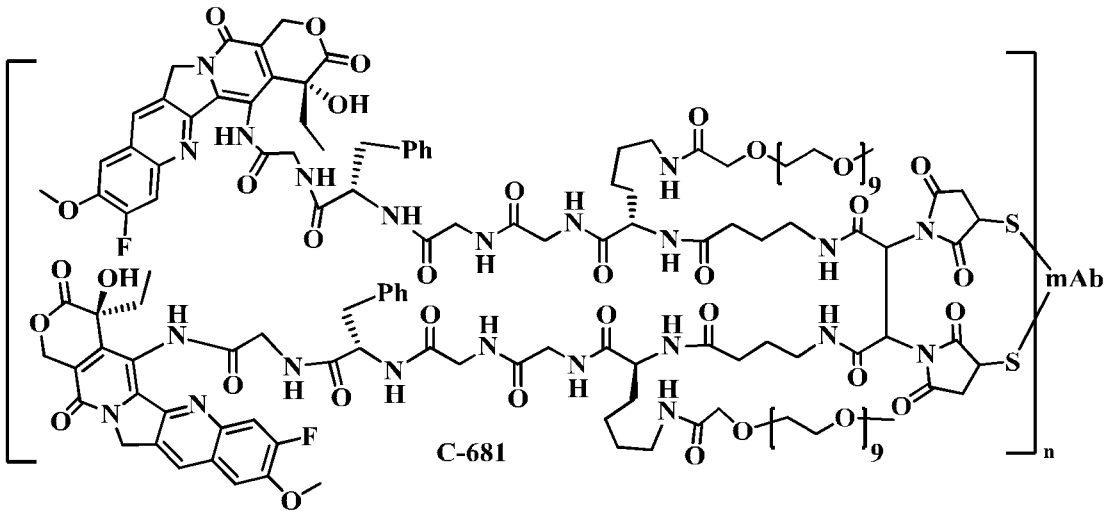
C-678



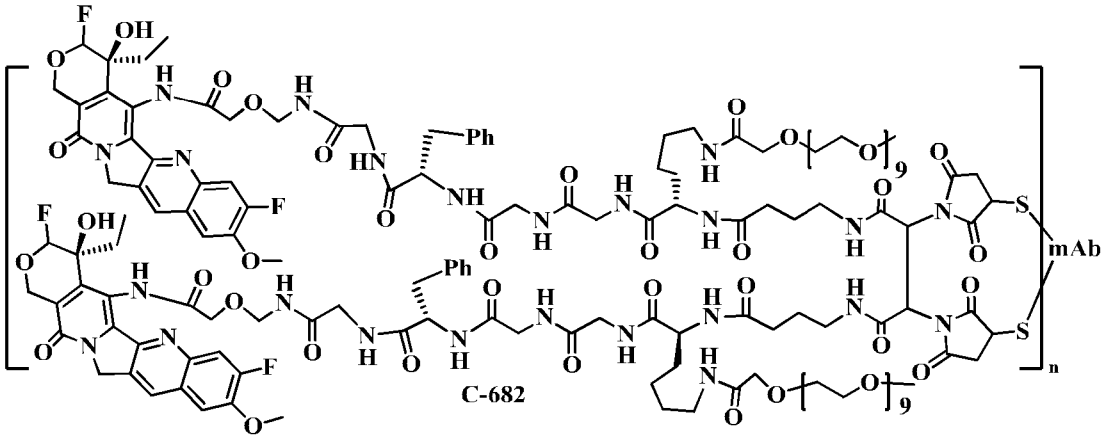
C-679



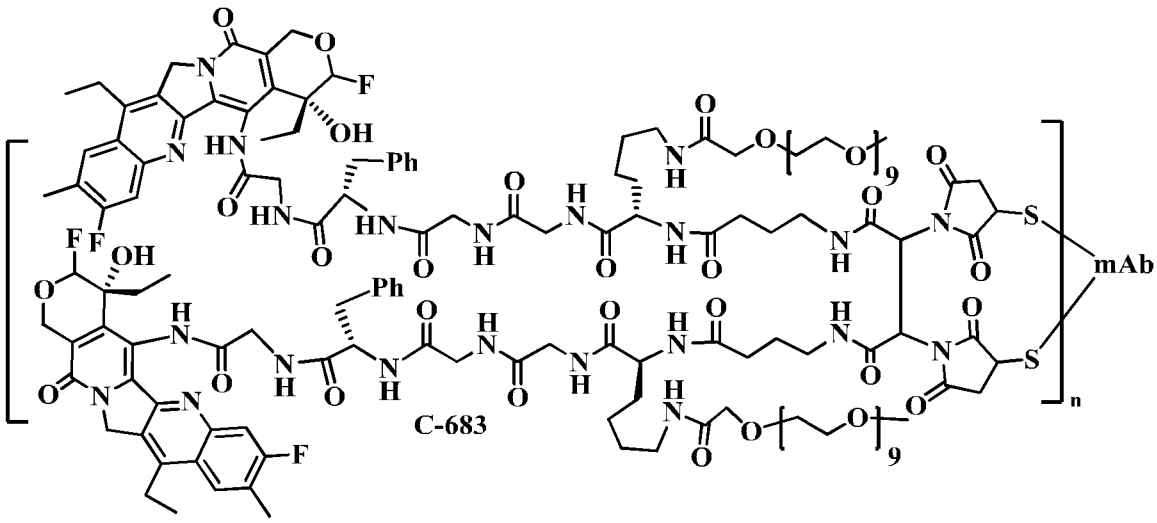
C-680

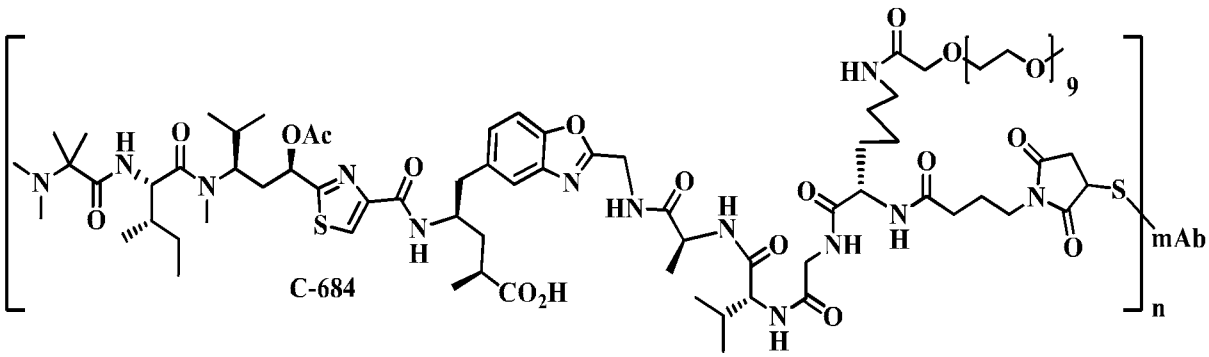


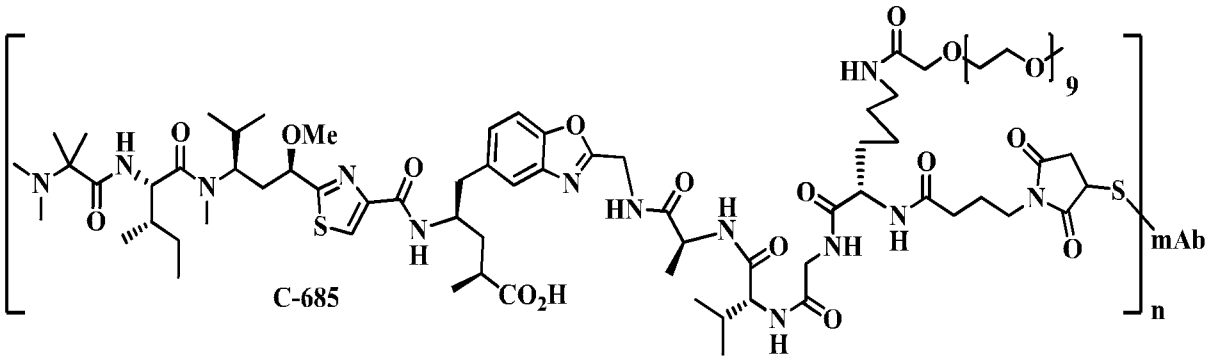
C-681

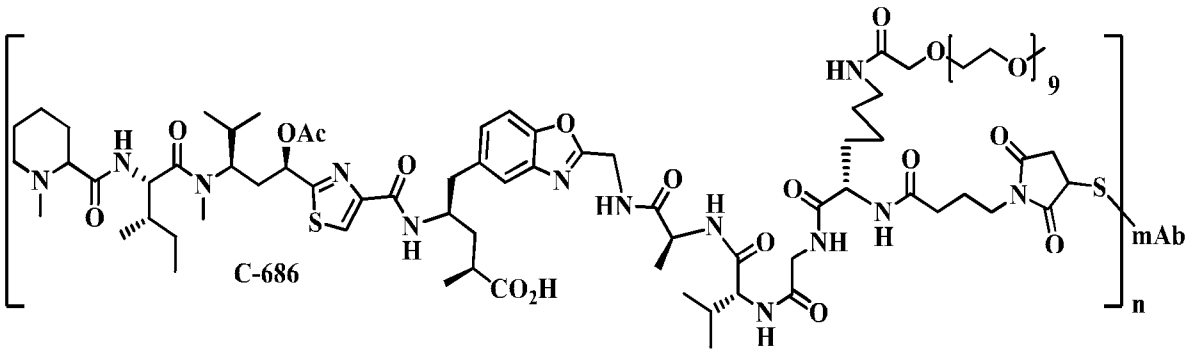


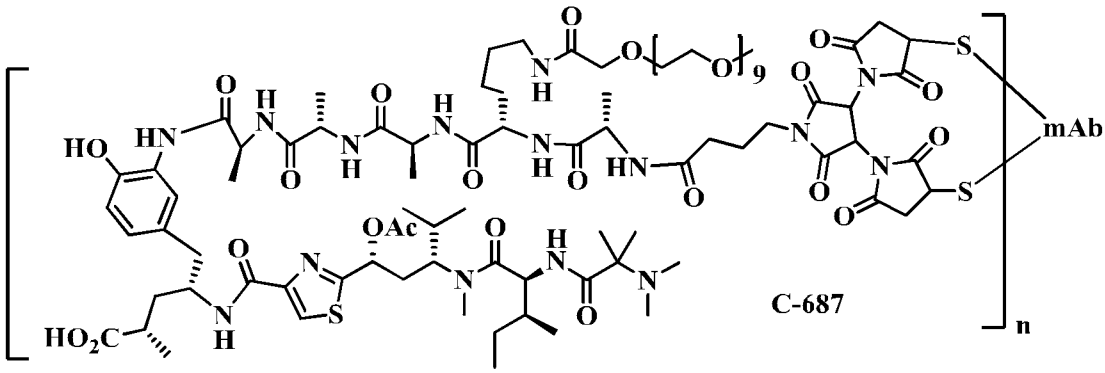
C-682


C-683
mAb
n

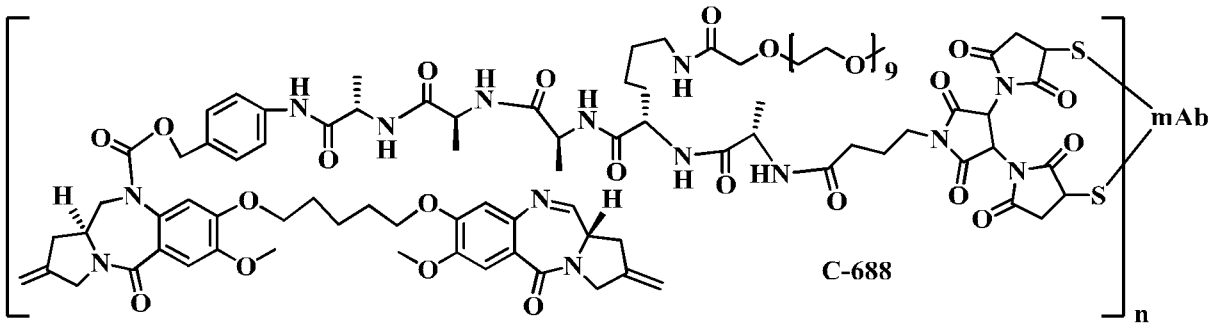

C-684
OAc
CO2H
mAb
n


C-685
OMe
CO2H
mAb
n

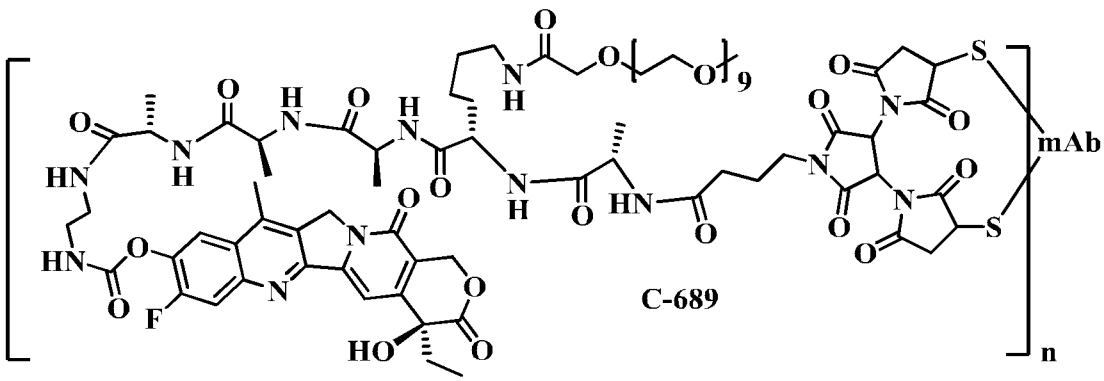

C-686
OAc
CO2H
mAb
n



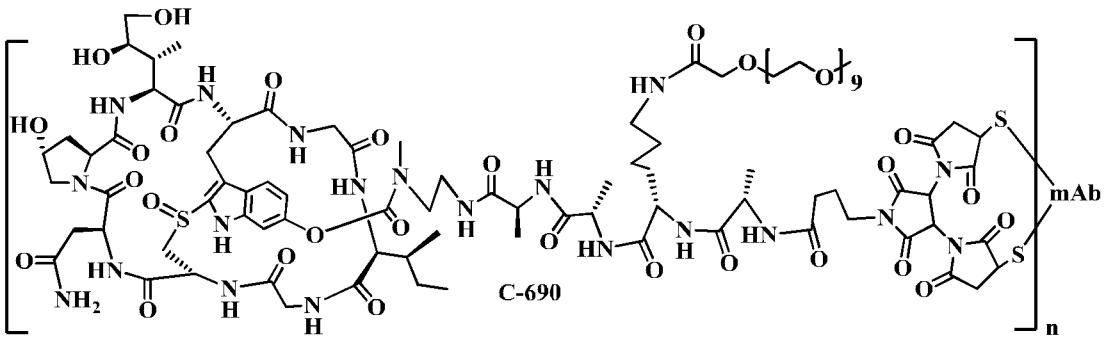
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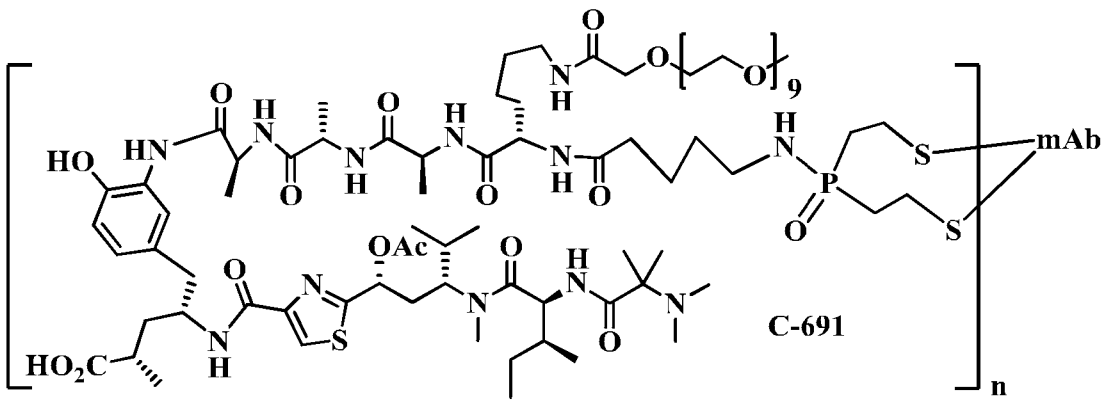
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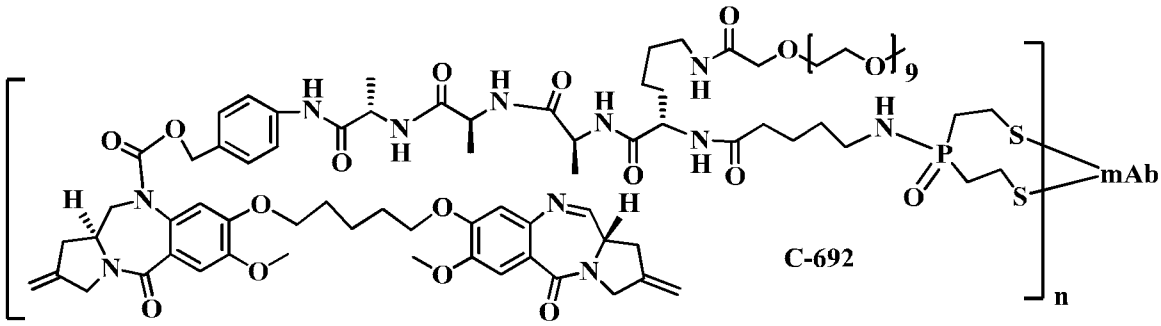
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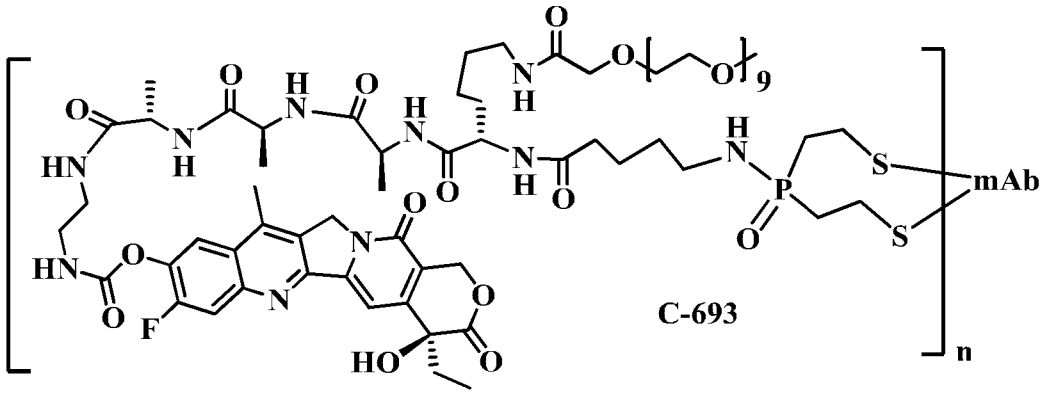
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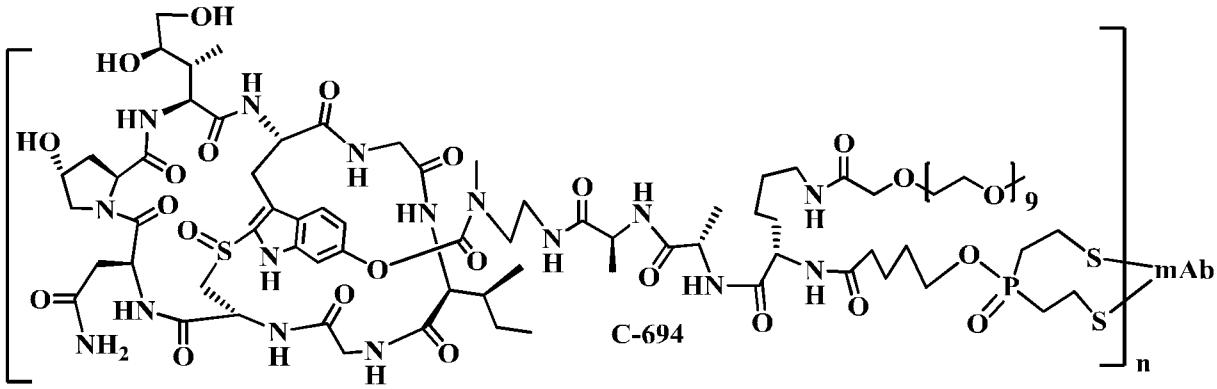
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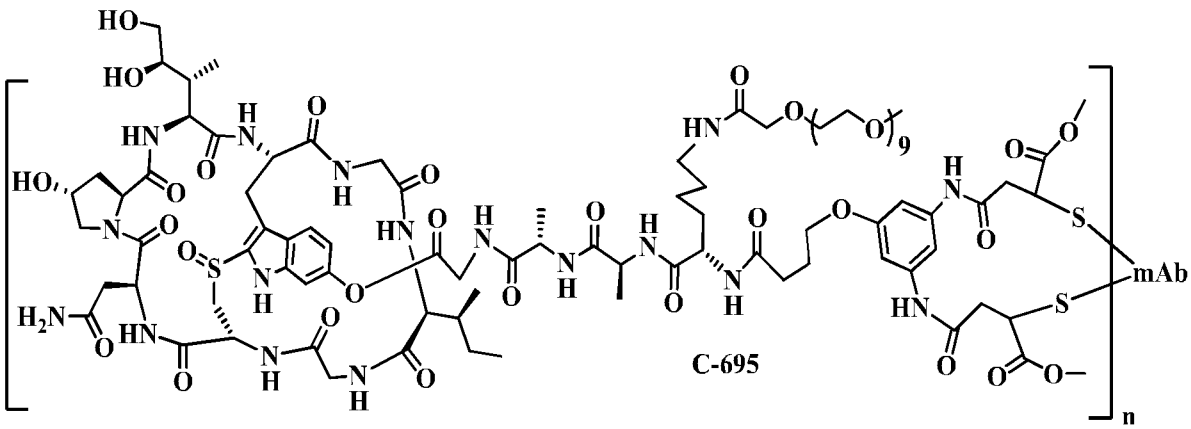
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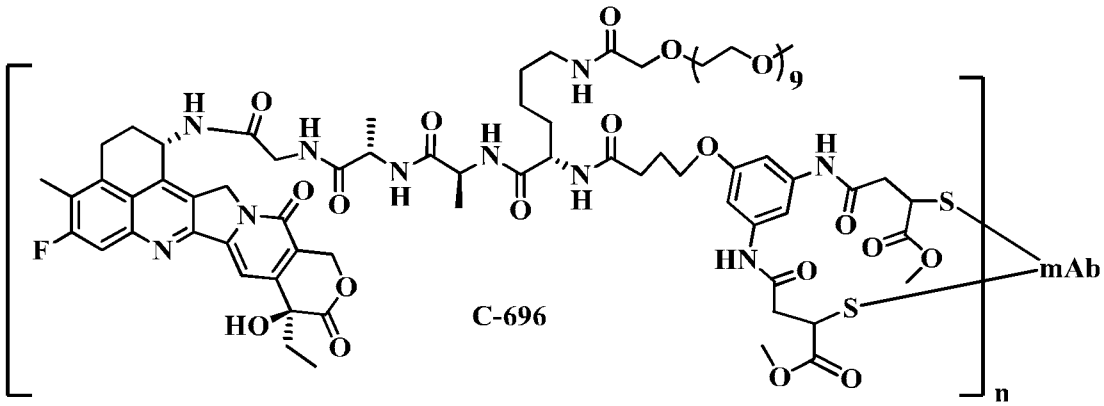
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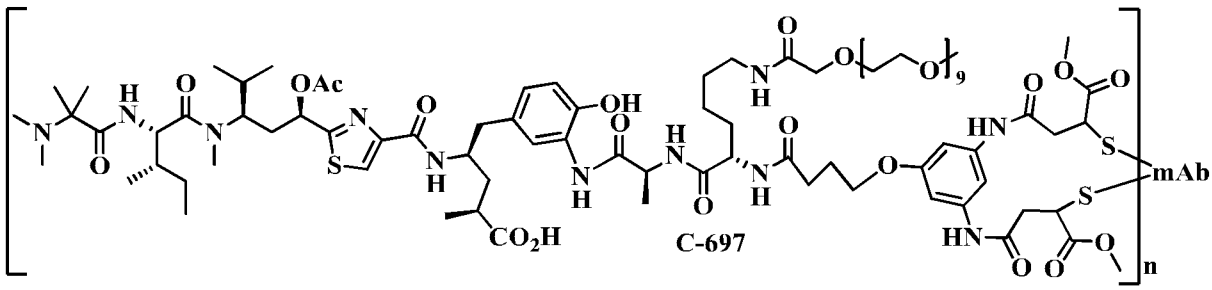
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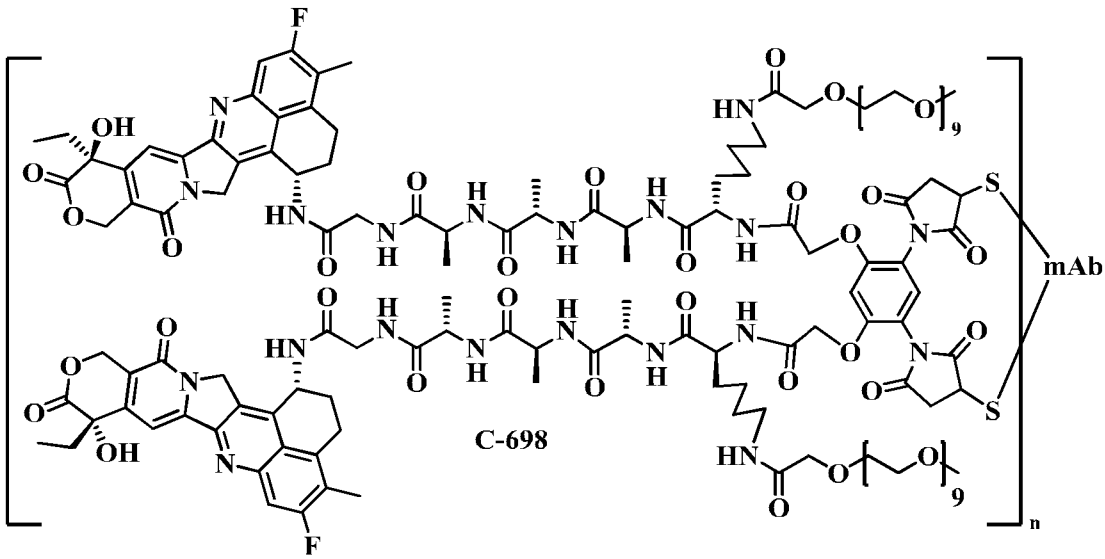
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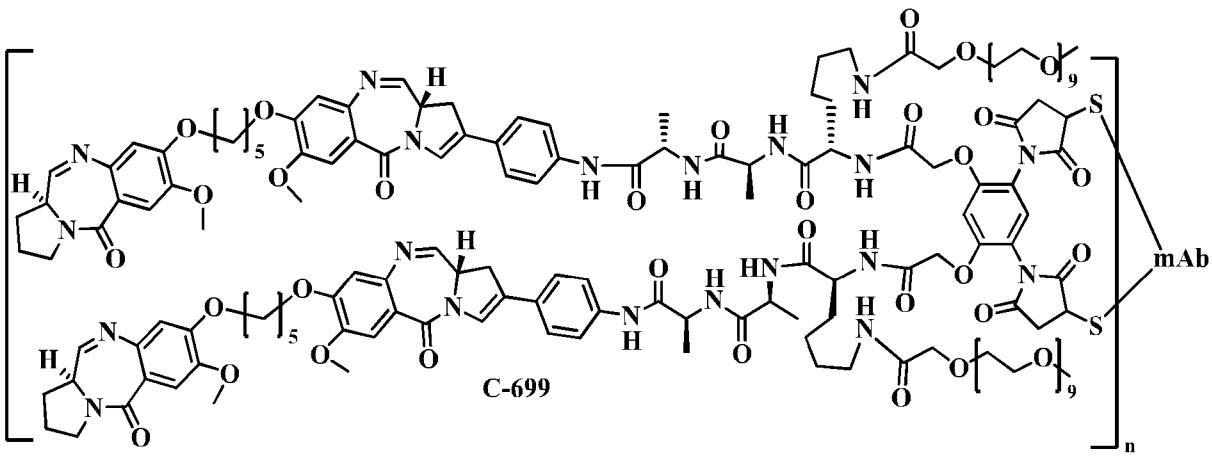
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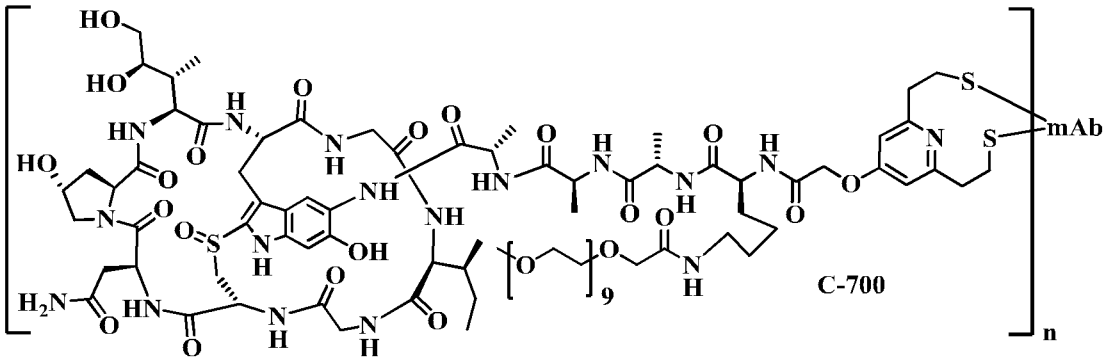
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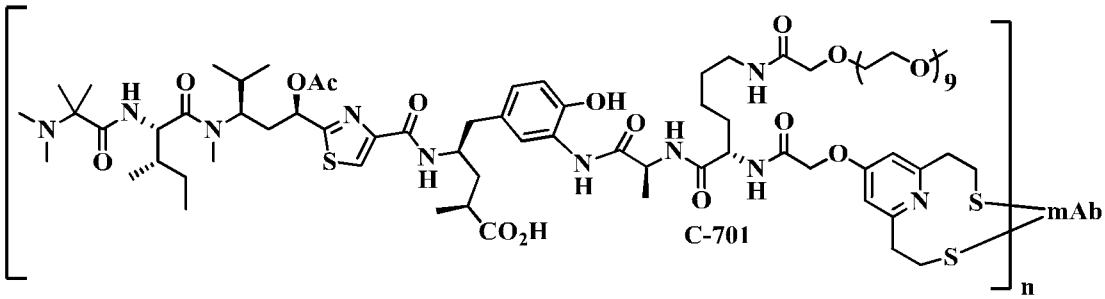
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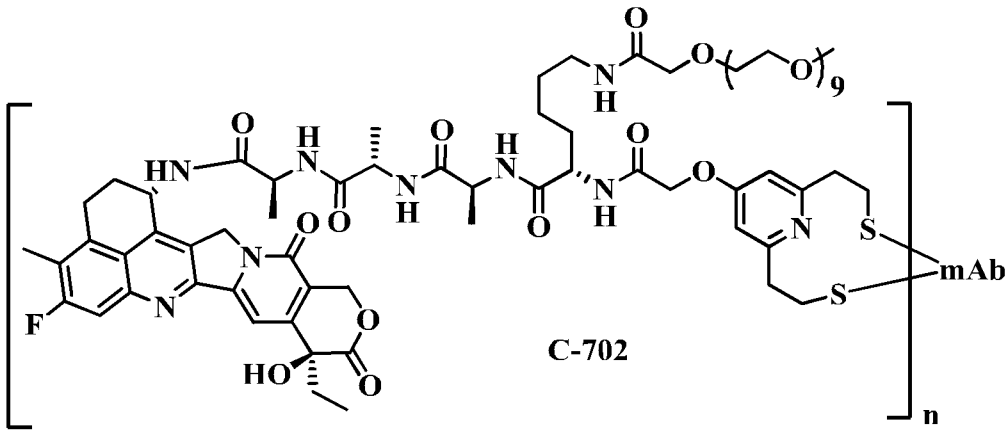
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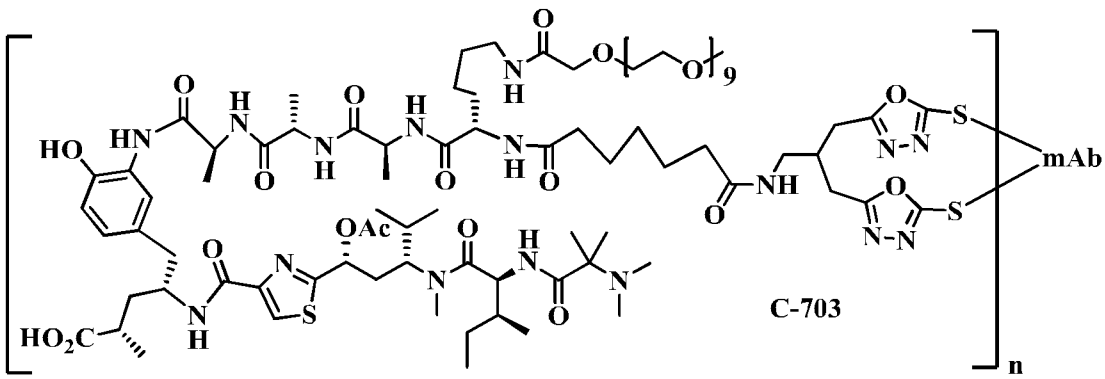
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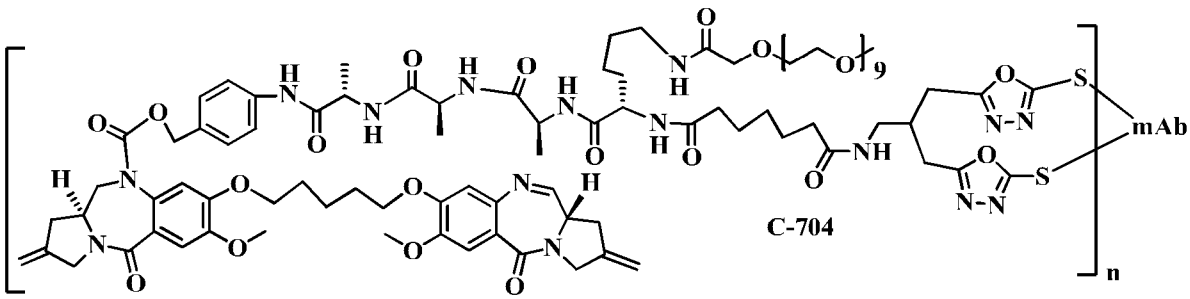
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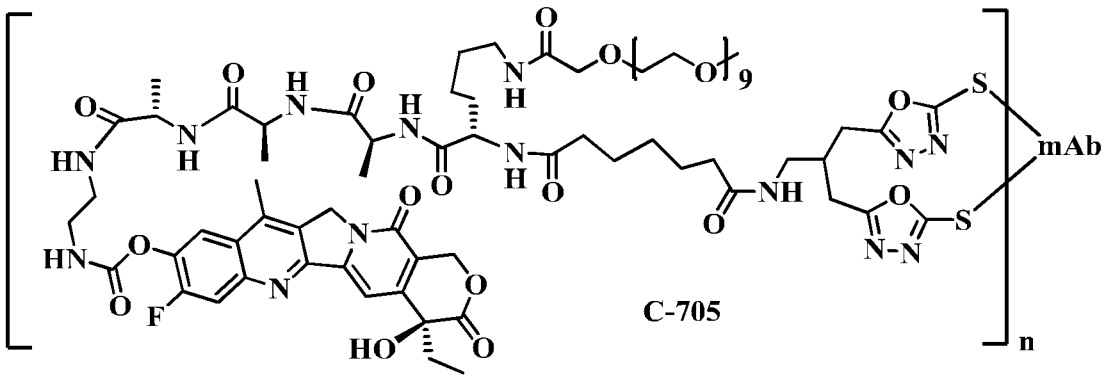
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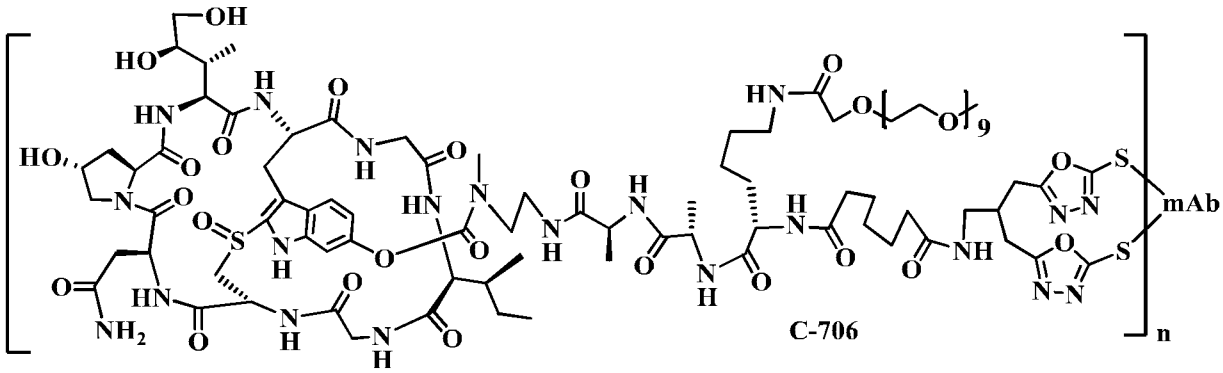
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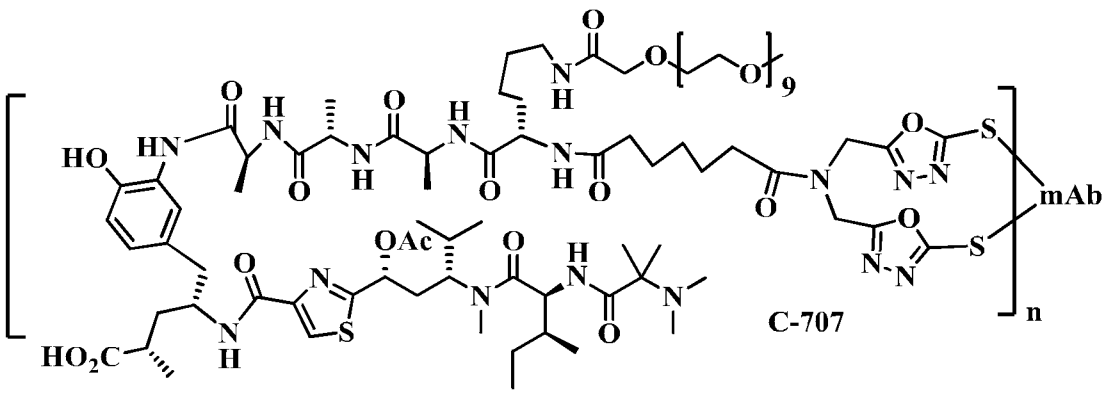
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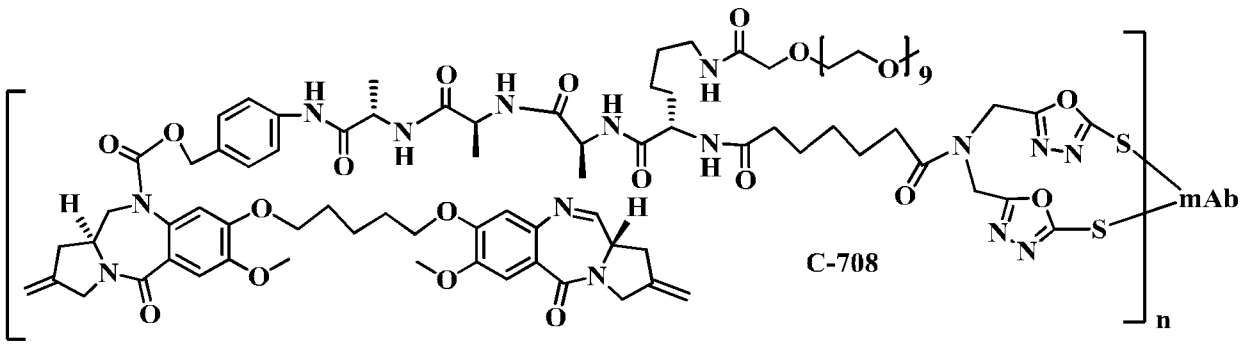
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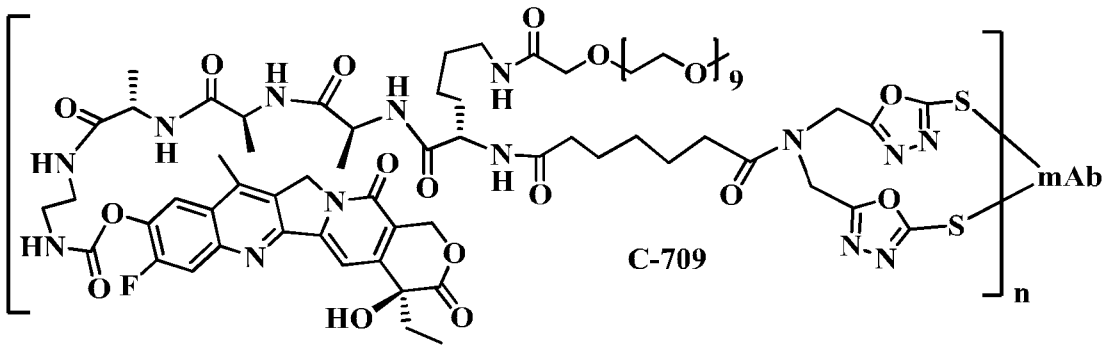
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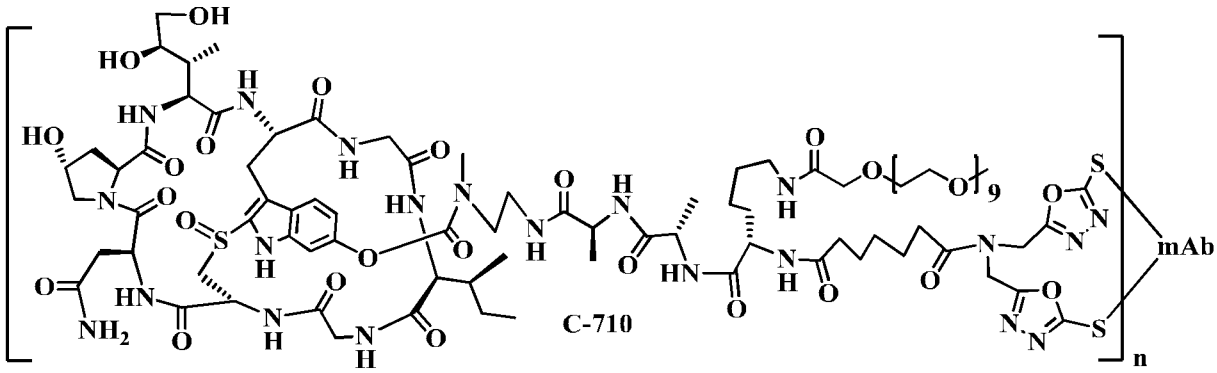
C-707



C-708

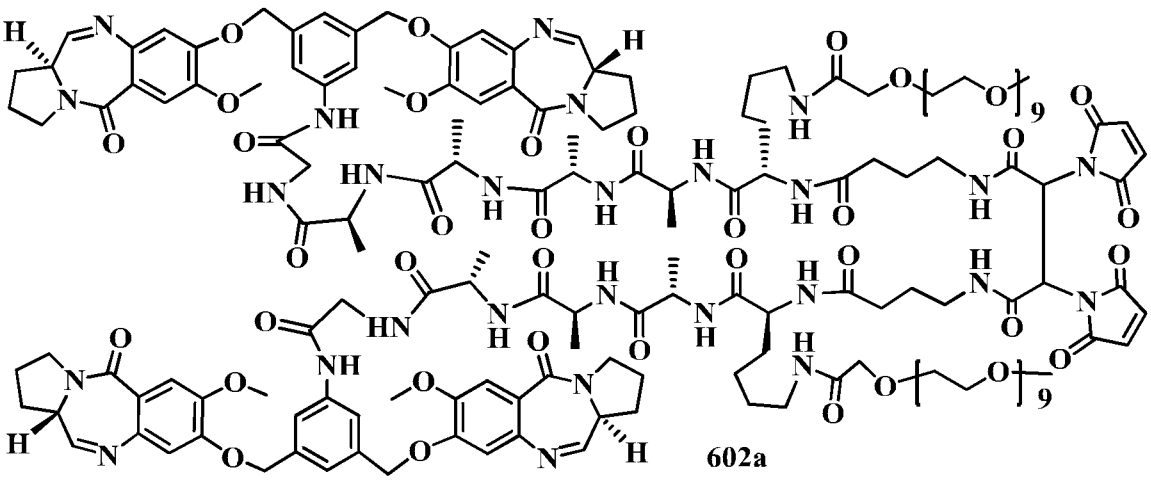


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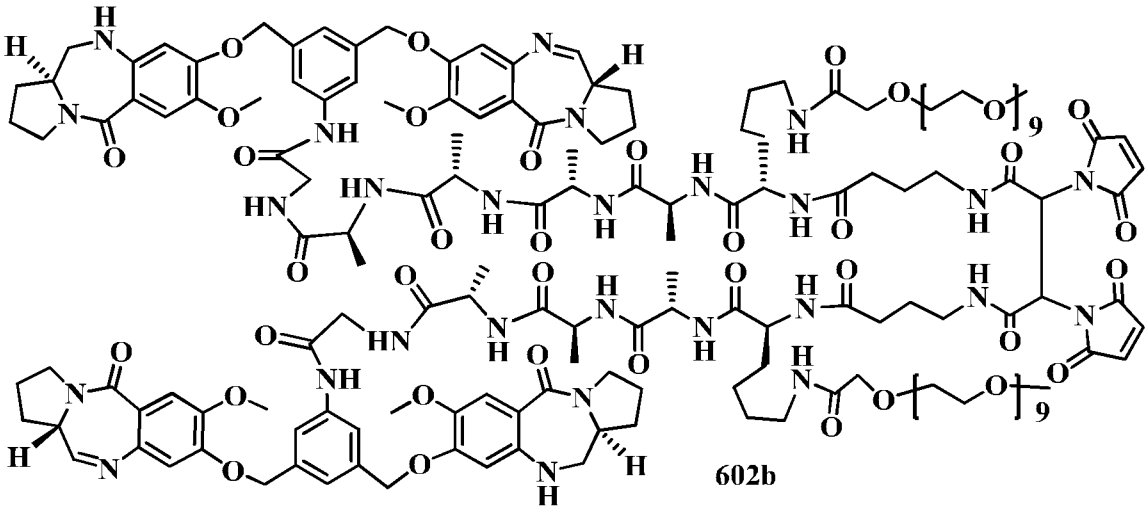


or one or more isotope of chemical elements, pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein n is 1 -10.

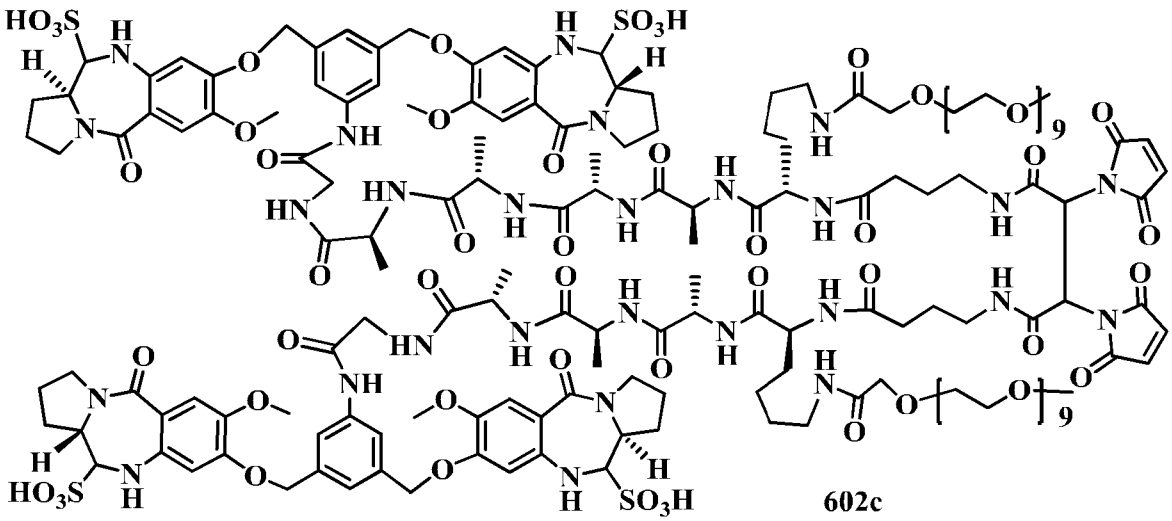
In other certain embodiments, examples of preferred drug/linker complex of formula (I), (II), (III) and (IV) which can be used under the process of the invention to achieve over 75% of the total drugs linked to the sites of cysteines between heavy-light chains (Fabs) of an antibody are illustrated in the sections of Figures and Examples as compounds of 25, 30, 36, 45, 51, 58a, 68a, 68b, 68c, 72a, 72b, 73, 83, 88, 91, 96, 102, 115, 120, 121, 126, 130, 137, 140, 152, 157, 168, 178, 181a, 181b, 181c, 181d, 190, 192, 195, 202, 209, 215, 221, 221b, 227, 233, 241, 255, 267, 277, 283, 284, 290, 300, 306, 309, 310, 312, 319, 322, 326, 328, 330, 332, 334, 335, 336, 337, 338, 361, 362, 372, 373, 374, 375, 384, 385, 390, 396, 399, 400, 402, 406, 409, 416, 417, 427, 432, 437, 441, 450, 455, 462, 464, 470, 478, 481, 484, 487, 488, 494, 498, 499, 500, 501, 504, 508, 511, 514, 518, 519, 521, 527, 531, 532, 534, 537, 542, 543, 544, 546, 550, 553, 560, 561, 562, 565, 570, 573, 577, 581, 582, 587, 591, 595, 599. The other examples of preferred drug/linker complex of formula (I), (II), (III) and (IV) are shown below:



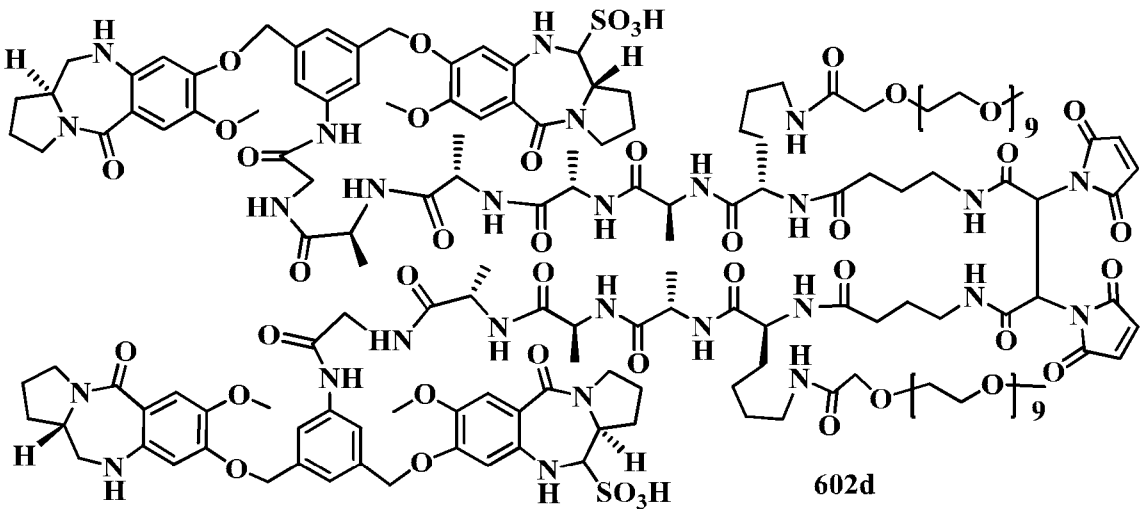
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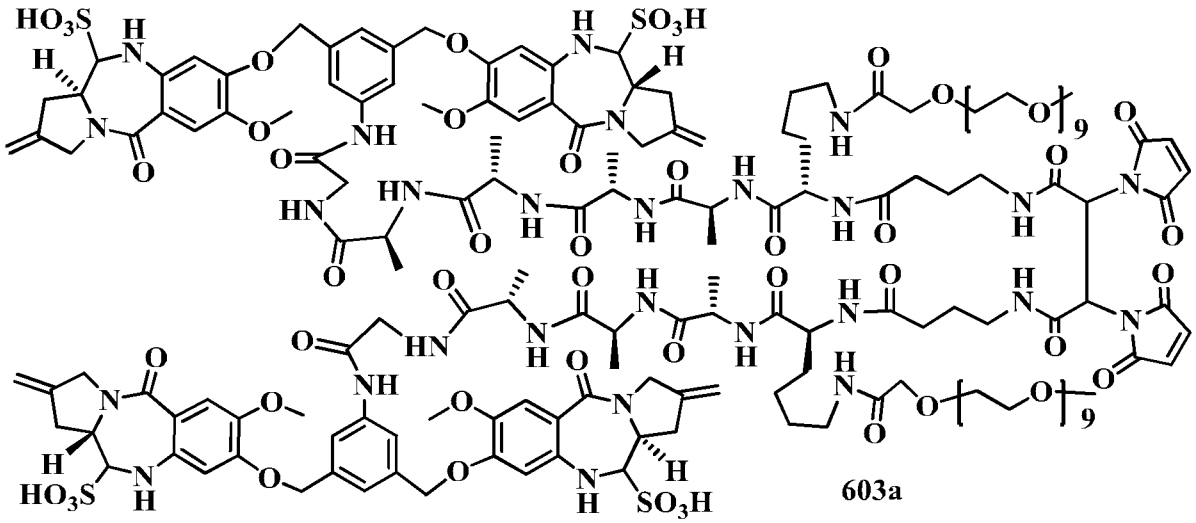
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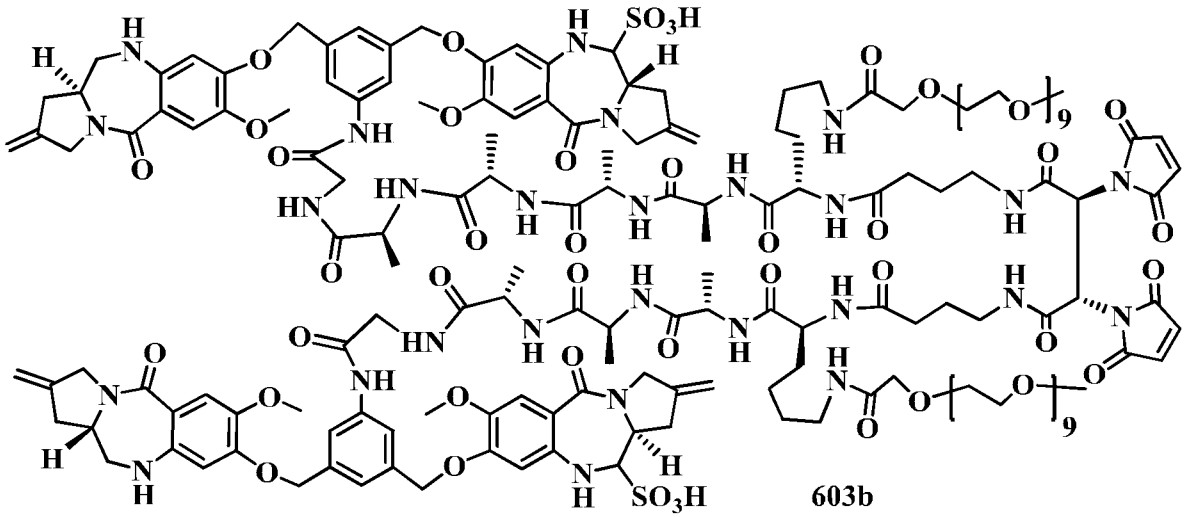
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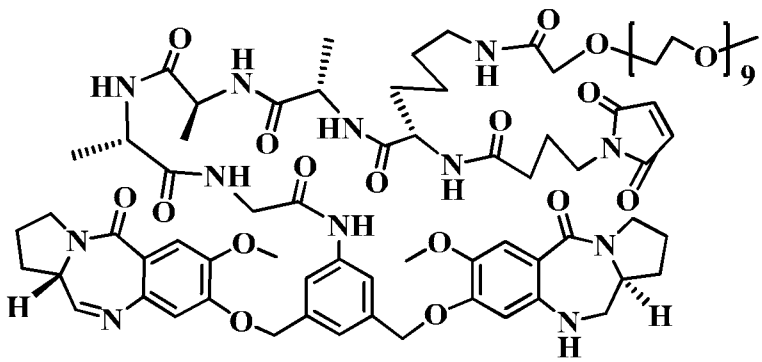
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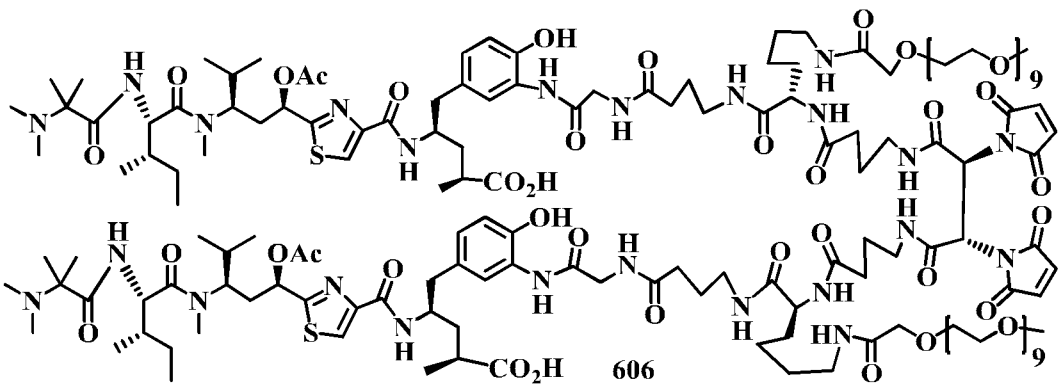
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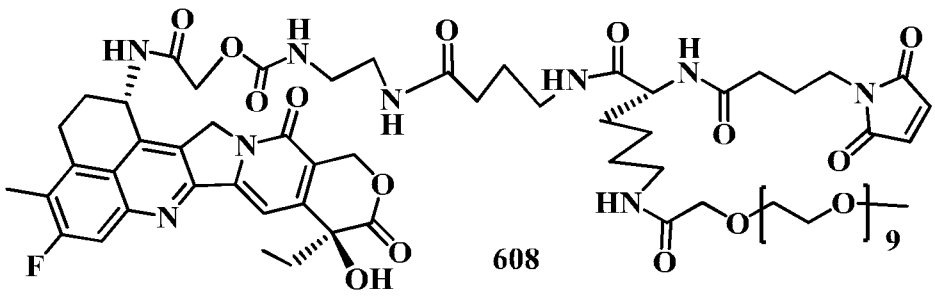
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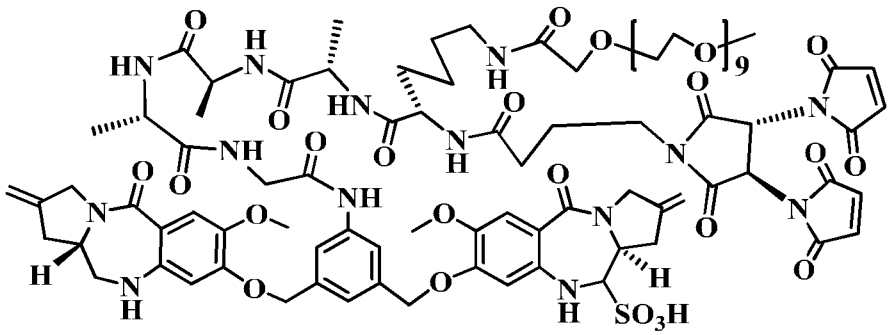


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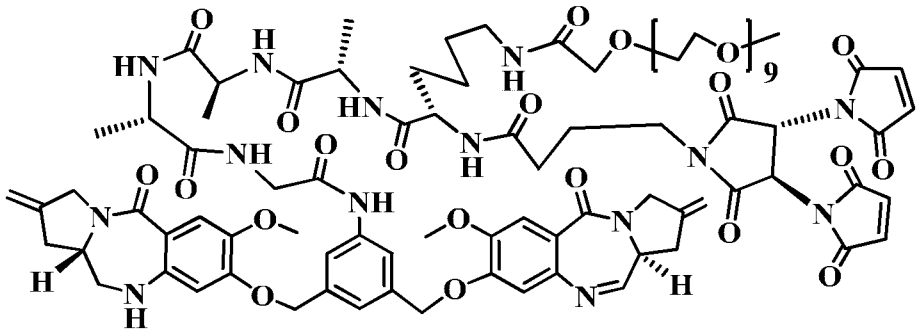


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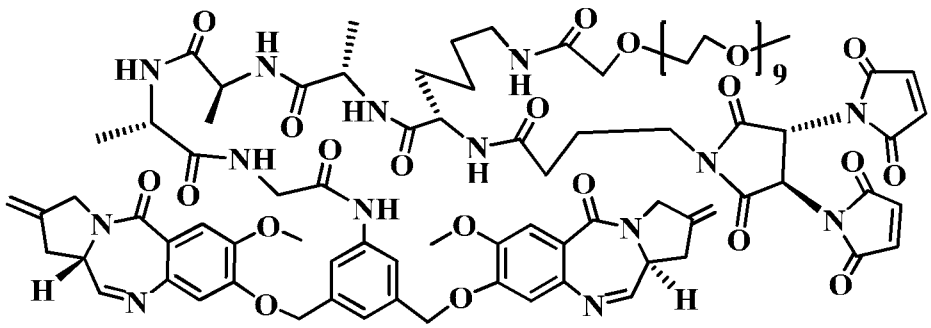
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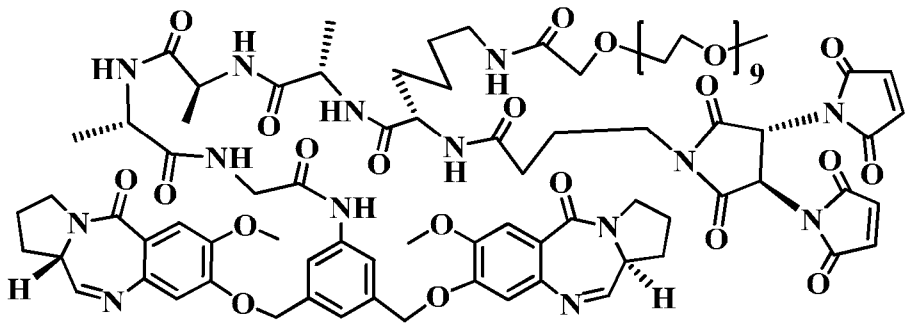
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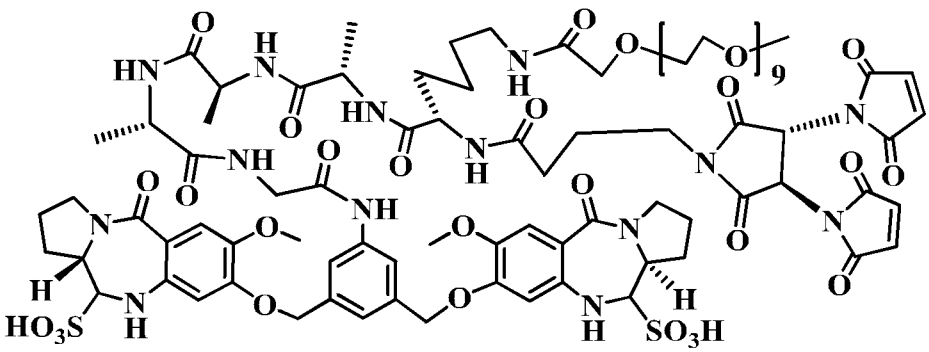
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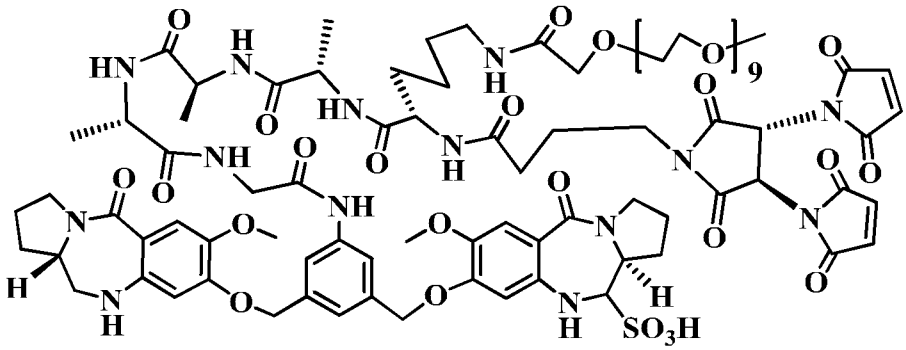
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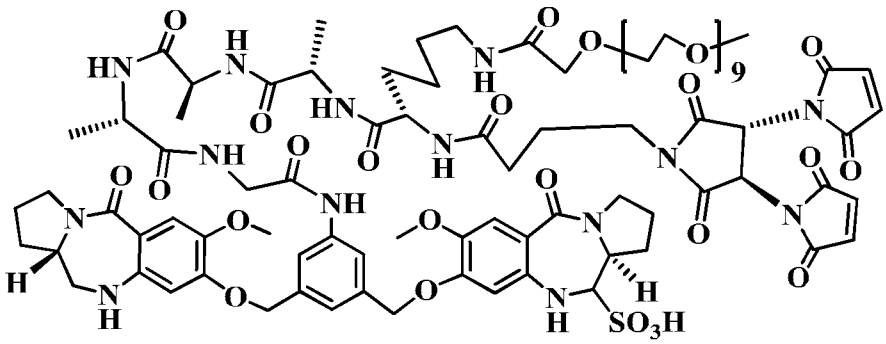
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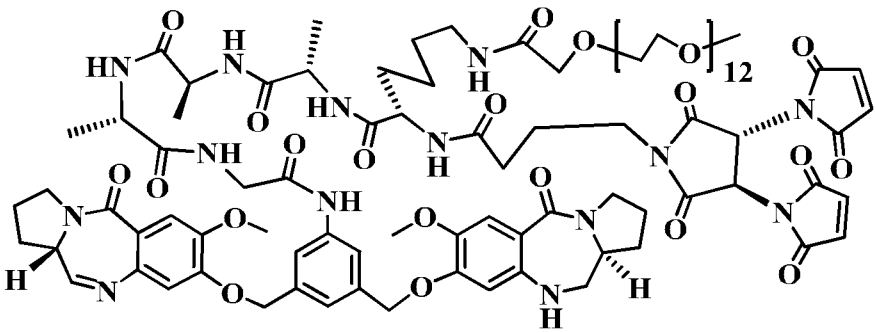
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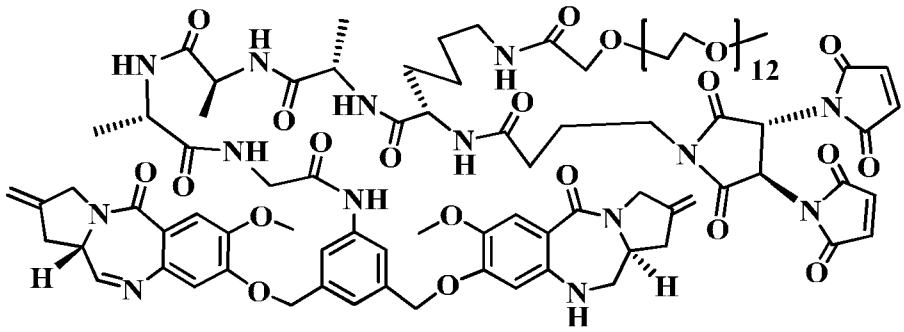
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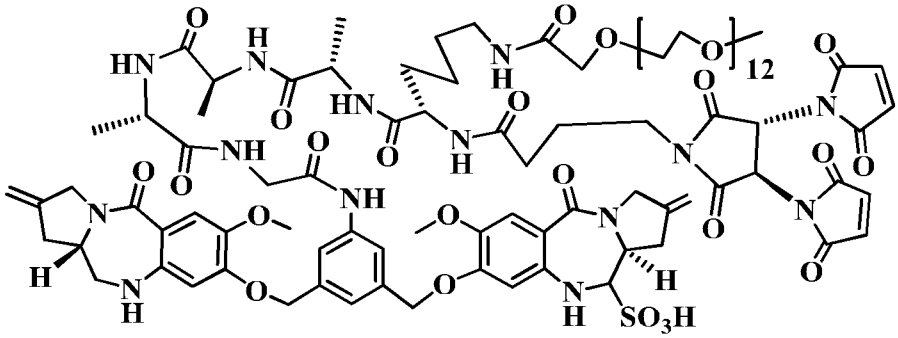
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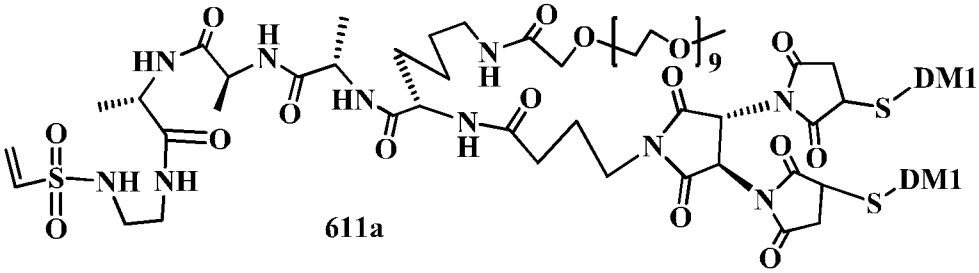
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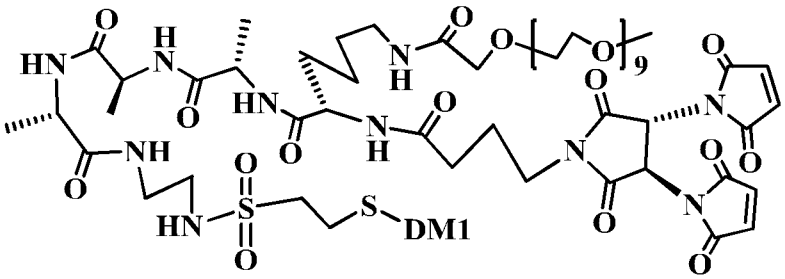


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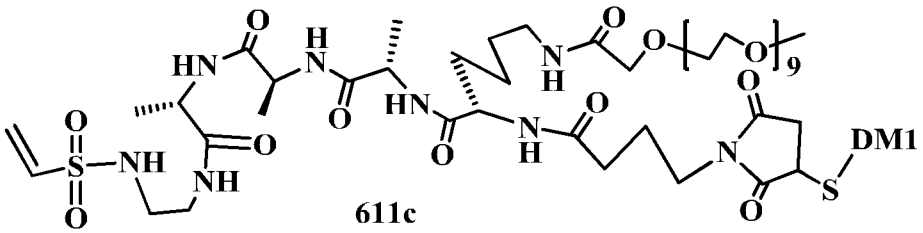
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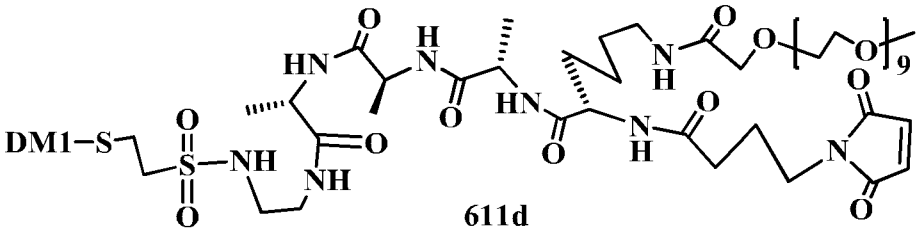
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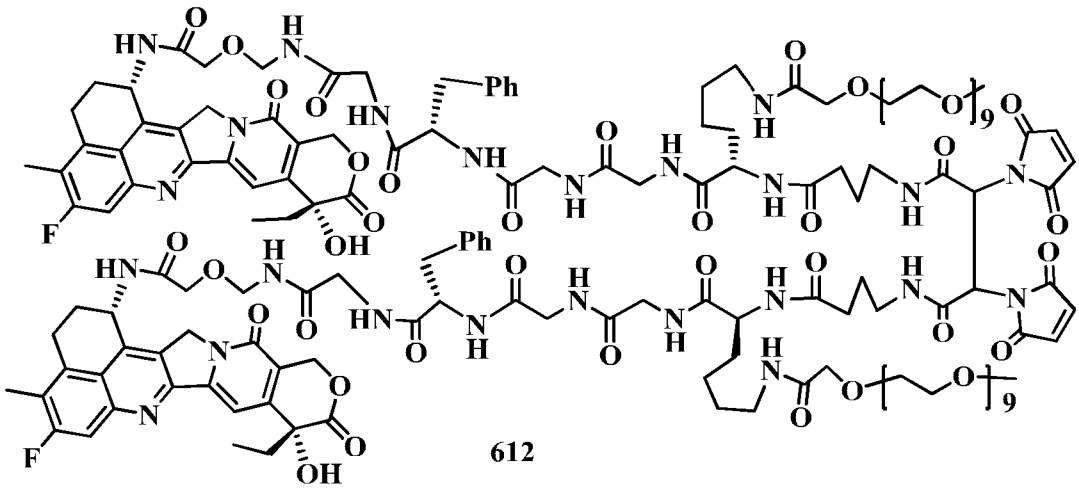


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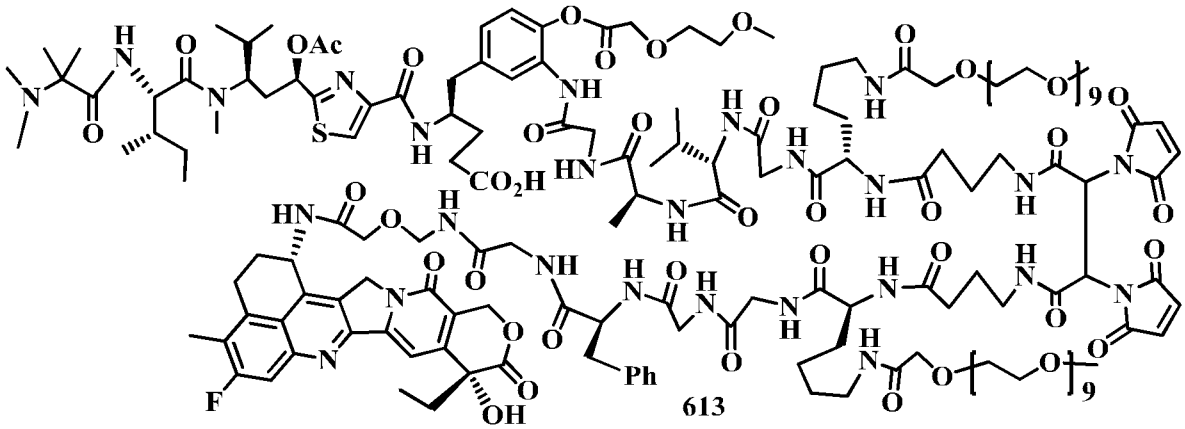
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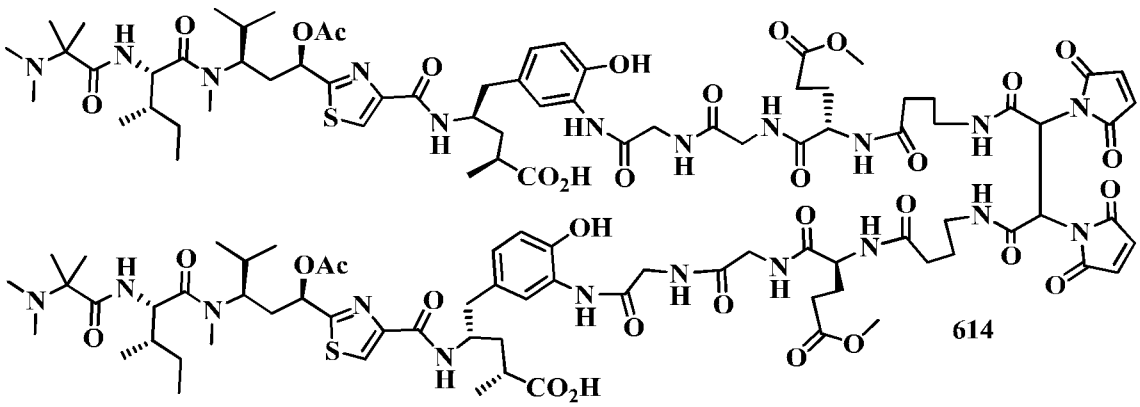
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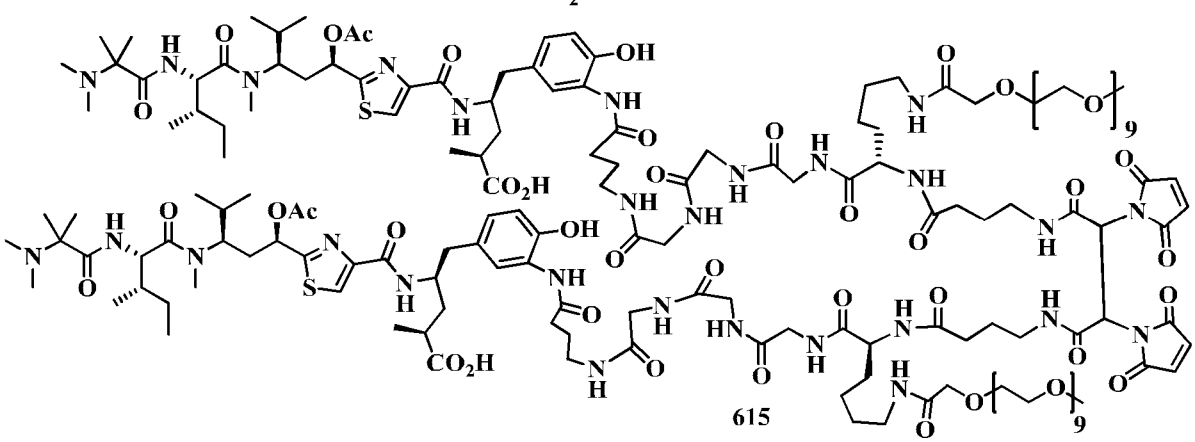
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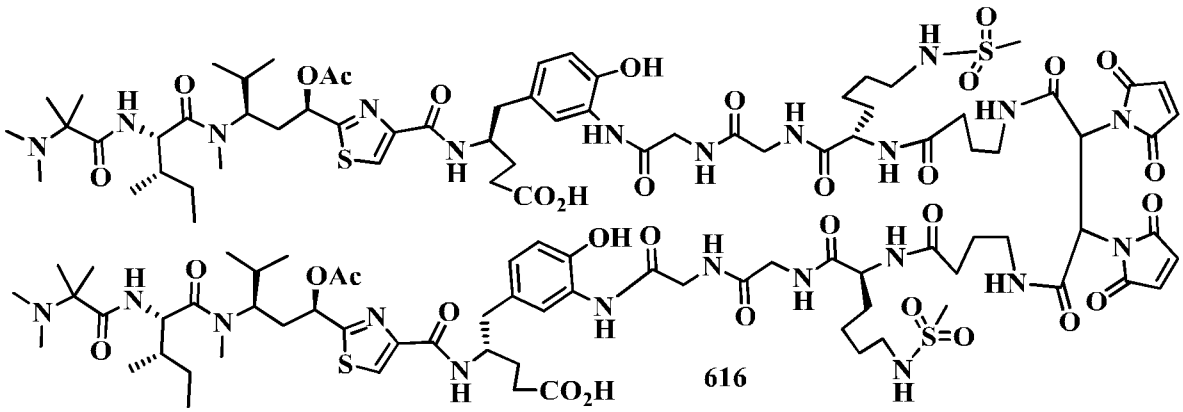
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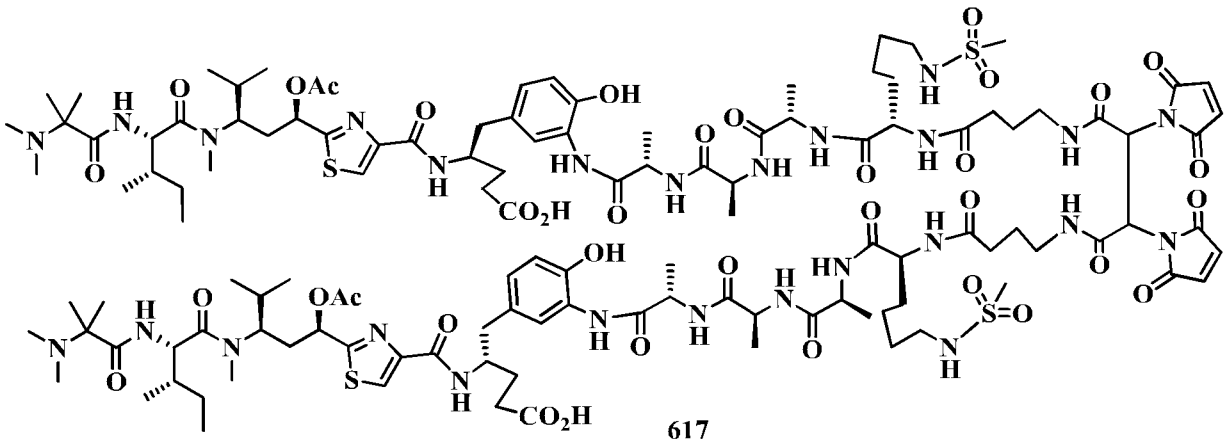
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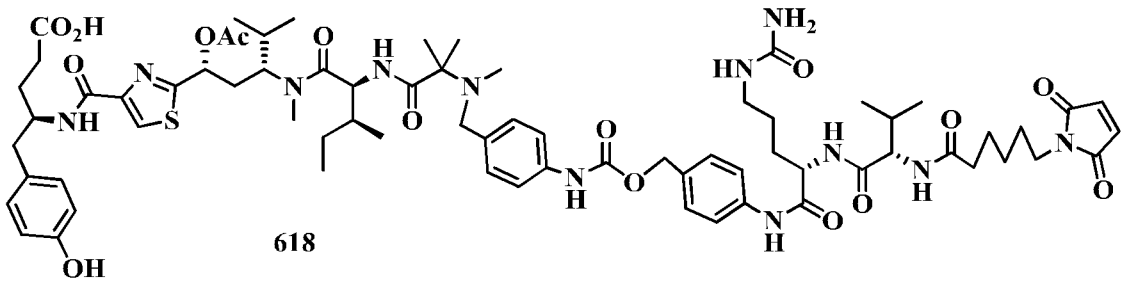
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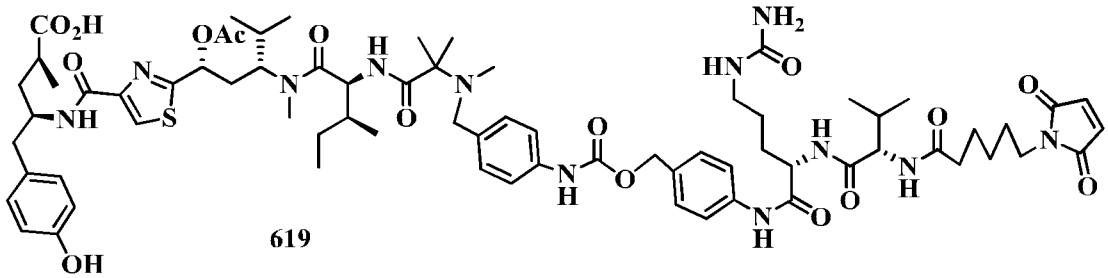
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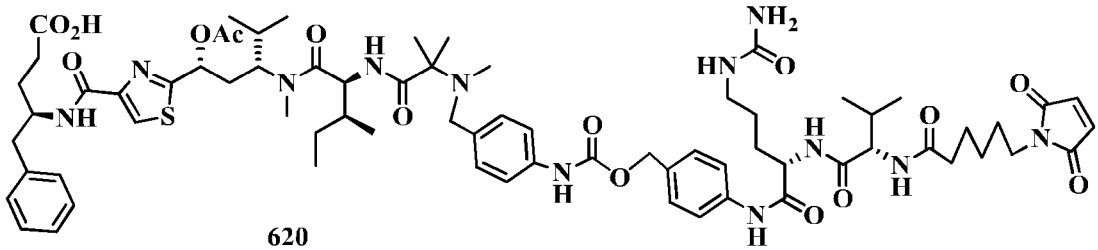
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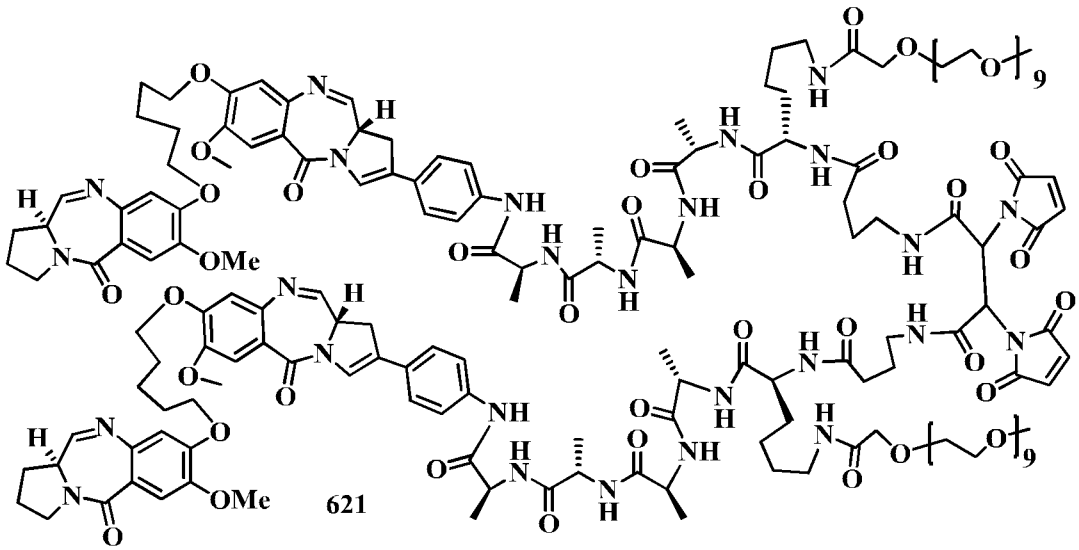
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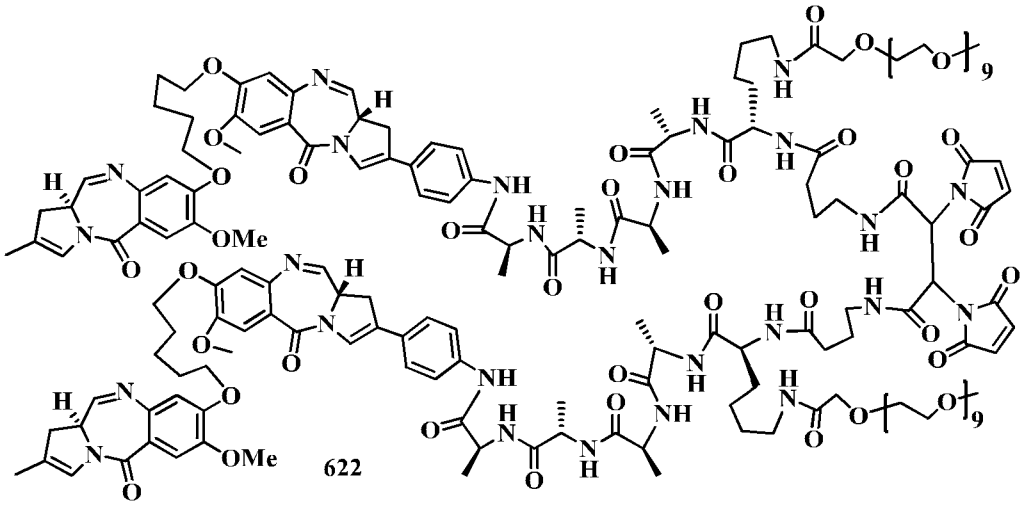
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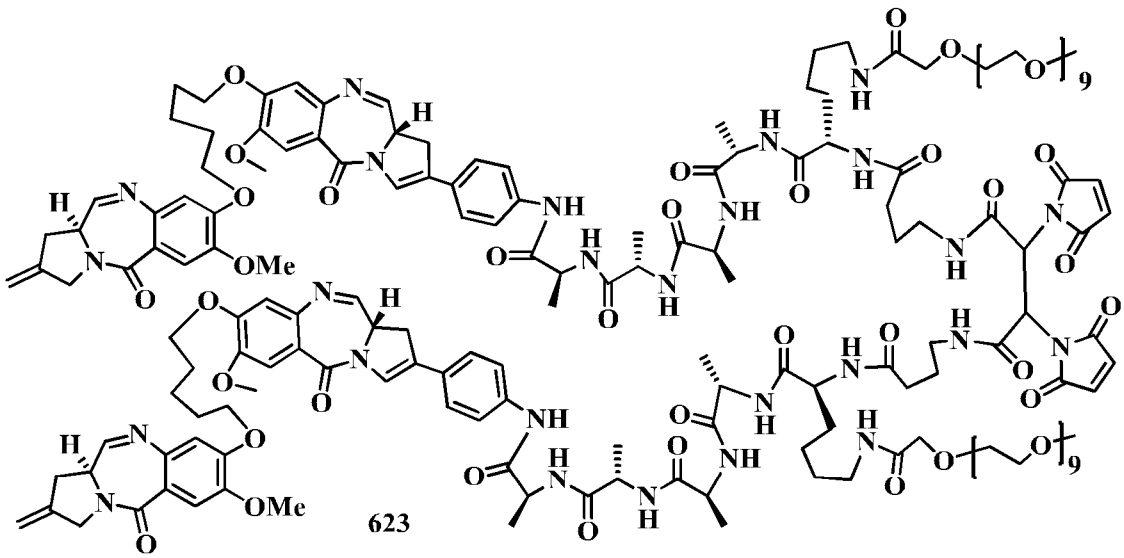
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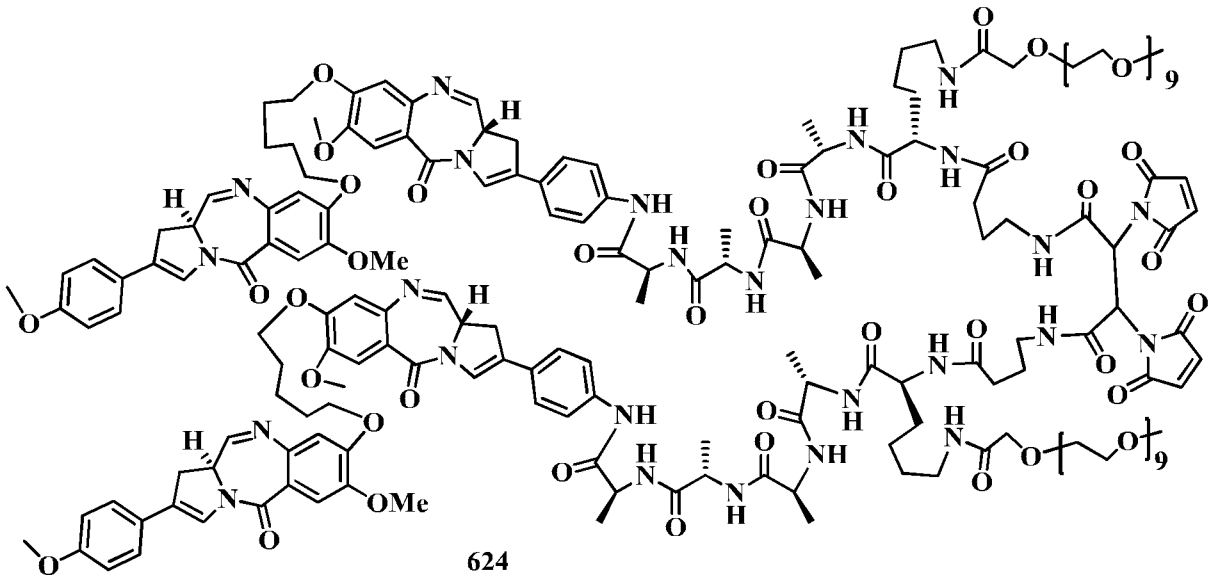
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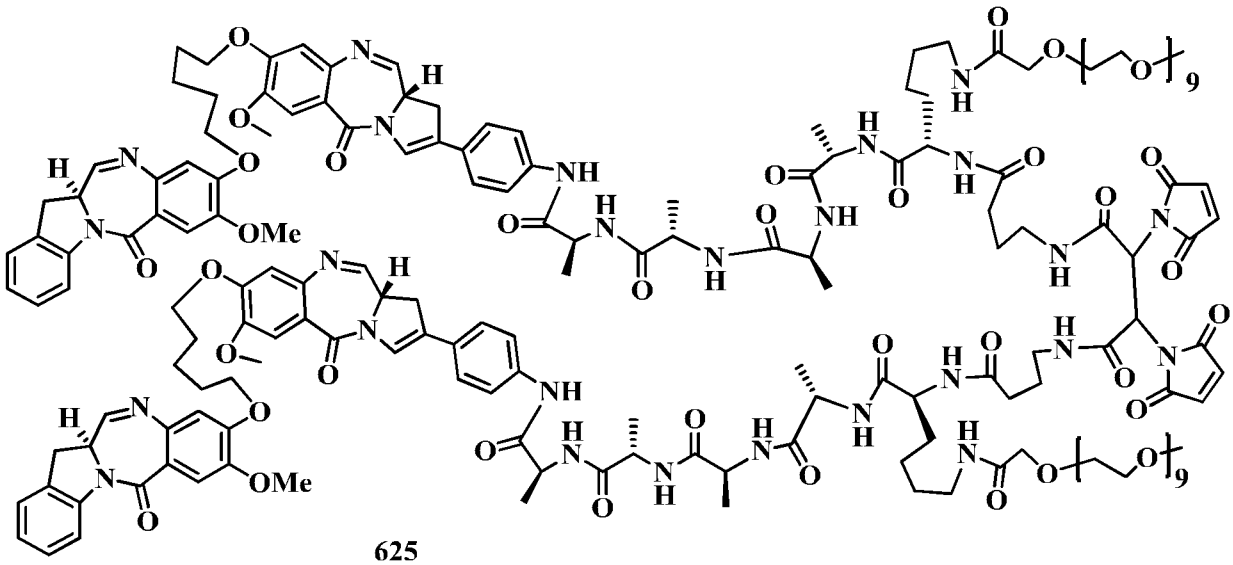
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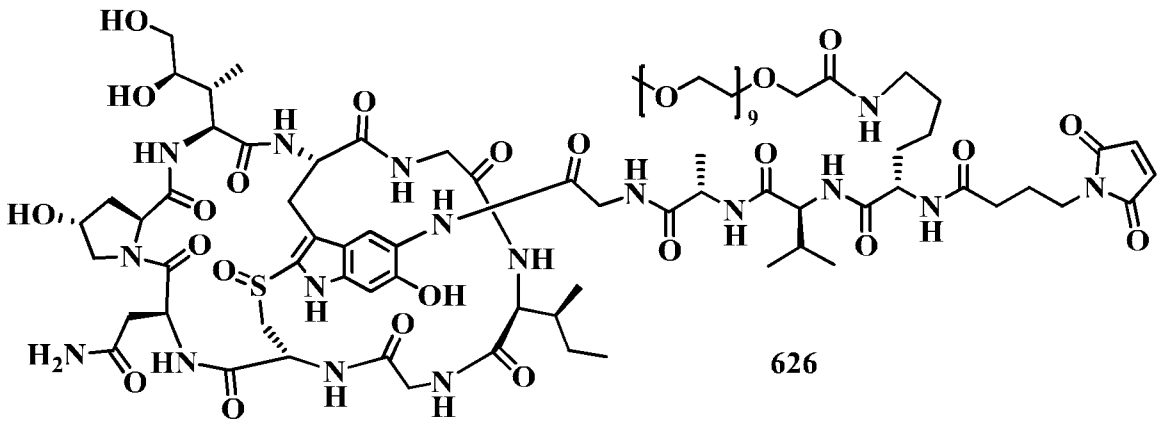
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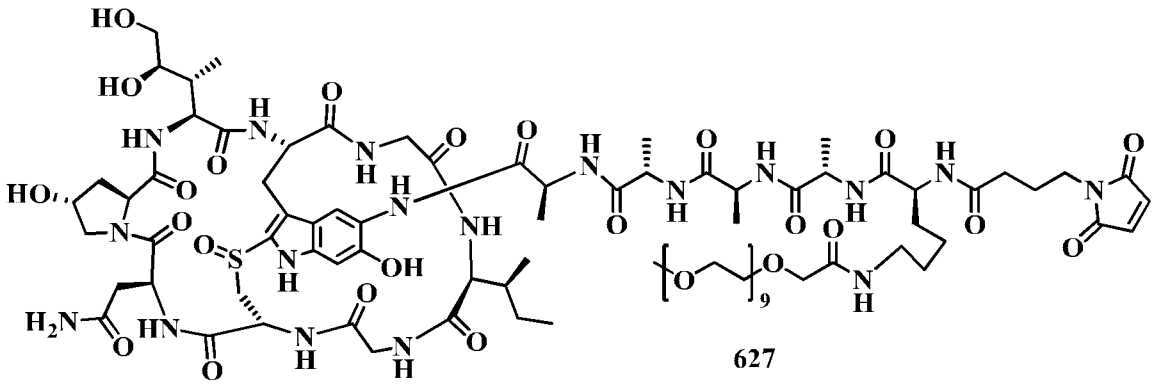
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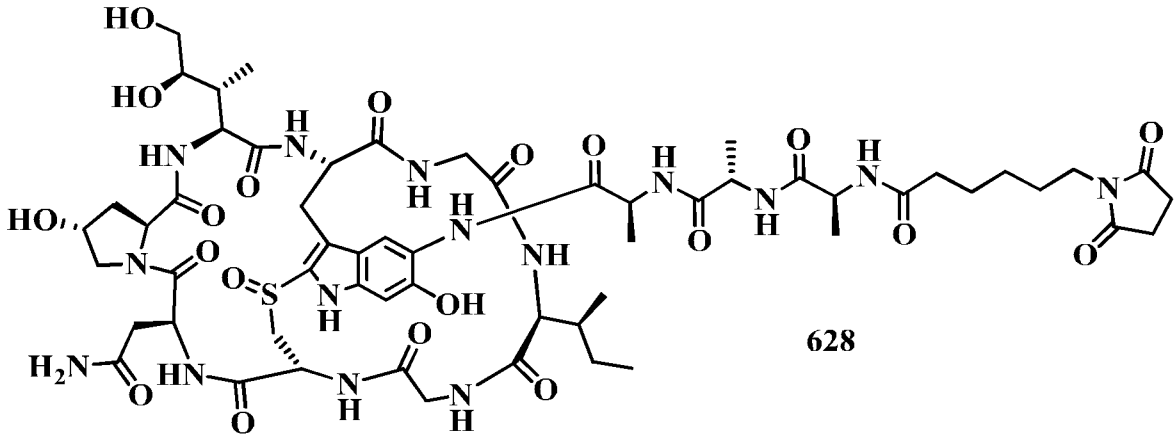
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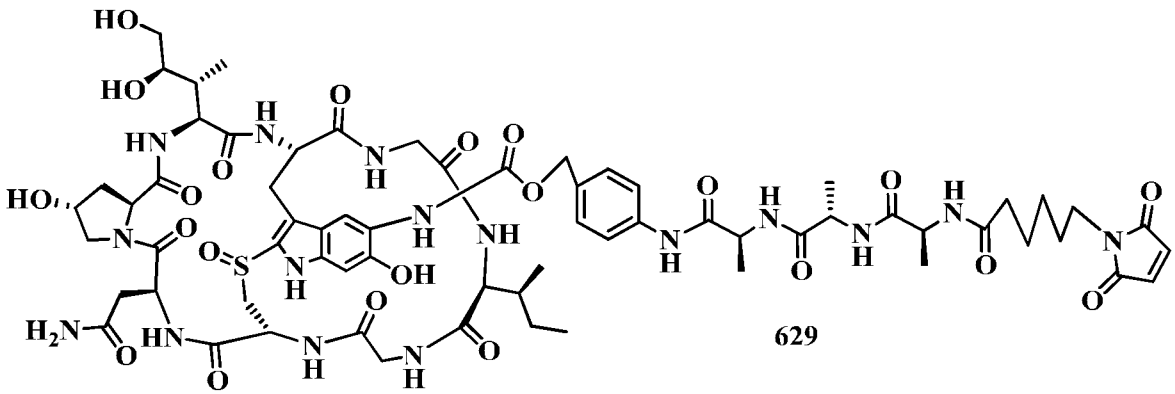
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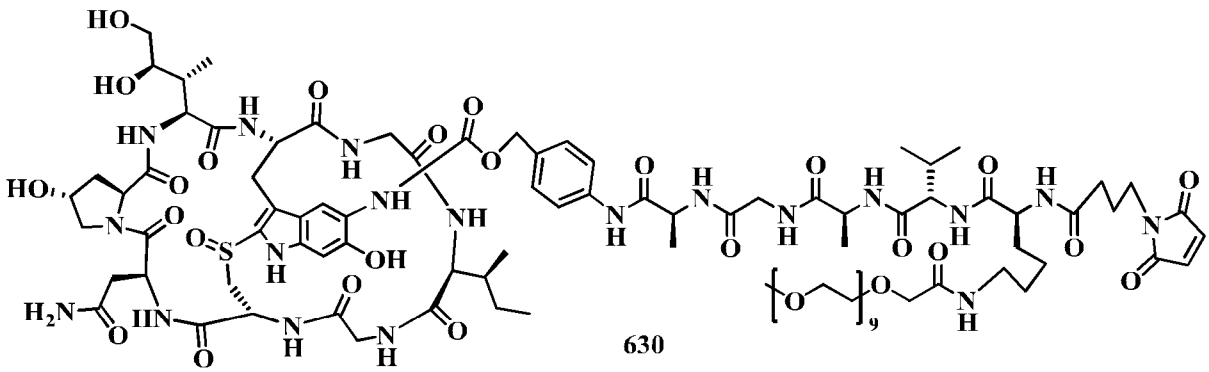
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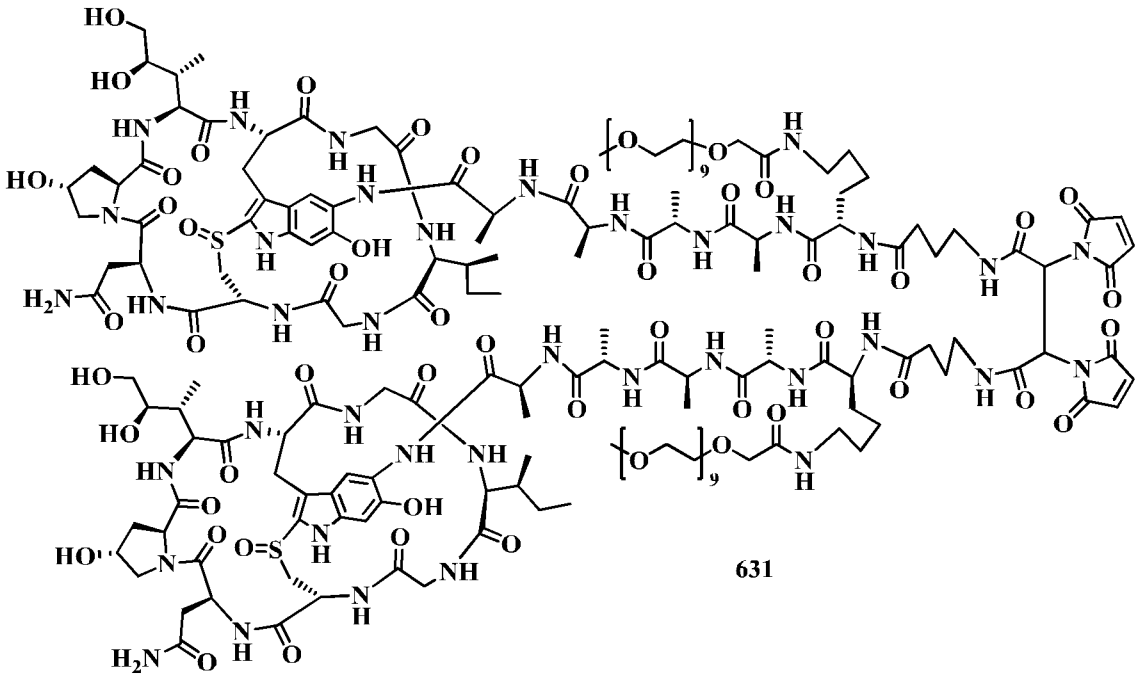
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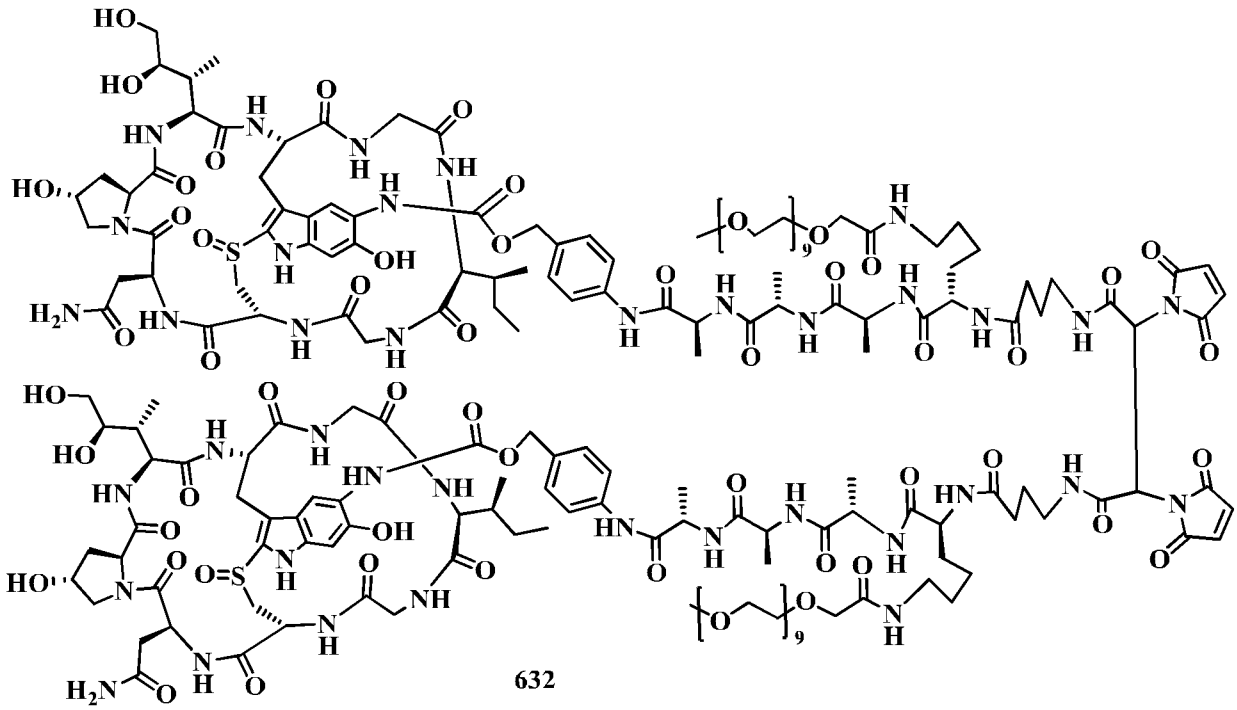
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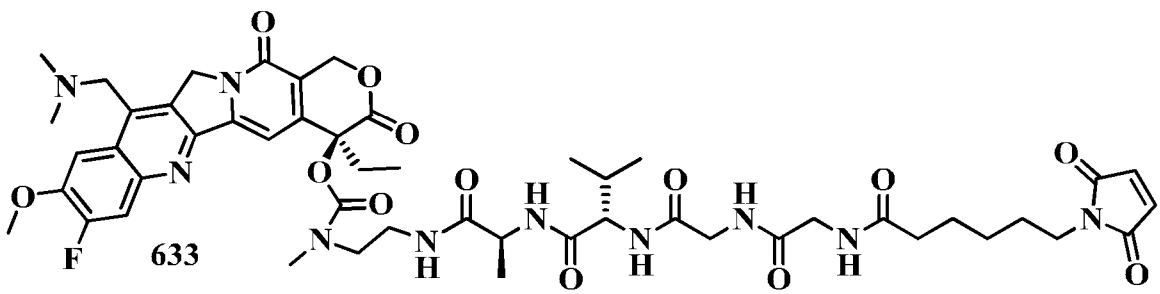
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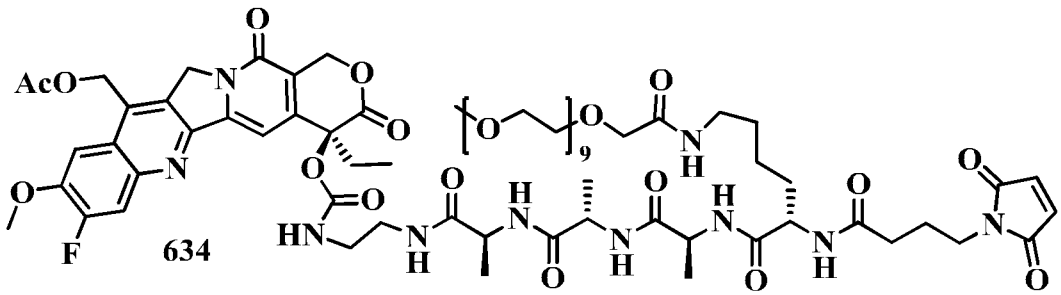
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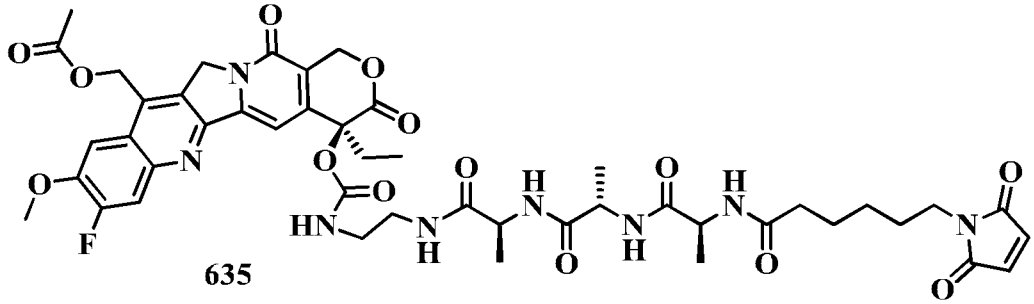
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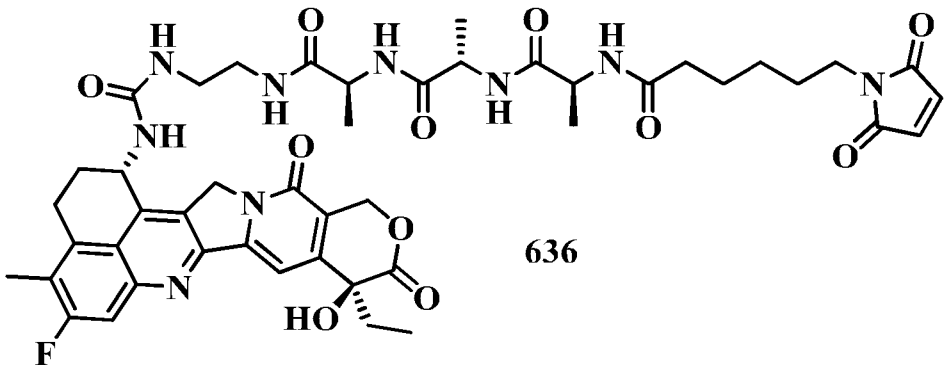
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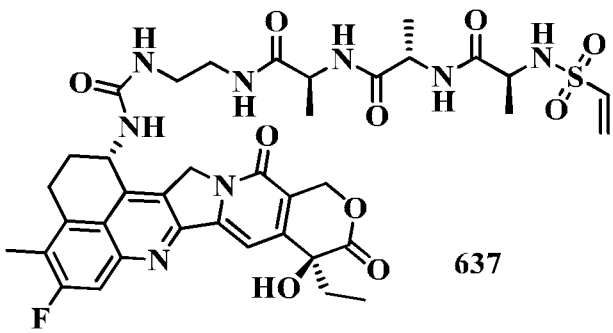
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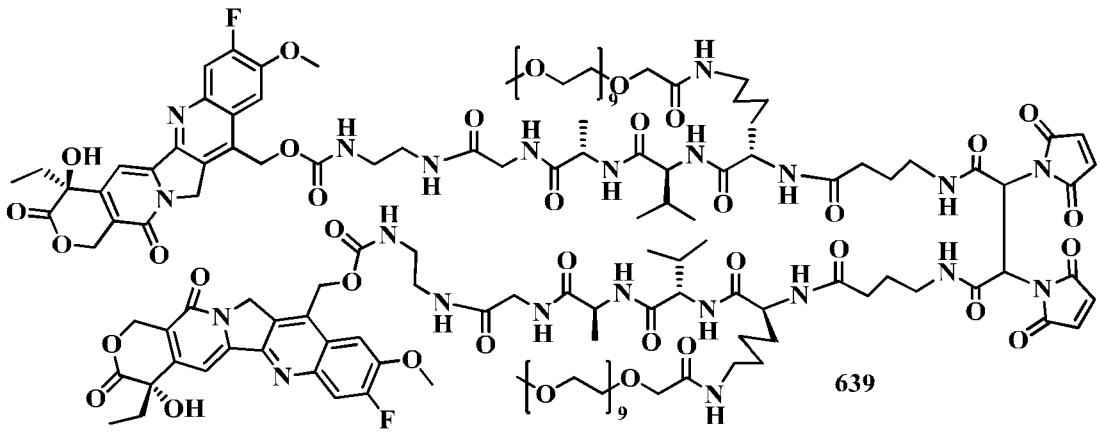
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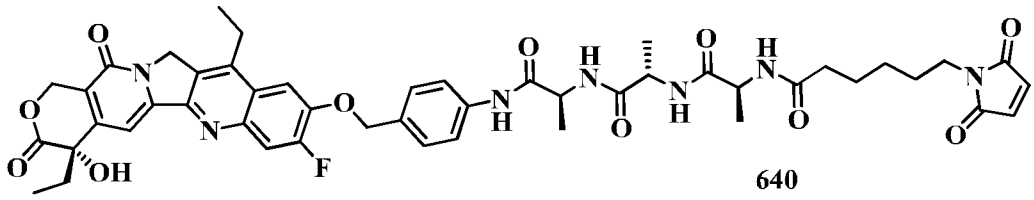
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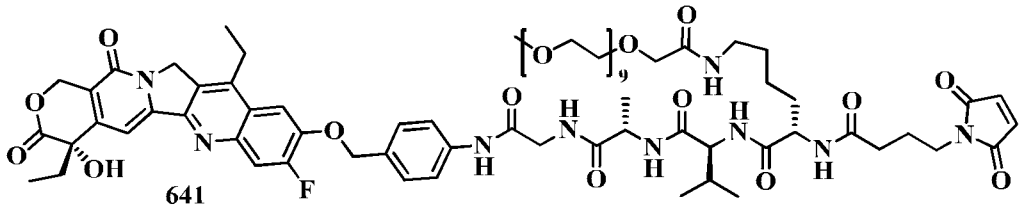
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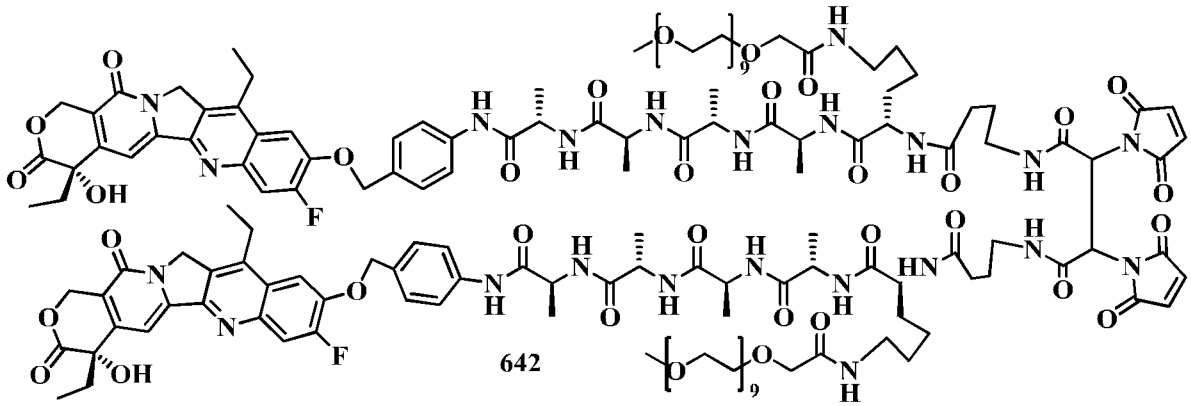
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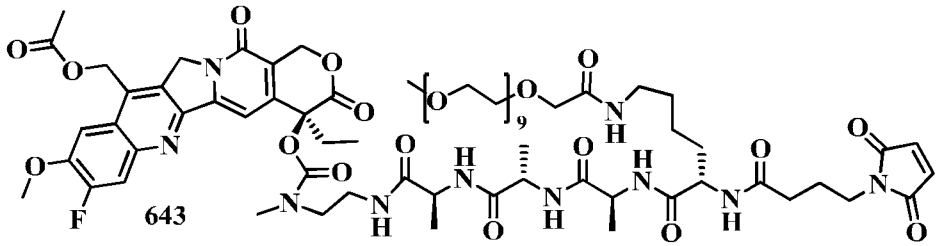
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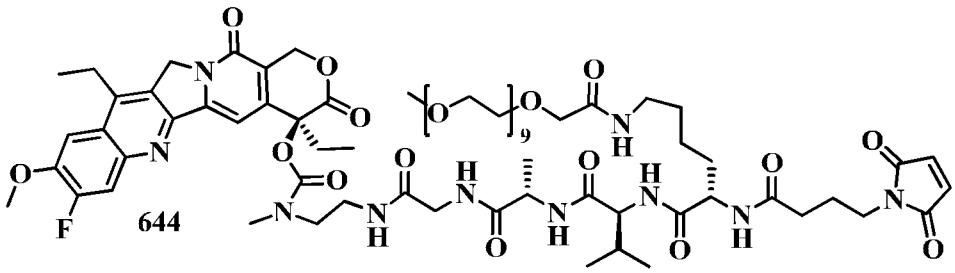
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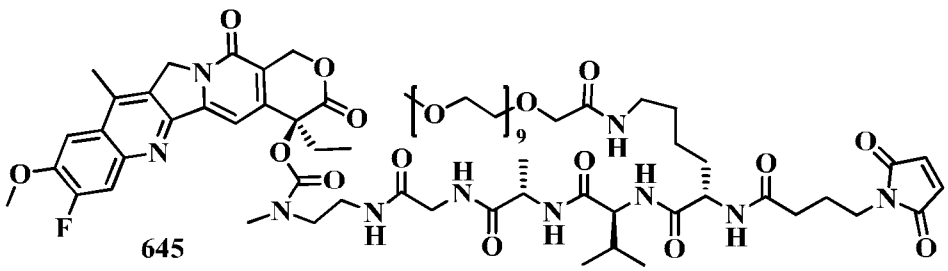
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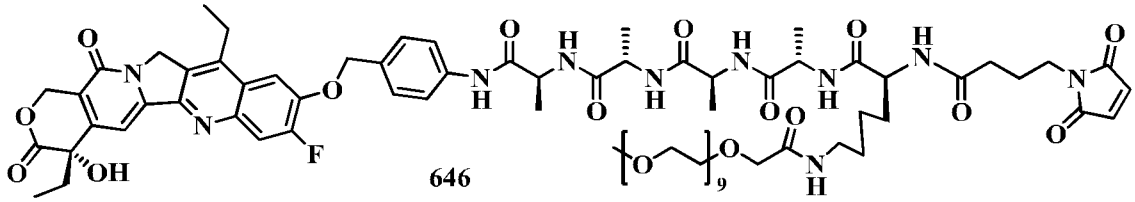
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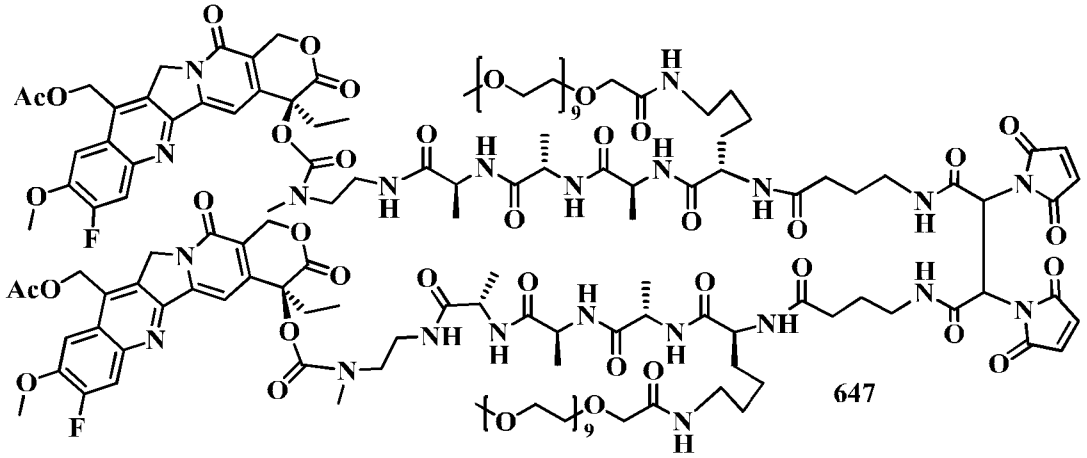
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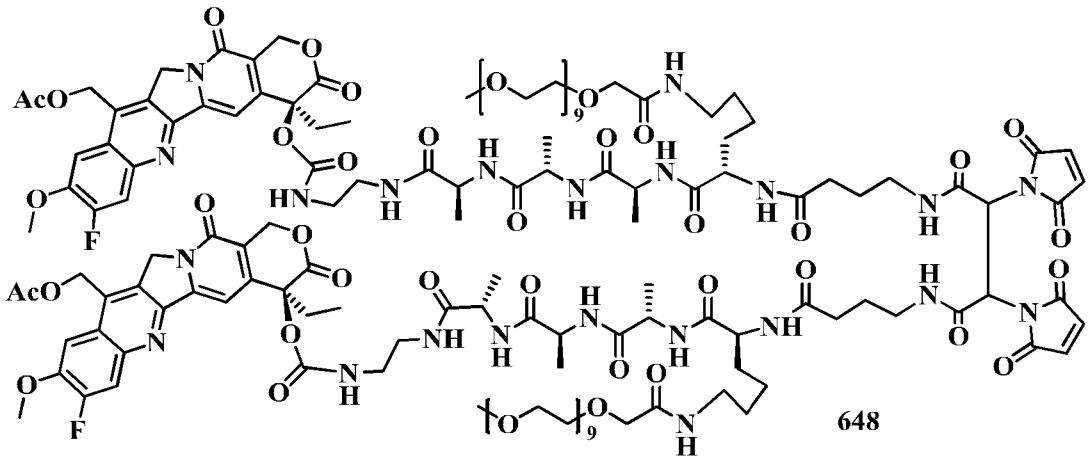
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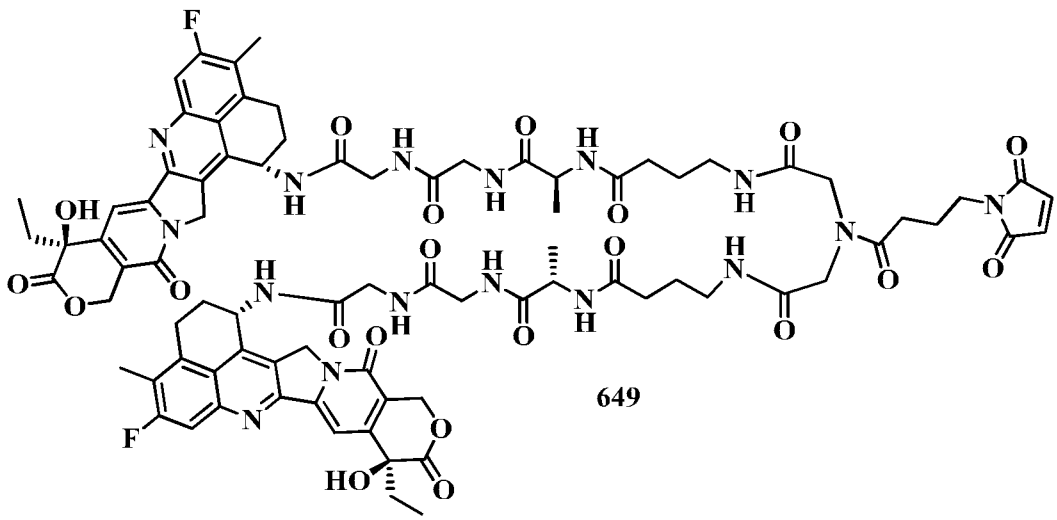
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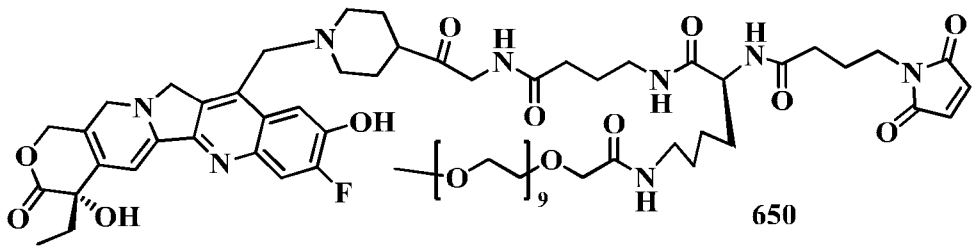
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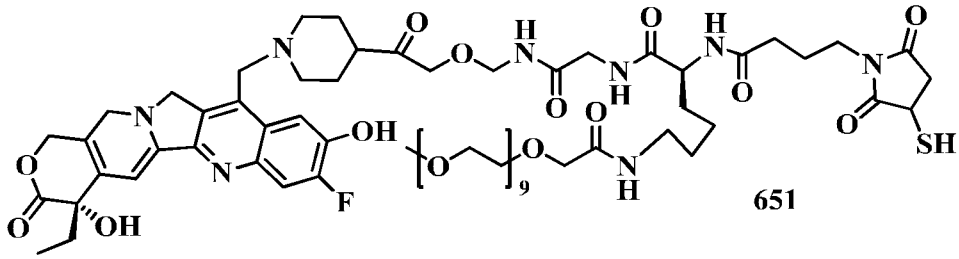
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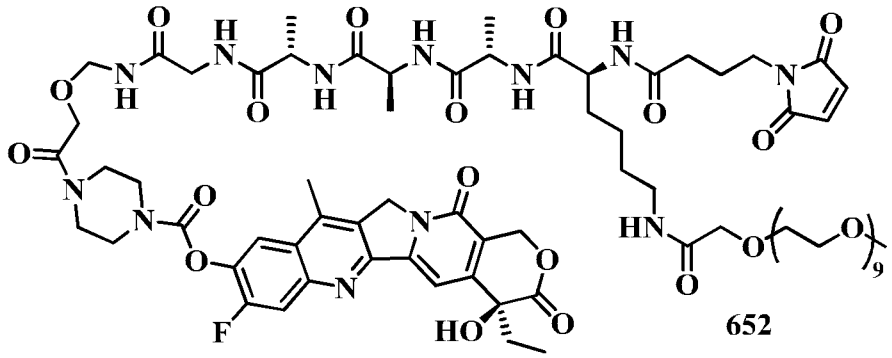
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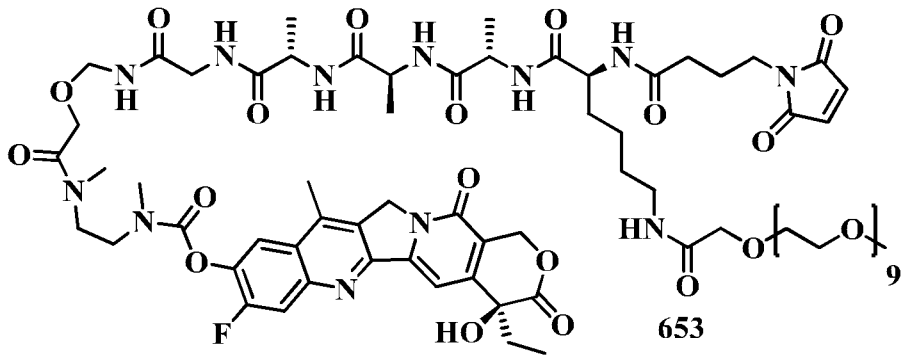
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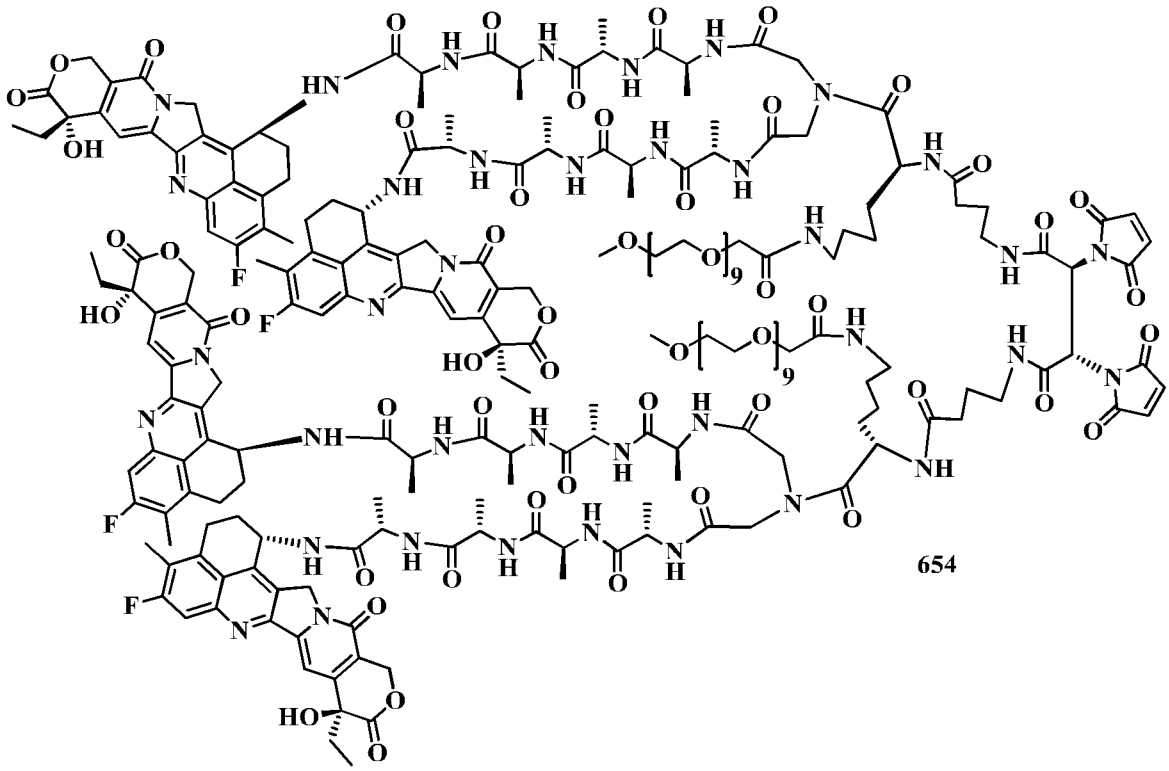
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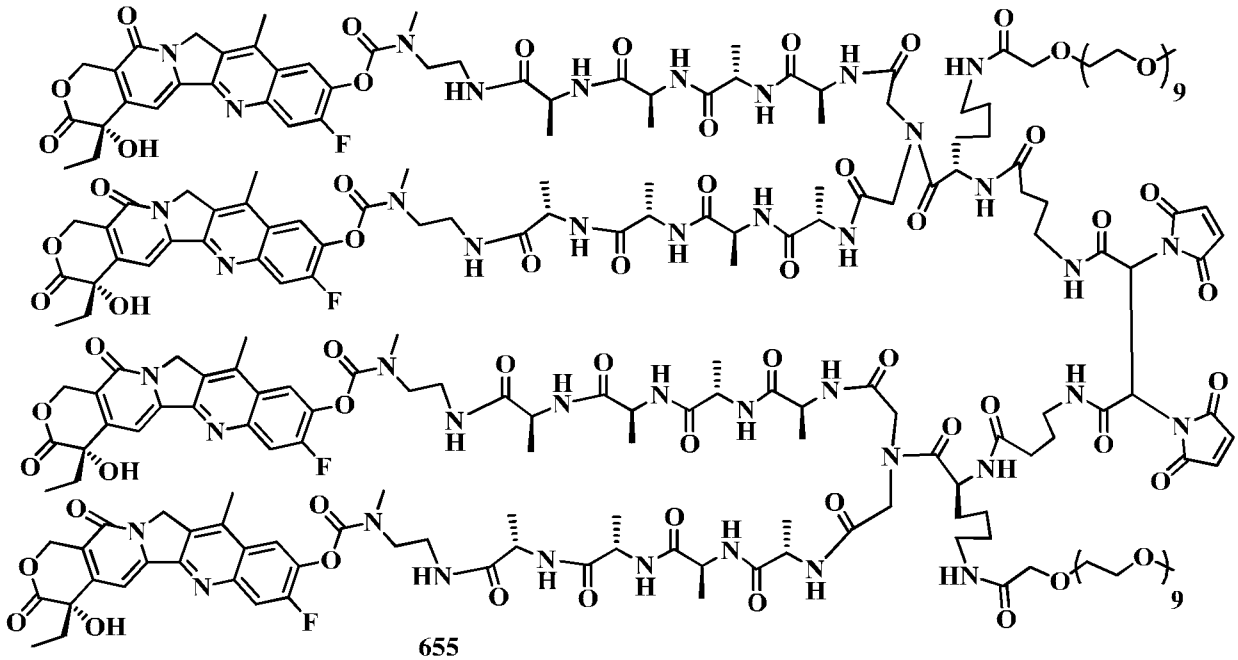
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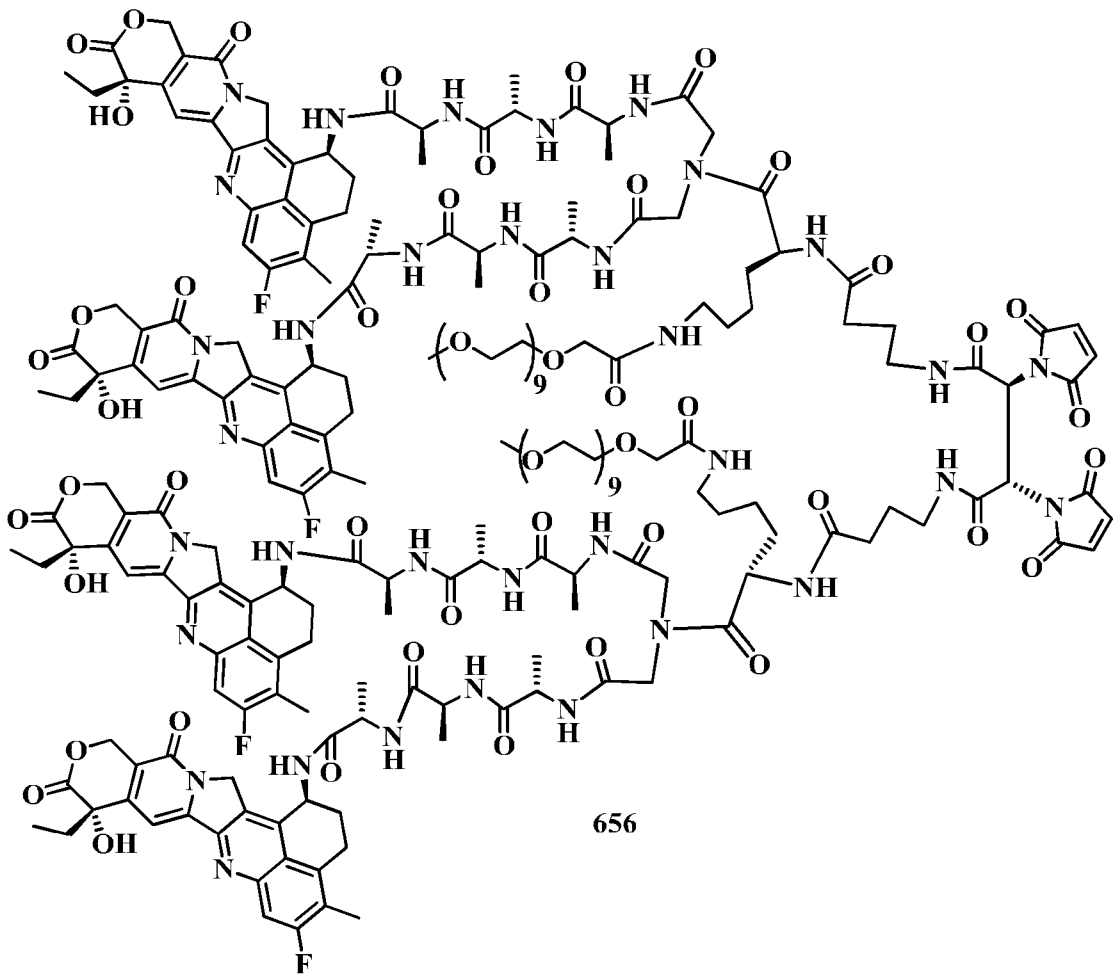
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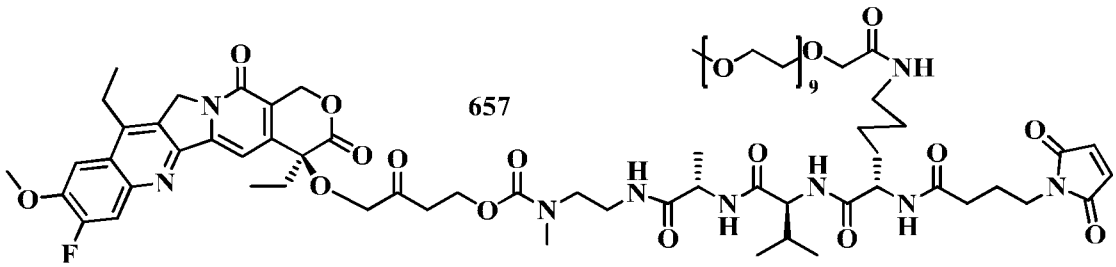
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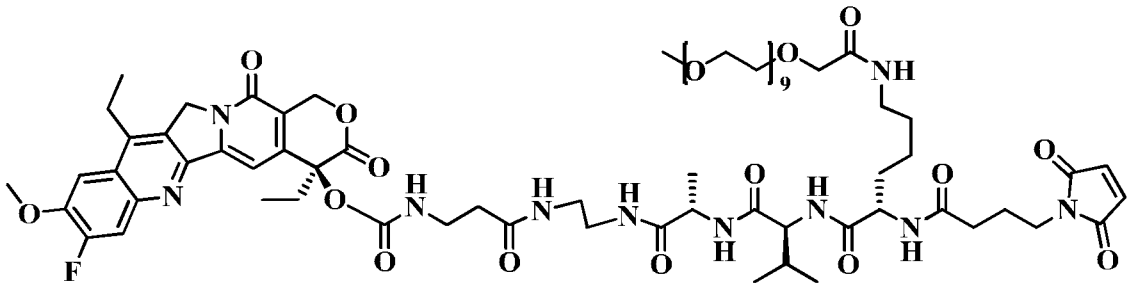
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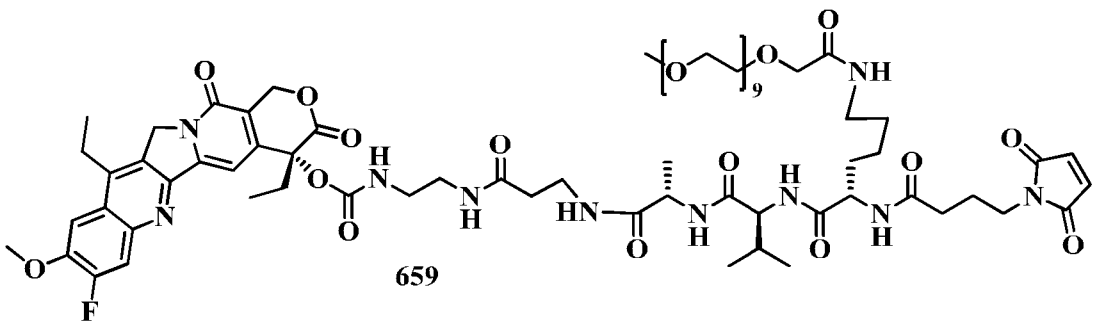
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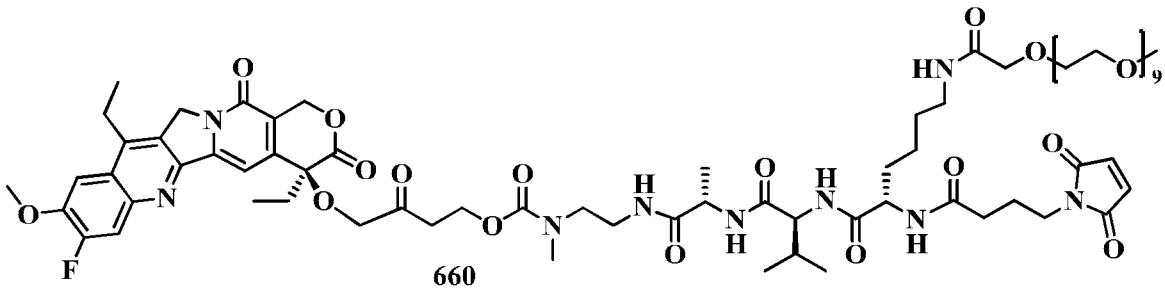
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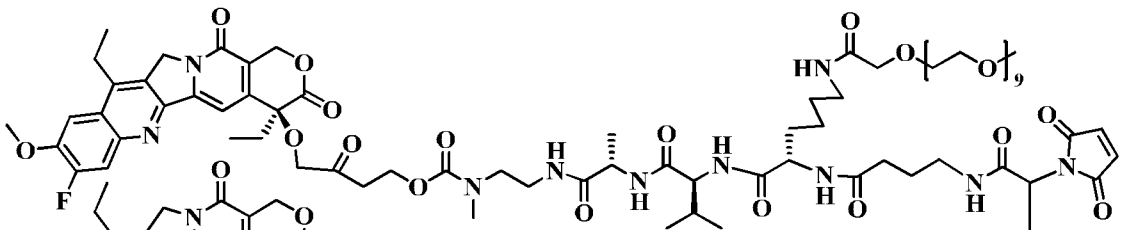
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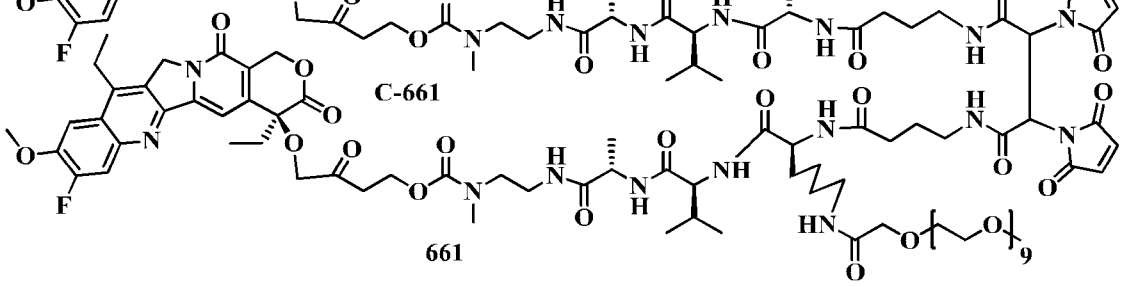
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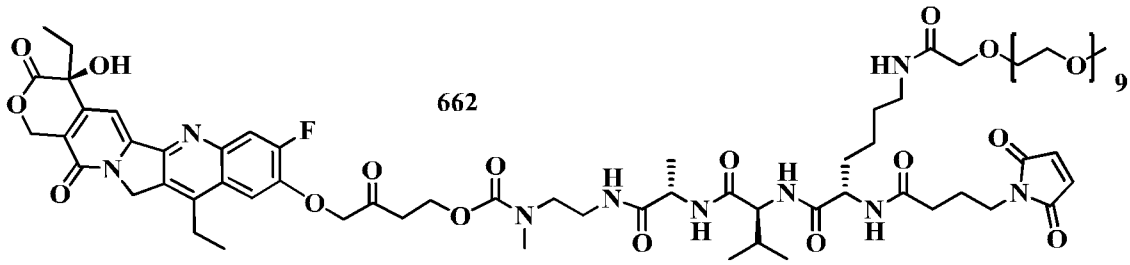
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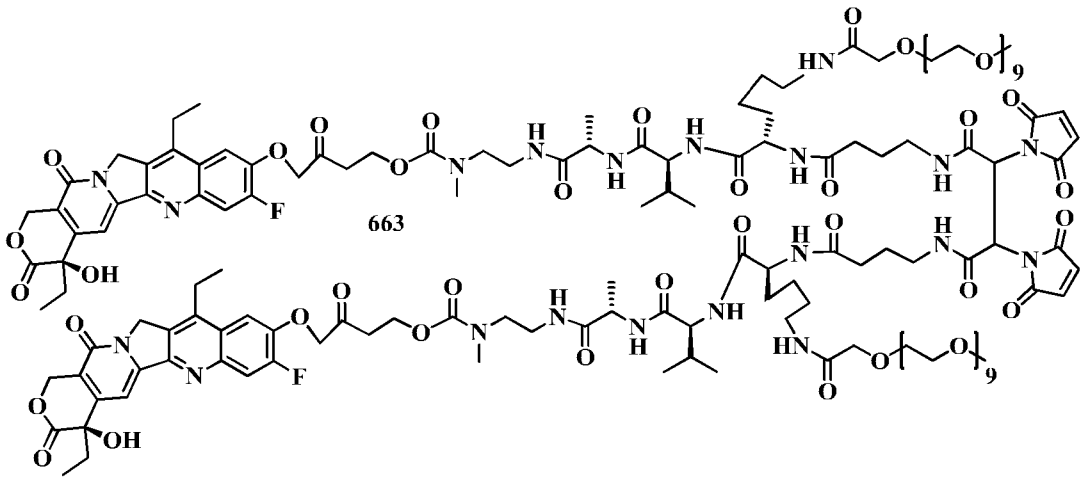
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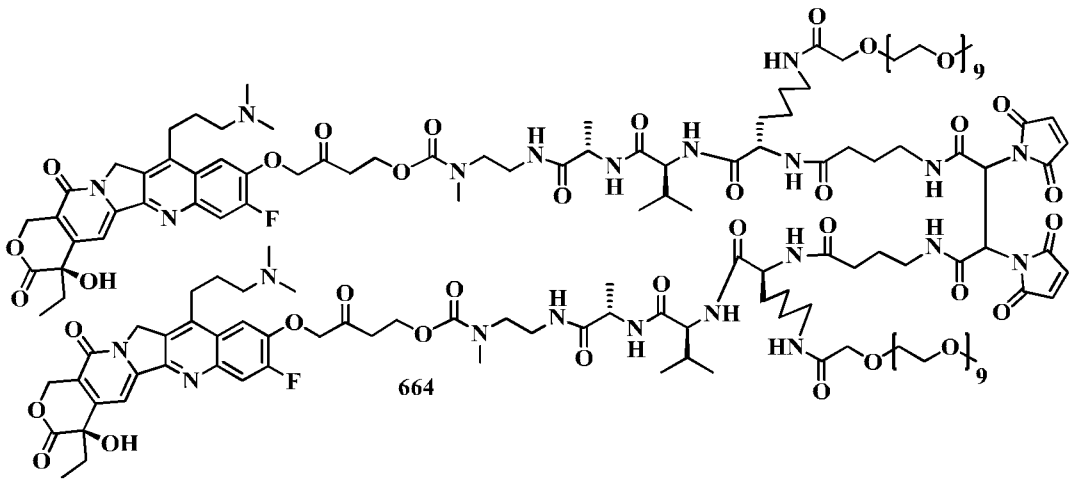
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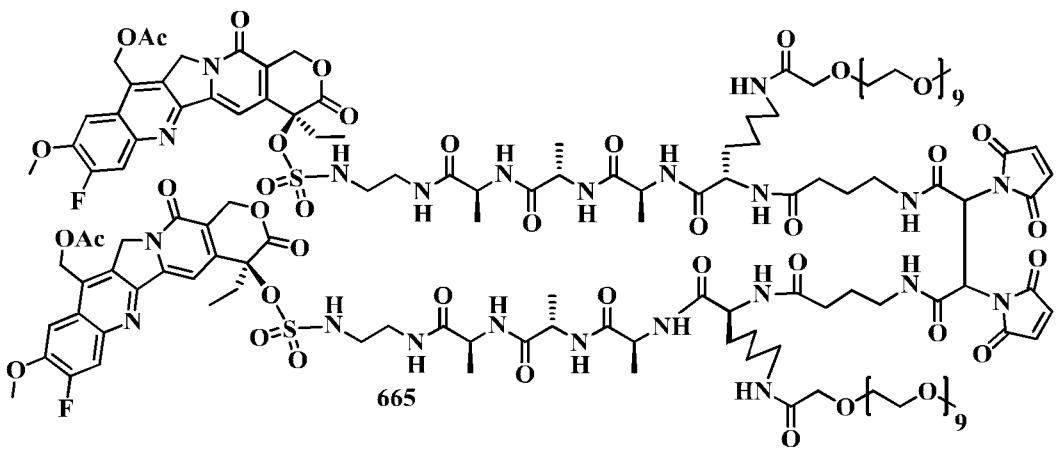
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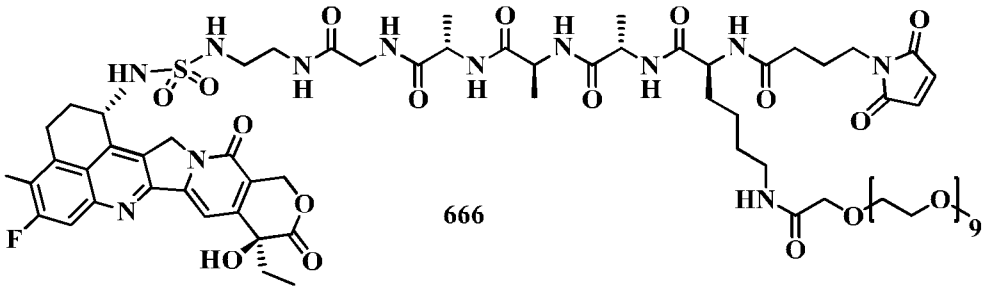
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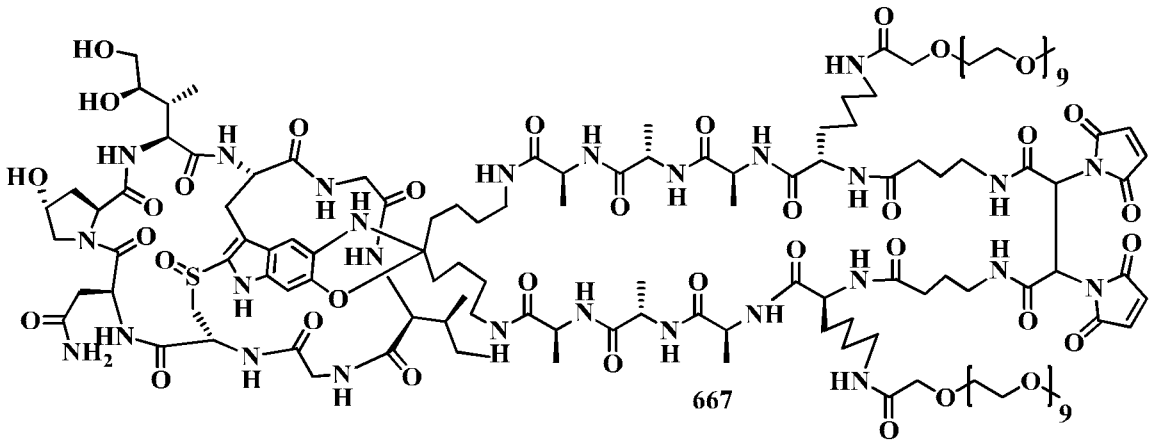
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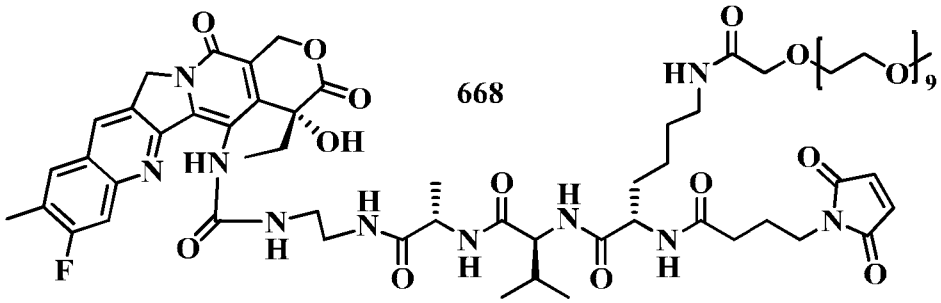
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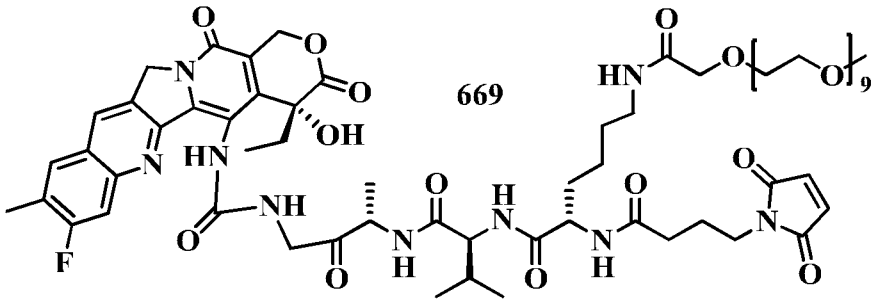
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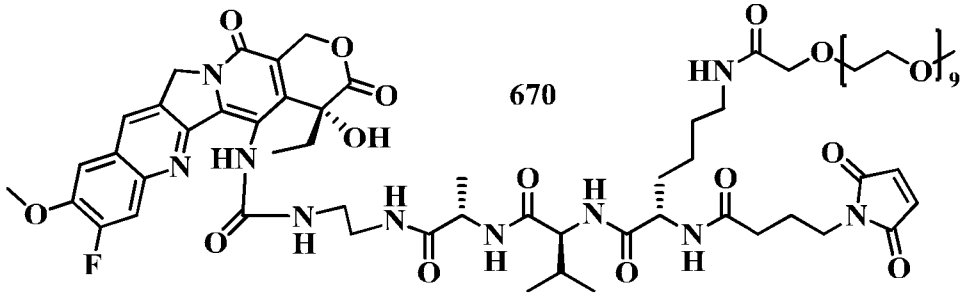
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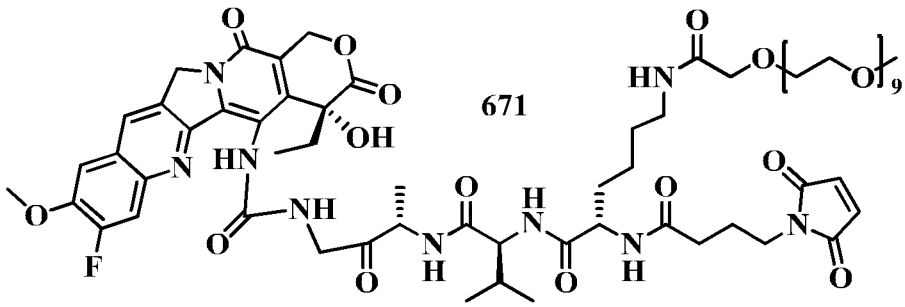
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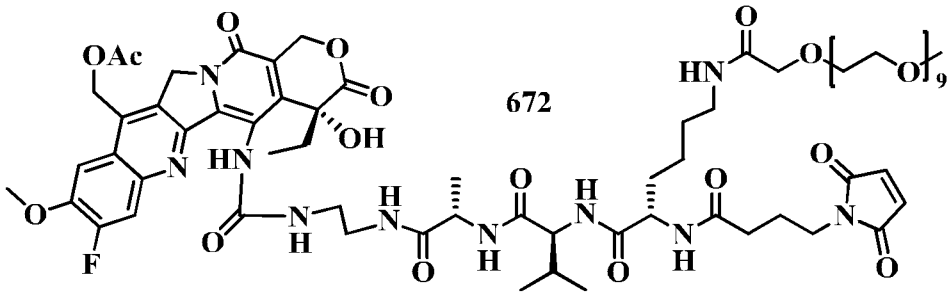
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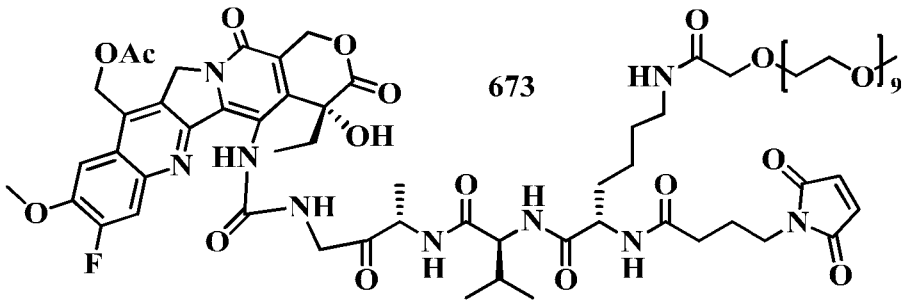
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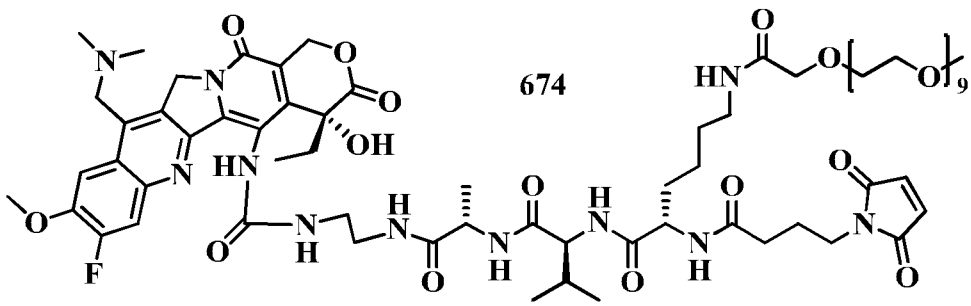
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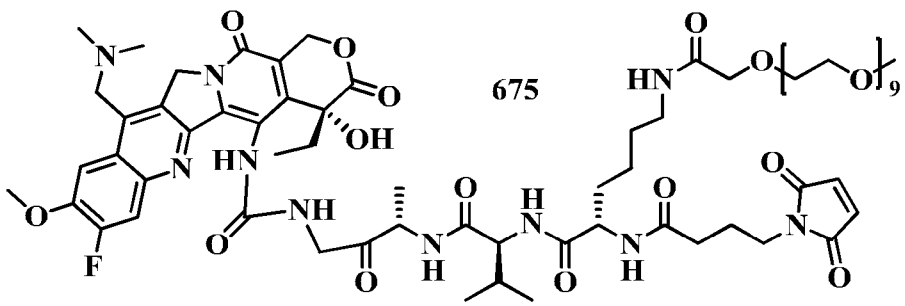
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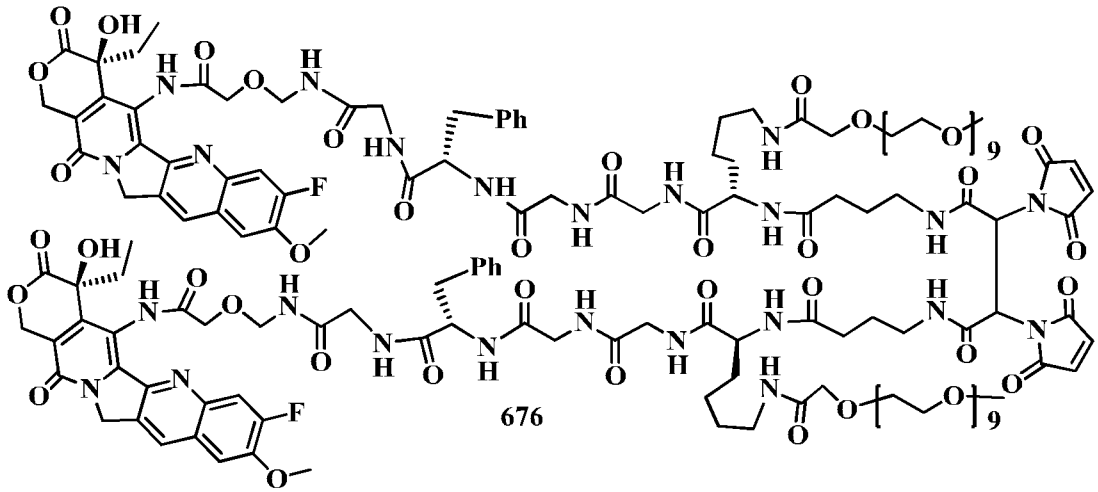
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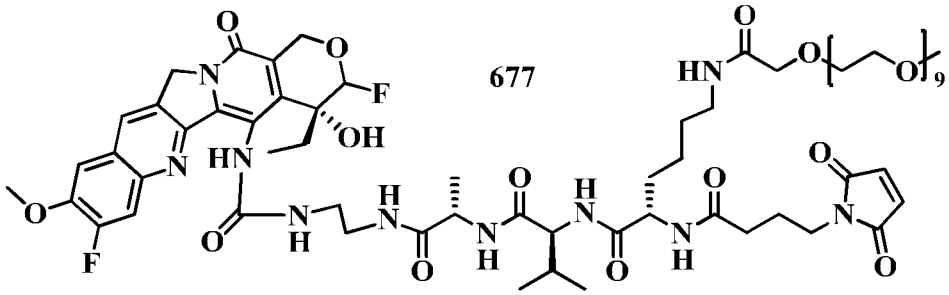
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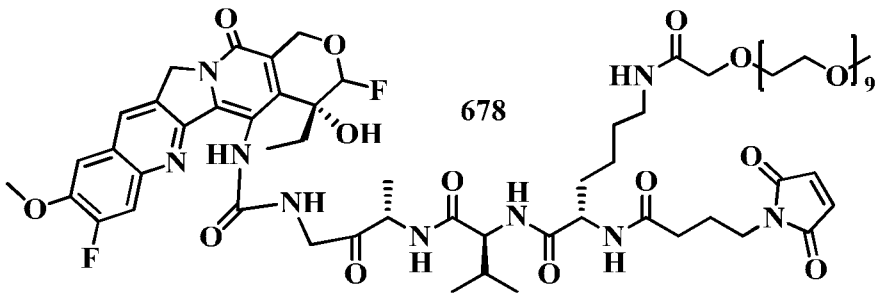
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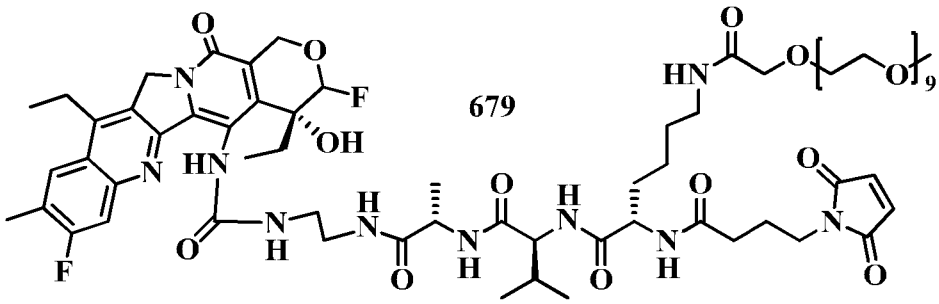
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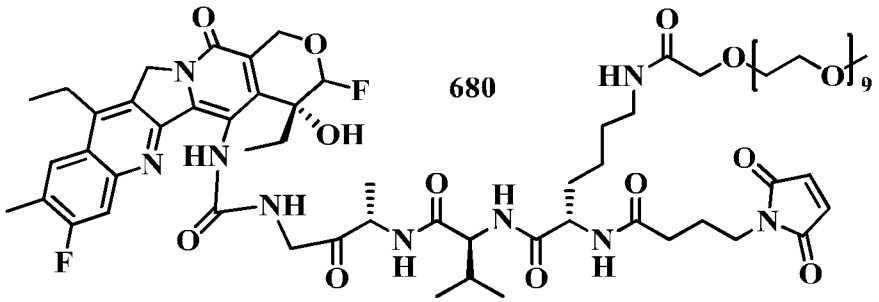
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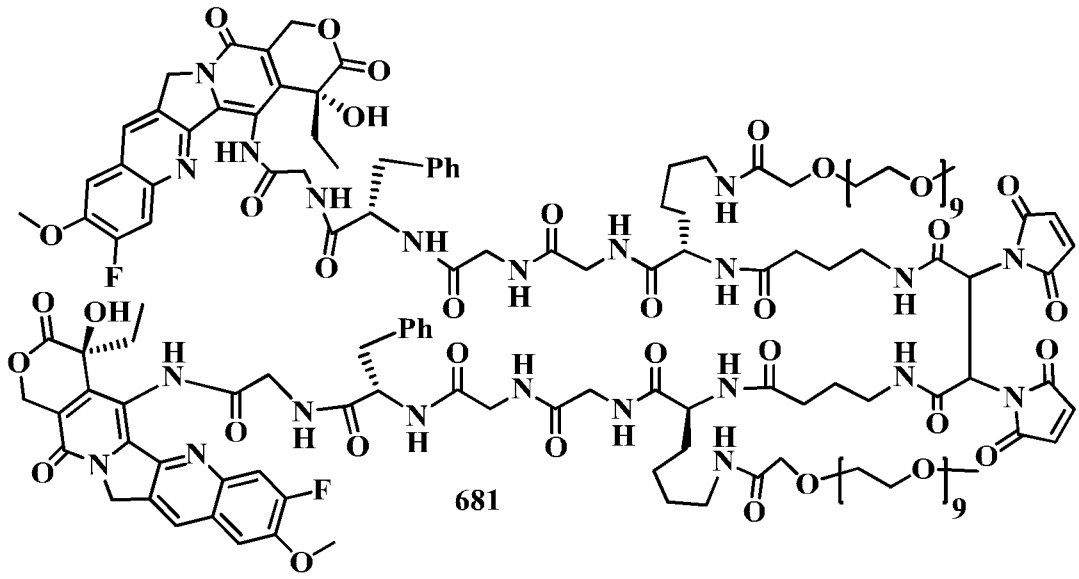
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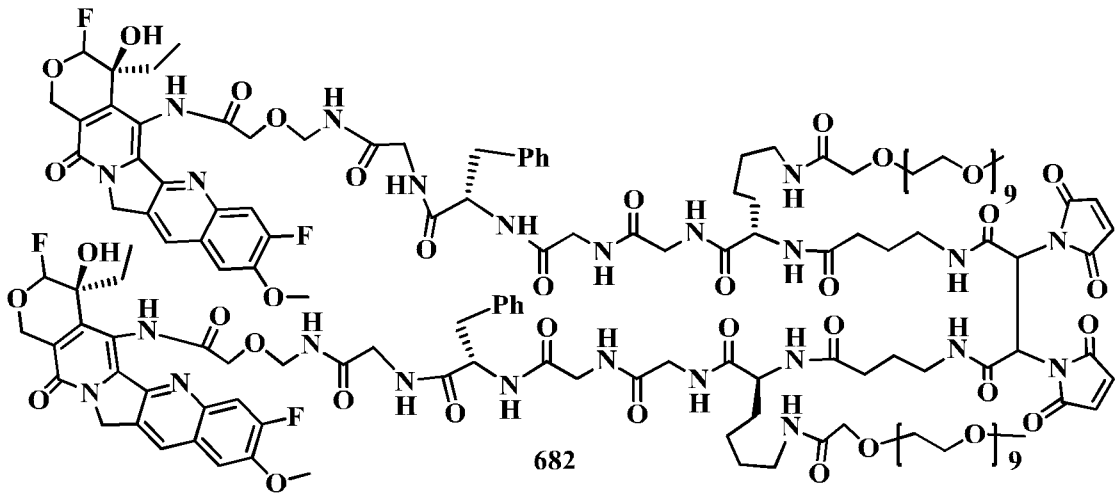
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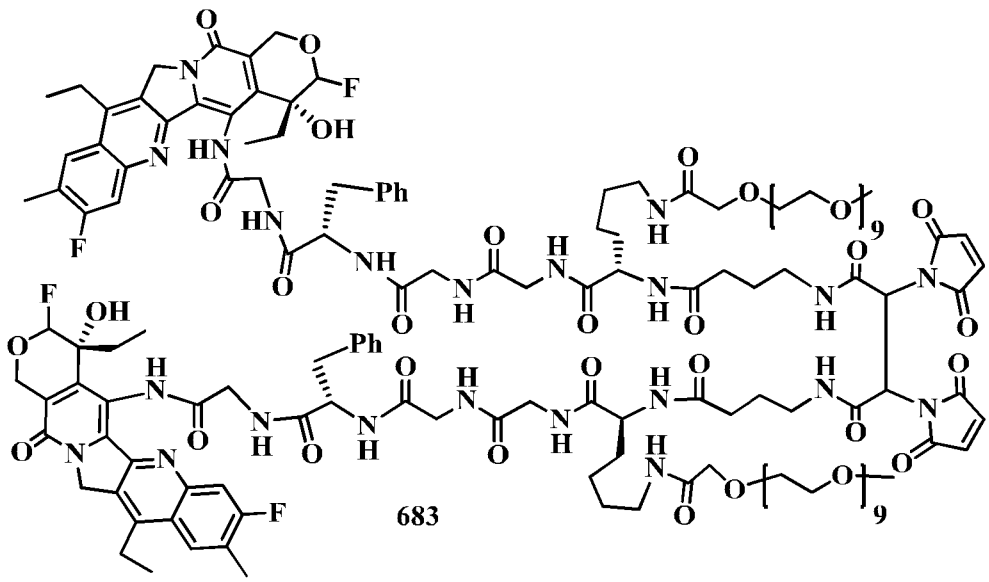
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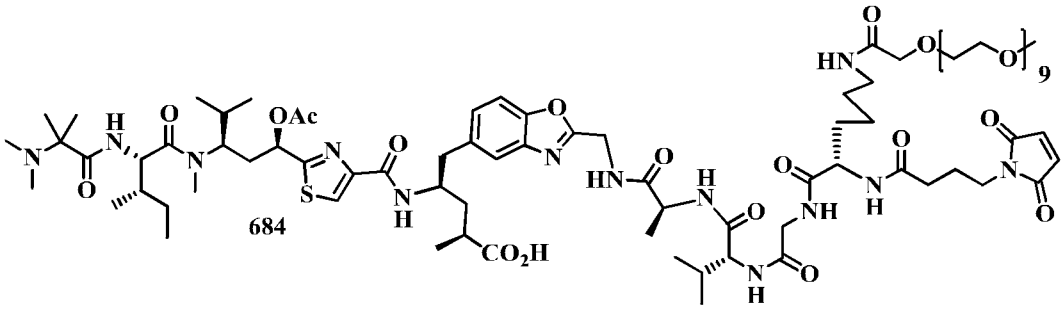
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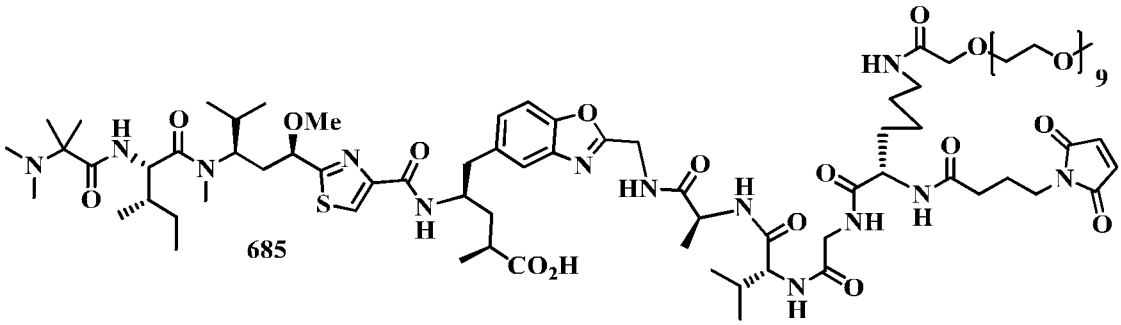
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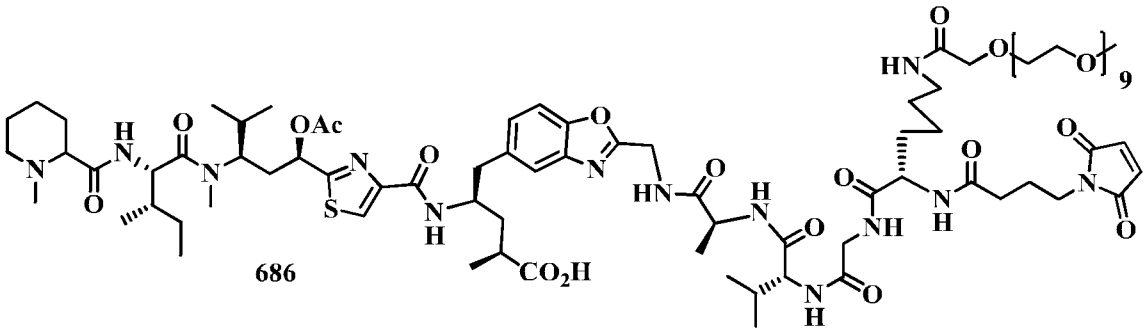
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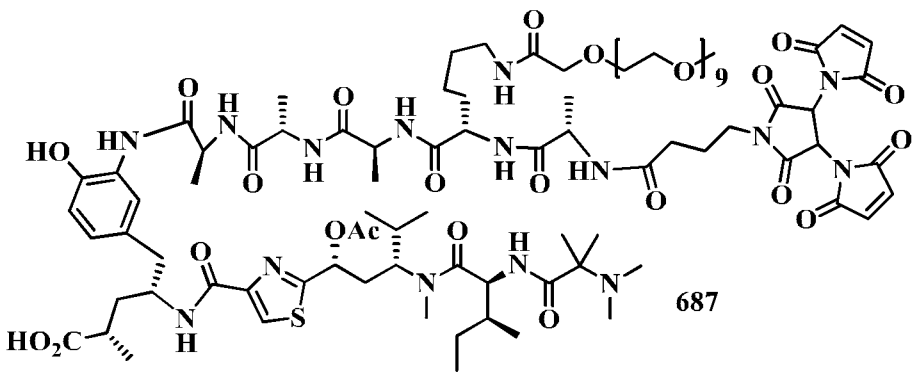
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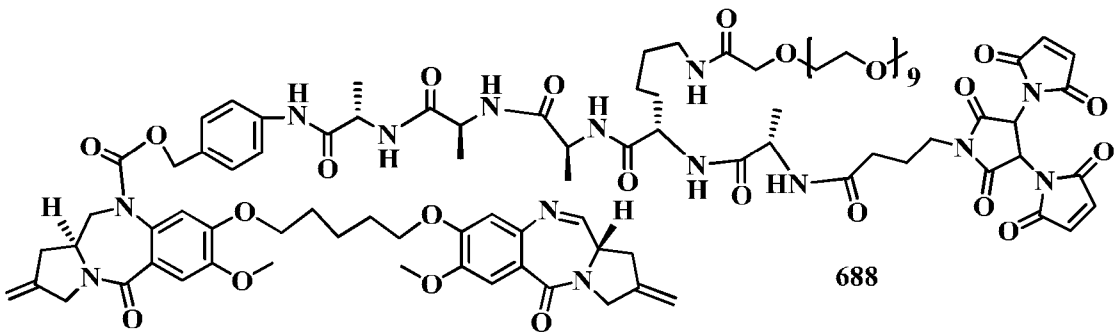
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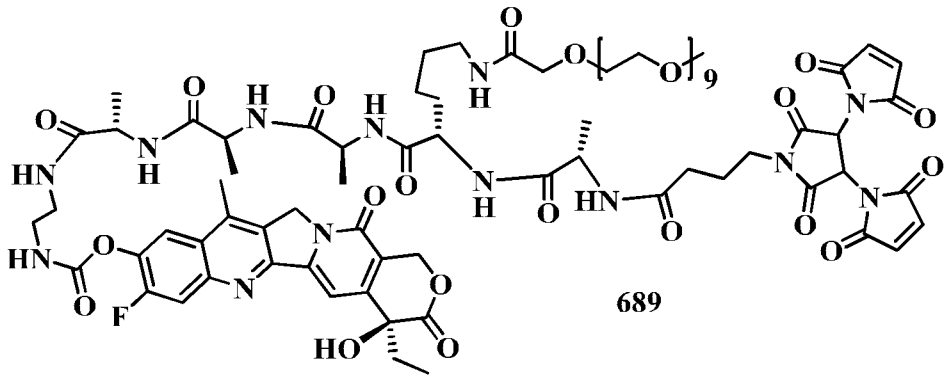
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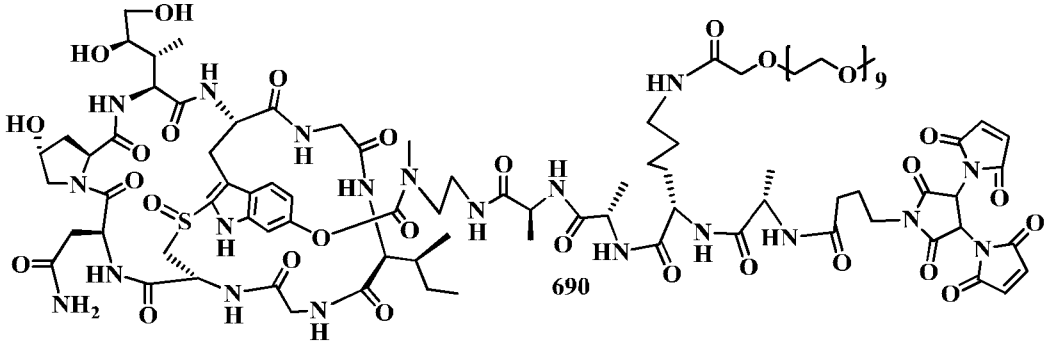
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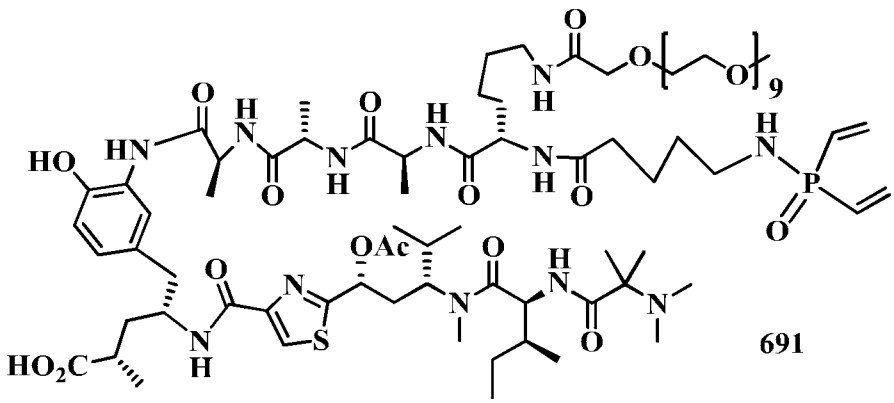
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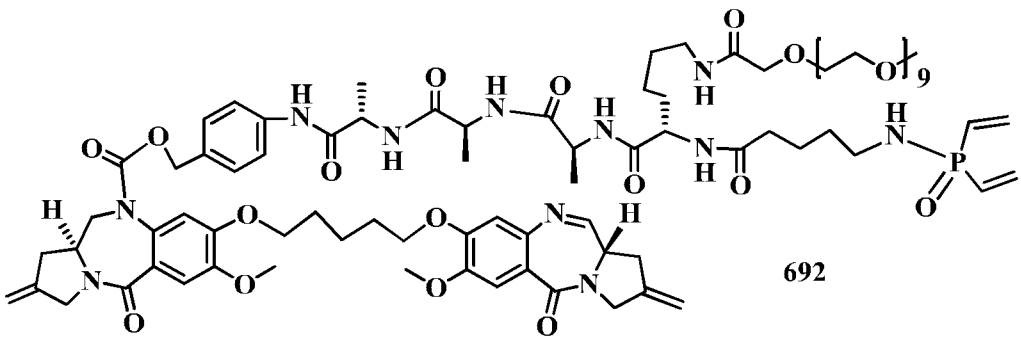
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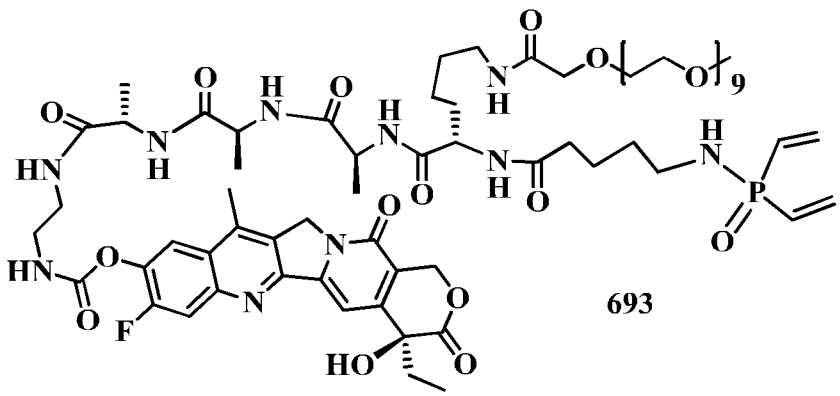
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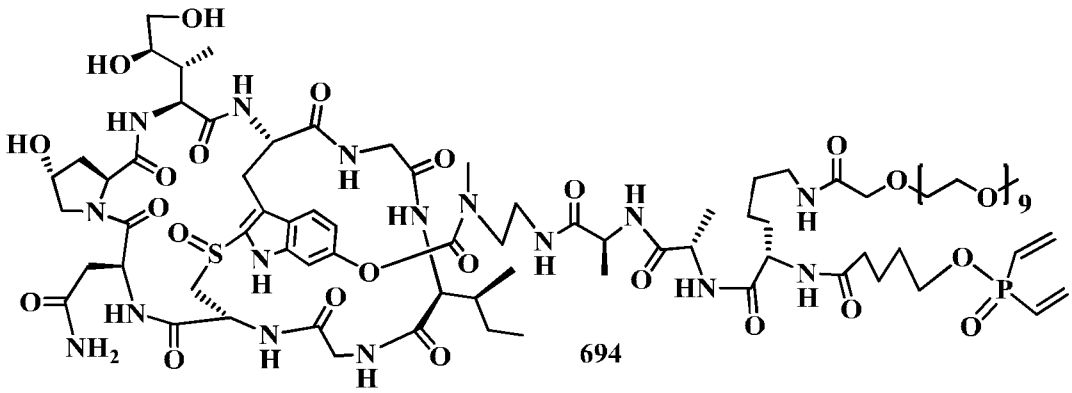
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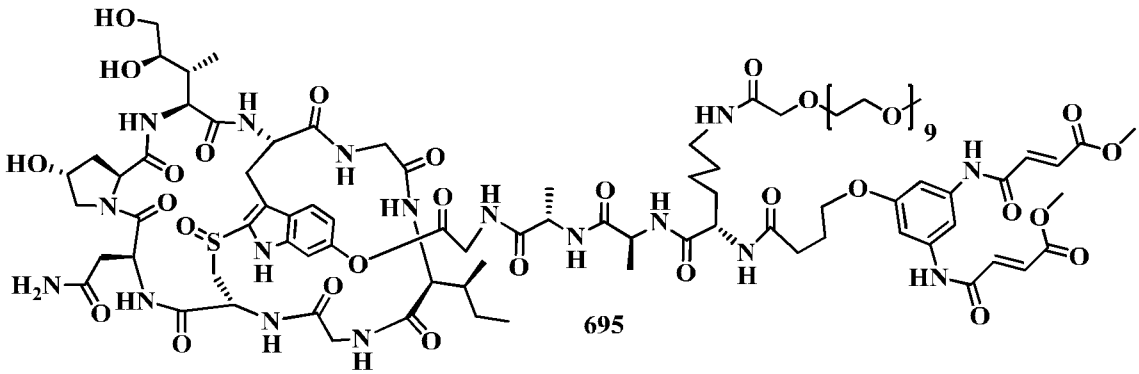
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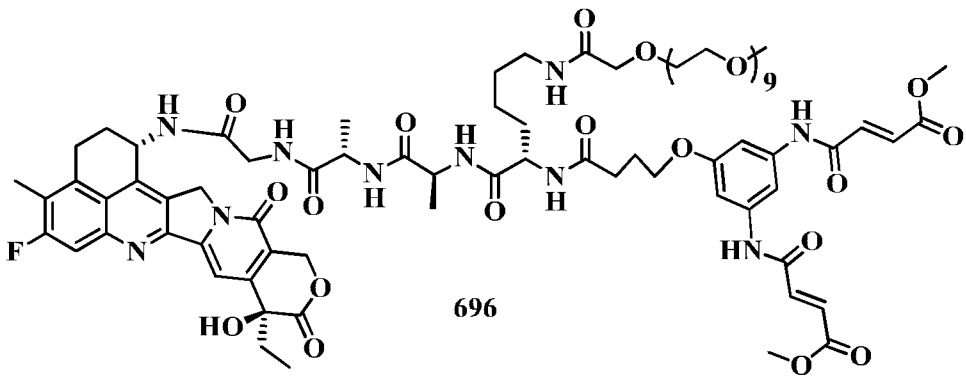
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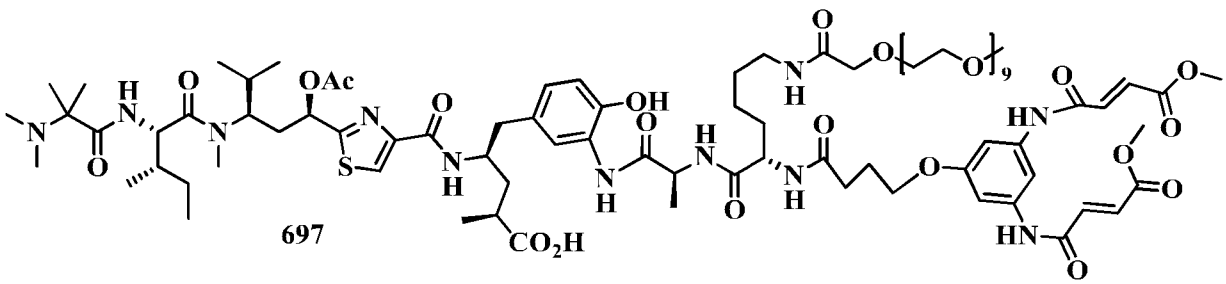
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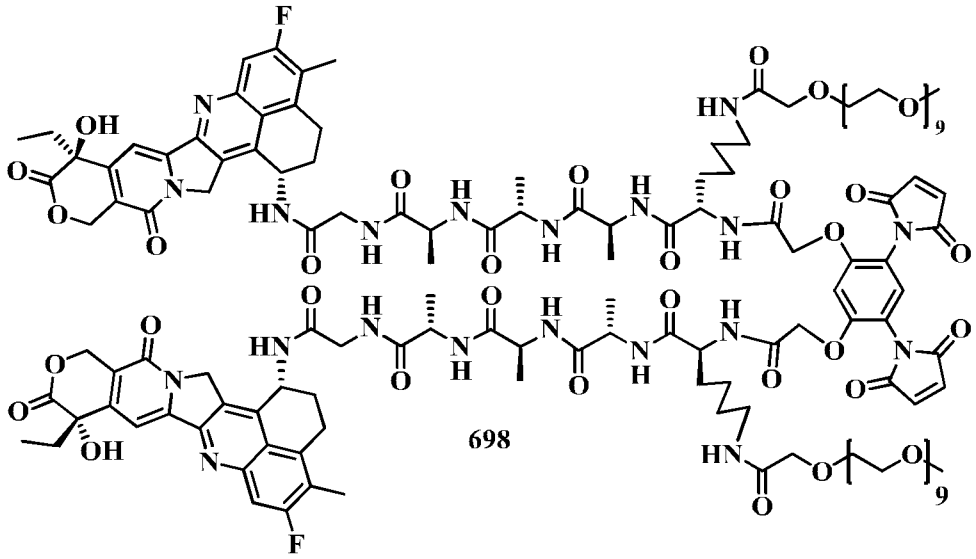
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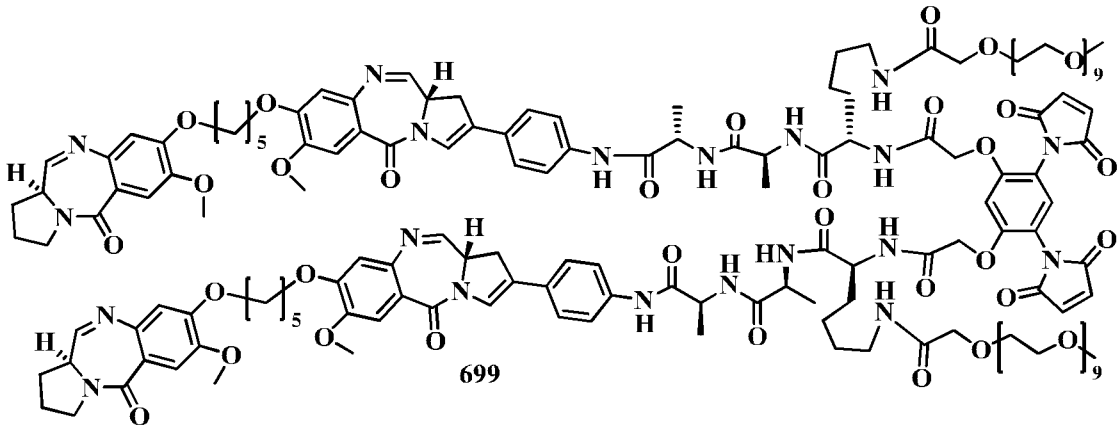
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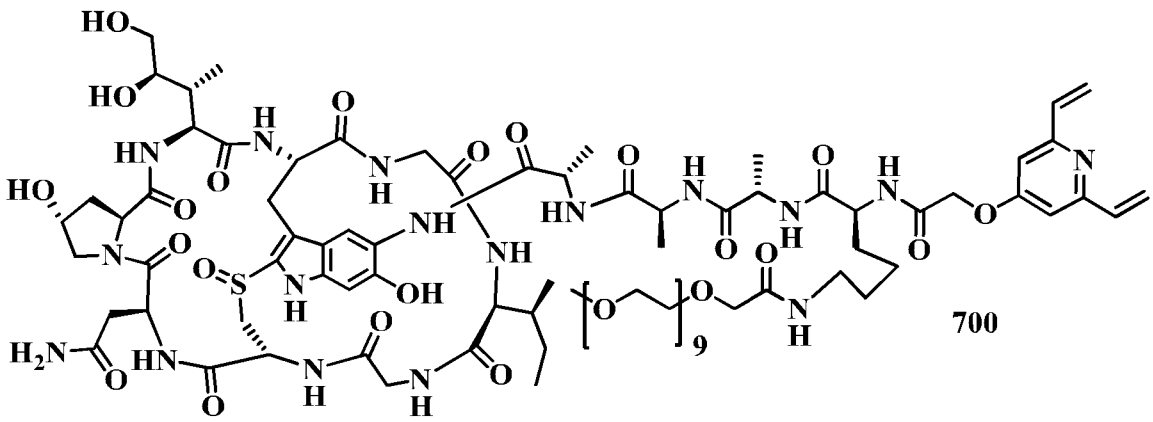
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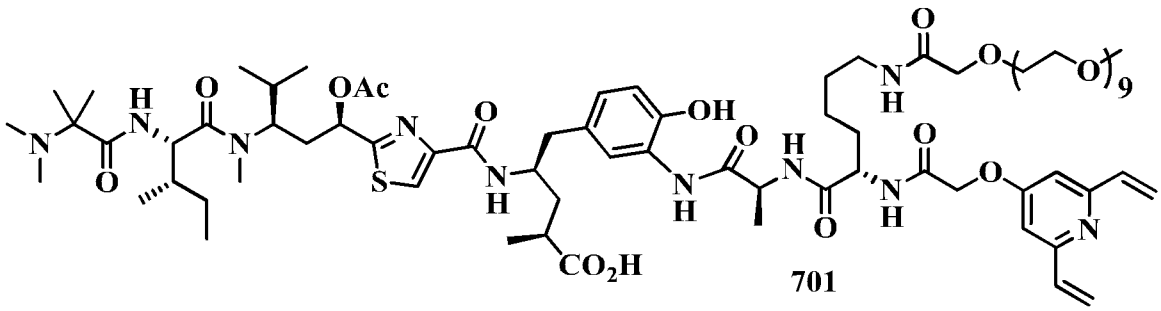
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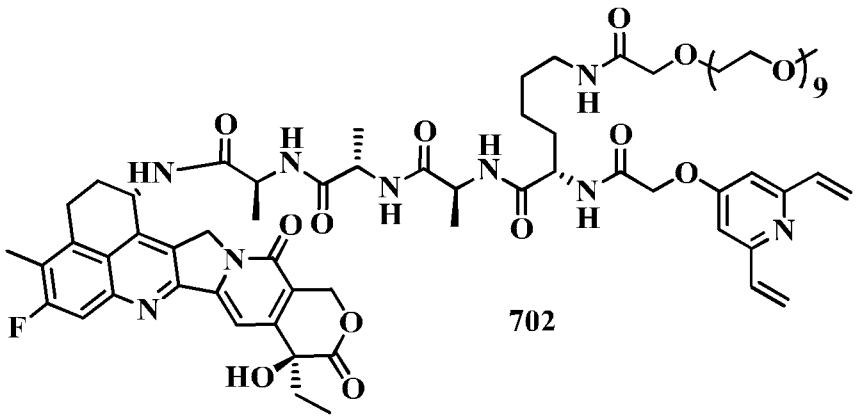
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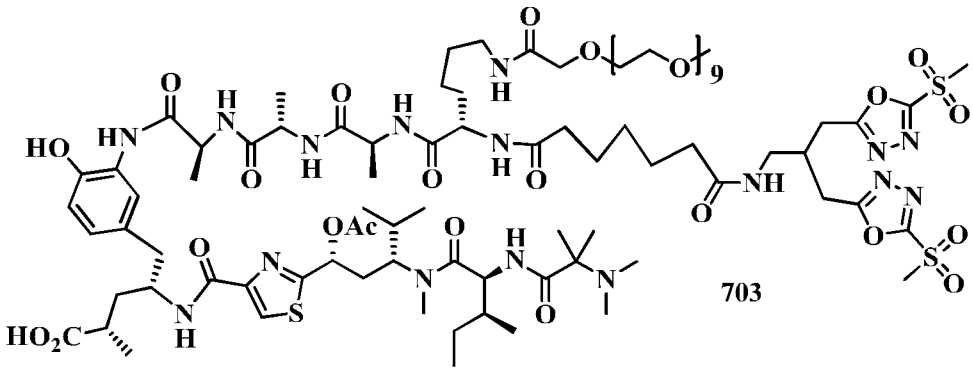
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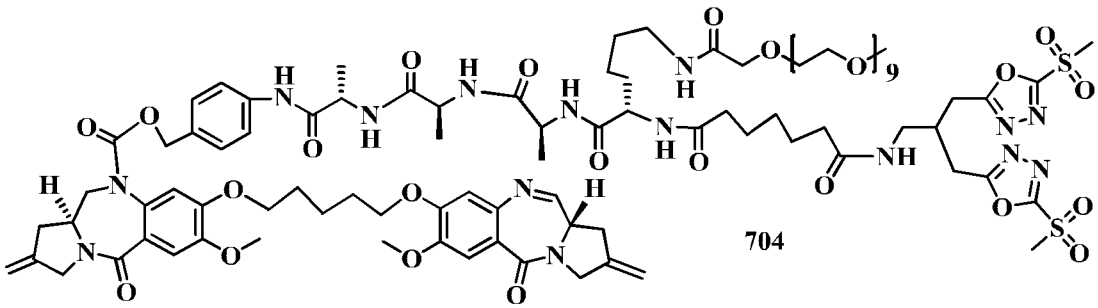
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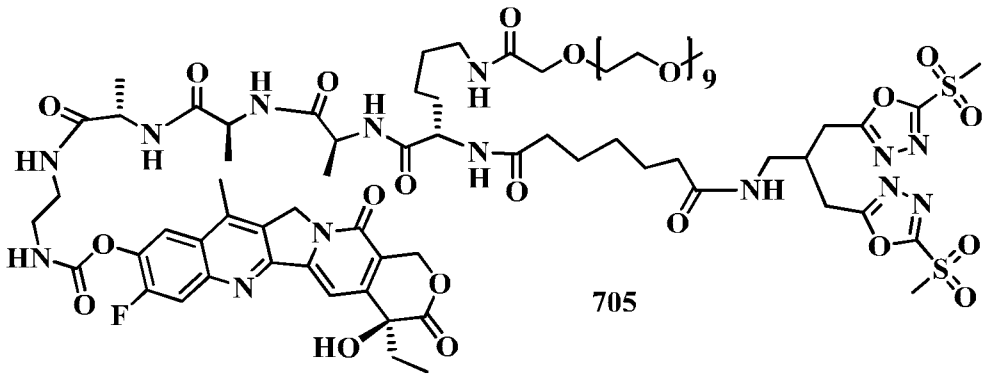
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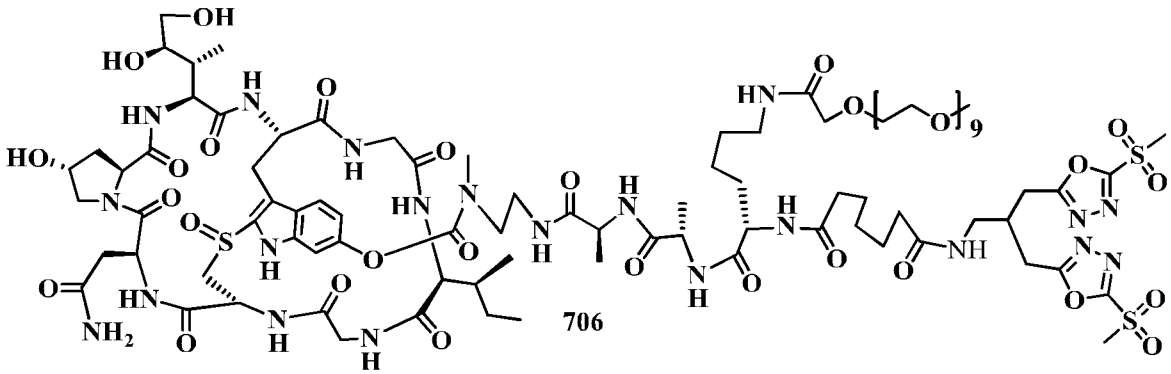
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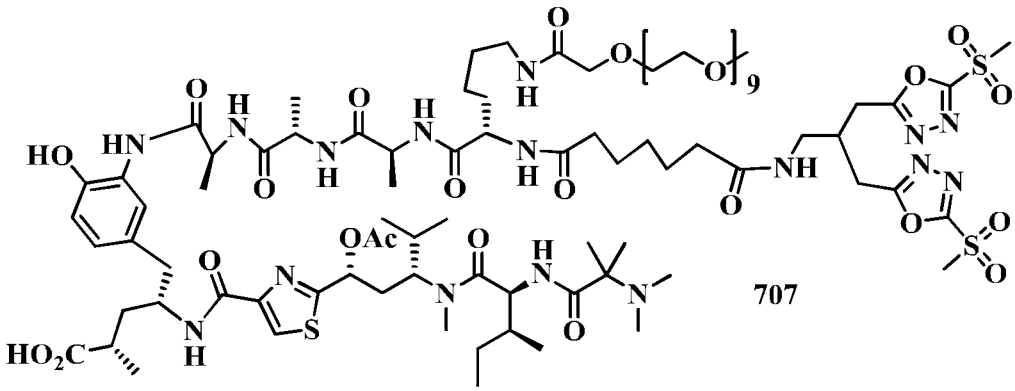
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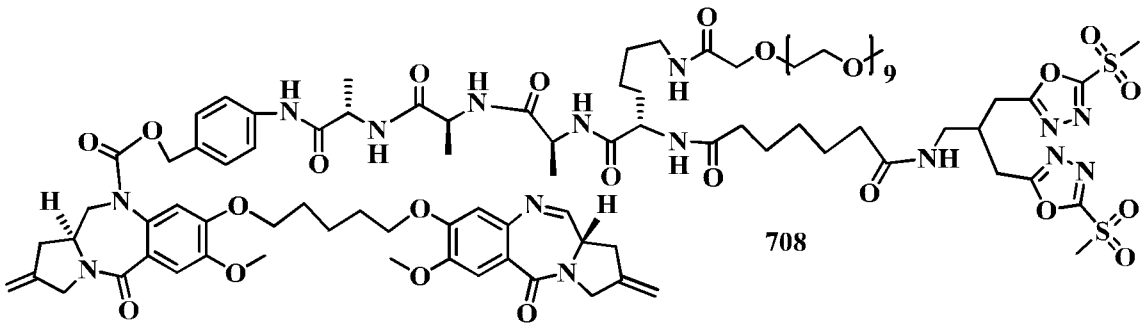
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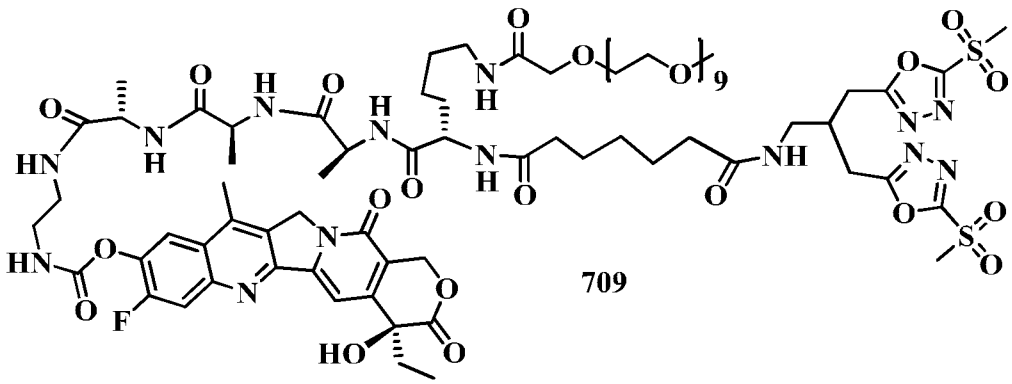
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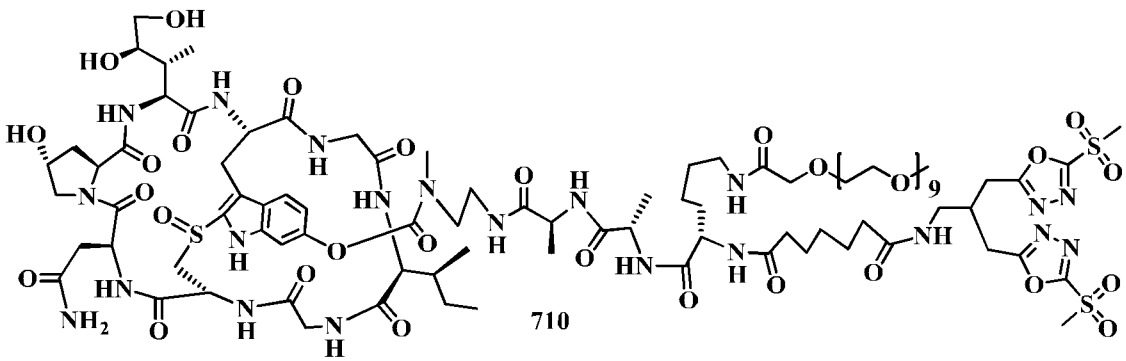
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708



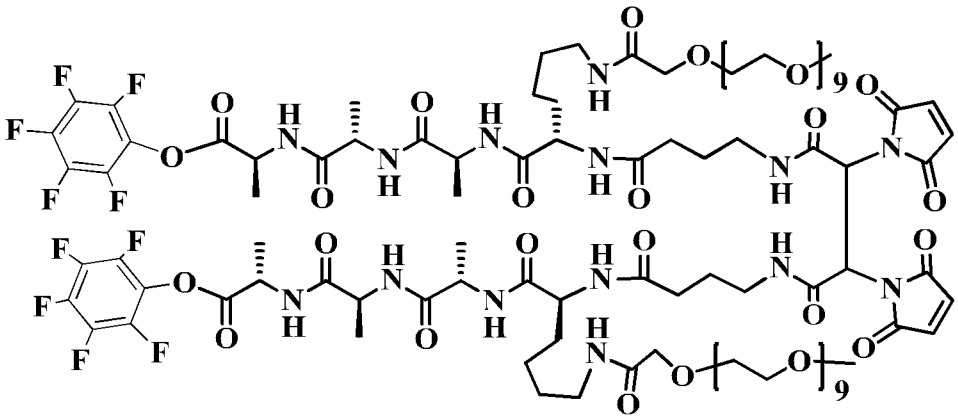
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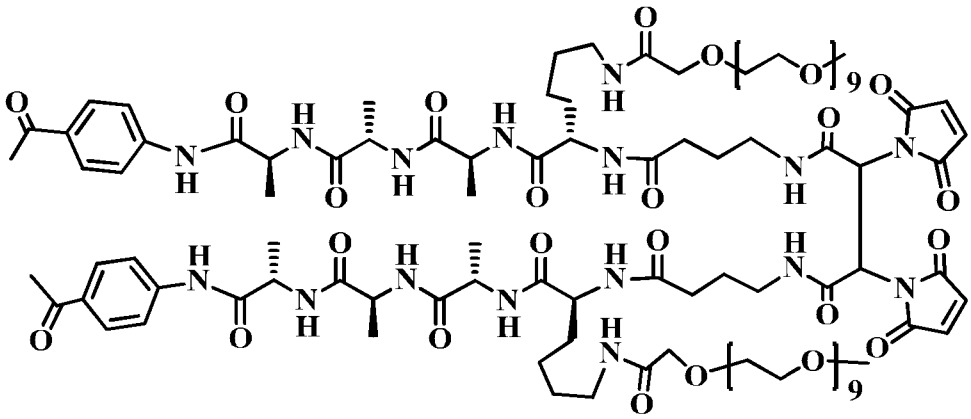
710

or a derivative with one or more isotopes, a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof; wherein n is 1 -10.

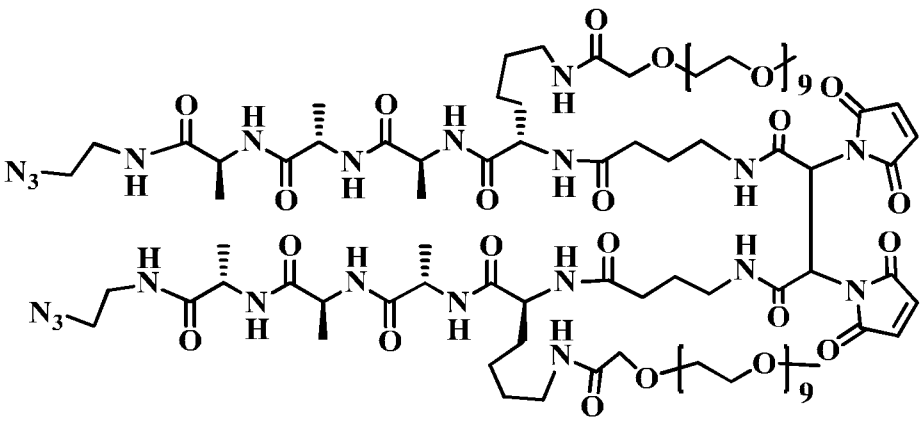
In further embodiments, examples of linker molecules of formula (X), (XI), (XII), or (XIII) are demonstrated below as:



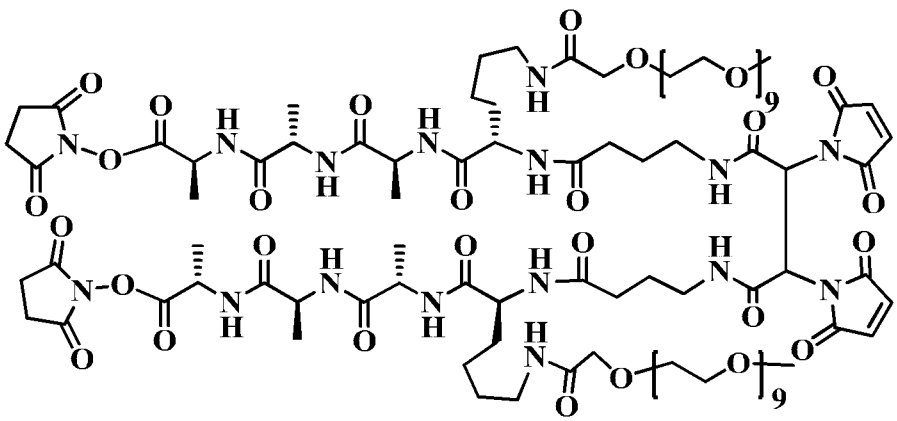
(XII-01),



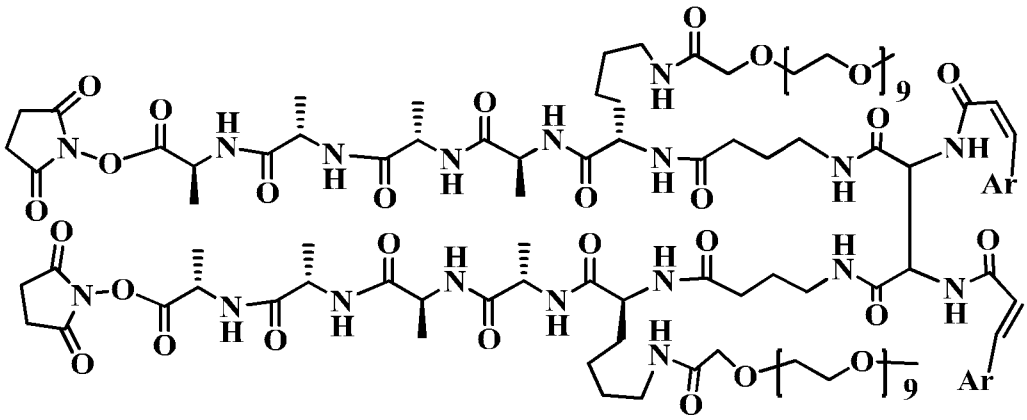
(XII-02),



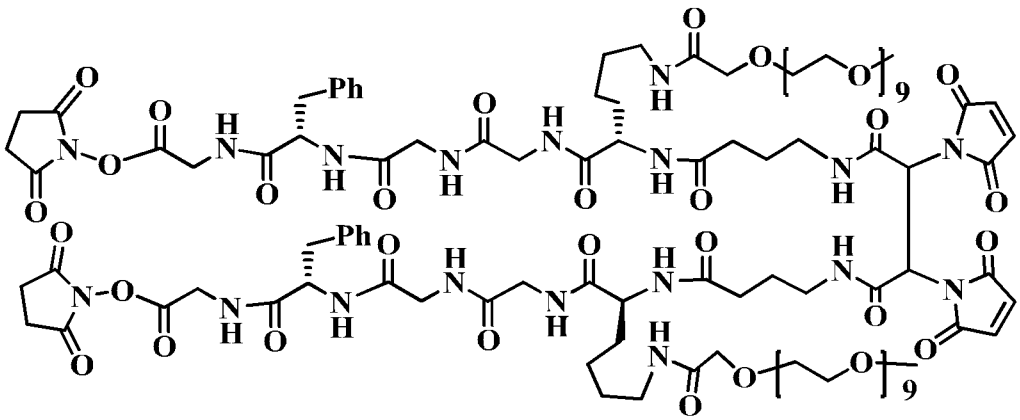
(XII-03),



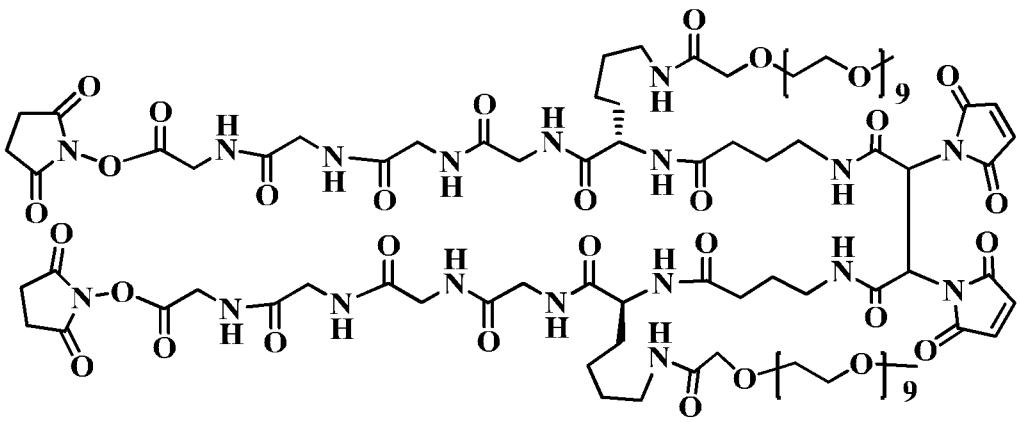
(XII-04),



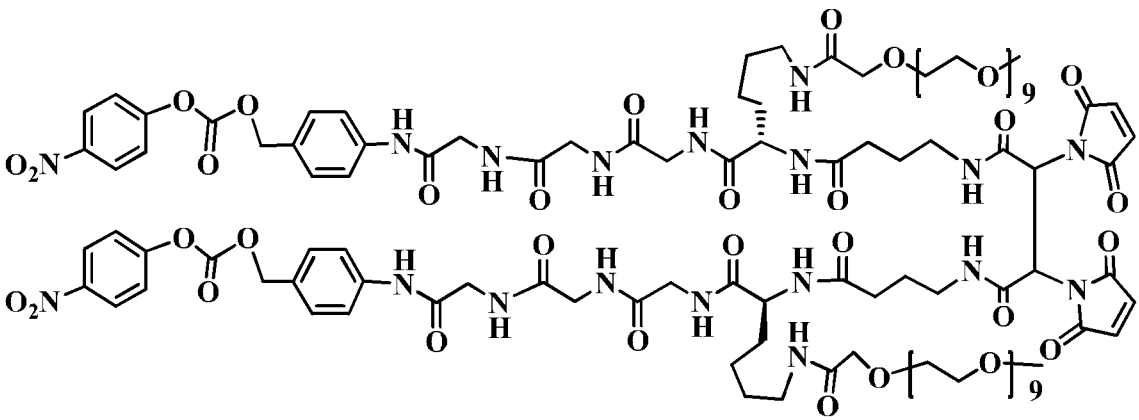
(XII-05),



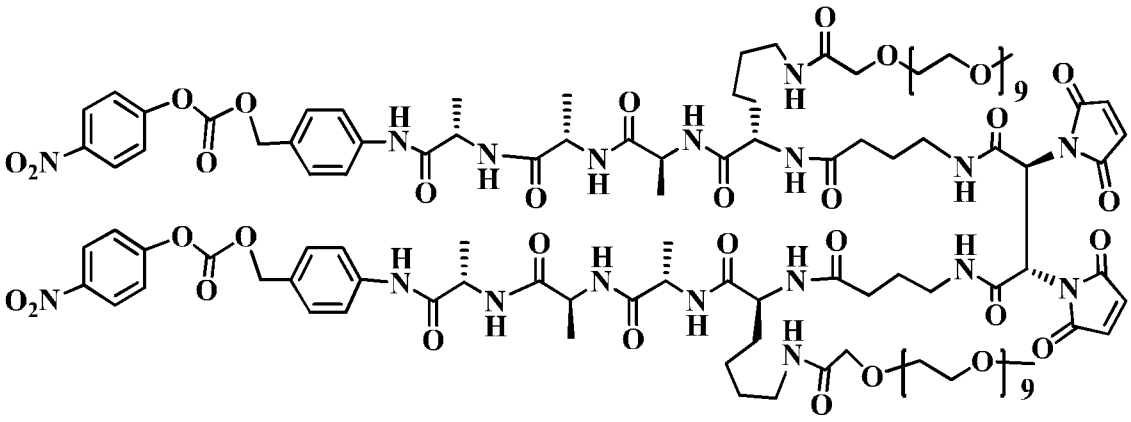
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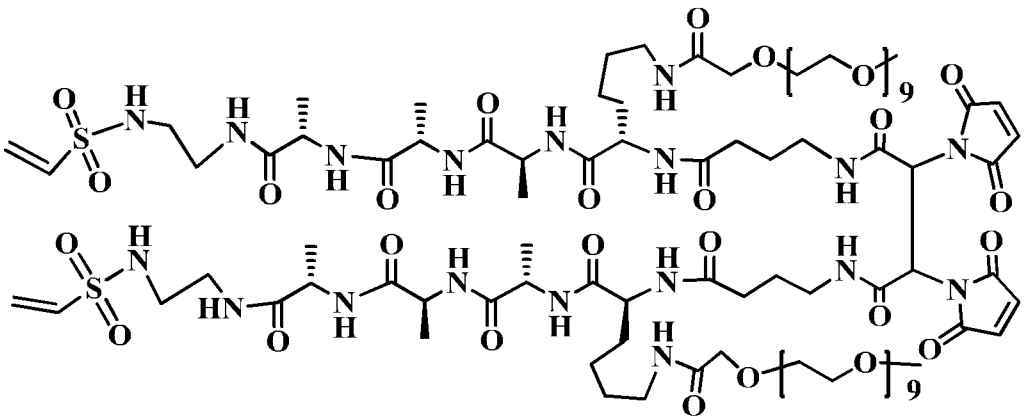
(XII-07),



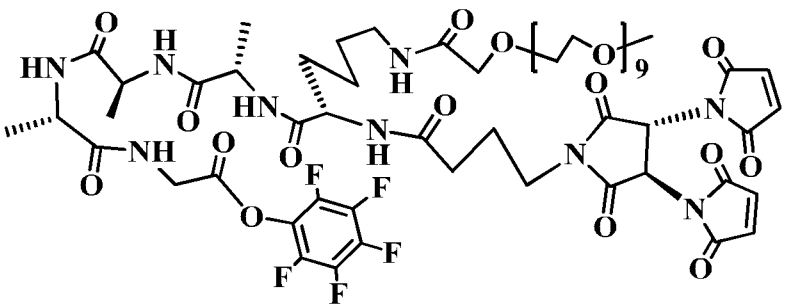
(XII-08)



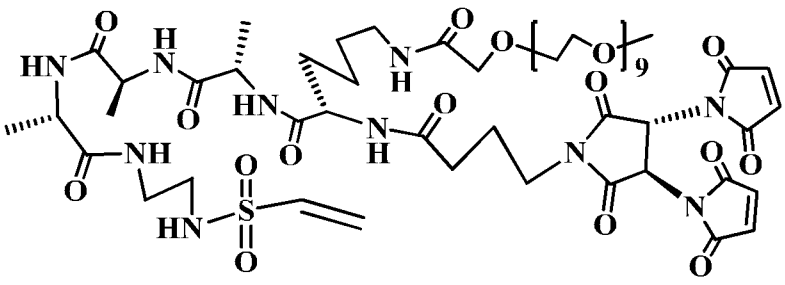
(XII-09),



(XII-10)

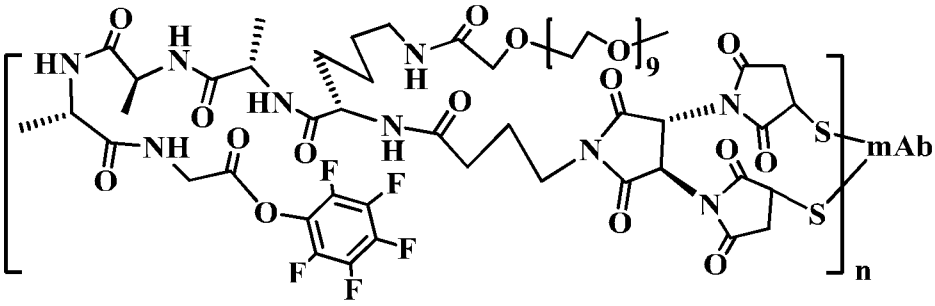


(XI-01),

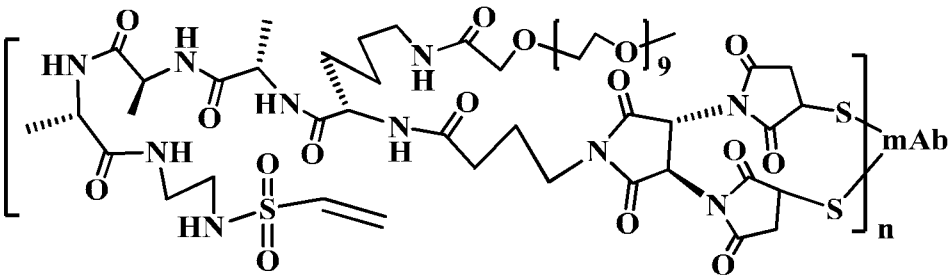


(XI-02).

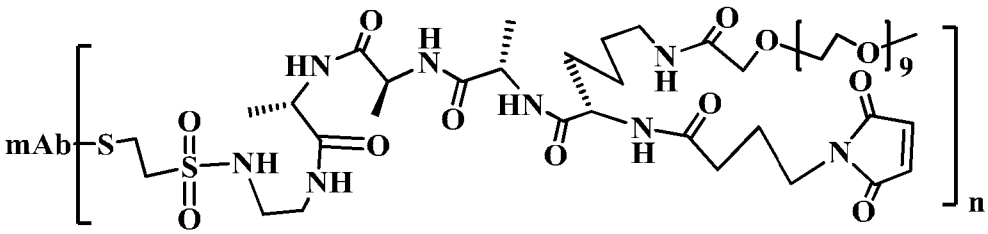
In further embodiments, examples of linker molecules of formula (XIV), (XV), (XVI) or (XVII) a are demonstrated below as:



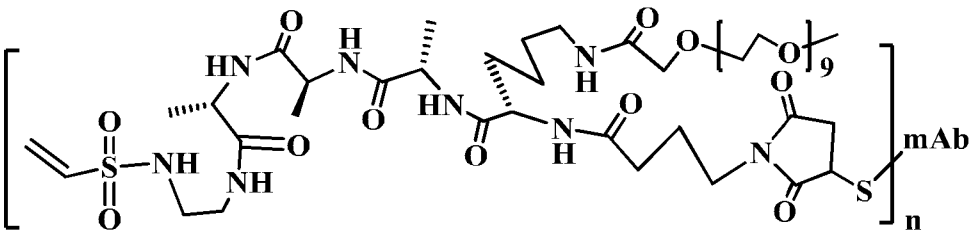
(XV-01),



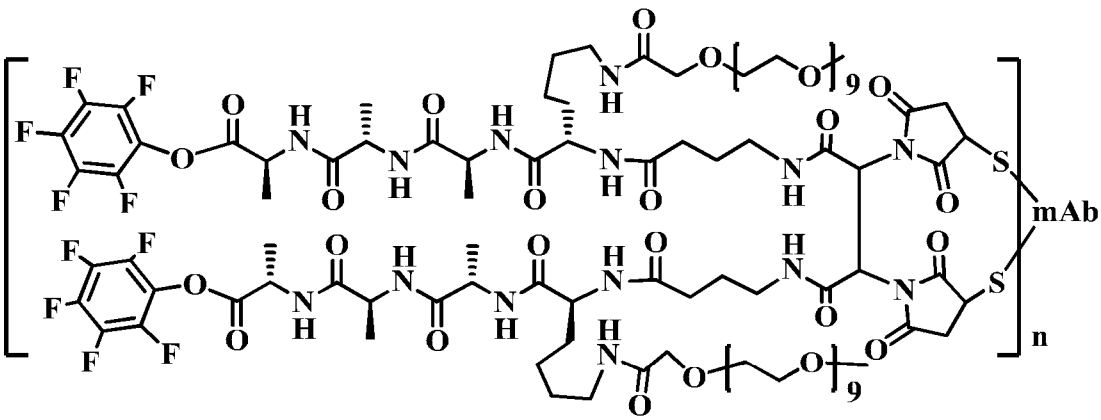
(XV-02),



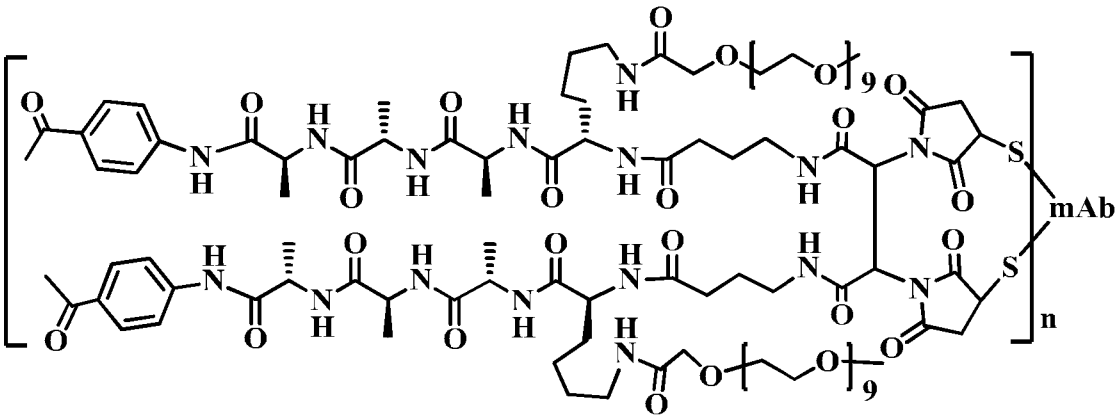
(XIV-01),



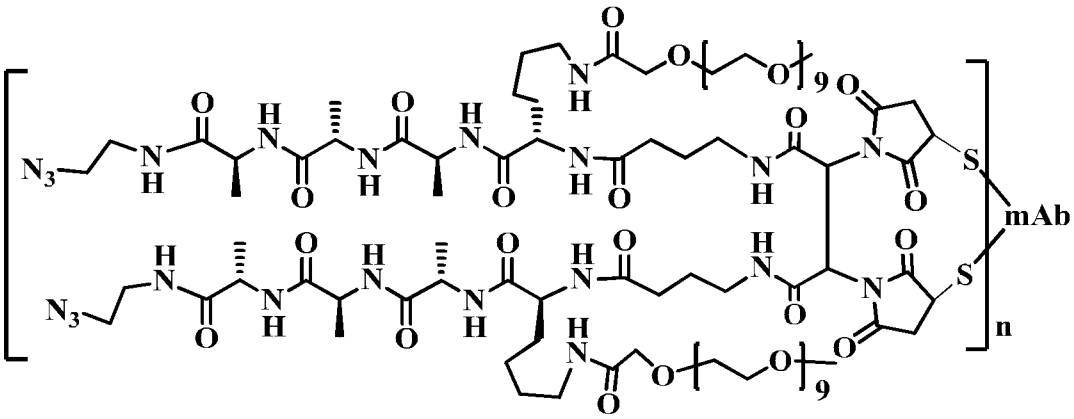
(XIV-02),



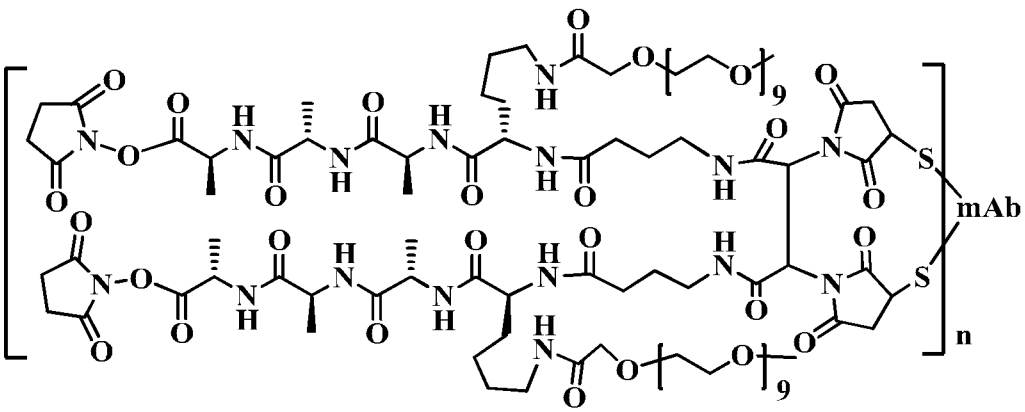
(XVI-01),



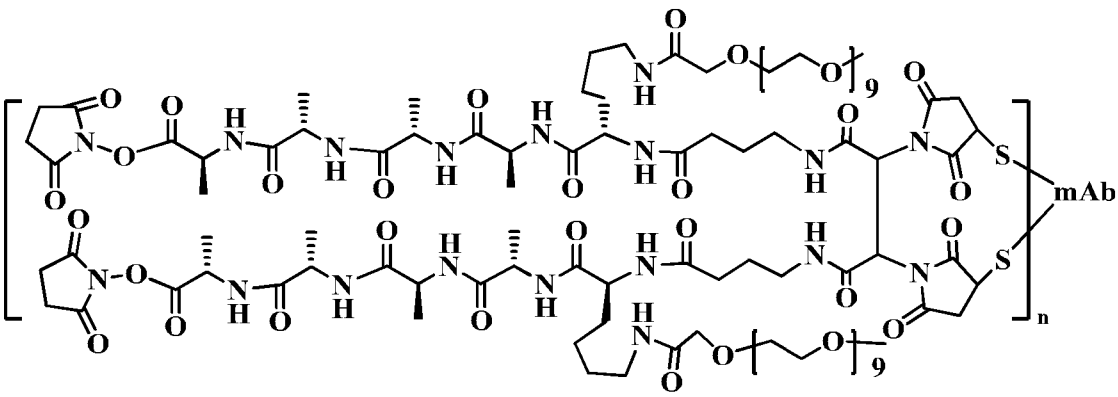
(XVI-02),



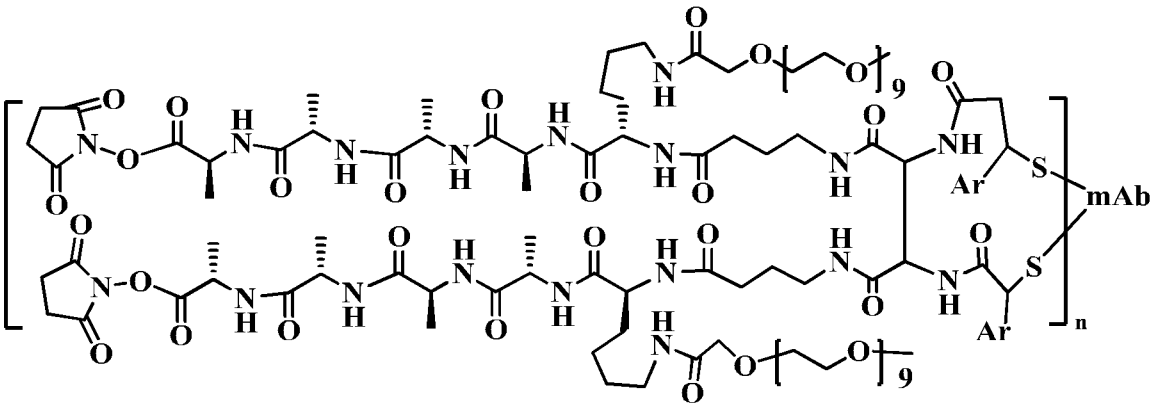
(XVI-03),



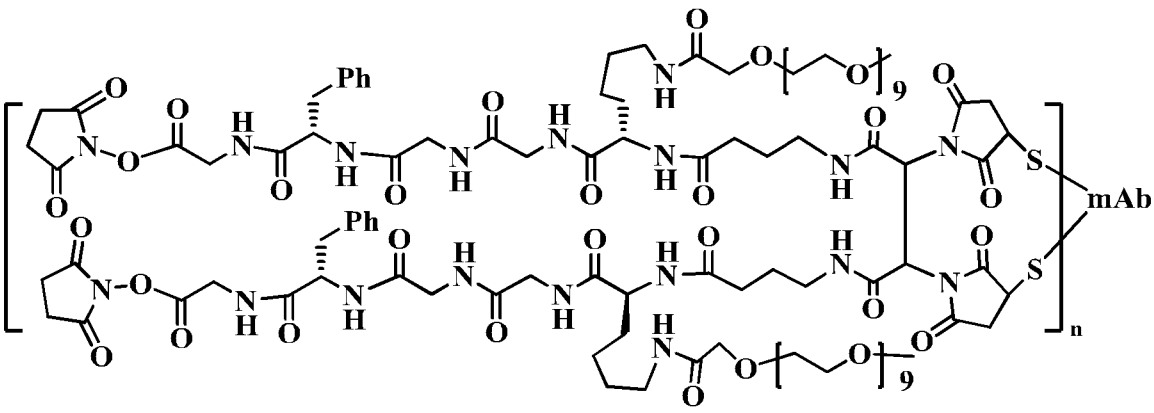
(XVI-04),



(XVI-05)



(XVI-06),



(XVI-07).

Many of the structures of the linker compounds of formula (X), (XI), (XII), (XIII), (XIV), (XV), (XVI) or (XVII) have been incorporated in the synthesis in the sections of Examples and Figures. One skilled in the art of conjugation of drugs/cytotoxic agents will readily understand that each of the linker compounds described or incorporated in the conjugates of examples of formula (VI), (VII), (VIII) or (IX) herein can be modified in such a manner for swift conjugation. The skilled artisan will also understand that many of linker compounds which are well known in art can be used in place of the linker compounds described herein for homogeneous conjugation of antibodies with this invention.

THE ANTIBODY

The antibody (mAb) used for the conjugation process is preferred a cell-binding antibody or antibody-like protein molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified.

For convenience in this section and elsewhere, "antibody" should be understood to include "antibody-like protein and peptide" except where the context requires otherwise. Suitable antibody-like proteins which may be present in the conjugates of the invention include for example peptides, polypeptides, antibodies, antibody fragments, enzymes, cytokines, chemokines, receptors, blood factors, peptide hormones, toxin, transcription antibody-like proteins, or multimeric antibody-like proteins, wherein they have interchain disulfide bonds structurally.

Enzymes include carbohydrate-specific enzymes, proteolytic enzymes and the like, for example the oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases disclosed by U.S. Pat. No. 4, 179, 337. Specific enzymes of interest include asparaginase, arginase, adenosine deaminase, superoxide dismutase, catalase, chymotrypsin, lipase, uricase, bilirubin oxidase, glucose oxidase, glucuronidase, galactosidase, glucocerbrosidase, and glutaminase.

Blood antibody-like proteins include albumin, transferrin, Factor VII, Factor VIII or Factor IX, von Willebrand factor, insulin, ACTH, glucagen, somatostatin, somatotropins, thymosin, parathyroid hormone, pigmentary hormones, somatomedins, erythropoietin, luteinizing hormone, hypothalamic releasing factors, antidiuretic hormones, prolactin, interleukins, interferons, for example IFN-α. or IFN-β, colony stimulating factors, haemoglobin, cytokines, antibodies, antibody fragments, chorionicgonadotropin, follicle-stimulating hormone, thyroid stimulating hormone and tissue plasminogen activator.

Other antibody-like proteins of interest are allergen antibody-like proteins disclosed by Dreborg et al Crit. Rev. Therap. Drug Carrier Syst. (1990) 6 315-365 as having reduced allergenicity when conjugated with a polymer such as poly(alkylene oxide) and consequently are suitable for use as tolerance inducers. Among the allergens disclosed are Ragweed antigen E, honeybee venom, mite allergen and the like.

Glycopolypeptides such as immunoglobulins, ovalbumin, lipase, glucocerebrosidase, lectins, tissue plasminogen activator and glycosylated interleukins, interferons and colony stimulating factors are of interest, as are immunoglobulins such as IgG, IgE, IgM, IgA, IgD and fragments thereof. Of particular interest are receptor and ligand binding antibody-like proteins and antibodies and antibody fragments which are used in clinical medicine for diagnostic and therapeutic purposes.

The antibody herein is preferred (A)**:** the group consisting of an antibody, a antibody-like protein molecule, probody, nanobody, peptides, an antibody coating on polymeric micelle, an antibody-liposome, a lipoprotein-based drug carrier, an antibody coating on nano-particle, an antibody-dendrimer, and a particle said above coated or linked with an antibody-like protein (antibody), or a combination of said above thereof;

(B): an antibody, full-length antibodies (polyclonal antibodies, monoclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibody, trispecific antibody, or tetraspecific antibody); single chain antibodies; an antibody fragment that binds to the target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that binds the target cell, a chimeric antibody, a chimeric antibody fragment that binds to the target cell, a domain antibody, a domain antibody fragment that binds to the target cell, a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment that binds to the target cell, a

humanized antibody or a resurfaced antibody, a humanized single chain antibody, or a humanized antibody fragment that binds to the target cell, anti-idiotypic (anti-Id) antibodies, CDR's, diabody, triabody, tetrabody, miniantibody, a probody, a probody fragment, small immune antibody-like proteins (SIP), a lymphokine antibody-like protein, a hormone type antibody-like protein, a growth factor antibody-like protein, a colony stimulating factor antibody-like protein, a nutrient-transport antibody-like protein, large molecular weight antibody-like proteins, fusion antibody-like proteins, a kinase inhibitor antibody-like protein, gene-targeting antibody-like protein, antibody-like protein coated on nanoparticles or polymers modified with antibodies or large molecular weight antibody-like proteins;

The fragments of antibodies include Fab, Fab', $F(ab')_2$, F_v, [Parham, J. Immunol. 131, 2895-902 (1983)], fragments produced by a Fab expression library, and epitope-binding fragments of any of the above which immuno-specifically bind to cancer cell antigens, viral antigens, microbial antigens or an antibody-like protein generated by the immune system that is capable of recognizing, binding to a specific antigen or exhibiting the desired biological activity (Miller et al (2003) J. of Immunology 170: 4854-61); interferons (such as type I, II, III); peptides; lymphokines such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, GM-CSF, interferon-gamma (IFN-γ); hormones such as insulin, TRH (thyrotropin releasing hormones), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens, melanocyte-stimulating hormone (MSH); growth factors and colony-stimulating factors such as epidermal growth factors (EGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factors (TGF), such as TGFα, TGFβ, insulin and insulin like growth factors (IGF-I, IGF-II) G-CSF, M-CSF and GM-CSF [Burgess, Immunology Today, 5, 155-8 (1984)]; vaccinia growth factors (VGF); fibroblast growth factors (FGFs); smaller molecular weight antibody-like proteins, poly-peptide, peptides and peptide hormones, such as bombesin, gastrin, gastrin-releasing peptide; platelet-derived growth factors; interleukin and cytokines, such as interleukin-2 (IL-2), interleukin-6 (IL-6), leukemia inhibitory factors, granulocyte-macrophage colony-stimulating factor (GM-CSF); vitamins, such as folate; apoproteins and glycoproteins, such as transferrin [O'Keefe et al, 260 J. Biol. Chem. 932-7 (1985)]; sugar-binding proteins or lipoproteins, such as lectins; cell nutrient-transport molecules; and small molecular inhibitors, such as prostate-specific membrane antigen (PSMA) inhibitors and small molecular tyrosine kinase inhibitors (TKI), non-peptides or any other cell binding molecule or substance, such as bioactive polymers (Dhar, et al, Proc. Natl. Acad. Sci. 2008, 105, 17356-61); bioactive dendrimers (Lee, et al, Nat. Biotechnol. 2005, 23, 1517-26; Almutairi, et al; Proc. Natl. Acad. Sci. 2009, 106, 685-90); nanoparticles (Liong, et al, ACS Nano, 2008, 2, 1309-12; Medarova, et al, Nat. Med. 2007, 13, 372-7; Javier, et al, Bioconjugate Chem. 2008, 19, 1309-12); liposomes

(Medinai, et al, Curr. Phar. Des. 2004, 10, 2981-9); viral capsides (Flenniken, et al, Viruses Nanotechnol. 2009, 327, 71-93).

In general, a monoclonal antibody is preferred as a cell-surface binding agent if an appropriate one is available. And the antibody may be murine, human, humanized, chimeric, or derived from other species.

Production of antibodies used in the present invention involves in vivo or in vitro procedures or combinations thereof. Methods for producing polyclonal anti-receptor peptide antibodies are well-known in the art, such as in U.S. Pat. No. 4, 493, 795 (to Nestor et al). A monoclonal antibody is typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen (Köhler, G.; Milstein, C. (1975). Nature 256: 495-7). The detailed procedures are described in "Antibodies--A Laboratory Manual", Harlow and Lane, eds., Cold Spring Harbor Laboratory Press, New York (1988), which is incorporated herein by reference. Particularly monoclonal antibodies are produced by immunizing mice, rats, hamsters or any other mammal with the antigen of interest such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus, and viral proteins. Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT (hypoxanthine-aminopterin-thymine). Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact specified receptors or inhibit receptor activity on target cells.

A monoclonal antibody used in the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques, such as using protein-A affinity chromatography; anion, cation, hydrophobic, or size exclusive chromatographies (particularly by affinity for the specific antigen after protein A, and sizing column chromatography); centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., Virol. 8, 396 (1959)) supplemented with 4.5 gm/l glucose, 0~20 mM glutamine, 0~20% fetal calf serum, several ppm amount of heavy metals, such as Cu, Mn, Fe, or Zn, etc, or/and the other heavy metals added in their salt forms, and with an anti-foaming agent, such as polyoxyethylene-polyoxypropylene block copolymer.

In addition, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with an oncovirus, such as Epstein-Barr virus (EBV, also called human herpesvirus 4 (HHV-4)) or Kaposi's sarcoma-associated herpesvirus (KSHV). See, U.S. Pat. Nos. 4, 341, 761; 4, 399, 121; 4, 427, 783; 4, 444, 887; 4, 451, 570; 4, 466, 917; 4, 472, 500; 4, 491, 632; 4, 493, 890. A monoclonal antibody may also be produced via an anti-receptor peptide or peptides containing the carboxyl terminal as described well-known in the art. See Niman et al., Proc. Natl. Acad. Sci. USA, 80: 4949-53 (1983); Geysen et al., Proc. Natl. Acad. Sci. USA, 82: 178-82 (1985); Lei et al. Biochemistry 34(20): 6675-88, (1995). Typically, the anti-receptor peptide or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen for producing anti-receptor peptide monoclonal antibodies.

There are also a number of other well-known techniques for making monoclonal antibodies as binding molecules in this invention. Particularly useful are methods of making fully human antibodies. One method is phage display technology which can be used to select a range of human antibodies binding specifically to the antigen using methods of affinity enrichment. Phage display has been thoroughly described in the literature and the construction and screening of phage display libraries are well known in the art, see, e.g., Dente et al, Gene. 148(1):7-13 (1994); Little et al, Biotechnol Adv. 12(3): 539-55 (1994); Clackson et al., Nature 352: 264-8 (1991); Huse et al., Science 246: 1275-81 (1989).

Monoclonal antibodies derived by hybridoma technique from another species than human, such as mouse, can be humanized to avoid human anti-mouse antibodies when infused into humans. Among the more common methods of humanization of antibodies are complementarity-determining region grafting and resurfacing. These methods have been extensively described, see e.g. U.S. Pat. Nos. 5, 859, 205 and 6, 797, 492; Liu et al, Immunol Rev. 222: 9-27 (2008); Almagro et al, Front Biosci. 13: 1619-33 (2008); Lazar et al, Mol Immunol. 44(8): 1986-98 (2007); Li et al, Proc. Natl. Acad. Sci. U S A. 103(10): 3557-62 (2006) each incorporated herein by reference. Fully human antibodies can also be prepared by immunizing transgenic mice, rabbits, monkeys, or other mammals, carrying large portions of the human immunoglobulin heavy and light chains, with an immunogen. Examples of such mice are: the Xenomouse. (Abgenix/Amgen), the HuMAb-Mouse (Medarex/BMS), the VelociMouse (Regeneron), see also U.S. Pat. Nos. 6, 596, 541, 6, 207, 418, 6, 150, 584, 6, 111, 166, 6, 075, 181, 5, 922, 545, 5, 661, 016, 5, 545, 806, 5, 436, 149 and 5, 569, 825. In human therapy, murine variable regions and human constant regions can also be fused to construct called "chimeric antibodies" that are considerably less immunogenic in man than murine mAbs (Kipriyanov et al, Mol Biotechnol. 26: 39-60 (2004); Houdebine, Curr Opin Biotechnol. 13:

625-9 (2002) each incorporated herein by reference). In addition, site-directed mutagenesis in the variable region of an antibody can result in an antibody with higher affinity and specificity for its antigen (Brannigan et al, Nat Rev Mol Cell Biol. 3: 964-70, (2002)); Adams et al, J Immunol Methods. 231: 249-60 (1999)) and exchanging constant regions of a mAb can improve its ability to mediate effector functions of binding and cytotoxicity.

Antibodies immunospecific for a malignant cell antigen can also be obtained commercially or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies immune-specific for a malignant cell antigen can be obtained commercially, e.g., from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing.

Apart from an antibody, an antibody like peptide or protein that bind/block/target or in some other way interact with the epitopes or corresponding receptors on a targeted cell can be used as a binding molecule. These antibody like peptides or proteins could be any random peptide or proteins that have an affinity for the epitopes or corresponding receptors and they don't necessarily have to be of the immune-globulin family. These peptides can be isolated by similar techniques as for phage display antibodies (Szardenings, J Recept Signal Transduct Res. 2003, 23(4): 307-49). The use of peptides from such random peptide libraries can be similar to antibodies and antibody fragments. The binding molecules of antibody like peptides or proteins may be conjugated on or linked to a large molecules or materials, such as, but is not limited, an albumin, a polymer, a liposome, a nano particle, a dendrimer, as long as such attachment permits the peptide or protein to retain its antigen binding specificity.

Examples of antibodies used for conjugation of drugs of this prevention for treating cancer, autoimmune disease, and/or infectious disease include, but are not limited to, 3F8 (anti-GD2), Abagovomab (anti CA-125), Abciximab (anti CD41 (integrin alpha-IIb), Adalimumab (anti-TNF-α), Adecatumumab (anti-EpCAM, CD326), Afelimomab (anti-TNF-α); Afutuzumab (anti-CD20), Alacizumab pegol (anti-VEGFR2), ALD518 (anti-IL-6), Alemtuzumab (Campath, MabCampath, anti- CD52), Altumomab (anti-CEA), Anatumomab (anti-TAG-72), Anrukinzumab (IMA-638, anti-IL-13), Apolizumab (anti-HLA-DR), Arcitumomab (anti-CEA), Aselizumab (anti-L-selectin (CD62L), Atlizumab (tocilizumab, Actemra, RoActemra, anti-IL-6 receptor), Atorolimumab (anti-Rhesus factor), Bapineuzumab (anti-beta amyloid), Basiliximab (Simulect, antiCD25 (α chain of IL-2 receptor), Bavituximab (anti-phosphatidylserine), Bectumomab (LymphoScan, anti-CD22), Belimumab (Benlysta, LymphoStat-B, anti-BAFF), Benralizumab (anti-CD125), Bertilimumab (anti-CCL11 (eotaxin-1)), Besilesomab (Scintimun, anti-CEA-related antigen), Bevacizumab (Avastin, anti-VEGF-A), Biciromab (FibriScint, anti-fibrin II beta chain), Bivatuzumab (anti-CD44

v6), Blinatumomab (BiTE, anti-CD19), Brentuximab (cAC10, anti-CD30 TNFRSF8), Briakinumab (anti-IL-12, IL-23) Canakinumab (Ilaris, anti-IL-1), Cantuzumab (C242, anti-CanAg), Capromab, Catumaxomab (Removab, anti-EpCAM, anti-CD3), CC49 (anti-TAG-72), Cedelizumab (anti-CD4), Certolizumab pegol (Cimzia anti-TNF-α), Cetuximab (Erbitux, IMC-C225, anti-EGFR), Citatuzumab bogatox (anti-EpCAM), Cixutumumab (anti-IGF-1), Clenoliximab (anti-CD4), Clivatuzumab (anti-MUC1), Conatumumab (anti-TRAIL-R2), CR6261 (anti-Influenza A hemagglutinin), Dacetuzumab (anti-CD40), Daclizumab (Zenapax, anti-CD25 (α chain of IL-2 receptor)), Daratumumab (anti-CD38 (cyclic ADP ribose hydrolase), Denosumab (Prolia, anti-RANKL), Detumomab (anti-B-lymphoma cell), Dorlimomab, Dorlixizumab, Ecromeximab (anti-GD3 ganglioside), Eculizumab (Soliris, anti-C5), Edobacomab (anti-endotoxin), Edrecolomab (Panorex, MAb17-1A, anti-EpCAM), Efalizumab (Raptiva, anti-LFA-1 (CD11a), Efungumab (Mycograb, anti-Hsp90), Elotuzumab (anti-SLAMF7), Elsilimomab (anti-IL-6), Enlimomab pegol (anti-ICAM-1 (CD54)), Epitumomab (anti-episialin), Epratuzumab (anti-CD22), Erlizumab (anti-ITGB2 (CD18)), Ertumaxomab (Rexomun, anti-HER2/neu, CD3), Etaracizumab (Abegrin, anti-integrin $\alpha_v\beta_3$), Exbivirumab (anti-hepatitis B surface antigen), Fanolesomab (NeutroSpec, anti-CD15), Faralimomab (anti-interferon receptor), Farletuzumab (anti-folate receptor 1), Felvizumab (anti-respiratory syncytial virus), Fezakinumab (anti-IL-22), Figitumumab (anti-IGF-1 receptor), Fontolizumab (anti-IFN-γ), Foravirumab (anti-rabies virus glycoprotein), Fresolimumab (anti-TGF-β), Galiximab (anti-CD80), Gantenerumab (anti- beta amyloid), Gavilimomab (anti-CD147 (basigin)), Gemtuzumab (anti-CD33), Girentuximab (anti-carbonic anhydrase 9), Glembatumumab (CR011, anti-GPNMB), Golimumab (Simponi, anti-TNF-α), Gomiliximab (anti-CD23 (IgE receptor)), Ibalizumab (anti-CD4), Ibritumomab (anti-CD20), Igovomab (Indimacis-125, anti-CA-125), Imciromab (Myoscint, anti-cardiac myosin), Infliximab (Remicade, anti-TNF-α), Intetumumab (anti-CD51), Inolimomab (anti-CD25 (α chain of IL-2 receptor)), Inotuzumab (anti-CD22), Ipilimumab (anti-CD152), Iratumumab (anti- CD30 (TNFRSF8)), Keliximab (anti-CD4), Labetuzumab (CEA-Cide, anti-CEA), Lebrikizumab (anti- IL-13), Lemalesomab (anti-NCA-90 (granulocyte antigen)), Lerdelimumab (anti-TGF beta 2), Lexatumumab (anti-TRAIL-R2), Libivirumab (anti-hepatitis B surface antigen), Lintuzumab (anti-CD33), Lucatumumab (anti-CD40), Lumiliximab (anti- CD23 (IgE receptor), Mapatumumab (anti-TRAIL-R1), Maslimomab (anti- T-cell receptor), Matuzumab (anti-EGFR), Mepolizumab (Bosatria, anti-IL-5), Metelimumab (anti-TGF beta 1), Milatuzumab (anti-CD74), Minretumomab (anti-TAG-72), Mitumomab (BEC-2, anti-GD3 ganglioside), Morolimumab (anti-Rhesus factor), Motavizumab (Numax, anti-respiratory syncytial virus), Muromonab-CD3 (Orthoclone OKT3, anti-CD3), Nacolomab (anti-C242), Naptumomab (anti-5T4), Natalizumab (Tysabri, anti-integrin α_4), Nebacumab (anti-endotoxin),

Necitumumab (anti-EGFR), Nerelimomab (anti-TNF-α), Nimotuzumab (Theracim, Theraloc, anti-EGFR), Nofetumomab, Ocrelizumab (anti-CD20), Odulimomab (Afolimomab, anti-LFA-1 (CD11a)), Ofatumumab (Arzerra, anti-CD20), Olaratumab (anti-PDGF-R α), Omalizumab (Xolair, anti-IgE Fc region), Oportuzumab (anti-EpCAM), Oregovomab (OvaRex, anti-CA-125), Otelixizumab (anti-CD3), Pagibaximab (anti-lipoteichoic acid), Palivizumab (Synagis, Abbosynagis, anti-respiratory syncytial virus), Panitumumab (Vectibix, ABX-EGF, anti-EGFR), Panobacumab (anti- Pseudomonas aeruginosa), Pascolizumab (anti-IL-4), Pemtumomab (Theragyn, anti-MUC1), Pertuzumab (Omnitarg, 2C4, anti-HER2/neu), Pexelizumab (anti-C5), Pintumomab (anti-adenocarcinoma antigen), Priliximab (anti-CD4), Pritumumab (anti-vimentin), PRO 140 (anti-CCR5), Racotumomab (1E10, anti-(N-glycolylneuraminic acid (NeuGc, NGNA)-gangliosides GM3)), Rafivirumab (anti-rabies virus glycoprotein), Ramucirumab (anti-VEGFR2), Ranibizumab (Lucentis, anti-VEGF-A), Raxibacumab (anti-anthrax toxin, protective antigen), Regavirumab (anti-cytomegalovirus glycoprotein B), Reslizumab (anti-IL-5), Rilotumumab (anti-HGF), Rituximab (MabThera, Rituxanmab, anti-CD20), Robatumumab (anti-IGF-1 receptor), Rontalizumab (anti-IFN-α), Rovelizumab (LeukArrest, anti-CD11, CD18), Ruplizumab (Antova, anti-CD154 (CD40L)), Satumomab (anti-TAG-72), Sevirumab (anti-cytomegalovirus), Sibrotuzumab (anti-FAP), Sifalimumab (anti-IFN-α), Siltuximab (anti-IL-6), Siplizumab (anti-CD2), (Smart) MI95 (anti-CD33), Solanezumab (anti-beta amyloid), Sonepcizumab (anti-sphingosine-1-phosphate), Sontuzumab (anti-episialin), Stamulumab (anti-myostatin), Sulesomab (LeukoScan, (anti-NCA-90 (granulocyte antigen), Tacatuzumab (anti-alpha-fetoprotein), Tadocizumab (anti-integrin $\alpha_{IIb}\beta_3$), Talizumab (anti-IgE), Tanezumab (anti-NGF), Taplitumomab (anti-CD19), Tefibazumab (Aurexis, (anti-clumping factor A), Telimomab, Tenatumomab (anti-tenascin C), Teneliximab (anti-CD40), Teplizumab (anti-CD3), TGN1412 (anti-CD28), Ticilimumab (Tremelimumab, (anti-CTLA-4), Tigatuzumab (anti-TRAIL-R2), TNX-650 (anti-IL-13), Tocilizumab (Atlizumab, Actemra, RoActemra, (anti-IL-6 receptor), Toralizumab (anti-CD154 (CD40L)), Tositumomab (anti-CD20), Trastuzumab (Herceptin, (anti-HER2/neu), Tremelimumab (anti-CTLA-4), Tucotuzumab celmoleukin (anti-EpCAM), Tuvirumab (anti-hepatitis B virus), Urtoxazumab (anti- Escherichia coli), Ustekinumab (Stelara, anti-IL-12, IL-23), Vapaliximab (anti-AOC3 (VAP-1)), Vedolizumab, (anti-integrin $\alpha_4\beta_7$), Veltuzumab (anti-CD20), Vepalimomab (anti-AOC3 (VAP-1), Visilizumab (Nuvion, anti-CD3), Vitaxin (anti-vascular integrin avb3), Volociximab (anti-integrin $\alpha_5\beta_1$), Votumumab (HumaSPECT, anti-tumor antigen CTAA16.88), Zalutumumab (HuMax-EGFr, (anti-EGFR), Zanolimumab (HuMax-CD4, anti-CD4), Ziralimumab (anti-CD147 (basigin)), Zolimomab (anti-CD5), Etanercept (Enbrel®), Alefacept (Amevive®), Abatacept (Orencia®), Rilonacept (Arcalyst), 14F7 [anti-IRP-2 (Iron Regulatory Protein 2)], 14G2a (anti-GD2 ganglioside, from Nat.

Cancer Inst. for melanoma and solid tumors), J591 (anti-PSMA, Weill Cornell Medical School for prostate cancers), 225.28S [anti-HMW-MAA (High molecular weight-melanoma-associated antigen), Sorin Radiofarmaci S.R.L. (Milan, Italy) for melanoma], COL-1 (anti-CEACAM3, CGM1, from Nat. Cancer Inst. USA for colorectal and gastric cancers), CYT-356 (Oncoltad®, for prostate cancers), HNK20 (OraVax Inc. for respiratory syncytial virus), ImmuRAIT (from Immunomedics for NHL), Lym-1 (anti-HLA-DR10, Peregrine Pharm. for Cancers), MAK-195F [anti-TNF (tumor necrosis factor; TNFA, TNF-alpha; TNFSF2), from Abbott / Knoll for Sepsis toxic shock], MEDI-500 [T10B9, anti-CD3, TRαβ (T cell receptor alpha/beta), complex, from MedImmune Inc for Graft-versus-host disease], RING SCAN [anti-TAG 72 (tumour associated glycoprotein 72), from Neoprobe Corp. for Breast, Colon and Rectal cancers], Avicidin (anti-EPCAM (epithelial cell adhesion molecule), anti-TACSTD1 (Tumor-associated calcium signal transducer 1), anti-GA733-2 (gastrointestinal tumor-associated protein 2), anti-EGP-2 (epithelial glycoprotein 2); anti-KSA; KS1/4 antigen; M4S; tumor antigen 17-1A; CD326, from NeoRx Corp. for Colon, Ovarian, Prostate cancers and NHL]; LymphoCide (Immunomedics, NJ), Smart ID10 (Protein Design Labs), Oncolym (Techniclone Inc, CA), Allomune (BioTransplant, CA), anti-VEGF (Genentech, CA); CEAcide (Immunomedics, NJ), IMC-1C11 (ImClone, NJ) and Cetuximab (ImClone, NJ) .

Other antibodies as cell binding molecules/ligands include, but are not limited to, are antibodies against the following antigens: Aminopeptidase N (CD13), Annexin A1, B7-H3 (CD276, various cancers), CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), CD2 (Hodgkin's disease, NHL lymphoma, multiple myeloma), CD3 epsilon (T cell lymphoma, lung, breast, gastric, ovarian cancers, autoimmune diseases, malignant ascites), CD19 (B cell malignancies), CD20 (non-Hodgkin's lymphoma), CD22 (leukemia, lymphoma, multiple myeloma, SLE), CD30 (Hodgkin's lymphoma), CD33 (leukemia, autoimmune diseases), CD38 (multiple myeloma), CD40 (lymphoma, multiple myeloma, leukemia (CLL)), CD51 (Metastatic melanoma, sarcoma), CD52 (leukemia), CD56 (small cell lung cancers, ovarian cancer, Merkel cell carcinoma, and the liquid tumor, multiple myeloma), CD66e (cancers), CD70 (metastatic renal cell carcinoma and non-Hodgkin lymphoma), CD74 (multiple myeloma), CD80 (lymphoma), CD98 (cancers), mucin (carcinomas), CD221 (solid tumors), CD227 (breast, ovarian cancers), CD262 (NSCLC and other cancers), CD309 (ovarian cancers), CD326 (solid tumors), CEACAM3 (colorectal, gastric cancers), CEACAM5 (carcinoembryonic antigen; CEA, CD66e) (breast, colorectal and lung cancers), DLL3 or DLL4 (delta-like-3 or delta-like-4), EGFR (Epidermal Growth Factor Receptor, various cancers), CTLA4

(melanoma), CXCR4 (CD184, Heme-oncology, solid tumors), Endoglin (CD105, solid tumors), EPCAM (epithelial cell adhesion molecule, bladder, head, neck, colon, NHL prostate, and ovarian cancers), ERBB2 (Epidermal Growth Factor Receptor 2; lung, breast, prostate cancers), FCGR1 (autoimmune diseases), FOLR (folate receptor, ovarian cancers), GD2 ganglioside (cancers), G-28 (a cell surface antigen glyvolipid, melanoma), GD3 idiotype (cancers), Heat shock proteins (cancers), HER1 (lung, stomach cancers), HER2 (breast, lung and ovarian cancers), HLA-DR10 (NHL), HLA-DRB (NHL, B cell leukemia), human chorionic gonadotropin (carcinoma), IGF1R (insulin-like growth factor 1 receptor, solid tumors, blood cancers), IL-2 receptor (interleukin 2 receptor, T-cell leukemia and lymphomas), IL-6R (interleukin 6 receptor, multiple myeloma, RA, Castleman's disease, IL6 dependent tumors), Integrins (αvβ3, α5β1, α6β4, αIIβ3, α5β5, αvβ5, for various cancers), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE 4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A member 1, Non-Hodgkin's B cell lymphoma, leukemia), MUC1 or MUC1-KLH (breast, ovarian, cervix, bronchus and gastrointestinal cancer), MUC16 (CA125) (Ovarian cancers), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), MPG (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A, small cell lung cancers, NHL), Nucleolin, Neu oncogene product (carcinomas), P21 (carcinomas), Paratope of anti-(N-glycolylneuraminic acid, Breast, Melanoma cancers), PLAP-like testicular alkaline phosphatase (ovarian, testicular cancers), PSMA (prostate tumors), PSA (prostate), ROBO4, TAG 72 (tumour associated glycoprotein 72, AML, gastric, colorectal, ovarian cancers), T cell transmembrane protein (cancers), Tie (CD202b), TNFRSF10B (tumor necrosis factor receptor superfamily member 10B, cancers), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B, multiple myeloma, NHL, other cancers, RA and SLE), TPBG (trophoblast glycoprotein, Renal cell carcinoma), TRAIL-R1 (Tumor necrosis apoprosis Inducing ligand Receptor 1, lymphoma, NHL, colorectal, lung cancers), VCAM-1 (CD106, Melanoma), VEGF, VEGF-A, VEGF-2 (CD309) (various cancers). Some other tumor associated antigens recognized by antibodies have been reviewed (Gerber, et al, mAbs 1:3, 247-53 (2009); Novellino et al, Cancer Immunol Immunother. 54(3), 187-207 (2005). Franke, et al, Cancer Biother Radiopharm. 2000, 15, 459-76).

The antibody-like protein, more preferred an IgG antibody that is able to against tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune disease cells, activated tumor cells, myeloid cells, activated T-cells, an affecting B cells, or melanocytes. More specifically the antibody is able to against abnormal cells expressing any one of the following antigens or receptors: CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CD12w,

CD14, CD15, CD16, CD16a, CD16b, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD32a, CD32b, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD49c, CD49d, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD65s, CD66, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD67, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD76, CD77, CD78, CD79, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, CD85a, CD85b, CD85c, CD85d, CD85e, CD85f, CD85g, CD85g, CD85i, CD85j, CD85k, CD85m, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD120a, CD120b, CD121, CD121a, CD121b, CD122, CD123, CD123a, CD124, CD125, CD126, CD127, CD128, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140, CD140a, CD140b, CD141, CD142, CD143, CD144, CD145, CDw145, CD146, CD147, CD148, CD149, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD156a, CD156b, CD156c, CD156d, CD157, CD158, CD158a, CD158b1, CD158b2, CD158c, CD158d, CD158e1, CD158e2, CD158f2, CD158g, CD158h, CD158i, CD158j, CD158k, CD159, CD159a, CD159b, CD159c, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD167, CD167a, CD167b, CD168, CD169, CD170, CD171, CD172, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CDw186, CD187, CD188, CD189, CD190, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CD198, CD199, CDw198, CDw199, CD200, CD201, CD202, CD202（a, b）, CD203, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CD210, CDw210a, CDw210b, CD211, CD212, CD213, CD213a1, CD213a2, CD214, CD215, CD216, CD217, CD218, CD218a, CD218, CD21b9, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235, CD235a, CD235b, CD236, CD237, CD238, CD239, CD240, CD240ce, CD240d, CD241, CD242, CD243, CD244, CD245, CD246, CD247, CD248, CD249, CD250, CD251, CD252, CD253, CD254, CD255, CD256, CD257, CD258, CD259, CD260, CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD270, CD271, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD281, CD282, CD283, CD284, CD285, CD286, CD287, CD288, CD289, CD290, CD291, CD292, CD293, CD294, CD295, CD296, CD297, CD298, CD299, CD300, CD300a, CD300b, CD300c, CD301, CD302, CD303, CD304, CD305, CD306, CD307, CD307a, CD307b, CD307c, CD307d, CD307e, CD307f, CD308, CD309,

CD310, CD311, CD312, CD313, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD323, CD324, CD325, CD326, CD327, CD328, CD329, CD330, CD331, CD332, CD333, CD334, CD335, CD336, CD337, CD338, CD339, CD340, CD341, CD342, CD343, CD344, CD345, CD346, CD347, CD348, CD349, CD350, CD351, CD352, CD353, CD354, CD355, CD356, CD357, CD358, CD359, CD360, CD361, CD362, CD363, CD364, CD365, CD366, CD367, CD368, CD369, CD370, CD371, CD372, CD373, CD374, CD375, CD376, CD377, CD378, CD379, CD381, CD382, CD383, CD384, CD385, CD386, CD387, CD388, CD389, CRIPTO, CRIPTO, CR, CR1, CRGF, CRIPTO, CXCR5, LY64, TDGF1, 4-1BB, APO2, ASLG659, BMPR1B, 4-1BB, 5AC, 5T4 (Trophoblastic glycoprotein, TPBG, 5T4, Wnt-Activated Inhibitory Factor 1 or WAIF1), Adenocarcinoma antigen, AGS-5, AGS-22M6, Activin receptor-like kinase 1, AFP, AKAP-4, ALK, Alpha integrin, Alpha v beta6, Amino-peptidase N, Amyloid beta, Androgen receptor, Angiopoietin 2, Angiopoietin 3, Annexin A1, Anthrax toxin protective antigen, Anti-transferrin receptor, AOC3 (VAP-1), B7-H3, Bacillus anthracis anthrax, BAFF (B-cell activating factor), BCMA, B-lymphoma cell, bcr-abl, Bombesin, BORIS, C5, C242 antigen, CA125 (carbohydrate antigen 125, MUC16), CA-IX (or CAIX, carbonic anhydrase 9), CALLA, CanAg, Canis lupus familiaris IL31, Carbonic anhydrase IX, Cardiac myosin, CCL11(C-C motif chemokine 11), CCR4 (C-C chemokine receptor type 4), CCR5, CD3E (epsilon), CEA (Carcinoembryonic antigen), CEACAM3, CEACAM5 (carcino-embryonic antigen), CFD (Factor D), Ch4D5, Cholecystokinin 2 (CCK2R), CLDN18 (Claudin-18), Clumping factor A, cMet, CRIPTO, FCSF1R (Colony stimulating factor 1 receptor), CSF2 (colony stimulating factor 2, Granulocyte-macrophage colony-stimulating factor (GM-CSF)), CSP4, CTLA4 (cytotoxic T-lymphocyte-associated protein 4), CTAA16.88 tumor antigen, CXCR4, C-X-C chemokine receptor type 4, cyclic ADP ribose hydrolase, Cyclin B1, CYP1B1, Cytomegalovirus, Cytomegalovirus glycoprotein B, Dabigatran, DLL3 (delta-like-ligand 3), DLL4 (delta-like-ligand 4), DPP4 (Dipeptidyl-peptidase 4), DR5 (Death receptor 5), E. coli shiga toxin type-1, E. coli shiga toxin type-2, ED-B, EGFL7 (EGF-like domain-containing protein 7), EGFR, EGFRII, EGFRvIII, Endoglin, Endothelin B receptor, Endotoxin, EpCAM (epithelial cell adhesion molecule), EphA2, Episialin, ERBB2 (Epidermal Growth Factor Receptor 2), ERBB3, ERG (TMPRSS2 ETS fusion gene), Escherichia coli, ETV6-AML, FAP (Fibroblast activation protein alpha), FCGR1, alpha-Fetoprotein, Fibrin II, beta chain, Fibronectin extra domain-B, FOLR (folate receptor), Folate receptor alpha, Folate hydrolase, Fos-related antigen 1F protein of respiratory syncytial virus, Frizzled receptor, Fucosyl GM1, GD2 ganglioside, G-28 (a cell surface antigen glyvolipid), GD3 idiotype, GloboH, Glypican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α-chain, Growth differentiation factor 8, GP100, GPNMB (Trans-membrane glycoprotein NMB), GUCY2C (Guanylate cyclase 2C, guanylyl cyclase C(GC-C), intestinal Guanylate cyclase,

Guanylate cyclase-C receptor, Heat-stable enterotoxin receptor (hSTAR)), Heat shock proteins, Hemagglutinin, Hepatitis B surface antigen, Hepatitis B virus, HER1 (human epidermal growth factor receptor 1), HER2, HER2/neu, HER3 (ERBB-3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB , HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGF1R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN-γ, Influenza hemagglutinin, IgE, IgE Fc region, IGHE, interleukins (comprising IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-6R, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17, IL-17A, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-27, or IL-28), IL31RA, ILGF2 (Insulin-like growth factor 2), Integrins (α4, $\alpha_{IIb}\beta_3$, αvβ3, $\alpha_4\beta_7$, α5β1, α6β4, α7β7, αIIβ3, α5β5, αvβ5), Interferon gamma-induced protein, ITGA2, ITGB2, KIR2D, Kappa Ig, LCK, Le, Legumain, Lewis-Y antigen, LFA-1 (Lymphocyte function-associated antigen 1, CD11a), LHRH, LINGO-1, Lipoteichoic acid, LIV1A, LMP2, LTA, MAD-CT-1, MAD-CT-2, MAGE-1, MAGE-2, MAGE-3, MAGE A1, MAGE A3, MAGE 4, MART1, MCP-1, MIF (Macrophage migration inhibitory factor, or glycosylation-inhibiting factor (GIF)), MS4A1 (membrane-spanning 4-domains subfamily A member 1), B7H3, B7H4, MSLN (mesothelin), MUC1(Mucin 1, cell surface associated (MUC1) or polymorphic epithelial mucin (PEM)), MUC1-KLH, MUC16 (CA125), MCP1(monocyte chemotactic protein 1), MelanA/MART1, ML-IAP, MPG, MS4A1 (membrane-spanning 4-domains subfamily A), MYCN, Myelin-associated glycoprotein, Myostatin, NA17, NARP-1, NCA-90 (granulocyte antigen), Nectin-4 (ASG-22ME), NGF, Neural apoptosis-regulated proteinase 1, NOGO-A, Notch receptor, Nucleolin, Neu oncogene product, NY-BR-1, NY-ESO-1, OX-40, OxLDL (Oxidized low-density lipoprotein), OY-TES1, P21, p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolylneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1), PDGF-Rα (Alpha-type platelet-derived growth factor receptor), PDGFR-β, PDL-1, PLAC1, PLAP-like testicular alkaline phosphatase, Platelet-derived growth factor receptor beta, Phosphate-sodium co-transporter, PMEL 17, Polysialic acid, Proteinase3 (PR1), Prostatic carcinoma, PS (Phosphatidylserine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhPI)), Rhesus factor, RANKL, RhoC, Ras mutant, RGS5, ROBO4, Respiratory syncytial virus, RON, ROR1, Sarcoma translocation breakpoints, SART3, Sclerostin, SLAMF7 (SLAM family member 7), Selectin P, SDC1 (Syndecan 1), sLe(a), Somatomedin C, SIP (Sphingosine-1-phosphate), Somatostatin, Sperm protein 17, SSX2, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), STEAP2, STn, TAG-72 (tumor associated glycoprotein 72), Survivin, T-cell receptor, T cell transmembrane protein, TEM1 (Tumor endothelial marker 1), TENB2, Tenascin C (TN-C),

TGF-α, TGF-β (Transforming growth factor beta), TGF-β1, TGF-β2 (Transforming growth factor-beta 2), Tie (CD202b), Tie2, TIM-1 (CDX-014), Tn, TNF, TNF-α, TNFRSF8, TNFRSF10B (tumor necrosis factor receptor superfamily member 10B), TNFRSF-13B (tumor necrosis factor receptor superfamily member 13B), TPBG (trophoblast glycoprotein), TRAIL-R1 (Tumor necrosis apoptosis Inducing ligand Receptor 1), TRAILR2 (Death receptor 5 (DR5)), tumor-associated calcium signal transducer 2, tumor specific glycosylation of MUC1, TWEAK receptor, TYRP1(glycoprotein 75), TRP-1 (Trop1), TRP-2 (Trop2), Tyrosinase, VCAM-1, VEGF, VEGF-A, VEGF-2, VEGFR-1, VEGFR2, or vimentin, WT1, XAGE 1, or cells expressing any insulin growth factor receptors, or any epidermal growth factor receptors.

Many of tumor-associated antigens (TAA) or tumor cell receptors are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides or glycoproteins that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via the ADCs of this application. Examples of the TAA and cognate antibodies with their known in art are:

(1) BMPR1B (Bone Morphogenetic Protein Receptor-Type IB):

Nucleotide: Genbank accession no. NM_001203; Genbank version no. NM_001203.2 GI:169790809. Polypeptide: Genbank accession no. NP_001194; Genbank version no. NP_001194.1 GI:4502431.

Cross-References: Attisano L, et al, Cell. 75(4):671-80 (1993); ten Dijke, P., et al Science 264 (5155): 101-104 (1994), Ide H., et al, Oncogene 14 (11):1377-1382 (1997); WO2004/063362 (Claim 2); WO2003/042661 (Claim 12); US2003/134790-A1 (Page 38-39); WO2002/102235 (Claim 13; Page 296); WO2003/055443 (Page 91-92); WO2002/99122 (Example 2; Page 528-530); WO2003/029421 (Claim 6); WO2003/024392 (Claim 2; FIG. 112); WO2002/98358 (Claim 1; Page 183); WO2002/54940 (Page 100-101); WO2002/59377 (Page 349-350); WO2002/30268 (Claim 27; Page 376); 15 WO2001/48204 (Example; FIG. 4); NP_001194 bone morphogenetic protein receptor, type IB/pid=NP_001194.1; MIM:603248; AY065994

(2) E16 (LAT1, SLC7A5):

Nucleotide: Genbank accession no. NM_003486; Genbank version no. NM_003486.5 GI:71979931. Polypeptide: Genbank accession no. NP_003477; Genbank version no. NP_003477.4 GI:71979932.

Cross References: Prasad, P. D. et al, *Biochem. Biophys. Res. Commun.* 255 (2), 283-288 (1999), Mastroberardino, L., et al, *Nature* 395 (6699):288-291 (1998), Pfeiffer R, et al, EMBO J. 18(1):49-57 (1999); Gaugitsch, H. W., et al, *J. Biol. Chem.* 267 (16):11267-11273 (1992); WO2004/048938 (Example 2); WO2004/032842 (Example IV); WO2003/042661 (Claim 12); WO2003/016475 (Claim 1); WO2002/78524 (Example 2); WO2002/99074 (Claim 19; Page 127-129); WO2002/86443 (Claim 27; Pages 222, 393); WO2003/003906 (Claim 10; Page 293); WO2002/64798 (Claim 33; Page 93-95); WO2000/14228 (Claim 5; Page 133-136); US2003/224454 (FIG. 3); 25 WO2003/025138 (Claim 12; Page 150); NP_003477 solute carrier family 7 (cationic amino acid transporter, y+system), member 5/pid=NP_003477.3—*Homo sapiens*; MIM:600182; NM_015923.

(3) STEAP1 (Six Transmembrane Epithelial Antigen of Prostate):

Nucleotide: Genbank accession no. NM_012449; Genbank version no. NM_012449.2 GI:22027487. Polypeptide: Genbank accession no. NP_036581; Genbank version no. NP_036581.1 GI:9558759.

Cross References: Tang, D. et al, *Cancer Res.* 61 (15), 5857-5860 (2001); Hubert, R. S., et al, *Proc. Natl. Acad. Sci. U.S.A.* 96 (25):14523-14528 (1999); WO2004/065577 (Claim 6); WO2004/027049 (FIG. 1L); EP1394274 (Example 11); WO2004/016225 (Claim 2); WO2003/042661 (Claim 12); US2003/157089 (Example 5); US2003/185830 (Example 5); US2003/064397 (FIG. 2); WO2002/89747 (Example 5; Page 618-619); WO2003/022995 (Example 9; FIG. 13A, 35 Example 53; Page 173, Example 2; FIG. 2A); six transmembrane epithelial antigen of the prostate; MIM:604415.

(4) 0772P (CA125, MUC16):

Nucleotide: Genbank accession no. AF361486; Genbank version no. AF361486.3 GI:34501466. Polypeptide: Genbank accession no. AAK74120; Genbank version no. AAK74120.3 GI:34501467.

Cross References: Yin, B. W., Lloyd, K. O. *J. Biol. Chem.* 276 (29): 27371-5 (2001); Bouanène H, Miled A. Bull Cancer. 96(5):597-601 (2009); Felder M, et al, Mol Cancer. 2014, 13:129; WO2004/045553 (Claim 14); WO2002/92836 (Claim 6; FIG. 12); WO2002/83866 (Claim 15; Page 116-121); US2003/124140 (Example 16); GI:34501467;

(5) MPF (MPF, MSLN, SMR, Megakaryocyte Potentiating Factor, Mesothelin):

Nucleotide: Genbank accession no. NM_005823; Genbank version no. NM_005823.5 GI:293651528. Polypeptide: Genbank accession no. NP_005814; Genbank version no. NP_005814.2 GI:53988378.

Cross References: Hassan R, et al, Clin Cancer Res. 10(12 Pt 1):3937-42(2004), Yamaguchi, N., et al *Biol. Chem.* 269 (2), 805-808 (1994), Scholler, N., *et al, Proc. Natl. Acad. Sci. U.S.A.* 96 (20):11531-11536 (1999), Chang, K., Pastan, I., *Proc. Natl. Acad. Sci. U.S.A.* 93 (1):136-140 (1996), Kojima T., et al, *J. Biol. Chem.* 270 (37):21984-21990 (1995); WO2003/101283 (Claim 14); (WO2002/102235 (Claim 13; Page 287-288); WO2002/101075 (Claim 4; Page 308-309); WO2002/71928 (Page 320-321); WO94/10312 (Page 52-57); IM:601051.

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, Solute Carrier Family 34 (Sodium Phosphate), Member 2, Type II Sodium-Dependent Phosphate Transporter 3b)

Nucleotide: Genbank accession no. NM_006424; Genbank version no. NM_006424.2 GI:110611905. Polypeptide: Genbank accession no. NP_006415; Genbank version no. NP_006415.2 GI:110611906.

Cross References: Hiroko Segawa, et al, *J. Biol. Chem.* 277 (22):19665-19672 (2002), Xu H, et al, *Genomics* 62 (2):281-284 (1999), Feild, J. A., et al *Biochem. Biophys. Res. Commun.* 258 (3):578-582(1999); WO2004/022778 (Claim 2); EP1394274 (Example 11); WO2002/102235 (Claim 13; Page 326); EP0875569 (Claim 1; Page 17-19); WO2001/57188 (Claim 20; Page 329); WO2004/032842 (Example IV); WO2001/75177 (Claim 24; Page 139-140); MIM:604217.

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, 25 Sema Domain, Seven Thrombospondin Repeats (Type 1 and Type 1-Like), Transmembrane Domain™ and Short Cytoplasmic Domain, (Semaphorin) 5B)

Nucleotide: Genbank accession no. AB040878; Genbank version no. AB040878.1 GI:7959148. Polypeptide: Genbank accession no. BAA95969; Genbank version no. BAA95969.1 GI:7959149.

Cross References: Nagase T., et al *DNA Res.* 7 (2):143-150 (2000); WO2004/000997 (Claim 1); WO2003/003984 (Claim 1); WO2002/06339 (Claim 1; Page 50); WO2001/88133 (Claim 1; Page 41-43, 48-58); WO2003/054152 (Claim 20); WO2003/101400 (Claim 11); Accession: 30 Q9P283; Genew; HGNC:10737.

(8) PSCA hlg (2700050C12Rik, C530008016Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene):

Nucleotide: Genbank accession no. AY358628; Genbank version no. AY358628.1 GI:37182377. Polypeptide: Genbank accession no. AAQ88991; Genbank version no. AAQ88991.1 GI:37182378.

Cross References：Ross et al *Cancer Res.* 62:2546-2553(2002); US2003/129192 (Claim 2); US2004/044180 (Claim 12); US2004/044179 (Claim 11); US2003/096961 (Claim 11); US2003/232056 (Example 5); WO2003/105758 16 (Claim 12); US2003/206918 (Example 5); EP1347046 (Claim 1); WO2003/025148 (Claim 20); GI:37182378.

(9) ETBR (Endothelin Type B Receptor)

Nucleotide：Genbank accession no. AY275463；Genbank version no. AY275463.1 GI:30526094. Polypeptide: Genbank accession no. AAP32295; Genbank version no. AAP32295.1 GI:30526095.

Cross References: Nakamuta M., et al *Biochem. Biophys. Res. Commun.* 177, 34-39(1991); Ogawa Y., et al *Biochem. Biophys. Res. Commun.* 178, 248-255(1991); Arai H., et al *Jpn. Circ. J.* 56, 1303-1307(1992); Arai H., et al *J. Biol. Chem.* 268, 3463-3470(1993); Sakamoto A., Yanagisawa M., et al *Biochem. Biophys. Res. Commun.* 178, 656-663(1991); Elshourbagy N. A., et al *J. Biol. Chem.* 268, 3873-3879(1993); Haendler B., et al *J. Cardiovasc. Pharmacol.* 20, S1-S4(1992); Tsutsumi M., et al *Gene* 228, 43-49(1999); Strausberg R. L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903(2002); Bourgeois C., et al *J. Clin. Endocrinol. Metab.* 82, 3116-3123(1997); Okamoto Y., et al *Biol. Chem.* 272, 21589-21596(1997); Verheij J. B., et al *Am. J. Med. Genet.* 108, 223-225(2002); Hofstra R. M. W., et al *Eur. J. Hum. Genet.* 5, 180-185(1997); Puffenberger E. G., et al *Cell* 79, 1257-1266(1994); Attie T., et al, *Hum. Mol. Genet.* 4, 2407-2409(1995); Auricchio A., et al *Hum. Mol. Genet.* 5:351-354(1996); Amiel J., et al *Hum. Mol. Genet.* 5, 355-357(1996); Hofstra R. M. W., et al *Nat. Genet.* 12, 445-447(1996); Svensson P. J., et al *Hum. Genet.* 103, 145-148(1998); Fuchs S., et al *Mol. Med.* 7, 115-124(2001); Pingault V., et al *Hum. Genet.* 111, 198-206(2002); WO2004/045516 (Claim 1); WO2004/048938 (Example 2); WO2004/040000 (Claim 151); WO2003/087768 (Claim 1); 20 WO2003/016475 (Claim 1); WO2003/016475 (Claim 1); WO2002/61087 (FIG. 1); WO2003/016494 (FIG. 6); WO2003/025138 (Claim 12; Page 144); WO2001/98351 (Claim 1; Page 124-125); EP0522868 (Claim 8; FIG. 2); WO2001/77172 (Claim 1; Page 297-299); US2003/109676; U.S. Pat. No. 6,518,404 (FIG. 3); U.S. Pat. No. 5,773,223 (Claim 1a; Col 31-34); WO2004/001004.

(10) MSG783 (RNF124, Hypothetical Protein FLJ20315)

Nucleotide: Genbank accession no. NM_017763; Genbank version no. NM_017763.4 GI:167830482. Polypeptide: Genbank accession no. NP_060233; Genbank version no. NP_060233.3 GI:56711322.

Cross References: WO2003/104275 (Claim 1); WO2004/046342 (Example 2); WO2003/042661 (Claim 12); WO2003/083074 (Claim 14; Page 61); WO2003/018621 (Claim 1); WO2003/024392 (Claim 2; FIG. 93); WO2001/66689 (Example 6); LocusID:54894.

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, Prostate Cancer Associated Gene 1, Prostate Cancer Associated Protein 1, Six Transmembrane Epithelial Antigen of Prostate 2, Six Transmembrane Prostate Protein)

Nucleotide: Genbank accession no. AF455138; Genbank version no. AF455138.1 GI:22655487. Polypeptide: Genbank accession no. AAN04080; Genbank version no. AAN04080.1 GI:22655488.

Cross References: Porkka, K P., et al, Lab. Invest. 82 (11):1573-1582 (2002); WO2003/087306; US2003/064397 (Claim 1; FIG. 1); WO2002/72596 (Claim 13; Page 54-55); WO2001/72962 (Claim 1; FIG. 4B); WO2003/104270 (Claim 11); WO2003/104270 (Claim 16); US2004/005598 (Claim 22); WO2003/042661 (Claim 12); US2003/060612 (Claim 12; FIG. 10); WO2002/26822 (Claim 23; FIG. 2); WO2002/16429 (Claim 12; FIG. 10); GI:22655488.

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, Transient Receptor Potential Cation 5 Channel, Subfamily M, Member 4)

Nucleotide: Genbank accession no. NM_017636; Genbank version no. NM_017636.3 GI:304766649. Polypeptide: Genbank accession no. NP_060106; Genbank version no. NP_060106.2 GI:21314671.

Cross References: Xu, X. Z., et al *Proc. Natl. Acad. Sci. U.S.A.* 98 (19):10692-10697 (2001), Launay P., et al *Cell* 109 (3):397-407 (2002), Nilius B., et al *J. Biol. Chem.* 278 (33):30813-30820 (2003); US2003/143557 (Claim 4); WO2000/40614 (Claim 14; Page 100-103); WO2002/10382 (Claim 1; FIG. 9A); WO2003/042661 (Claim 12); WO2002/30268 (Claim 27; Page 391); US2003/219806 (Claim 4); WO2001/62794 (Claim 14; FIG. 1A-D); MIM:606936.

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, Teratocarcinoma-Derived Growth Factor)

Nucleotide: Genbank accession no. NM_003212; Genbank version no. NM_003212.3 GI:292494881. Polypeptide: Genbank accession no. NP_003203; Genbank version no. NP_003203.1 GI:4507425.

Cross References: Ciccodicola, A., et al *EMBO J.* 8 (7):1987-1991 (1989), Dono R., et al, *Am. J. Hum. Genet.* 49 (3):555-565 (1991); US2003/224411 (Claim 1); WO2003/083041 (Example 1); WO2003/034984 (Claim 12); WO2002/88170 (Claim 2; Page 52-53); WO2003/024392 (Claim 2; FIG. 58); WO2002/16413 (Claim 1; Page 94-95, 105); WO2002/22808 (Claim 2; FIG. 1); U.S. Pat. No. 5,854,399 (Example 2; Col 17-18); U.S. Pat. No. 5,792,616 (FIG. 2); MIM:187395.

(14) CD21 (CR2 (Complement Receptor 2) or C3DR (C3d/Epstein Barr Virus Receptor) or Hs. 73792)

Nucleotide: Genbank accession no M26004; Genbank version no. M26004.1 GI:181939. Polypeptide: Genbank accession no. AAA35786; Genbank version no. AAA35786.1 GI:181940.

Cross References: Fujisaku et al *J. Biol. Chem.* 264 (4):2118-2125(1989); Weis J. J., et al *J. Exp. Med.* 167, 1047-1066(1988); Moore M., et al *Proc. Natl. Acad. Sci. U.S.A.* 84, 9194-9198(1987); Barel M., et al *Mol. Immunol.* 35, 1025-1031(1998); Weis J. J., et al *Proc. Natl. Acad. Sci. U.S.A.* 83, 5639-5643(1986); Sinha S. K., et al *J. Immunol.* 150, 5311-5320(1993); WO2004/045520 (Example 4); US2004/005538 (Example 1); WO2003/062401 (Claim 9); WO2004/045520 (Example 4); WO91/02536 (FIGS. 9.1-9.9); WO2004/020595 (Claim 1); Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) CD79b (CD79B, CD793, IGb (Immunoglobulin-Associated Beta), B29)

Nucleotide: Genbank accession no NM_000626; Genbank version no. NM_000626.2 GI:90193589. Polypeptide: Genbank accession no. NP_000617; Genbank version no. NP_000617.1 GI:11038674.

Cross References: Gordon MS., et al *Proc. Natl. Acad. Sci. U.S.A.* 100 (7):4126-4131 (2003), *Cragg MS., et al Blood* 100 (9):3068-3076 (2002), Muller et al *Eur. J. Immunol.* 22 (6):1621-1625(1992); WO2004/016225 (claim 2, FIG. 140); WO2003/087768, US2004/101874 (claim 1, page 102); WO2003/062401 (claim 9); WO2002/78524 (Example 2); US2002/150573 (claim 35 5, page 15); U.S. Pat. No. 5,644,033; WO2003/048202 (claim 1, pages 306 and 309); WO 99/58658, U.S. Pat. No. 6,534,482 (claim 13, FIG. 17A/B); WO2000/55351 (claim 11, pages 1145-1146); MIM:147245

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 Domain Containing Phosphatase Anchor Protein 5 La), SPAP1B, SPAP1C)

Nucleotide: Genbank accession no NM_030764; Genbank version no. NM_030764.3 GI:227430280. Polypeptide: Genbank accession no. NP_110391; Genbank version no. NP_110391.2 GI:19923629.

Cross References: AY358130); Clark HF., et al *Genome Res.* 13 (10):2265-2270 (2003), Guselnikov SV., et al *Immunogenetics* 54 (2):87-95 (2002), Miller I., et al *Blood* 99 (8):2662-2669 (2002), Davis RS., et al *Proc. Natl. Acad. Sci. U.S.A.* 98 (17):9772-9777 (2001), Xu, M. J., et al *Biochem. Biophys. Res. Commun.* 280 (3):768-775(2001); WO2004/016225 (Claim 2); WO2003/077836; WO2001/38490 (Claim 5; FIG. 18D-1-18D-2); WO2003/097803 (Claim 12); 10 WO2003/089624 (Claim 25); MIM:606509.

(17) HER2 (ErbB2):

Nucleotide: Genbank accession no M11730; Genbank version no. M11730.1 GI:183986. Polypeptide: Genbank accession no. AAA75493; Genbank version no. AAA75493.1 GI:306840.

Cross References: Coussens L., et al *Science* 230(4730):1132-1139(1985); Yamamoto T., et al *Nature* 319, 230-234(1986); Semba K., et al *Proc. Natl. Acad. Sci. U.S.A.* 82, 6497-6501(1985);

Swiercz J. M., et al *J. Cell Biol.* 165, 869-880(2004); Kuhns J. J., et al *J. Biol. Chem.* 274, 36422-36427(1999); Cho H.-S., et al *Nature* 421, 756-760(2003); Ehsani A., et al *Genomics* 15, 426-429(1993); WO2004/048938 (Example 2); WO2004/027049 (FIG. 11); WO2004/009622; WO2003/081210; WO2003/089904 (Claim 9); WO2003/016475 (Claim 1); US2003/118592; WO2003/008537 (Claim 1); WO2003/055439 (Claim 29; FIG. 1A-B); WO2003/025228 (Claim 37; FIG. 5C); 20 WO2002/22636 (Example 13; Page 95-107); WO2002/12341 (Claim 68; FIG. 7); WO2002/13847 (Page 71-74); WO2002/14503 (Page 114-117); WO2001/53463 (Claim 2; Page 41-46); WO2001/41787 (Page 15); WO2000/44899 (Claim 52; FIG. 7); WO2000/20579 (Claim 3; FIG. 2); U.S. Pat. No. 5,869,445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004/043361 (Claim 7); WO2004/022709; WO2001/00244 25 (Example 3; FIG. 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1

Antibodies: Abbott: US20110177095, For example, an antibody comprising CDRs having overall at least 80% sequence identity to CDRs having amino acid sequences of SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:104 and/or SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2), and SEQ ID NO:8 (CDR-L3), wherein the anti-HER2 antibody or anti-HER2 binding fragment has reduced immunogenicity as compared to an antibody having a VH of SEQ ID NO:1 and a VL of SEQ ID NO:2.

Biogen: US20100119511； For example, ATCC accession numbers: PTA-10355, PTA-10356, PTA-10357, PTA-10358

For example, a purified antibody molecule that binds to HER2 comprising all six CDR's from an antibody selected from the group consisting of BIIB71F10 (SEQ ID NOs:11, 13), B11B69A09 (SEQ ID NOs:15, 17); BIIB67F10 (SEQ ID NOs:19, 21); BIIB67F11 (SEQ ID NOs:23, 25), BIIB66A12 (SEQ ID NOs:27, 29), BIIB66C01 (SEQ ID NOs:31, 33), BIIB65C10 (SEQ ID NOs:35, 37), BIIB65H09 (SEQ ID NOs:39, 41) and B11B65B03 (SEQ ID NOs:43, 45), or CDRs which are identical or which have no more than two alterations from said CDRs.

Herceptin (Genentech): U.S. Pat. No. 6,054,297; ATCC accession no. CRL-10463 (Genentech);

Pertuzumab (Genentech): US20110117097. For example, see SEQ IDs No. 15&16, SEQ IDs No. 17&18, SEQ IDs No. 23&24 & ATCC accession numbers HB-12215, HB-12216, CRL 10463, HB-12697. US20090285837, US20090202546. For example, ATCC accession numbers: HB-12215, HB-12216, CRL 10463, HB-12698. US20060088523. For example, ATCC accession numbers: HB-12215, HB-12216. For example, an antibody comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively. For example, an antibody comprising a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24 US20060018899. For example, ATCC accession

numbers: (7C2) HB-12215, (7F3) HB-12216, (4D5) CRL-10463, (2C4) HB-12697. For example, an antibody comprising the amino acid sequence in SEQ ID No. 23, or a deamidated and/or oxidized variant thereof. US2011/0159014. For example, an antibody having a light chain variable domain comprising the hypervariable regions of SEQ ID NO: 1". For example, an antibody having a heavy chain variable domain comprising the hypervariable regions of SEQ ID NO: 2. US20090187007

Glycotope: TrasGEX antibody http://www.glycotope.com/pipeline. For example, see International Joint Cancer Institute and Shanghai Hospital Cancer Cent: HMTI-Fc Ab—Gao J., et al *BMB Rep.*; 42(10):636-41(2009).

Symphogen: US20110217305, Union Stem Cell &Gene Engineering, China—Liu HQ., et al *Xi Bao Yu Fen Zi Mian YiXue Za Zhi.*; 26(5):456-8(2010).

(18) NCA (CEACAM6):

Nucleotide: Genbank accession no M18728; Genbank version no. M18728.1 GI:189084. Polypeptide: Genbank accession no. AAA59907; Genbank version no. AAA59907.1 GI:189085.

Cross References: Barnett T., et al *Genomics* 3, 59-66(1988); Tawaragi Y., et al *Biochem. Biophys. Res. Commun.* 150, 89-96(1988); Strausberg R. L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99:16899-16903(2002); WO2004/063709; EP1439393 (Claim 7); WO2004/044178 (Example 4); WO2004/031238; WO2003/042661 (Claim 12); WO2002/78524 (Example 2); WO2002/86443 (Claim 27; Page 427); WO2002/60317 (Claim 2); Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728.

(19) MDP (DPEP1):

Nucleotide: Genbank accession no BC017023; Genbank version no. BC017023.1 GI:16877538. Polypeptide: Genbank accession no. AAH17023; Genbank version no. AAH17023.1 GI:16877539.

Cross References: Strausberg RL., et al *Proc. Natl. Acad. Sci. U.S.A.* 99 (26):16899-16903 (2002); WO2003/016475 (Claim 1); WO2002/64798 (Claim 33; Page 85-87); JP05003790 (FIG. 6-8); WO99/46284 (FIG. 9); MIM:179780.

(20) IL20R-Alpha (IL20Ra, ZCYTOR7):

Nucleotide: Genbank accession no AF184971; Genbank version no. AF184971.1 GI:6013324. Polypeptide: Genbank accession no. AAF01320; Genbank version no. AAF01320.1 GI:6013325.

Cross References: Clark H. F., et al *Genome Res.* 13, 2265-2270(2003); Mungall A. J., et al *Nature* 425, 805-811(2003); Blumberg H., et al *Cell* 104, 9-19(2001); Dumoutier L., et al *J. Immunol.* 167, 3545-3549(2001); Parrish-Novak J., et al *J. Biol. Chem.* 277, 47517-47523(2002); Pletnev S., et al 10 *Biochemistry* 42:12617-12624 (2003); Sheikh F., et al *J. Immunol.* 172, 2006-2010(2004); EP1394274 (Example 11); US2004/005320 (Example 5); WO2003/029262 (Page 74-75); WO2003/002717 (Claim 2; Page 63); WO2002/22153 (Page 45-47); US2002/042366 (Page 20-

21); WO2001/46261 (Page 57-59); WO2001/46232 (Page 63-65); WO98/37193 (Claim 1; Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) Brevican (BCAN, BEHAB):

Nucleotide: Genbank accession no AF229053; Genbank version no. AF229053.1 GI:10798902. Polypeptide: Genbank accession no. AAG23135. Genbank version no. AAG23135.1 GI:10798903.

Cross References: Gary S. C., et al *Gene* 256, 139-147(2000); Clark H. F., et al *Genome Res.* 13, 2265-2270(2003); Strausberg R. L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903(2002); US2003/186372 (Claim 11); US2003/186373 (Claim 11); US2003/119131 (Claim 1; FIG. 52); US2003/119122 (Claim 1; FIG. 52); US2003/119126 (Claim 1); US2003/119121 (Claim 1; FIG. 52); US2003/119129 (Claim 1); US2003/119130 (Claim 1); US2003/119128 (Claim 1; FIG. 52); US2003/119125 (Claim 1); WO2003/016475 (Claim 1); WO2002/02634 (Claim 1)

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5):

Nucleotide: Genbank accession no NM_004442; Genbank version no. NM_004442.6 GI:111118979. Polypeptide: Genbank accession no. NP_004433; Genbank version no. NP_004433.2 GI:21396504.

Cross References: Chan, J. and Watt, V. M., Oncogene 6 (6), 1057-1061 (1991), Fox GM., et al, Oncogene 10 (5):897-905 (1995), Flanagan JG., et al, Annu. Rev. Neurosci. 21:309-345 (1998), Wilkinson DG, Int. Rev. Cytol. 196:177-244 (2000); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42); MIM:600997.

(23) ASLG659 (B7h):

Nucleotide: Genbank accession no. AX092328; Genbank version no. AX092328.1 GI:13444478.

Cross References: US2004/0101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (FIG. 3); US2003/165504 (Claim 1); US2003/124140 (Example 2); US2003/065143 (FIG. 60); WO2002/102235 (Claim 13; Page 299); US2003/091580 (Example 2); WO2002/10187 (Claim 6; FIG. 10); WO2001/94641 (Claim 12; FIG. 7b); WO2002/02624 (Claim 13; FIG. 1A-1B); US2002/034749 (Claim 54; Page 45-46); WO2002/06317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO2002/71928 (Page 468-469); WO2002/02587 (Example 1; FIG. 1); WO2001/40269 (Example 3; Pages 190-192); WO2000/36107 (Example 2; Page 205-207); WO2004/053079 (Claim 12); WO2003/004989 (Claim 1); WO2002/71928 (Page 233-234, 452-453); WO 01/16318.

(24) PSCA (Prostate Stem Cell Antigen Precursor):

Nucleotide: Genbank accession no AJ297436; Genbank version no. AJ297436.1 GI:9367211. Polypeptide: Genbank accession no. CAB97347; Genbank version no. CAB97347.1 GI:9367212.

Cross References: Reiter R. E., et al *Proc. Natl. Acad. Sci. U.S.A.* 95, 1735-1740(1998); Gu Z., et al *Oncogene* 19, 1288-1296(2000); *Bahrenberg G., et al, Biochem. Biophys. Res. Commun.* 275(3):783-788(2000); WO2004/022709; EP1394274 (Example 11); US2004/018553 (Claim 17); WO2003/008537 (Claim 1); WO2002/81646 (Claim 1; Page 164); WO2003/003906 (Claim 10; Page 288); WO2001/40309 (Example 1; FIG. 17); US2001/055751 (Example 1; FIG. 1 b); WO2000/32752 (Claim 18; FIG. 1); WO98/51805 (Claim 17; Page 97); WO98/51824 (Claim 10; Page 94); WO98/40403 (Claim 2; FIG. 1B); Accession: 043653; EMBL; AF043498; AAC39607.1

(25) GEDA:

Nucleotide: Genbank accession no AY260763; Genbank version no. AY260763.1 GI:30102448. Polypeptide: Genbank accession no. AAP14954; Genbank version no. AAP14954.1 GI:30102449.

Cross References: AP14954 lipoma HMGIC fusion-partnerlike protein/pid=AAP14954.1—*Homo sapiens* (human); WO2003/054152 (Claim 20); WO2003/000842 (Claim 1); WO2003/023013 (Example 3, Claim 20); US2003/194704 (Claim 45); GI:30102449.

(26) BAFF-R (B Cell-Activating Factor Receptor, BLyS Receptor 3, BR3):

Nucleotide: Genbank accession no AF116456; Genbank version no. AF116456.1 GI:4585274. Polypeptide: Genbank accession no. AAD25356; Genbank version no. AAD25356.1 GI:4585275.

Cross References: BAFF receptor/pid=NP_443177.1—*Homo sapiens*: Thompson, J. S., et al *Science* 293 (5537), 2108-2111 (2001); WO2004/058309; WO2004/011611; WO2003/045422 (Example; Page 32-33); WO2003/014294 (Claim 35; FIG. 6B); WO2003/035846 (Claim 70; Page 615-616); WO2002/94852 (Col 136-137); WO2002/38766 (Claim 3; Page 133); WO2002/24909 (Example 3; FIG. 3); MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) CD22 (B-Cell Receptor CD22-B Isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814):

Nucleotide: Genbank accession no AK026467; Genbank version no. AK026467.1 GI:10439337. Polypeptide: Genbank accession no. BAB15489; Genbank version no. BAB15489.1 G1:10439338.

Cross References: Wilson et al *J. Exp. Med.* 173:137-146(1991); WO2003/072036 (Claim 1; FIG. 1); IM:107266; NP_001762.1; NM_001771_1.

(27A) Cd22 (Cd22 Molecule):

Nucleotide: Genbank accession no X52785; Genbank version no. X52785.1 G1:29778. Polypeptide: Genbank accession no. CAA36988; Genbank version no. CAA36988.1 G1:29779.

Cross References: Stamenkovic I. et al., *Nature* 345 (6270), 74-77 (1990).

Other Information: Official Symbol: CD22; Other Aliases: SIGLEC-2, SIGLEC2 Other Designations: B-cell receptor CD22; B-lymphocyte cell adhesion molecule; BL-CAM; CD22 antigen; T-cell surface antigen Leu-14; sialic acid binding Ig-like lectin 2; sialic acid-binding Ig-like lectin 2

Antibodies: G5/44 (Inotuzumab): DiJoseph J F., et al *Cancer Immunol Immunother.*54(1):11-24(2005). Epratuzumab—Goldenberg DM., et al *Expert Rev Anticancer Ther.* 6(10): 1341-53(2006).

(28) CD79a (CD79A, CD79alpha), Immunoglobulin-Associated Alpha, a B Cell-Specific Protein that Covalently Interacts with Ig Beta (CD79B) and Forms a Complex on the Surface with Ig M 35 Molecules, Transduces a Signal Involved in B-Cell Differentiation), Pl: 4.84, MW: 25028 Tm: 2 [P] Gene Chromosome: 19q13.2):

Nucleotide: Genbank accession no NM_001783; Genbank version no. NM_001783.3 GI:90193587. Polypeptide: Genbank accession no. NP_001774; Genbank version no. NP_001774.1 GI:4502685.

Cross References: WO2003/088808, US2003/0228319; WO2003/062401 (claim 9); US2002/150573 (claim 4, pages 13-14); WO99/58658 (claim 13, FIG. 16); WO92/07574 (FIG. 1); U.S. Pat. No. 5,644,033; Ha et al *J. Immunol.* 148(5):1526-1531 (1992); Müller et al *Eur. J. Immunol.* 22:1621-1625(1992); Hashimoto et al *Immunogenetics* 40(4):287-295 (1994); Preud'homme et al Clin. Exp. 5 *Immunol.* 90(1):141-146(1992); Yu et al *J. Immunol.* 148(2) 633-637(1992); Sakaguchi et al *EMBO J.* 7(11):3457-3464(1988).

(29) CXCR5 (Burkitt's Lymphoma Receptor 1, a G Protein-Coupled Receptor that is Activated by the CXCL13 Chemokine, Functions in Lymphocyte Migration and Humoral Defense, Plays a 10 Role in HIV-2 Infection and Perhaps Development of AIDS, Lymphoma, Myeloma, and Leukemia); 372 Aa, Pl: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3:

Nucleotide: Genbank accession no NM_001716; Genbank version no. NM_001716.4 GI:342307092. Polypeptide: Genbank accession no. NP_001707; Genbank version no. NP_001707.1 GI:4502415.

Cross References: WO2004/040000; WO2004/015426; US2003/105292 (Example 2); U.S. Pat. No. 6,555,339 (Example 2); WO2002/61087 (FIG. 1); WO2001/57188 (Claim 20, page 269); WO2001/72830 (pages 12-13); WO2000/22129 (Example 1, pages 152-153, Example 2, pages 254-256); WO99/28468 (claim 1, page 38); U.S. Pat. No. 5,440,021 (Example 2, col 49-52); WO94/28931 (pages 56-58); WO92/17497 (claim 7, FIG. 5); Dobner et al *Eur. J. Immunol.* 22:2795-2799(1992); Barella et al *Biochem. J.* 309:773-779(1995).

(30) HLA-DOB (Beta Subunit of MHC Class II Molecule (La Antigen) that Binds Peptides and 20 Presents them to CD4+T Lymphocytes); 273 Aa, Pl: 6.56, MW: 30820. TM: 1 [P] Gene Chromosome: 6p21.3):

Nucleotide: Genbank accession no NM_002120; Genbank version no. NM_002120.3 GI:118402587. Polypeptide: Genbank accession no. NP_002111; Genbank version no. NP_002111.1 GI:4504403.

Cross References: Tonnelle C., et al *EMBO J.* 4(11):2839-2847 (1985); Jonsson AK., et al *Immunogenetics* 29(6):411-413 (1989); Beck S., et al *J. Mol. Biol.* 228:433-441 (1992); Strausberg RL., et al *Proc. Natl. Acad. Sci USA* 99:16899-16903(2002); Servenius B., et al *J. Biol. Chem.* 262:8759-8766 (1987); Beck S., et al *J. Mol. Biol.* 255:1-13(1996); Naruse TK., et al *Tissue Antigens* 59:512-519 (2002); WO99/58658 (claim 13, FIG. 15); U.S. Pat. No. 6,153,408 (Col 35-38); U.S. Pat. No. 5,976,551 (col 168-170); U.S. Pat. No. 6,011,146 (col 145-146); Kasahara M., et al *Immunogenetics* 30(1):66-68(1989); Larhammar D., et al *J. Biol. Chem.* 260(26):14111-14119(1985).

(31) P2X5 (Purinergic Receptor P2X Ligand-Gated Ion Channel 5, an Ion Channel Gated by Extracellular ATP, May be Involved in Synaptic Transmission and Neurogenesis, Deficiency May Contribute to the Pathophysiology of Idiopathic Detrusor Instability); 422 Aa), Pl: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3):

Nucleotide: Genbank accession no NM_002561; Genbank version no. NM_002561.3 GI:325197202. Polypeptide: Genbank accession no. NP_002552; Genbank version no. NP_002552.2 GI:28416933.

Cross References: Le KT., et al *FEBS Lett.* 418(1-2):195-199(1997); WO2004/047749; WO2003/072035 (claim 10); Touchman JW., et al *Genome Res.* 10:165-173(2000); WO2002/22660 (claim 20); WO2003/093444 (claim 1); WO2003/087768 (claim 1); WO2003/029277 (page 82)

(32) CD72 (B-Cell Differentiation Antigen CD72, Lyb-2); 359 Aa, Pl: 8.66, MW: 40225, TM: 1 5 [P] Gene Chromosome: 9p13.3):

Nucleotide: Genbank accession no NM_001782; Genbank version no. NM_001782.2 GI:194018444. Polypeptide: Genbank accession no. NP_001773; Genbank version no. NP_001773.1 GI:4502683.

Cross References: WO2004042346 (claim 65); WO2003/026493 (pages 51-52, 57-58); WO2000/75655 (pages 105-106); Von Hoegen et al *J. Immunol.* 144(12):4870-4877 (1990); Strausberg RL et al *Proc. Natl. Acad. Sci USA* 99:16899-16903(2002).

(33) LY64 (Lymphocyte Antigen 64 (RP105), Type I Membrane Protein of the Leucine Rich Repeat (LRR) Family, Regulates B-Cell Activation and Apoptosis, Loss of Function is Associated

with Increased Disease Activity in Patients with Systemic Lupus Erythematosis); 661 Aa, pl: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12):

Nucleotide: Genbank accession no NM_005582; Genbank version no. NM_005582.2 GI:167555126. Polypeptide: Genbank accession no. NP_005573; Genbank version no. NP_005573.2 GI:167555127.

Cross References: US2002/193567; WO97/07198 (claim 11, pages 39-42); Miura Y et al *Genomics* 38(3):299-304(1996); Miura Y et al *Blood* 92:2815-2822(1998); WO2003/083047; WO97/44452 (claim 8, pages 57-61); WO2000/12130 (pages 24-26).

(34) FcRH1 (Fc Receptor-Like Protein 1, a Putative Receptor for the Immunoglobulin Fc Domain that Contains C2 Type Ig-Like and ITAM Domains, May have a Role in B-Lymphocyte 20 Differentiation); 429 Aa, Pl: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22)

Nucleotide: Genbank accession no NM_052938; Genbank version no. NM_052938.4 GI:226958543. Polypeptide: Genbank accession no. NP_443170; Genbank version no. NP_443170.1 GI:16418419.

Cross References: WO2003/077836; WO2001/38490 (claim 6, FIG. 18E-1-18-E-2); Davis RS et al *Proc. Natl. Acad. Sci USA* 98(17):9772-9777(2001); WO2003/089624 (claim 8); EP1347046 (claim 1); WO2003/089624 (claim 7).

(35) IRTA2 (Immunoglobulin Superfamily Receptor Translocation Associated 2, a Putative Immunoreceptor with Possible Roles in B Cell Development and Lymphomagenesis; Deregulation of the Gene by Translocation Occurs in Some B Cell Malignancies); 977 Aa, pl: 6.88, MW: 106468, TM: 1 [P] Gene Chromosome: 1q21):

Nucleotide: Genbank accession no AF343662; Genbank version no. AF343662.1 GI:13591709. Polypeptide: Genbank accession no. AAK31325; Genbank version no. AAK31325.1 GI:13591710.

Cross References: AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse: AK089756, AY158090, AY506558; NP_112571.1; WO2003/024392 (claim 2, FIG. 97); Nakayama Y et al *Biochem. Biophys. Res. Commun.* 277(1):124-127(2000); WO2003/077836; WO2001/38490 (claim 3, FIG. 18B-1-18B-2).

(36) TENB2 (TMEFF2, Tomoregulin, TPEF, HPP1, TR, Putative Transmembrane 35 Proteoglycan, Related to the EGF/Heregulin Family of Growth Factors and Follistatin); 374 Aa):

Nucleotide: Genbank accession no AF179274; Genbank version no. AF179274.2 GI:12280939. Polypeptide: Genbank accession no. AAD55776; Genbank version no. AAD55776.2 GI:12280940.

Cross References: NCBI Accession: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; AY358907, CAF85723, CQ782436; WO2004/074320; JP2004113151; WO2003/042661; WO2003/009814; EP1295944

(pages 69-70); WO2002/30268 (page 329); WO2001/90304; US2004/249130; US2004/022727; WO2004/063355; US2004/197325; US2003/232350; US2004/005563; US2003/124579; Horie M et al *Genomics* 67:146-152(2000); Uchida T et al *Biochem. Biophys. Res. Commun.* 266:593-602(1999); Liang G et al *Cancer Res.* 60:4907-12 (2000); Glynne-Jones E et al *Int J Cancer*; 94(2):178-84(2001).

(37) PSMA-FOLH1 (Folate Hydrolase (Prostate-Specific Membrane Antigen) 1):

Nucleotide: Genbank accession no M99487; Genbank version no. M99487.1 G1:190663. Polypeptide: Genbank accession no. AAA60209; Genbank version no. AAA60209.1 GI:190664.

Cross References: Israeli R. S., et al *Cancer Res.* 53 (2), 227-230 (1993)

Other Information: Official Symbol: FOLH1; Other Aliases: GIG27, FGCP, FOLH, GCP2, GCPII, NAALAD1, NAALAdase, PSM, PSMA, mGCP; Other Designations: N-acetylated alpha-linked acidic dipeptidase 1; N-acetylated-alpha-linked acidic dipeptidase I; NAALADase I; cell growth-inhibiting gene 27 protein; folylpoly-gamma-glutamate carboxypeptidase; glutamate carboxylase II; glutamate carboxypeptidase 2; glutamate carboxypeptidase II; membrane glutamate carboxypeptidase; prostate specific membrane antigen variant F; pteroylpoly-gamma-glutamate carboxypeptidase.

Antibodies: U.S. Pat. No. 7,666,425; Antibodies produces by Hybridomas having the following ATCC references: ATCC accession No. HB-12101, ATCC accession No. HB-12109, ATCC accession No. HB-12127 and ATCC accession No. HB-12126.

Proscan: a monoclonal antibody selected from the group consisting of 8H12, 3E11, 17G1, 29B4, 30C1 and 20F2 (U.S. Pat. No. 7,811,564; Moffett S., et al Hybridoma (Larchmt) 26(6):363-72(2007)).

Cytogen: monoclonal antibodies 7E11-C5 (ATCC accession No. HB 10494) and 9H10-A4 (ATCC accession No. HB11430)—U.S. Pat. No. 5,763,202.

GlycoMimetics: NUH2—ATCC accession No. HB 9762 (U.S. Pat. No. 7,135,301).

Human Genome Science: HPRAJ70—ATCC accession No. 97131 (U.S. Pat. No. 6,824,993); Amino acid sequence encoded by the cDNA clone (HPRAJ70) deposited as American Type Culture Collection ("ATCC") Deposit No. 97131.

Medarex: Anti-PSMA antibodies that lack fucosyl residues—U.S. Pat. No. 7,875,278.

Mouse anti-PSMA antibodies include the 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6, 4C8B9, and monoclonal antibodies. Hybridomas secreting 3F5.4G6, 3D7.1.1, 4E10-1.14, 3E11, 4D8, 3E6, 3C9, 2C7, 1G3, 3C4, 3C6, 4D4, 1G9, 5C8B9, 3G6 or 4C8B9 have been publicly deposited and are described in U.S. Pat. No. 6,159,508. Relevant hybridomas have been publicly deposited and are described in U.S. Pat. No. 6,107,090.

Moreover, humanized anti-PSMA antibodies, including a humanized version of J591, are described in further detail in PCT Publication WO 02/098897.

Other mouse anti-human PSMA antibodies have been described in the art, such as mAb 107-1A4 (Wang, S. et al. Int. J. Cancer 92:871-876 (2001)) and mAb 2C9 (Kato, K. et al. Int. J. Urol. 10:439-444 (2003)).

Examples of human anti-PSMA monoclonal antibodies include the 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 antibodies, isolated and structurally characterized as originally described in PCT Publications WO 01/09192 and WO 03/064606 and in U.S. Provisional Application Ser. No. 60/654,125, entitled "Human Monoclonal Antibodies to Prostate Specific Membrane Antigen (PSMA)", filed on Feb. 18, 2005. The V.sub.H amino acid sequences of 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 are shown in SEQ ID NOs: 1-9, respectively. The V.sub.L amino acid sequences of 4A3, 7F12, 8C12, 8A11, 16F9, 2A10, 2C6, 2F5 and 1C3 are shown in SEQ ID NOs: 10-18, respectively.

Other human anti-PSMA antibodies include the antibodies disclosed in PCT Publication WO 03/034903 and US Application No. 2004/0033229.

NW Biotherapeutics: A hybridoma cell line selected from the group consisting of 3F5.4G6 having ATCC accession number HB12060, 3D7-1.1. having ATCC accession number HB12309, 4E10-1.14 having ATCC accession number HB12310, 3E11 (ATCC HB12488), 4D8 (ATCC HB12487), 3E6 (ATCC HB12486), 3C9 (ATCC HB12484), 2C7 (ATCC HB12490), 1G3 (ATCC HB12489), 3C4 (ATCC HB12494), 3C6 (ATCC HB12491), 4D4 (ATCC HB12493), 1G9 (ATCC HB12495), 5C8B9 (ATCC HB12492) and 3G6 (ATCC HB12485)—see U.S. Pat. No. 6,150,508.

PSMA Development Company/Progenics/Cytogen—Seattle Genetics: mAb 3.9, produced by the hybridoma deposited under ATCC Accession No. PTA-3258 or mAb 10.3, produced by the hybridoma deposited under ATCC Accession No. PTA-3347—U.S. Pat. No. 7,850,971.

PSMA Development Company—Compositions of PSMA antibodies (US 20080286284, Table 1) This application is a divisional of U.S. patent application Ser. No. 10/395,894, filed on Mar. 21, 2003 (U.S. Pat. No. 7,850,971).

University Hospital Freiburg, Germany—mAbs 3/A12, 3/E7, and 3/F11 (Wolf P., et al *Prostate.*; 70(5):562-9 (2010)).

(38) SST (Somatostatin Receptor; Note that there are 5 Subtypes):

(38.1) SSTR2 (Somatostatin Receptor 2):

Nucleotide: Genbank accession no NM_001050; Genbank version no. NM_001050.2 GI:44890054. Polypeptide: Genbank accession no. NP_001041; Genbank version no. NP_001041.1 GI:4557859.

Cross References: Yamada Y., et al Proc. Natl. Acad. Sci. U.S.A. 89 (1), 251-255 (1992); Susini C., et al Ann Oncol. 17(12):1733-42(2006)

Other Information: Official Symbol: SSTR2; Other Designations: SRIF-1; SS2R; somatostatin receptor type 2.

(38.2) SSTR5 (Somatostatin Receptor 5):

Nucleotide: Genbank accession no D16827; Genbank version no. D16827.1 GI:487683. Polypeptide: Genbank accession no. BAA04107; Genbank version no. BAA04107.1 GI:487684.

Cross References: Yamada, Y., et al *Biochem. Biophys. Res. Commun.* 195 (2), 844-852 (1993).

Other Information: Official Symbol: SSTR5; Other Aliases: SS-5-R; Other Designations: Somatostatin receptor subtype 5; somatostatin receptor type 5.

(38.3) SSTR1; (38.4) SSTR3; (38.5) SSTR4; AvB6—Both Subunits (39+40).

(39) ITGAV (Integrin, Alpha V:

Nucleotide: Genbank accession no M14648 J02826 M18365; Genbank version no. M14648.1 GI:340306. Polypeptide: Genbank accession no. AAA36808; Genbank version no. AAA36808.1 GI:340307.

Cross References: Suzuki S., et al *Proc. Natl. Acad. Sci. U.S.A.* 83 (22), 8614-8618 (1986).

Other Information: Official Symbol: ITGAV; Other Aliases: CD51, MSK8, VNRA, VTNR

Other Designations: antigen identified by monoclonal antibody L230; integrin alpha-V; integrin alphaVbeta3; integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51); vitronectin receptor subunit alpha.

(40) ITGB6 (Integrin, Beta 6):

Nucleotide: Genbank accession no NM_000888; Genbank version no. NM_000888.3 GI:9966771. Polypeptide: Genbank accession no. NP_000879; Genbank version no. NP_000879.2 GI:9625002.

Cross References: Sheppard D. J., et al *Biol. Chem.* 265 (20), 11502-11507 (1990)

Other Information: Official Symbol: ITGB6; Other Designations: integrin beta-6.

Antibodies:

Biogen: U.S. Pat. No. 7,943,742—Hybridoma clones 6.3G9 and 6.8G6 were deposited with the ATCC, accession numbers ATCC PTA-3649 and -3645, respectively.

Biogen: U.S. Pat. No. 7,465,449—In some embodiments, the antibody comprises the same heavy and light chain polypeptide sequences as an antibody produced by hybridoma 6.1A8, 6.3G9, 6.8G6, 6.2B1, 6.2B10, 6.2A1, 6.2E5, 7.1G10, 7.7G5, or 7.1C5.

Centocor (J&J): U.S. Pat. Nos. 7,550,142; 7,163,681. For example in U.S. Pat. No. 7,550,142—an antibody having human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 7 and SEQ ID NO: 8.

Seattle Genetics: 15H3 (Ryan M C., et al Cancer Res Apr. 15, 2012; 72(8 Supplement): 4630).

(41) CEACAM5 (Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5):

Nucleotide: Genbank accession no M17303; Genbank version no. M17303.1 GI:178676. Polypeptide: Genbank accession no. AAB59513; Genbank version no. AAB59513.1 G1:178677.

Cross References: Beauchemin N., et al *Mol. Cell. Biol.* 7 (9), 3221-3230 (1987).

Other Information: Official Symbol: CEACAM5; Other Aliases: CD66e, CEA;

Other Designations: meconium antigen 100.

Antibodies:

AstraZeneca-MedImmune: US 20100330103; US20080057063;

US20020142359. For example an antibody having complementarity determining regions (CDRs) with the following sequences: heavy chain; CDR1—DNYMH, CDR2—WIDPENGDTE YAPKFRG, CDR3—LIYAGYLAMD Y; and light chain CDR1—SASSSVTYMH, CDR2—STSNLAS, CDR3—QQRSTYPLT. [0218] Hybridoma 806.077 deposited as European Collection of Cell Cultures (ECACC) deposit no. 96022936.

Research Corporation Technologies, Inc.: U.S. Pat. No. 5,047,507.

Bayer Corporation: U.S. Pat. No. 6,013,772.

BioAlliance: U.S. Pat. Nos. 7,982,017; 7,674,605; U.S. Pat. No. 7,674,605 an antibody comprising the heavy chain variable region sequence from the amino acid sequence of SEQ ID NO: 1, and the light chain variable region sequence from the amino acid sequence of SEQ ID NO:2. an antibody comprising the heavy chain variable region sequence from the amino acid sequence of SEQ ID NO:5, and the light chain variable region sequence from the amino acid sequence of SEQ ID NO:6.

Celltech Therapeutics Limited: U.S. Pat. No. 5,877,293.

The Dow Chemical Company: U.S. Pat. Nos. 5,472,693; 6,417,337; 6,333,405; U.S. Pat. No. 5,472,693—for example, ATCC No. CRL-11215; U.S. Pat. No. 6,417,337—for example, ATCC CRL-12208; U.S. Pat. No. 6,333,405—for example, ATCC CRL-12208.

Immunomedics, Inc: U.S. Pat. Nos. 7,534,431; 7,230,084; 7,300,644; 6,730,300; US20110189085; an antibody having CDRs of the light chain variable region comprise: CDR1 comprises KASQDVGTSVA (SEQ ID NO: 20); CDR2 comprises WTSTRHT (SEQ ID NO: 21); and CDR3 comprises QQYSLYRS (SEQ ID NO: 22); and the CDRs of the heavy chain variable region of said anti-CEA antibody comprise: CDR1 comprises TYWMS (SEQ ID NO: 23); CDR2

comprises EIHPDSSTINYAPSLKD (SEQ ID NO: 24); and CDR3 comprises LYFGFPWFAY (SEQ ID NO: 25). US20100221175; US20090092598; US20070202044; US20110064653; US20090185974; US20080069775.

(42) MET (Met Proto-Oncogene; Hepatocyte Growth Factor Receptor):

Nucleotide: Genbank accession no M35073; Genbank version no. M35073.1 GI:187553. Polypeptide: Genbank accession no. AAA59589; Genbank version no. AAA59589.1 GI:553531.

Cross References: Dean M., et al *Nature* 318 (6044), 385-388 (1985).

Other Information: Official Symbol: MET; Other Aliases: AUTS9, HGFR, RCCP2, c-Met.

Other Designations: HGF receptor; HGF/SF receptor; SF receptor; hepatocyte growth factor receptor; met proto-oncogene tyrosine kinase; proto-oncogene c-Met; scatter factor receptor; tyrosine-protein kinase Met.

Antibodies:

Abgenix/Pfizer: US20100040629, For example, the antibody produced by hybridoma 13.3.2 having American Type Culture Collection (ATCC) accession number PTA-5026; the antibody produced by hybridoma 9.1.2 having ATCC accession number PTA-5027; the antibody produced by hybridoma 8.70.2 having ATCC accession number PTA-5028; or the antibody produced by hybridoma 6.90.3 having ATCC accession number PTA-5029.

Amgen/Pfizer: US20050054019, For example, an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 2 where X2 is glutamate and X4 is serine and a light chain having the amino acid sequence set forth in SEQ ID NO: 4 where X8 is alanine, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 6 and a light chain having the amino acid sequence set forth in SEQ ID NO: 8, without the signal sequences; an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 10 and a light chain having the amino acid sequence set forth in SEQ ID NO: 12, without the signal sequences; or an antibody comprising a heavy chain having the amino acid sequences set forth in SEQ ID NO: 14 and a light chain having the amino acid sequence set forth in SEQ ID NO: 16, without the signal sequences.

Agouron Pharmaceuticals (Now Pfizer): US20060035907.

Eli Lilly: US20100129369.

Genentech: U.S. Pat. No. 5,686,292; US20100028337; US20100016241; US20070129301; US20070098707; US20070092520, US20060270594; US20060134104; US20060035278; US20050233960; US20050037431; U.S. Pat. No. 5,686,292—for example, ATCC HB-11894 and ATCC HB-11895; US 20100016241—for example, ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6).

National Defense Medical Center, Taiwan: Lu R M., et al Biomaterials. 2011 April; 32(12):3265-74.

Novartis: US20090175860.

For example, an antibody comprising the sequences of CDR1, CDR2 and CDR3 of heavy chain 4687, wherein the sequences of CDR1, CDR2, and CDR3 of heavy chain 4687 are residues 26-35, 50-65, and 98-102, respectively, of SEQ ID NO: 58; and the sequences of CDR1, CDR2, and CDR3 of light chain 5097, wherein the sequences of CDR1, CDR2, and CDR3 of light chain 5097 are residues 24-39,55-61, and 94-100 of SEQ ID NO: 37.

Pharmacia Corporation: US20040166544.

Pierre Fabre: US20110239316, US20110097262, US20100115639.

Sumsung: US 20110129481—for example a monoclonal antibody produced from a hybridoma cell having accession number KCLRF-BP-00219 or accession number of KCLRF-BP-00223.

Samsung: US 20110104176—for example an antibody produced by a hybridoma cell having Accession Number: KCLRF-BP-00220.

University of Turin Medical School: DN-30 Pacchiana G., et al *J Biol Chem.*; 285(46):36149-57(2010).

Van Andel Research Institute: Jiao Y., et al *Mol Biotechnol.*; 31(1):41-54(2005).

(43) MUC1 (Mucin 1, Cell Surface Associated):

Nucleotide: Genbank accession no J05581; Genbank version no. J05581.1 GI:188869. Polypeptide: Genbank accession no. AAA59876; Genbank version no. AAA59876.1 G1:188870.

Cross References: Gendler S. J., et al *J. Biol. Chem.* 265 (25), 15286-15293 (1990)

Other Information: Official Symbol: MUC1; Other Aliases: RP11-263K19.2, CD227, EMA, H23AG, KL-6, MAM6, MUC-1, MUC-1/SEC, MUC-1/X, MUC1/ZD, PEM, PEMT, PUM.

Other Designations: DF3 antigen; H23 antigen; breast carcinoma-associated antigen DF3; carcinoma-associated mucin; episialin; krebs von den Lungen-6; mucin 1, transmembrane; mucin-1; peanut-reactive urinary mucin; polymorphic epithelial mucin; tumor associated epithelial mucin; tumor-associated epithelial membrane antigen; tumor-associated mucin.

Antibodies:

AltaRex—Quest Pharma Tech: U.S. Pat. No. 6,716,966—for example an Alt-1 antibody produced by the hybridoma ATCC No PTA-975.

AltaRex—Quest Pharma Tech: U.S. Pat. No. 7,147,850.

CRT: 5E5—Sorensen AL., et al *Glycobiology*. 16(2) 96-107(2006); HMFG2—Burchell J., et al *Cancer Res.*, 47, 5476-5482 (1987); see WO2015/159076

Glycotope GT-MAB: GT-MAB 2.5-GEX (Website: http://www.glycotope.com/pipeline/pankomab-gex)

Immunogen: U.S. Pat. No. 7,202,346. For example, antibody MJ-170: hybridoma cell line MJ-170 ATCC accession no. PTA-5286Monoclonal antibody MJ-171: hybridoma cell line MJ-171 ATCC accession no. PTA-5287; monoclonal antibody MJ-172: hybridoma cell line MJ-172 ATCC accession no. PTA-5288; or monoclonal antibody MJ-173: hybridoma cell line MJ-173 ATCC accession no. PTA-5302.

Immunomedics: U.S. Pat. No. 6,653,104.

Ramot Tel Aviv Uni: U.S. Pat. No. 7,897,351.

Regents Uni. CA: U.S. Pat. No. 7,183,388; US20040005647; US20030077676.

Roche GlycArt: U.S. Pat. No. 8,021,856.

Russian National Cancer Research Center: Imuteran—Ivanov P K., et al *Biotechnol J.*; 2(7):863-70(2007).

Technische Univ Braunschweig: (IIB6, HT186-B7, HT186-D11, HT186-G2, HT200-3A-C1, HT220-M-D1, HT220-M-G8)—Thie H., et al *PLoS One.*; 6(1):e15921(2011).

(44) CA9 (Carbonic Anhydrase IX):

Nucleotide: Genbank accession no. X66839; Genbank version no. X66839.1 GI:1000701. Polypeptide: Genbank accession no. CAA47315; Genbank version no. CAA47315.1 G1:1000702.

Cross References: Pastorek J., et al *Oncogene* 9 (10), 2877-2888 (1994).

Other Information: Official Symbol: CA9; Other Aliases: CAIX, MN;

Other Designations: CA-IX; P54/58N; RCC-associated antigen G250; RCC-associated protein G250; carbonate dehydratase IX; carbonic anhydrase 9; carbonic dehydratase; membrane antigen MN; pMW1; renal cell carcinoma-associated antigen G250.

Antibodies:

Abgenix/Amgen: US20040018198.

Affibody: Anti-CAIX Affibody molecules (http://www.affibody.com/en/Product-Portfolio/Pipeline/).

Bayer: U.S. Pat. No. 7,462,696.

Bayer/Morphosys: 3ee9 mAb—Petrul H M., et al *Mol Cancer Ther.*; 11(2):340-9(2012)

Harvard Medical School: Antibodies G10, G36, G37, G39, G45, G57, G106, G119, G6, G27, G40 and G125. Xu C., et al *PLoS One.*; 5(3): e9625(2010).

Institute of Virology, Slovak Academy of Sciences (Bayer)—U.S. Pat. No. 5,955,075. For example, M75—ATCC Accession No. HB 11128 or MN12—ATCC Accession No. HB 11647.

Institute of Virology, Slovak Academy of Sciences: U.S. Pat. No. 7,816,493. For example the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128; or the V/10 monoclonal antibody secreted from the hybridoma V/10-VU, which was deposited at the International Depository Authority of the Belgian Coordinated Collection of Microorganisms (BCCM) at the Laboratorium voor Moleculaire Bioloqie-Plasmidencollectie (LMBP) at the Universeit Gent in Gent, Belgium, under Accession No. LMBP 6009CB.

Institute of Virology, Slovak Academy of Sciences US20080177046; US20080176310; US20080176258; US20050031623.

Novartis: US20090252738.

Wilex: U.S. Pat. No. 7,691,375—for example the antibody produced by the hybridoma cell line DSM ASC 2526.

Wilex: US20110123537; Rencarex: Kennett R H., et al *Curr Opin Mol Ther.*; 5(1):70-5(2003).

Xencor: US20090162382.

(45) EGFRvIII (Epidermal Growth Factor Receptor (EGFR), Transcript Variant 3):

Nucleotide: Genbank accession no. NM_201283; Genbank version no. NM_201283.1 G1:41327733. Polypeptide: Genbank accession no. NP_958440; Genbank version no. NP_958440.1 G1:41327734.

Cross-References: Batra SK., et al *Cell Growth Differ.*; 6(10):1251-1259(1995).

Antibodies: U.S. Pat. Nos. 7,628,986 and 7,736,644 (Amgen) For example, a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 142 and variants & a light chain variable region amino acid sequence selected from the group consisting of: SEQ ID NO: 144 and variants.

US20100111979 (Amgen); For example, an antibody comprising a heavy chain amino acid sequence comprising: CDR1 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR1 region of antibodies 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17); CDR2 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR2 region of antibodies 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17); and CDR3 consisting of a sequence selected from the group consisting of the amino acid sequences for the CDR3 region of antibodies 13.1.2

(SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

US20090240038 (Amgen): For example, an antibody having at least one of the heavy or light chain polypeptides comprises an amino acid sequence that is at least 90% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 19, SEQ ID NO: 142, SEQ ID NO: 144, and any combination thereof.

US20090175887 (Amgen): For example, an antibody having a heavy chain amino acid sequence selected from the group consisting of the heavy chain amino acid sequence of antibody 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

US20090156790 (Amgen): For example, antibody having heavy chain polypeptide and a light chain polypeptide, wherein at least one of the heavy or light chain polypeptides comprises an amino acid sequence that is at least 90% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 19, SEQ ID NO: 142, SEQ ID NO: 144, and any combination thereof.

US20090155282, US20050059087 and US20050053608 (Amgen): For example, an antibody heavy chain amino acid sequence selected from the group consisting of the heavy chain amino acid sequence of antibody 13.1.2 (SEQ ID NO: 138), 131 (SEQ ID NO: 2), 170 (SEQ ID NO: 4), 150 (SEQ ID NO: 5), 095 (SEQ ID NO: 7), 250 (SEQ ID NO: 9), 139 (SEQ ID NO: 10), 211 (SEQ ID NO: 12), 124 (SEQ ID NO: 13), 318 (SEQ ID NO: 15), 342 (SEQ ID NO: 16), and 333 (SEQ ID NO: 17).

MR1-1 (U.S. Pat. No. 7,129,332; Duke): For example, a variant antibody having the sequence of SEQ ID NO.18 with the substitutions S98P-T99Y in the CDR3 VH, and F92W in CDR3 VL.

L8A4, H10, Y10 (Wikstrand CJ., et al *Cancer Res.*; 55(14):3140-8(1995); Duke).

US20090311803 (Harvard University): For example, SEQ ID NO:9 for antibody heavy chain variable region, and SEQ ID NO: 3 for light chain variable region amino acid sequences

US20070274991 (EMD72000, also known as matuzumab; Harvard University) For example, SEQ ID NOs: 3 & 9 for light chain and heavy chain respectively.

U.S. Pat. No. 6,129,915 (Schering) For example, SEQ. ID NOs: 1, 2, 3, 4, 5 and 6.

mAb CH12—Wang H., et al *FASEB J.*; 26(1):73-80(2012) (Shanghai Cancer Institute).

RAbDMvIII—Gupta P., et al *BMC Biotechnol.*; 10:72 (2010)(Stanford University Medical Center).

mAb Ua30—Ohman L., et al Tumour Biol.; 23(2):61-9 (2002) (Uppsala University).

Han DG., et al *Nan Fang Yi Ke Da Xue Xue Bao.*; 30(1):25-9(2010) (Xi'an Jiaotong University).

(46) Cd33 (Cd33 Molecule):

Nucleotide: Genbank accession no. M_23197; Genbank version no. NM_23197.1 G1:180097. Polypeptide: Genbank accession no. AAA51948; Genbank version no. AAA51948.1 G1:188098.

Cross-References: Simmons D., et al *J. Immunol.* 141 (8), 2797-2800 (1988). CD33, a member of the sialic acid binding, immunoglobulin-like lectin family, is a 67-kDa glycosylated transmembrane protein. CD33 is expressed on most myeloid and monocytic leukemia cells in addition to committed myelomonocytic and erythroid progenitor cells. It is not seen on the earliest pluripotent stem cells, mature granulocytes, lymphoid cells, or nonhematopoietic cells (Sabbath et al., (1985) J. Clin. Invest. 75:756-56; Andrews et al., (1986) Blood 68: 1030-5). CD33 contains two tyrosine residues on its cytoplasmic tail, each of which is followed by hydrophobic residues similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM) seen in many inhibitory receptors.

Other Information: Official Symbol: CD33; Other Aliases: SIGLEC-3, SIGLEC3, p67.

Other Designations: CD33 antigen (gp67); gp67; myeloid cell surface antigen CD33; sialic acid binding Ig-like lectin 3; sialic acid-binding Ig-like lectin.

Antibodies: H195 (Lintuzumab)—Raza A., et al *Leuk Lymphoma.*; 50(8):1336-44 (2009); U.S. Pat. No. 6,759,045 (Seattle Genetics/Immunomedics).

mAb OKT9: Sutherland, D. R. et al. *Proc Natl Acad Sci USA* 78(7): 4515-4519 (1981), Schneider C., et al *J Biol Chem* 257(14), 8516-8522 (1982).

mAb E6: Hoogenboom, H. R., et al *J Immunol* 144(8), 3211-3217 (1990).

U.S. Pat. No. 6,590,088 (Human Genome Sciences) For example, SEQ ID NOs: 1 and 2 and ATCC accession no. 97521.

U.S. Pat. No. 7,557,189 (Immunogen) For example, an antibody or fragment thereof comprising a heavy chain variable region which comprises three CDRs having the amino acid sequences of SEQ ID NOs:1-3 and a light chain variable region comprising three CDRs having the amino acid sequences of SEQ ID NOs:4-6.

(47) Cd19 (Cd19 Molecule):

Nucleotide: Genbank accession no. NM_001178098; Genbank version no. NM_001178098.1 G1:296010920. Polypeptide: Genbank accession no. NP_001171569; Genbank version no. NP_001171569.1 G1:296010921.

Cross-References: Tedder T F., et al J. Immunol. 143 (2): 712-7 (1989)

Other Information: Official Symbol: CD19; Other Aliases: B4, CVID3.

Other Designations: B-lymphocyte antigen CD19; B-lymphocyte surface antigen B4; T-cell surface antigen Leu-12; differentiation antigen CD19.

Antibodies

Immunogen: HuB4—Al-Katib A M., et al *Clin Cancer Res.*; 15(12):4038-45(2009).

4G7: Kügler M., et al *Protein Eng Des Sel.*; 22(3):135-47(2009).

For example, sequences in FIG. 3 of Knappik, A. et al. *J Mol Biol.*; 296(1):57-86(2000).

AstraZeneca/MedImmune: MEDI-551—Herbst R., et al *J Pharmacol Exp Ther.*; 335(1):213-22(2010).

Glenmark Pharmaceuticals: GBR-401—Hou S., et al Mol Cancer Ther (Meeting Abstract Supplement) C164(2011).

U.S. Pat. No. 7,109,304 (Immunomedics): For example, an antibody comprising the sequence of hA19Vk (SEQ ID NO:7) and the sequence of hA19VH (SEQ ID NO:10).

U.S. Pat. No. 7,902,338 (Immunomedics): For example, an antibody or antigen-binding fragment thereof that comprises the light chain complementarity determining region CDR sequences CDR1 of SEQ ID NO: 16 (KASQSVDYDGDSYLN); CDR2 of SEQ ID NO: 17 (DASNLVS); and CDR3 of SEQ ID NO: 18 (QQSTEDPWT) and the heavy chain CDR sequences CDR1 of SEQ ID NO: 19 (SYWMN); CDR2 of SEQ ID NO: 20 (QIWPGDGDTNYNGKFKG) and CDR3 of SEQ ID NO: 21 (RETTTVGRYYYAMDY) and also comprises human antibody framework (FR) and constant region sequences with one or more framework region amino acid residues substituted from the corresponding framework region sequences of the parent murine antibody, and wherein said substituted FR residues comprise the substitution of serine for phenylalanine at Kabat residue 91 of the heavy chain variable region.

Medarex: MDX-1342—Cardarelli P M., et al *Cancer Immunol Immunother.*; 59(2):257-65(2010).

MorphoSys/Xencor: MOR-208/XmAb-5574—Zalevsky J., et al *Blood.*; 113(16):3735-43(2009)

U.S. Pat. No. 7,968,687 (Seattle Genetics) An antibody or antigen-binding fragment comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:9 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 24.

4G7 chim—Lang P., et al Blood.; 103(10):3982-5(2004) (University of Tübingen) For example, FIG. 6 and SEQ ID No: 80 of US20120082664.

Zhejiang University School of Medicine: 2E8—Zhang J., et al J Drug Target.; 18(9):675-8(2010).

(48) IL2RA (Interleukin 2 Receptor, Alpha); NCBI Reference Sequence: NM_000417.2):

Nucleotide: Genbank accession no. NM_000417; Genbank version no. NM_000417.2 GI:269973860. Polypeptide: Genbank accession no. NP_000408; Genbank version no. NP_000408.1 G1:4557667.

Cross-References: Kuziel W. A., et al *J. Invest. Dermatol.* 94 (6 SUPPL), 27S-32S (1990)

Other Information: Official Symbol: IL2RA; Other Aliases: RP11-536K7.1, CD25, IDDM10, IL2R, TCGFR; Other Designations: FIL-2 receptor subunit alpha; IL-2-RA; IL-2R subunit alpha; IL2-RA; TAC antigen; interleukin-2 receptor subunit alpha; p55.

Antibodies: U.S. Pat. No. 6,383,487 (Novartis/UCL: Baxilisimab [Simulect]) U.S. Pat. No. 6,521,230 (Novartis/UCL: Baxilisimab [Simulect]) For example, an antibody having an antigen binding site comprises at least one domain which comprises CDR1 having the amino acid sequence in SEQ. ID. NO: 7, CDR2 having the amino acid sequence in SEQ. ID. NO: 8, and CDR3 chaving the amino acid sequence in SEQ. ID. NO: 9; or said CDR1, CDR2 and CDR3 taken in sequence as a whole comprise an amino acid sequence which is at least 90% identical to SEQ. ID. NOs: 7, 8 and 9 taken in sequence as a whole.

Daclizumab—Rech A J., et al *Ann N Y Acad Sci.*; 1174:99-106(2009) (Roche).

(49) AXL (AXL Receptor Tyrosine Kinase):

Nucleotide: Genbank accession no. M76125; Genbank version no. M76125.1 G1:292869. Polypeptide: Genbank accession no. AAA61243; Genbank version no. AAA61243.1 G1:29870.

Cross-References: O'Bryan J. P., et al *Mol. Cell. Biol.* 11 (10), 5016-5031 (1991); Bergsagel P. L., et al *J. Immunol.* 148 (2), 590-596 (1992).

Other Information: Official Symbol: AXL; Other Aliases: JTK11, UFO.

Other Designations: AXL oncogene; AXL transforming sequence/gene; oncogene AXL; tyrosine-protein kinase receptor UFO.

Antibodies: YW327.6S2—Ye X., et al *Oncogene.*; 29(38):5254-64(2010). (Genentech); BergenBio: BGB324 (http://www.bergenbio.com/BGB324).

(50) CD30—TNFRSF8 (Tumor Necrosis Factor Receptor Superfamily, Member 8):

Nucleotide: Genbank accession no. M83554; Genbank version no. M83554.1 G1:180095. Polypeptide: Genbank accession no. AAA51947; Genbank version no. AAA51947.1 G1:180096.

Cross-References: Durkop H., et al *Cell* 68 (3), 421-427 (1992).

Other Information: Official Symbol: TNFRSF8; Other Aliases: CD30, D1S166E, Ki-1

Other Designations: CD30L receptor; Ki-1 antigen; cytokine receptor CD30; lymphocyte activation antigen CD30; tumor necrosis factor receptor superfamily member 8.

(51) BCMA (B-Cell Maturation Antigen)—TNFRSF17 (Tumor Necrosis Factor Receptor Superfamily, Member 17):

Nucleotide: Genbank accession no. Z29574; Genbank version no. Z29574.1 GI:471244. Polypeptide: Genbank accession no. CAA82690; Genbank version no. CAA82690.1 GI:471245.

Cross-References: Laabi Y., et al Nucleic Acids Res. 22 (7), 1147-1154 (1994).

Other Information: Official Symbol: TNFRSF17; Other Aliases: BCM, BCMA, CD269.

Other Designations: B cell maturation antigen; B-cell maturation factor; B-cell maturation protein; tumor necrosis factor receptor superfamily member 17.

(52) CT Ags—CTA (Cancer Testis Antigens):

Cross-References: Fratta E., et al. *Mol Oncol.*; 5(2):164-82(2011); Lim S H., et al Am J Blood Res.; 2(1):29-35(2012).

(53) CD174 (Lewis Y)—FUT3 (Fucosyltransferase 3 (Galactoside 3(4)-L-Fucosyltransferase, Lewis Blood Group):

Nucleotide: Genbank accession no. NM000149; Genbank version no. NM000149.3 GI:148277008. Polypeptide: Genbank accession no. NP_000140; Genbank version no. NP_000140.1 G1:4503809.

Cross-References: Kukowska-Latallo, J. F., et al *Genes Dev.* 4 (8), 1288-1303 (1990).

Other Information: Official Symbol: FUT3; Other Aliases: CD174, FT3B, FucT-III, LE, Les

Other Designations: Lewis FT; alpha-(1,3/1,4)-fucosyltransferase; blood group Lewis alpha-4-fucosyltransferase; fucosyltransferase III; galactoside 3(4)-L-fucosyltransferase.

(54) CLEC14A (C-Type Lectin Domain Family 14, Member a; Genbank Accession No. NM175060):

Nucleotide: Genbank accession no. NM175060; Genbank version no. NM175060.2 G1:371123930. Polypeptide: Genbank accession no. NP_778230; Genbank version no. NP_778230.1 G1:28269707.

Other Information: Official Symbol: CLEC14A; Other Aliases: UNQ236/PRO269, C14orf27, CEG1, EGFR-5.

Other Designations: C-type lectin domain family 14 member A; CIECT and EGF-like domain containing protein; epidermal growth factor receptor 5.

(55) GRP78—HSPA5 (Heat Shock 70 kDa Protein 5 (Glucose-Regulated Protein, 78 kDa):

Nucleotide: Genbank accession no. NM005347; Genbank version no. NM005347.4 G1:305855105. Polypeptide: Genbank accession no. NP_005338; Genbank version no. NP_005338.1 GI:16507237.

Cross-References: Ting J., et al DNA 7 (4), 275-286 (1988).

Other Information: Official Symbol: HSPA5; Other Aliases: BIP, GRP78, MIF2.

Other Designations: 78 kDa glucose-regulated protein; endoplasmic reticulum lumenal Ca(2+)-binding protein grp78; immunoglobulin heavy chain-binding protein.

(56) Cd70 (Cd70 Molecule) L08096:

Nucleotide: Genbank accession no. L08096; Genbank version no. L08096.1 GI:307127. Polypeptide: Genbank accession no. AAA36175; Genbank version no. AAA36175.1 GI:307128.

Cross-References: Goodwin R. G., et al *Cell* 73 (3), 447-456 (1993).

Other Information: Official Symbol: CD70; Other Aliases: CD27L, CD27LG, TNFSF7.

Other Designations: CD27 ligand; CD27-L; CD70 antigen; Ki-24 antigen; surface antigen CD70; tumor necrosis factor (ligand) superfamily, member 7; tumor necrosis factor ligand superfamily member 7.

Antibodies: MDX-1411 against CD70 (Medarex) h1F6 (Oflazoglu, E., et al, Clin Cancer Res.; 14(19):6171-80(2008); (Seattle Genetics) For example, see US20060083736 SEQ ID NOs: 1, 2, 11 and 12 and FIG. 1.

(57) Stem Cell Specific Antigens: For Example: 5T4 (see entry (63) below) ; CD25 (see entry (48) above); CD32 . Polypeptide: Genbank accession no. ABK42161; Genbank version no. ABK42161.1 GI:117616286; LGR5/GPR49. Nucleotide: Genbank accession no. NM_003667; Genbank version no. NM_003667.2 G1:24475886. Polypeptide: Genbank accession no. NP_003658; Genbank version no. NP_003658.1 G1:4504379. Prominin/CD133. Nucleotide: Genbank accession no. NM_006017; Genbank version no. NM_006017.2 G1:224994187. Polypeptide: Genbank accession no. NP_006008; Genbank version no. NP_006008.1 G1:5174387.

(58) ASG-5:

Cross-References: (Smith L. M., et. al *AACR* 2010 *Annual Meeting* (abstract #2590); Gudas J. M., et. al. *AACR* 2010 *Annual Meeting* (abstract #4393).

Antibodies Anti-AGS-5 Antibody: M6.131 (Smith, L. M., et. al *AACR* 2010 *Annual Meeting* (abstract #2590)).

(59) ENPP3 (Ectonucleotide Pyrophosphatase/Phosphodiesterase 3):

Nucleotide: Genbank accession no. AF005632; Genbank version no. AF005632.2 G1:4432589. Polypeptide: Genbank accession no. AAC51813; Genbank version no. AAC51813.1 G1:2465540.

Cross-References: Jin-Hua P., et al *Genomics* 45 (2), 412-415 (1997).

Other Information: Official Symbol: ENPP3; Other Aliases: RP5-988G15.3, B10, CD203c, NPP3, PD-IBETA, PDNP3.

Other Designations: E-NPP 3; dJ1005H11.3 (phosphodiesterase I/nucleotide pyrophos-phatase 3); dJ914N13.3 (phosphodiesterase I/nucleotide pyrophosphatase 3); ectonucleotide

pyrophosphatase/phosphodiesterase family member 3; gp130RB13-6; phosphodiesterase I beta; phosphodiesterase I/nucleotide pyrophosphatase 3; phosphodiesterase-1 beta.

(60) PRR4 (Proline Rich 4 (Lacrimal)):

Nucleotide: Genbank accession no. NM_007244; Genbank version no. NM_007244.2 G1:154448885. Polypeptide: Genbank accession no. NP_009175; Genbank version no. NP_009175.2 G1:154448886.

Cross-References: Dickinson D. P., et al *Invest. Ophthalmol. Vis. Sci.* 36 (10), 2020-2031 (1995).

Other Information: Official Symbol: PRR4; Other Aliases: LPRP, PROL4.

Other Designations: lacrimal proline-rich protein; nasopharyngeal carcinoma-associated proline-rich protein 4; proline-rich polypeptide 4; proline-rich protein 4.

(61) GCC—GUCY2C (Guanylate Cyclase 2C (Heat Stable Enterotoxin Receptor):

Nucleotide: Genbank accession no. NM_004963; Genbank version no. NM_004963.3 G1:222080082. Polypeptide: Genbank accession no. NP_004954; Genbank version no. NP_004954.2 G1:222080083.

Cross-References: De Sauvage F. J., et al *J. Biol. Chem.* 266 (27), 17912-17918 (1991); Singh S., et al *Biochem. Biophys. Res. Commun.* 179 (3), 1455-1463 (1991).

Other Information: Official Symbol: GUCY2C; Other Aliases: DIAR6, GUC2C, MUCIL, STAR.

Other Designations: GC-C; STA receptor; guanylyl cyclase C; hSTAR; heat-stable enterotoxin receptor; intestinal guanylate cyclase.

(62) Liv-1—SLC39A6 (Solute Carrier Family 39 (Zinc Transporter), Member 6):

Nucleotide: Genbank accession no. U41060; Genbank version no. U41060.2 GI:12711792. Polypeptide: Genbank accession no. AAA96258; Genbank version no. AAA96258.2 GI:12711793.

Cross-References: Taylor KM., et al *Biochim Biophys Acta.*; 1611(1-2):16-30(2003).

Other Information: Official Symbol: SLC39A6; Other Aliases: LIV-1.

Other Designations: LIV-1 protein, estrogen regulated; ZIP-6; estrogen-regulated protein LIV-1; solute carrier family 39 (metal ion transporter), member 6; solute carrier family 39 member 6; zinc transporter ZIP6; zrt- and Irt-like protein 6.

(63) 5T4, Trophoblast Glycoprotein, TPBG—TPBG (Trophoblast Glycoprotein):

Nucleotide: Genbank accession no. AJ012159; Genbank version no. AJ012159.1 GI:3805946. Polypeptide: Genbank accession no. CAA09930; Genbank version no. CAA09930.1 GI:3805947.

Cross-References: King K. W., et al *Biochim. Biophys. Acta* 1445 (3), 257-270 (1999).

Other Information: Official Symbol: TPBG Other Aliases: 5T4, 5T4AG, M6P1; Other Designations: 5T4 oncofetal antigen; 5T4 oncofetal trophoblast glycoprotein; 5T4 oncotrophoblast glycoprotein See WO2015/155345.

(64) CD56—NCMA 1 (Neural Cell Adhesion Molecule 1):

Nucleotide: Genbank accession no. NM_000615; Genbank version no. NM_000615.6 G1:336285433. Polypeptide: Genbank accession no. NP_000606; Genbank version no. NP_000606.3 G1:94420689.

Cross-References: Dickson, G., et al, *Cell* 50 (7), 1119-1130 (1987).

Other Information: Official Symbol: NCAM1; Other Aliases: CD56, MSK39, NCAM.

Other Designations: antigen recognized by monoclonal antibody 5.1H11; neural cell adhesion molecule, NCAM.

Antibodies: Immunogen: HuN901 (Smith S V., et al *Curr Opin Mol Ther.*; 7(4):394-401(2005); For example, see humanized from murine N901 antibody. See FIGS. 1b and 1e of Roguska, M. A., et al. *Proc Natl Acad Sci USA.*; 91:969-973(1994).

(65) CanAg (Tumor Associated Antigen CA242):

Cross-References: Haglund C., et al *Br J Cancer* 60:845-851(1989); Baeckstrom D., et al *J Biol Chem* 266:21537-21547(1991).

Antibodies: huC242 (Tolcher A W et al., *J Clin Oncol.*; 21(2):211-22(2003); (Immunogen) For example, see US20080138898A1 SEQ ID NO: 1 and 2.

(66) FOLR1 (Folate Receptor 1):

Nucleotide: Genbank accession no. J05013; Genbank version no. J05013.1 G1:182417. Polypeptide: Genbank accession no. AAA35823; Genbank version no. AAA35823.1 G1:182418.

Cross-References: Elwood P. C., et al *J. Biol. Chem.* 264 (25), 14893-14901 (1989).

Other Information: Official Symbol: FOLR1; Other Aliases: FBP, FOLR.

Other Designations: FR-alpha; KB cells FBP; adult folate-binding protein; folate binding protein; folate receptor alpha; folate receptor, adult; ovarian tumor-associated antigen MOv18.

Antibodies M9346A—Whiteman K R., et al *Cancer Res*, 72(8 Supplement): 4628(2012) (Immunogen).

(67) GPNMB (Glycoprotein (Transmembrane) Nmb):

Nucleotide: Genbank accession no. X76534; Genbank version no. X76534.1 G1:666042. Polypeptide: Genbank accession no. CAA54044; Genbank version no. CAA54044.1 GI:666043.

Cross-References: Weterman M. A., et al *Int. J. Cancer* 60 (1), 73-81 (1995).

Other Information: Official Symbol: GPNMB; Other Aliases: UNQ1725/PRO9925, HGFIN, NMB.

Other Designations: glycoprotein NMB; glycoprotein nmb-like protein; osteoactivin; transmembrane glycoprotein HGFIN; transmembrane glycoprotein NMB

Antibodies: Celldex Therapeutics: CR011 (Tse K F., et al *Clin Cancer Res.*; 12(4):1373-82(2006)); For example, see EP1827492B1 SEQ ID NO: 22, 24, 26, 31, 33 and 35.

(68) TIM-1—HAVCR1 (Hepatitis a Virus Cellular Receptor 1):

Nucleotide: Genbank accession no. AF043724; Genbank version no. AF043724.1 GI:2827453. Polypeptide: Genbank accession no. AAC39862; Genbank version no. AAC39862.1 GI:2827454.

Cross-References: Feigelstock D., et al *J. Virol.* 72 (8), 6621-6628 (1998).

Other Information: Official Symbol: HAVCR1; Other Aliases: HAVCR, HAVCR-1, KIM-1, KIM1, TIM, TIM-1, TIM1, TIMD-1, TIMD1.

Other Designations: T cell immunoglobin domain and mucin domain protein 1; T-cell membrane protein 1; kidney injury molecule 1.

(69) RG-1/Prostate Tumor Target Mindin—Mindin/RG-1:

Cross-References: Parry R., et al Cancer Res.; 65(18):8397-405(2005).

(70) B7-H4—VTCN1 (V-Set Domain Containing T Cell Activation Inhibitor 1:

Nucleotide: Genbank accession no. BX648021; Genbank version no. BX648021.1 G1:34367180.

Cross-References: Sica G L., et al *Immunity.*; 18(6):849-61(2003).

Other Information: Official Symbol: VTCN1.

Other Aliases: RP11-229A19.4, B7-H4, B7H4, B7S1, B7X, B7h.5, PRO1291, VCTN1.

Other Designations: B7 family member, H4; B7 superfamily member 1; T cell costimulatory molecule B7x; T-cell costimulatory molecule B7x; V-set domain-containing T-cell activation inhibitor 1; immune costimulatory protein B7-H4.

(71) PTK7 (PTK7 Protein Tyrosine Kinase 7):

Nucleotide: Genbank accession no. AF447176; Genbank version no. AF447176.1 G1:17432420. Polypeptide: Genbank accession no. AAL39062; Genbank version no. AAL39062.1 G1:17432421.

Cross-References: Park S. K., et al *J. Biochem.* 119 (2), 235-239 (1996).

Other Information: Official Symbol: PTK7; Other Aliases: CCK-4, CCK4.

Other Designations: colon carcinoma kinase 4; inactive tyrosine-protein kinase 7; pseudo tyrosine kinase receptor 7; tyrosine-protein kinase-like 7.

(72) Cd37 (Cd37 Molecule):

Nucleotide: Genbank accession no. NM_001040031; Genbank version no. NM_001040031.1 G1:91807109. Polypeptide: Genbank accession no. NP_001035120; Genbank version no. NP_001035120.1 G1:91807110.

Cross-References: Schwartz-Albiez R., et al *J. Immunol.* 140 (3), 905-914 (1988)

Other Information: Official Symbol: CD37; Other Aliases: GP52-40, TSPAN26

Other Designations: CD37 antigen; cell differentiation antigen 37; leukocyte antigen CD37; leukocyte surface antigen CD37; tetraspanin-26; tspan-26.

Antibodies: Boehringer Ingelheim: mAb 37.1 (Heider K H., et al *Blood.*; 118(15):4159-68(2011)).

Trubion: CD37-SMIP (G28-1 scFv-lg) ((Zhao X., et al *Blood.*; 110: 2569-2577(2007)) For example, see US20110171208A1 SEQ ID NO: 253 Immunogen: K7153A (Deckert J., et al Cancer Res; 72(8 Supplement): 4625(2012)).

(73) CD138—SDC1 (Syndecan 1):

Nucleotide: Genbank accession no. AJ551176; Genbank version no. AJ551176.1 G1:29243141. Polypeptide: Genbank accession no. CAD80245; Genbank version no. CAD80245.1 G1:29243142.

Cross-References: O'Connell FP., et al *Am J Clin Pathol.*; 121(2):254-63(2004)

Other Information: Official Symbol: SDC1. Other Aliases: CD138, SDC, SYND1, syndecan.

Other Designations: CD138 antigen; heparan sulfate proteoglycan fibroblast growth factor receptor; syndecan proteoglycan 1; syndecan-1.

Antibodies: Biotest: chimerized MAb (nBT062)—(Jagannath S., et al Poster ASH #3060, 2010; WIPO Patent Application WO/2010/128087)For example, see US20090232810 SEQ ID NO: 1 and 2; Immunogen: B-B4 (Tassone P., et al Blood 104_3688-3696) For example, see US20090175863A1 SEQ ID NO: 1 and 2.

(74) CD74 (CD74 Molecule, Major Histocompatibility Complex, Class II Invariant Chain):

Nucleotide: Genbank accession no. NM_004355; Genbank version no. NM_004355.1 G1:343403784. Polypeptide: Genbank accession no. NP_004346; Genbank version no. NP_004346.1 G1:10835071.

Cross-References: Kudo, J., et al *Nucleic Acids Res.* 13 (24), 8827-8841 (1985).

Other Information: Official Symbol: CD74; Other Aliases: DHLAG, HLADG, II, la-GAMMA.

Other Designations: CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); HLA class II histocompatibility antigen gamma chain; HLA-DR antigens-associated invariant chain; HLA-DR-gamma; la-associated invariant chain; MHC HLA-DR gamma chain; gamma chain of class II antigens; p33.

Antibodies: Immunomedics: hLL1 (Milatuzumab,)—Berkova Z., et al *Expert Opin Investig Drugs.*; 19(1):141-9(2010) For example, see US20040115193 SEQ ID NOs: 19, 20, 21, 22, 23 and 24. Genmab: HuMax-CD74 (see website).

(75) Claudins—CLs (Claudins):

Cross-References: Offner S., et al *Cancer Immunol Immunother.*; 54(5):431-45(2005), Suzuki H., et al *Ann N Y Acad Sci.*; 1258:65-70 (2012). In humans, 24 members of the family have been described—see literature reference.

(76) EGFR (Epidermal Growth Factor Receptor):

Nucleotide: Genbank accession no. NM_005228; Genbank version no. NM_005228.3 GI:41927737. Polypeptide: Genbank accession no. NP_005219; Genbank version no. NP_005219.2 GI:29725609.

Cross-References: Dhomen N S., et al *Crit Rev Oncog.*; 17(1):31-50 (2012).

Other Information: Official Symbol: EGFR; Other Aliases: ERBB, ERBB1, HER1, PIG61, mENA.

Other Designations: avian erythroblastic leukemia viral (v-erb-b) oncogene homolog; cell growth inhibiting protein 40; cell proliferation-inducing protein 61; proto-oncogene c-ErbB-1; receptor tyrosine-protein kinase erbB-1

Antibodies: BMS: Cetuximab (Erbitux)—Broadbridge VT., et al *Expert Rev Anticancer Ther.*;12(5):555-65(2012).

For example, see U.S. Pat. No. 6,217,866—ATTC deposit No. 9764.

Amgen: Panitumumab (Vectibix)—Argiles G., et al *Future Oncol.*; 8(4):373-89(2012).

For example, see U.S. Pat. No. 6,235,883 SEQ ID NOs: 23-38.

Genmab: Zalutumumab—Rivera F., et al *Expert Opin Biol Ther.*; 9(5):667-74(2009).

YM Biosciences: Nimotuzumab—Ramakrishnan M S., et al MAbs.; 1(1):41-8(2009).

For example, see U.S. Pat. No. 5,891,996 SEQ ID NOs: 27-34.

(77) Her3 (ErbB3)—ERBB3 (v-Erb-b2 Erythroblastic Leukemia Viral Oncogene Homolog 3 (Avian)):

Nucleotide: Genbank accession no. M34309; Genbank version no. M34309.1 G1:183990. Polypeptide: Genbank accession no. AAA35979; Genbank version no. AAA35979.1 G1:306841.

Cross-References: Plowman, G. D., et al., *Proc. Natl. Acad. Sci. U.S.A.* 87 (13), 4905-4909 (1990).

Other Information: Official Symbol: ERBB3; Other Aliases: ErbB-3, HER3, LCCS2, MDA-BF-1, c-erbB-3, c-erbB3, erbB3-S, p180-ErbB3, p45-sErbB3, p85-sErbB3.

Other Designations: proto-oncogene-like protein c-ErbB-3; receptor tyrosine-protein kinase erbB-3; tyrosine kinase-type cell surface receptor HER3.

Antibodies: Merimack Pharma: MM-121 (Schoeberl B., et al Cancer Res.; 70(6):2485-2494(2010)). For example, see US2011028129 SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7 and 8.

(78) RON—MST1R (Macrophage Stimulating 1 Receptor (c-Met-Related Tyrosine Kinase)):

Nucleotide: Genbank accession no. X70040; Genbank version no. X70040.1 G1:36109. Polypeptide: Genbank accession no. CCA49634; Genbank version no. CCA49634.1 G1:36110.

Cross-References: Ronsin C., et al *Oncogene* 8 (5), 1195-1202 (1993).

Other Information: Official Symbol: MST1R; Other Aliases: CD136, CDw136, PTK8, RON.

Other Designations: MSP receptor; MST1R variant RON30; MST1R variant RON62; PTK8 protein tyrosine kinase 8; RON variant E2E3; c-met-related tyrosine kinase; macrophage-stimulating protein receptor; p185-Ron; soluble RON variant 1; soluble RON variant 2; soluble RON variant 3; soluble RON variant 4.

(79) EPHA2 (EPH Receptor A2):

Nucleotide: Genbank accession no. BC037166; Genbank version no. BC037166.2 G1:33879863. Polypeptide: Genbank accession no. AAH37166; Genbank version no. AAH37166.1 G1:22713539.

Cross-References: Strausberg R. L., et al *Proc. Natl. Acad. Sci. U.S.A.* 99 (26), 16899-16903 (2002).

Other Information: Official Symbol: EPHA2; Other Aliases: ARCC2, CTPA, CTPP1, ECK.

Other Designations: ephrin type-A receptor 2; epithelial cell receptor protein tyrosine kinase; soluble EPHA2 variant 1; tyrosine-protein kinase receptor ECK.

Antibodies: Medimmune: 1C1 (Lee J W., et al *Clin Cancer Res.*; 16(9):2562-2570(2010).

For example, see US20090304721A1 FIGS. 7 and 8.

(80) CD20—MS4A1 (Membrane-Spanning 4-Domains, Subfamily a, Member 1):

Nucleotide: Genbank accession no. M27394; Genbank version no. M27394.1 G1:179307. Polypeptide: Genbank accession no. AAA35581; Genbank version no. AAA35581.1 G1:179308.

Cross-References: Tedder T. F., et al *Proc. Natl. Acad. Sci. U.S.A.* 85 (1), 208-212 (1988).

Other Information: Official Symbol: MS4A1; Other Aliases: B1, Bp35, CD20, CVID5, LEU-16, MS4A2, S7.

Other Designations: B-lymphocyte antigen CD20; B-lymphocyte cell-surface antigen B1; CD20 antigen; CD20 receptor; leukocyte surface antigen Leu-16.

Antibodies: Genentech/Roche: Rituximab—Abdulla N E., et al *BioDrugs.*; 26(2):71-82(2012). For example, see U.S. Pat. No. 5,736,137, ATCC deposit No. HB-69119.

GSK/Genmab: Ofatumumab—Nightingale G., et al Ann Pharmacother.; 45(10):1248-55(2011). For example, see US20090169550A1 SEQ ID NOs: 2, 4 and 5.

Immunomedics: Veltuzumab—Goldenberg DM., et al *Leuk Lymphoma.*; 51(5):747-55(2010). For example, see U.S. Pat. No. 7,919,273B2 SEQ ID NOs: 1, 2, 3, 4, 5 and 6.

(81) Tenascin C— TNC (Tenascin C):

Nucleotide: Genbank accession no. NM_002160; Genbank version no. NM_002160.3 G1:340745336. Polypeptide: Genbank accession no. NP_002151; Genbank version no. NP_002151.2 G1:153946395.

Cross-References: Nies D. E., et al J. Biol. Chem. 266 (5), 2818-2823 (1991); Siri A., et al Nucleic Acids Res. 19 (3), 525-531 (1991).

Other Information: Official Symbol: TNC; Other Aliases: 150-225, GMEM, GP, HXB, JI, TN, TN-C.

Other Designations: GP 150-225; cytotactin; glioma-associated-extracellular matrix antigen; hexabrachion (tenascin); myotendinous antigen; neuronectin; tenascin; tenascin-C isoform 14/AD1/16.

Antibodies: Philogen: G11 (von Lukowicz T., et al *J Nuci Med.*; 48(4):582-7 (2007) and F16 (Pedretti M., et al Lung Cancer.; 64(1):28-33(2009). For example, see U.S. Pat. No. 7,968,685 SEQ ID NOs: 29, 35, 45 and 47.

(82) FAP (Fibroblast Activation Protein, Alpha):

Nucleotide: Genbank accession no. U09278; Genbank version no. U09278.1 G1:1888315. Polypeptide: Genbank accession no. AAB49652; Genbank version no. AAB49652.1 G1:1888316.

Cross-References: Scanlan, M. J., et al *Proc. Natl. Acad. Sci. U.S.A.* 91 (12), 5657-5661 (1994).

Other Information: Official Symbol: FAP; Other Aliases: DPPIV, FAPA.

Other Designations: 170 kDa melanoma membrane-bound gelatinase; integral membrane serine protease; seprase.

(83) DKK-1 (Dickkopf 1 Homolog (*Xenopus laevis*):

Nucleotide: Genbank accession no. NM_012242; Genbank version no. NM_012242.2 G1:61676924. Polypeptide: Genbank accession no. NP_036374; Genbank version no. NP_036374.1 G1:7110719.

Cross-References: Fedi P. et al *J. Biol. Chem.* 274 (27), 19465-19472 (1999).

Other Information: Official Symbol: DKK1; Other Aliases: UNQ492/PRO1008, DKK-1, SK.

Other Designations: dickkopf related protein-1; dickkopf-1 like; dickkopf-like protein 1; dickkopf-related protein 1; hDkk-1.

Antibodies: Novartis: BHQ880 (Fulciniti M., et al *Blood.*; 114(2):371-379 (2009)). For example, see US20120052070A1 SEQ ID NOs: 100 and 108.

(84) Cd52 (Cd52 Molecule):

Nucleotide: Genbank accession no. NM_001803; Genbank version no. NM_001803.2 G1:68342029. Polypeptide: Genbank accession no. NP_001794; Genbank version no. NP_001794.2 G1:68342030.

Cross-References: Xia M. Q., et al *Eur. J. Immunol.* 21 (7), 1677-1684 (1991).

Other Information: Official Symbol: CD52; Other Aliases: CDW52.

Other Designations: CAMPATH-1 antigen; CD52 antigen (CAMPATH-1 antigen); CDW52 antigen (CAMPATH-1 antigen); cambridge pathology 1 antigen; epididymal secretory protein E5; he5; human epididymis-specific protein 5.

Antibodies: Alemtuzumab (Campath)—Skoetz N., et al *Cochrane Database Syst Rev.*; 2:CD008078(2012). For example, see Drugbank Acc. No. DB00087 (BIOD00109, BTD00109).

(85) CS1—SLAMF7 (SLAM Family Member 7):

Nucleotide: Genbank accession no. NM_021181; Genbank version no. NM_021181.3 GI:1993571. Polypeptide: Genbank accession no. NP_067004; Genbank version no. NP_067004.3 GI:19923572.

Cross-References: Boles K. S., et al *Immunogenetics* 52 (3-4), 302-307 (2001).

Other Information: Official Symbol: SLAMF7; Other Aliases: UNQ576/PRO1138, 19A, CD319, CRACC, CS1.

Other Designations: 19A24 protein; CD2 subset 1; CD2-like receptor activating cytotoxic cells; CD2-like receptor-activating cytotoxic cells; membrane protein FOAP-12; novel LY9 (lymphocyte antigen 9) like protein; protein 19A.

Antibodies: BMS: elotuzumab/HuLuc63 (Benson DM., et al *J Clin Oncol.*; 30(16):2013-2015(2012)) For example, see US20110206701 SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15 and 16.

(86) Endoglin—ENG (Endoglin):

Nucleotide: Genbank accession no. AF035753; Genbank version no. AF035753.1 GI:3452260. Polypeptide: Genbank accession no. AAC32802; Genbank version no. AAC32802.1 GI:3452261.

Cross-References: Rius C., et al *Blood* 92 (12), 4677-4690 (1998).

Official Symbol: ENG.

Other Information: Other Aliases: RP11-228B15.2, CD105, END, HHT1, ORW, ORW1.

Other Designations: CD105 antigen.

(87) PMEL17 (silver homolog; SILV; D12S53E; PMEL17; SI; SIL); ME20; gp100) BC001414; BT007202; M32295; M77348; NM_006928;

Cross-References: McGlinchey, R. P. et al. (2009) Proc. Natl. Acad. Sci. U.S.A. 106 (33), 13731-13736; Kummer, M. P. et al. (2009) J. Biol. Chem. 284 (4), 2296-2306.

(88) TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains 1; Tomoregulin-1); H7365; C9orf2; C90RF2; U19878; X83961; NM_080655; NM_003692;

Cross-References: Harms, P. W. (2003) Genes Dev. 17 (21), 2624-2629; Gery, S. et al. (2003) Oncogene 22 (18):2723-2727.

(89) GDNF-Ral (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETLi; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1); U95847; BC014962; NM 145793 NM_005264;

Cross-References: Kim, M. H. et al. (2009) Mol. Cell. Biol. 29 (8), 2264-2277; Treanor, J. J. et al. (1996) Nature 382 (6586):80-83.

(90) Ly6E (lymphocyte antigen 6 complex, locus E; Ly67, RIG-E, SCA-2, TSA-1); NP_002337.1; NM_002346.2;

Cross-References: de Nooij-van Dalen, A G. et al. (2003) Int. J. Cancer 103 (6), 768-774; Zammit, D. J. et al. (2002) Mol. Cell. Biol. 22 (3):946-952.

(91) TMEM46 (shisa homolog 2 (*Xenopus laevis*); SHISA2); NP_001007539.1; NM_001007538.1;

Cross-References: Furushima, K. et al. (2007) Dev. Biol. 306 (2), 480-492; Clark, H. F. et al. (2003) Genome Res. 13 (10):2265-2270.

(92) Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1); NP_067079.2; NM_021246.2;

Cross-References: Mallya, M. et al. (2002) Genomics 80 (1): 113-123; Ribas, G. et al. (1999) J. Immunol. 163 (1):278-287.

(93) LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67); NP_003658.1; NM_003667.2;

Cross-References: Salanti, G. et al. (2009) Am. J. Epidemiol. 170 (5):537-545; Yamamoto, Y. et al. (2003) Hepatology 37 (3):528-533.

(94) RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; PTC; CDHF12; Hs.168114; RET51; RET-ELE1); NP_066124.1; NM_020975.4;

Cross-References: Tsukamoto, H. et al. (2009) Cancer Sci. 100 (10): 1895-1901; Narita, N. et al. (2009) Oncogene 28 (34):3058-3068.

(95) LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226); NP_059997.3; NM_017527.3;

Cross-References: Ishikawa, N. et al. (2007) Cancer Res. 67 (24): 11601-11611; de Nooij-van Dalen, A G. et al. (2003) Int. J. Cancer 103 (6):768-774.

(96) GPR19 (G protein-coupled receptor 19; Mm.4787); NP_006134.1; NM_006143.2; Cross-References: Montpetit, A. and Sinnett, D. (1999) Hum. Genet. 105 (1-2): 162-164; O'Dowd, B. F. et al. (1996) FEBS Lett. 394 (3):325-329.

(97) GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12); NP_115940.2; NM_032551.4;

Cross-References: Navenot, J. M. et al. (2009) Mol. Pharmacol. 75 (6): 1300-1306; Hata, K. et al. (2009) Anticancer Res. 29 (2):617-623.

(98) ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982); NP_859069.2; NM_181718.3;

Cross-References: Gerhard, D. S. et al. (2004) Genome Res. 14 (10B):2121-2127.

(99) Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3); NP_000363.1; NM_000372.4;

Cross-References: Bishop, D. T. et al. (2009) Nat. Genet. 41 (8):920-925; Nan, H. et al. (2009) Int. J. Cancer 125 (4):909-917.

(100) TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627); NP_001103373.1; NM_001109903.1;

Cross-References: Clark, H. F. et al. (2003) Genome Res. 13 (10):2265-2270; Scherer, S. E. et al. (2006) Nature 440 (7082):346-351.

(101) GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ertd747e); NP_078807.1; NM_024531.3;

Cross-References: Ericsson, T. A. et al. (2003) Proc. Natl. Acad. Sci. U.S.A. 100 (1 1):6759-6764; Takeda, S. et al. (2002) FEBS Lett 520 (1-3):97-101.

(102) CLL-1 (CLEC12A, MICL, and DCAL2), encodes a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily. Members of this family share a common protein fold and have diverse functions, such as cell adhesion, cell-cell signaling, glycoprotein turnover, and roles in inflammation and immune response. The protein encoded by this gene is a negative regulator of granulocyte and monocyte function. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of some of these variants has not been determined. This gene is closely linked to other CTL/CTLD superfamily members in the natural killer gene complex region on chromosome 12p13 (Drickamer K (1999) Curr. Opin. Struct. Biol. 9 (5):585-90; van Rhenen A, et al, (2007) Blood 110 (7):2659-66; Chen C H, et al. (2006) Blood 107 (4): 1459-67; Marshall A S, et al. (2006) Eur. J. Immunol. 36 (8):2159-69; Bakker A B, et al. (2005) Cancer Res. 64 (22): 8443-50; Marshall A S, et al. (2004) J. Biol. Chem. 279 (15): 14792-802). CLL-1 has been shown to be a type II transmembrane receptor comprising a single C-type lectin-like domain (which

is not predicted to bind either calcium or sugar), a stalk region, a transmembrane domain and a short cytoplasmic tail containing an ITIM motif.

(103) Annexin A1—ANXA1 (Annexin A1):

Nucleotide: Genbank accession no. X05908; Genbank version no. X05908.1 GI:34387.

Polypeptide: Genbank accession no. CCA29338; Genbank version no. CCA29338.1 GI:34388.

Cross-References: Wallner B. P., et al *Nature* 320 (6057), 77-81 (1986).

Other Information: Official Symbol: ANXA1; Other Aliases: RP11-71A24.1, ANX1, LPC1

Other Designations: annexin I (lipocortin I); annexin-1; calpactin II; calpactin-2; chromobindin-9; lipocortin I; p35; phospholipase A2 inhibitory protein.

(104) V-CAM (CD106)—VCAM1 (Vascular Cell Adhesion Molecule 1):

Nucleotide: Genbank accession no. M60335; Genbank version no. M60335.1 GI:340193.

Polypeptide: Genbank accession no. AAA61269; Genbank version no. AAA61269.1 GI:340194.

Cross-References: Hession C., et al *J. Biol. Chem.* 266 (11), 6682-6685 (1991).

Other Information: Official Symbol VCAM1; Other Aliases: CD106, INCAM-100.

Other Designations: CD106 antigen; vascular cell adhesion protein 1.

(105) B7H3 (CD276 or B7RP-2, a member of the B7 ligand family, has two isoforms: 2Ig- and 4Ig-B7H3 with molecular weights of approximately 45-kDa and 100-kDa, respectively.)

Nucleotide：Genbank accession no.NM_001329628.2; Genbank version no. NM_001024736.2, Genbank version no. NM_025240.3; Genbank version no. NM_001329629.2

Polypeptide：Genbank accession no.NP_001019907.1; Genbank version no.NP_001316557.1 Genbank version no.NP_001316558.1; Genbank version no.NP_079516.1

Cross-References: Chen, Y-W; et al (2008) *Current Cancer Drug Targets, 8(5), 404–13;* Zhou, W.-T. and Jin, W. L., (2021) Front Immunol. 12: 701006.

(106) CD54（also Known as：BB2; CD54; P3.58, This gene encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. It binds to integrins of type CD11a / CD18, or CD11b / CD18 and is also exploited by Rhinovirus as a receptor. [provided by RefSeq, Jul 2008]）

Nucleotide：Genbank accession no.NM_000201.3; Polypeptide：Genbank accession no.NP_000192.2

(107) CA19-9 (Carbohydrate antigen 19-9 (CA 19-9) is a cell surface glycoprotein complex most commonly associated with pancreatic ductal adenocarcinoma (PDAC), It was first described in 1979 using a mouse monoclonal antibody (1116-NS-19-9) in a colorectal carcinoma cell line (SW1116))

(108) Tissue Factor (coagulation factor III, tissue factor, TF; TFA; CD142)

Nucleotide: Genbank accession no.NM_001178096.2 , Genbank version no. NM_001993.5; Polypeptide： Genbank accession no.NP_001171567.1 , Genbank version no.NP_001984.1 .

(109) ROR1 (receptor tyrosine kinase like orphan receptor 1, NTRKR1; dJ537F10.1);

Nucleotide： Genbank accession no.NM_001083592.2 , Genbank version no.NM_005012.4; Polypeptide: Genbank accession no. NP_001077061.1, Genbank version no. NP_005003.2.

(110) Claudin18 (SFTA5; SFTPJ)

Nucleotide： Genbank accession no.NM_001002026.3; Polypeptide： Genbank accession no. NP_001002026.1.

(111) FGFR2(fibroblast growth factor receptor 2, BEK; JWS; BBDS; CEK3; CFD1; ECT1; KGFR; TK14; TK25; BFR-1; CD332; K-SAM)

Nucleotide: Genbank accession no.NM_000141.5 , Genbank version no. NM_001144913.1, Genbank version no. NM_001144914.1, Genbank version no. NM_001144915.2, Genbank version no. NM_001144916.2, Genbank version no. NM_001144917.2, Genbank version no. NM_001144918.2, Genbank version no. NM_001144919.2, Genbank version no. NM_001320654.2, Genbank version no. NM_001320658.2, Genbank version no. NM_022970.3, Genbank version no. NM_023029.2; Polypeptide： Genbank accession no.NP_000132.3, Genbank version no. NP_001138385.1, Genbank version no. NP_001138386.1, Genbank version no. NP_001138387.1, Genbank version no. NP_001138388.1, Genbank version no. NP_001138389.1, Genbank version no. NP_001138390.1, Genbank version no. NP_001138391.1, Genbank version no. NP_001307583.1, Genbank version no. NP_001307587.1, Genbank version no. NP_075259.4, Genbank version no. NP_075418.1.

(112) FGFR3 (fibroblast growth factor receptor 3, ACH; CEK2; JTK4; CD333; HSFGFR3EX)

Nucleotide: Genbank accession no. XM_006713868.2, Genbank version no. XM_006713869.2, Genbank version no.XM_006713870.2, Genbank version no.XM_047449821.1, Genbank version no.XM_006713871.2, Genbank version no.XM_011513420.2, Genbank version no. XM_011513422.2, Genbank version no.XM_006713873.2, Genbank version no. XM_047449820.1, Genbank version no.XM_047449822.1, Genbank version no. XM_047449823.1, Genbank version no.XM_047449824.1; Polypeptide： Genbank accession no. XP_006713931.1, Genbank version no.XP_006713932.1, Genbank version no.XP_006713933.1 , Genbank version no. XP_047305777.1 , Genbank version no.XP_006713934.1, Genbank version no.XP_011511722.1, Genbank version no.XP_011511724.1, Genbank version no.XP_006713936.1, Genbank version no.XP_047305776.1, Genbank version no.XP_047305778.1, Genbank version no.XP_047305779.1, Genbank version no.XP_047305780.1.

(113) FGFR4 (fibroblast growth factor receptor 4, TKF; JTK2; CD334)

Nucleotide: Genbank accession no. NM_001291980.2, Genbank version no. NM_001354984.2, Genbank version no. NM_002011.5, Genbank version no. NM_022963.3, Genbank version no. NM_213647.3; Polypeptide: Genbank accession no. NP_001278909.1, Genbank version no. NP_001341913.1, Genbank version no.NP_002002.3, Genbank version no. NP_075252.2, Genbank version no.NP_998812.1.

(114) FGFR1 (fibroblast growth factor receptor 1, CEK; FLG; HH2; OGD; ECCL; FLT2; KAL2; BFGFR; CD331; FGFBR; FLT-2; HBGFR; N-SAM; FGFR-1; HRTFDS; bFGF-R-1)

Nucleotide: Genbank accession no.XM_006716310.4, Genbank version no. XM_047421569.1, Genbank version no. XM_006716303. 4, Genbank version no. XM_047421574.1, Genbank version no. XM_006716314.3, Genbank version no. XM_047421573.1, Genbank version no. XM_006716311.1, Genbank version no. XM_047421571.1, Genbank version no. XM_006716307.2, Genbank version no. XM_047421570.1, Genbank version no. XM_006716304.2, Genbank version no. XM_017013221.2, Genbank version no. XM_047421572.1, Genbank version no. XM_017013227.2, Genbank version no. XM_011544449.2, Genbank version no. XM_011544450.3, Genbank version no. XM_017013226.2, Genbank version no. XM_011544448.2, Genbank version no. XM_017013219.2, Genbank version no. XM_017013220.2, Genbank version no. XM_011544444.2, Genbank version no. XM_011544445.3, XM_011544447.3, Genbank version no. XM_011544446.3, Genbank version no. XM_017013225.3, Genbank version no. XM_006716312.2, Genbank version no. XM_011544451.1, Genbank version no. XM_024447097.1, Genbank version no. XM_047421576.1, Genbank version no. XM_047421575.1, Genbank version no. XM_011544452.3, Genbank version no. XM_017013231.2; Polypeptide: Genbank accession no.XP_006716373.1, Genbank version no. XP_047277525.1, Genbank version no. XP_006716366.1, Genbank version no. XP_047277530.1, Genbank version no. XP_006716377.1, Genbank version no. XP_047277529.1, Genbank version no. XP_006716374.1, Genbank version no. XP_047277527.1, Genbank version no. XP_006716370.1, Genbank version no. XP_047277526.1, Genbank version no. XP_006716367.1, Genbank version no. XP_016868710.1 , Genbank version no. XP_047277528.1, Genbank version no. XP_016868716.1, Genbank version no. XP_011542751.1, Genbank version no. XP_011542752.1, Genbank version no. XP_016868715.1, Genbank version no. XP_011542750.1 , Genbank version no. XP_016868708.1, Genbank version no. XP_016868709.1, Genbank version no. XP_011542746.1, Genbank version no. XP_011542747.1, XP_011542749.1, Genbank version no. XP_011542748.1, Genbank version no. XP_016868714.1, Genbank version no. XP_006716375.1, Genbank version no.

XP_011542753.1, Genbank version no. XP_024302865.1, Genbank version no. XP_047277532.1, Genbank version no. XP_047277531.1, Genbank version no. XP_011542754.1, Genbank version no. XP_016868720.1

(115) ROR2 (receptor tyrosine kinase like orphan receptor 2, BDB; BDB1; NTRKR2)

Nucleotide: Genbank accession no. XM_005252008.5, Genbank version no. XM_047423436.1, Genbank version no. XM_047423435.1, Genbank version no. XM_047423437.1, Genbank version no. XM_047423434.1, Genbank version no. XM_017014762.2, Genbank version no. XM_006717121.4, Genbank version no. XM_005252009.4; Polypeptide：Genbank accession no. XP_005252065.1, Genbank version no. XP_047279392.1, Genbank version no. XP_047279391.1, Genbank version no.XP_047279393.1, Genbank version no. XP_047279390.1, Genbank version no. XP_016870251.1, Genbank version no. XP_006717184.1 ，Genbank version no.XP_005252066.1.

(116) SLC44A4 (SLC44A4 – solute carrier family 44 member 4， CTL4; NG22; TPPT; DFNA72; hTPPT1; C6orf29)

Nucleotide: Genbank accession no. NM_001178044.2, Genbank version no. NM_001178045.2, Genbank version no.NM_025257.3; Polypeptide: Genbank accession no. NP_001171515.1, Genbank version no.NP_001171516.1, Genbank version no.NP_079533.2

(117) DLL3 (delta like canonical Notch ligand 3, SCDO1)

Nucleotide: Genbank accession no. NM_016941.4 , Genbank version no. NM_203486.3;

Polypeptide：Genbank accession no. NP_982353.1, Genbank version no.NP_058637.1.

(118) DLL4 (delta like canonical Notch ligand 4, AOS6; delta4; hdelta2)

Nucleotide: Genbank accession no. NM_019074.4; Polypeptide：Genbank accession no. NP_061947.1.

(119) ALK (ALK receptor tyrosine kinase, ALK1; CD246; NBLST3)

Nucleotide: Genbank accession no. NM_001353765.2, Genbank version no. NM_004304.5

Polypeptide: Genbank accession no. NP_001340694.1, Genbank version no.NP_004295.2

(120) CLDN6 (claudin 6)

Nucleotide: Genbank accession no. NM_021195.5; Polypeptide: Genbank accession no. NP_067018.2

(121) CLDN3 (claudin 3, RVP1; HRVP1; C7orf1; CPE-R2; CPETR2)

Nucleotide: Genbank accession no. NM_001306.4;

Polypeptide: Genbank accession no.NP_001297.1

(122) EFNA4 (ephrin A4, EFL4; EPLG4; LERK4; LERK-4)

Nucleotide: Genbank accession no. NM_001406810.1 , Genbank version no. NM_005227.3, Genbank version no.NM_182689.2, Genbank version no.NM_182690.3;

Polypeptide： Genbank accession no. NP_001393739.1, Genbank version no.NP_005218.1, Genbank version no.NP_872631.1, Genbank version no.NP_872632.2.

(123) Notch1(notch receptor 1, hN1; AOS5; TAN1; AOVD1)

Nucleotide: Genbank accession no. NM_017617.5, Genbank version no. XM_011518717.3;

Polypeptide: Genbank accession no. NP_060087.3, Genbank version no. XP_011517019.2.

(124) Notch2 (notch receptor 2, hN2; AGS2; HJCYS)

Nucleotide: Genbank accession no.NM_001200001.2 , Genbank version no.NM_024408.4;

Polypeptide： Genbank accession no. NP_001186930.1 , Genbank version no. NP_077719.2.

(125) Notch3 (notch receptor 3, IMF2; LMNS; CASIL; CADASIL; CADASIL1)

Nucleotide: Genbank accession no.NM_000435.3 , Genbank version no.XM_005259924.5;

Polypeptide: Genbank accession no. NP_000426.2, Genbank version no.XP_005259981.1.

(126) LAMP-1 (lysosomal associated membrane protein 1, LAMPA; CD107a; LGP120)

Nucleotide: Genbank accession no. NM_005561.4 , Genbank version no. XM_047430302.1, Genbank version no. XM_011537494.3; Polypeptide: Genbank accession no. NP_005552.3, Genbank version no. XP_047286258.1, Genbank version no. XP_011535796.1.

In another specific embodiment, the antibody-drug conjugates of this invention are used for the targeted treatment of cancers. The targeted cancers include, but are not limited, Adrenocortical Carcinoma, Anal Cancer, Bladder Cancer, Brain Tumor (Adult, Brain Stem Glioma, Childhood, Cerebellar Astrocytoma, Cerebral Astrocytoma, Ependymoma, Medulloblastoma, Supratentorial Primitive Neuroectodermal and Pineal Tumors, Visual Pathway and Hypothalamic Glioma), Breast Cancer, Carcinoid Tumor, Gastrointestinal, Carcinoma of Unknown Primary, Cervical Cancer, Colon Cancer, Endometrial Cancer, Esophageal Cancer, Extrahepatic Bile Duct Cancer, Ewings Family of Tumors (PNET), Extracranial Germ Cell Tumor, Eye Cancer, Intraocular Melanoma, Gallbladder Cancer, Gastric Cancer (Stomach), Germ Cell Tumor, Extragonadal, Gestational Trophoblastic Tumor, Head and Neck Cancer, Hypopharyngeal Cancer, Islet Cell Carcinoma, Kidney Cancer (renal cell cancer), Laryngeal Cancer, Leukemia (Acute Lymphoblastic, Acute Myeloid, Chronic Lymphocytic, Chronic Myelogenous, Hairy Cell), Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer (Non-Small Cell, Small Cell, Lymphoma (AIDS-Related, Central Nervous System, Cutaneous T-Cell, Hodgkin's Disease, Non-Hodgkin's Disease, Malignant Mesothelioma, Melanoma, Merkel Cell Carcinoma, Metasatic Squamous Neck Cancer with Occult Primary, Multiple Myeloma, and Other Plasma Cell Neoplasms, Mycosis Fungoides, Myelodysplastic Syndrome, Myeloproli-ferative Disorders, Nasopharyngeal Cancer,

Neuroblastoma, Oral Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer (Epithelial, Germ Cell Tumor, Low Malignant Potential Tumor), Pancreatic Cancer (Exocrine, Islet Cell Carcinoma), Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pheochromocytoma Cancer, Pituitary Cancer, Plasma Cell Neoplasm, Prostate Cancer Rhabdomyosarcoma, Rectal Cancer, Renal Cell Cancer (kidney cancer), Renal Pelvis and Ureter (Transitional Cell), Salivary Gland Cancer, Sezary Syndrome, Skin Cancer, Skin Cancer (Cutaneous T-Cell Lymphoma, Kaposi's Sarcoma, Melanoma), Small Intestine Cancer, Soft Tissue Sarcoma, Stomach Cancer, Testicular Cancer, Thymoma (Malignant), Thyroid Cancer, Urethral Cancer, Uterine Cancer (Sarcoma), Unusual Cancer of Childhood, Vaginal Cancer, Vulvar Cancer, Wilms' Tumor.

In another specific embodiment, the the antibody-drug conjugates of this invention are used in accordance with the compositions and methods for the treatment or prevention of an autoimmune disease. The autoimmune diseases include, but are not limited, Achlorhydra Autoimmune Active Chronic Hepatitis, Acute Disseminated Encephalomyelitis, Acute hemorrhagic leukoencephalitis, Addison's Disease, Agammaglobulinemia, Alopecia areata, Amyotrophic Lateral Sclerosis, Ankylosing Spondylitis, Anti-GBM/TBM Nephritis, Antiphospholipid syndrome, Antisynthetase syndrome, Arthritis, Atopic allergy, Atopic Dermatitis, Autoimmune Aplastic Anemia, Autoimmune cardiomyopathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune pancreatitis, Autoimmune polyendocrine syndrome Types I, II, & III, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Bechets Syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous Pemphigoid, Castleman's disease, Chagas disease, Chronic Fatigue Immune Dysfunction Syndrome, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal ostomyelitis, Chronic lyme disease, Chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial Pemphigoid, Coeliac Disease, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Cranial arteritis, CREST syndrome, Crohns Disease (a type of idiopathic inflammatory bowel diseases), Cushing's Syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Discoid lupus erythematosus, Eczema, Endometriosis, Enthesitis-related arthritis, Eosinophilic fasciitis, Epidermolysis bullosa acquisita, Erythema nodosum, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibromyositis, Fibrosing aveolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis,

Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Haemolytic anaemia, Henoch-Schonlein purpura, Herpes gestationis, Hidradenitis suppurativa, Hughes syndrome (See Antiphospholipid syndrome), Hypogamma-globulinemia, Idiopathic Inflammatory Demyelinating Diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura (See Autoimmune thrombocytopenic purpura), IgA nephropathy (Also Berger's disease), Inclusion body myositis, Inflammatory demyelinating polyneuopathy, Interstitial cystitis, Irritable Bowel Syndrome , Juvenile idiopathic arthritis, Juvenile rheumatoid arthritis, Kawasaki's Disease, Lambert-Eaton myasthenic syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease (LAD), Lou Gehrig's Disease (Also Amyotrophic lateral sclerosis), Lupoid hepatitis, Lupus erythematosus, Majeed syndrome, Ménière's disease, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed Connective Tissue Disease, Morphea, Mucha-Habermann disease, Muckle–Wells syndrome, Multiple Myeloma, Multiple Sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's Disease), Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord thyroiditis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis, Pemphigus, Pemphigus vulgaris, Pernicious anaemia, Perivenous encephalomyelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progressive inflammatory neuropathy, Psoriasis, Psoriatic Arthritis, Pyoderma gangrenosum, Pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syndrome, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatoid fever, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Spondyloarthropathy, Sticky blood syndrome, Still's Disease, Stiff person syndrome, Subacute bacterial endocarditis, Susac's syndrome, Sweet syndrome, Sydenham Chorea, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis (giant cell arteritis), Tolosa-Hunt syndrome, Transverse Myelitis, Ulcerative Colitis (a type of idiopathic inflammatory bowel diseases), Undifferentiated connective tissue disease, Undifferentiated spondyloarthropathy, Vasculitis, Vitiligo, Wegener's granulomatosis, Wilson's syndrome, Wiskott-Aldrich syndrome

In another specific embodiment, the antibody-drug conjugates of this invention for the treatment or prevention of an autoimmune disease can be, but are not limited to, anti-elastin antibody; Abys against epithelial cells antibody; Anti-Basement Membrane Collagen Type IV Protein antibody; Anti-Nuclear Antibody; Anti ds DNA; Anti ss DNA, Anti Cardiolipin Antibody IgM, IgG; anti-celiac antibody; Anti Phospholipid Antibody IgK, IgG; Anti SM Antibody; Anti Mitochondrial

Antibody; Thyroid Antibody; Microsomal Antibody, T-cells antibody; Thyroglobulin Antibody, Anti SCL-70; Anti-Jo; Anti-U.sub.1RNP; Anti-La/SSB; Anti SSA; Anti SSB; Anti Perital Cells Antibody; Anti Histones; Anti RNP; C-ANCA; P-ANCA; Anti centromere; Anti-Fibrillarin, and Anti GBM Antibody, Anti-ganglioside antibody; Anti-Desmogein 3 antibody; Anti-p62 antibody; Anti-sp100 antibody; Anti-Mitochondrial(M2) antibody; Rheumatoid factor antibody; Anti-MCV antibody; Anti-topoisomerase antibody; Anti-neutrophil cytoplasmic(cANCA) antibody.

In certain preferred embodiments, the binding molecule for the conjugate in the present invention, can bind to both a receptor and a receptor complex expressed on an activated lymphocyte which is associated with an autoimmune disease. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member (e.g. CD2, CD3, CD4, CD8, CD19, CD20, CD22, CD28, CD30, CD33, CD37, CD38, CD56, CD70, CD79, CD79b, CD90, CD125, CD137, CD138, CD147, CD152/CTLA-4, PD-1, or ICOS), a TNF receptor superfamily member (e.g. CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, INF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3), an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin (C-type, S-type, or I-type), or a complement control protein.

In another specific embodiment, useful cell binding ligands that are immunospecific for a viral or a microbial antigen are humanized or human monoclonal antibodies. As used herein, the term "viral antigen" includes, but is not limited to, any viral peptide, polypeptide protein (e.g. HIV gp120, HIV nef, RSV F glycoprotein, influenza virus neuramimi-dase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g. gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., bacteria, fungi, pathogenic protozoa, or yeast polypeptides including, e.g., LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response. Examples of antibodies available l for the viral or microbial infection include, but are not limited to, Palivizumab which is a humanized anti-respiratory syncytial virus monoclonal antibody for the treatment of RSV infection; PRO542 which is a CD4 fusion antibody for the treatment of HIV infection; Ostavir which is a human antibody for the treatment of hepatitis B virus; PROTVIR which is a humanized IgG.sub.1 antibody for the treatment of cytomegalovirus; and anti-LPS antibodies.

The antibody-drug conjugates of this invention can be used in the treatment of infectious diseases. These infectious diseases include, but are not limited to, Acinetobacter infections, Actinomycosis, African sleeping sickness (African trypanosomiasis), AIDS (Acquired immune deficiency syndrome), Amebiasis, Anaplasmosis, Anthrax, Arcano-bacterium haemolyticum

infection, Argentine hemorrhagic fever, Ascariasis, Aspergillosis, Astrovirus infection, Babesiosis, Bacillus cereus infection, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystis hominis infection, Blastomycosis, Bolivian hemorrhagic fever, Borrelia infection, Botulism (and Infant botulism), Brazilian hemorrhagic fever, Brucellosis, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Campylobacteriosis, Candidiasis (Moniliasis; Thrush), Cat-scratch disease, Cellulitis, Chagas Disease (American trypanosomiasis), Chancroid, Chickenpox, Chlamydia, Chlamydophila pneumoniae infection, Cholera, Chromoblastomycosis, Clonorchiasis, Clostridium difficile infection, Coccidioido-mycosis, Colorado tick fever, Common cold (Acute viral rhinopharyngitis; Acute coryza), Creutzfeldt-Jakob disease, Crimean-Congo hemorrhagic fever, Cryptococcosis, Cryptosporidiosis, Cutaneous larva migrans, Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue fever, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Enterococcus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum, Fasciolopsiasis, Fasciolosis, Fatal familial insomnia, Filariasis, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann-Sträussler-Scheinker syndrome, Giardiasis, Glanders, Gnathosto-miasis, Gonorrhea, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group B streptococcal infection, Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome, Helicobacter pylori infection, Hemolytic-uremic syndrome, Hemorrhagic fever with renal syndrome, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Herpes simplex, Histoplasmosis, Hookworm infection, Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis, Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein-Barr Virus Infectious Mononucleosis (Mono), Influenza, Isosporiasis, Kawasaki disease, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Legionellosis (Legionnaires' disease), Legionellosis (Pontiac fever), Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever, Measles, Melioidosis (Whitmore's disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, Molluscum contagiosum, Mumps, Murine typhus (Endemic typhus), Mycoplasma pneumonia, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis,

Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (Pubic lice, Crab lice), Pelvic inflammatory disease, Pertussis (Whooping cough), Plague, Pneumococcal infection, Pneumocystis pneumonia, Pneumonia, Poliomyelitis, Prevotella infection, Primary amoebic meningoencephalitis, Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Rat-bite fever, Respiratory syncytial virus infection, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsial-pox, Rift Valley fever, Rocky mountain spotted fever, Rotavirus infection, Rubella, Salmonellosis, SARS (Severe Acute Respiratory Syndrome), Scabies, Schistosomiasis, Sepsis, Shigellosis (Bacillary dysentery), Shingles (Herpes zoster), Smallpox (Variola), Sporotrichosis, Staphylococcal food poisoning, Staphylococcal infection, Strongyloidiasis, Syphilis, Taeniasis, Tetanus (Lockjaw), Tinea barbae (Barber's itch), Tinea capitis (Ringworm of the Scalp), Tinea corporis (Ringworm of the Body), Tinea cruris (Jock itch), Tinea manuum (Ringworm of the Hand), Tinea nigra, Tinea pedis (Athlete's foot), Tinea unguium (Onychomycosis), Tinea versicolor (Pityriasis versicolor), Toxocariasis (Ocular Larva Migrans), Toxocariasis (Visceral Larva Migrans), Toxoplasmosis, Trichinellosis, Trichomoniasis, Trichuriasis (Whipworm infection), Tuberculosis, Tularemia, Ureaplasma urealyticum infection, Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, Viral pneumonia, West Nile Fever, White piedra (Tinea blanca), Yersinia pseudotuber-culosis infection, Yersiniosis, Yellow fever, Zygomycosis.

The cell binding molecule, which is more preferred to be an antibody described in this patent that are against pathogenic strains include, but are not limit, Acinetobacter baumannii, Actinomyces israelii, Actinomyces gerencseriae and Propionibacterium propionicus, Trypanosoma brucei, HIV (Human immunodeficiency virus), Entamoeba histolytica, Anaplasma genus, Bacillus anthracis, Arcanobacterium haemolyticum, Junin virus, Ascaris lumbricoides, Aspergillus genus, Astroviridae family, Babesia genus, Bacillus cereus, multiple bacteria, Bacteroides genus, Balantidium coli, Baylisascaris genus, BK virus, Piedraia hortae, Blastocystis hominis, Blastomyces dermatitides, Machupo virus, Borrelia genus, Clostridium botulinum, Sabia, Brucella genus, usually Burkholderia cepacia and other Burkholderia species, Mycobacterium ulcerans, Caliciviridae family, Campylobacter genus, usually Candida albicans and other Candida species, Bartonella henselae, Group A Streptococcus and Staphylococcus, Trypanosoma cruzi, Haemophilus ducreyi, Varicella zoster virus (VZV), Chlamydia trachomatis, Chlamydophila pneumoniae, Vibrio cholerae, Fonsecaea pedrosoi, Clonorchis sinensis, Clostridium difficile, Coccidioides immitis and Coccidioides posadasii, Colorado tick fever virus, rhinoviruses, coronaviruses, CJD prion, Crimean-Congo hemorrhagic fever virus, Cryptococcus neoformans, Cryptosporidium genus, Ancylostoma braziliense; multiple parasites, Cyclospora cayetanensis, Taenia solium, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4) – Flaviviruses, Dientamoeba fragilis, Corynebacterium

diphtheriae, Diphyllobothrium, Dracunculus medinensis, Ebolavirus, Echinococcus genus, Ehrlichia genus, Enterobius vermicularis, Enterococcus genus, Enterovirus genus, Rickettsia prowazekii, Parvovirus B19, Human herpesvirus 6 and Human herpesvirus 7, Fasciolopsis buski, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily, Clostridium perfringens, Fusobacterium genus, Clostridium perfringens; other Clostridium species, Geotrichum candidum, GSS prion, Giardia intestinalis, Burkholderia mallei, Gnathostoma spinigerum and Gnathostoma hispidum, Neisseria gonorrhoeae, Klebsiella granulomatis, Streptococcus pyogenes, Streptococcus agalactiae, Haemophilus influenzae, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71, Sin Nombre virus, Helicobacter pylori, Escherichia coli O157:H7, Bunyaviridae family, Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1, Herpes simplex virus 2, Histoplasma capsulatum, Ancylostoma duodenale and Necator americanus, Hemophilus influenzae, Human bocavirus, Ehrlichia ewingii, Anaplasma phagocytophilum, Human metapneumovirus, Ehrlichia chaffeensis, Human papillomavirus, Human parainfluenza viruses, Hymenolepis nana and Hymenolepis diminuta, Epstein-Barr Virus, Orthomyxoviridae family, Isospora belli, Kingella kingae, Klebsiella pneumoniae, Klebsiella ozaenas, Klebsiella rhinoscleromotis, Kuru prion, Lassa virus, Legionella pneumophila, Legionella pneumophila, Leishmania genus, Mycobacterium leprae and Mycobacterium lepromatosis, Leptospira genus, Listeria monocytogenes, Borrelia burgdorferi and other Borrelia species, Wuchereria bancrofti and Brugia malayi, Lymphocytic choriomeningitis virus (LCMV), Plasmodium genus, Marburg virus, Measles virus, Burkholderia pseudomallei, Neisseria meningitides, Metagonimus yokagawai, Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Rickettsia typhi, Mycoplasma pneumoniae, numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma), parasitic dipterous fly larvae, Chlamydia trachomatis and Neisseria gonorrhoeae, vCJD prion, Nocardia asteroides and other Nocardia species, Onchocerca volvulus, Paracoccidioides brasiliensis, Paragonimus westermani and other Paragonimus species, Pasteurella genus, Pediculus humanus capitis, Pediculus humanus corporis, Phthirus pubis, Bordetella pertussis, Yersinia pestis, Streptococcus pneumoniae, Pneumocystis jirovecii, Poliovirus, Prevotella genus, Naegleria fowleri, JC virus, Chlamydophila psittaci, Coxiella burnetii, Rabies virus, Streptobacillus moniliformis and Spirillum minus, Respiratory syncytial virus, Rhinosporidium seeberi, Rhinovirus, Rickettsia genus, Rickettsia akari, Rift Valley fever virus, Rickettsia rickettsii, Rotavirus, Rubella virus, Salmonella genus, SARS coronavirus, Sarcoptes scabiei, Schistosoma genus, Shigella genus, Varicella zoster virus, Variola major or Variola minor, Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Staphylococcus aureus, Streptococcus pyogenes, Strongyloides stercoralis, Treponema pallidum, Taenia genus, Clostridium

tetani, Trichophyton genus, Trichophyton tonsurans, Trichophyton genus, Epidermophyton floccosum, Trichophyton rubrum, and Trichophyton mentagrophytes, Trichophyton rubrum, Hortaea werneckii, Trichophyton genus, Malassezia genus, Toxocara canis or Toxocara cati, Toxoplasma gondii, Trichinella spiralis, Trichomonas vaginalis, Trichuris trichiura, Mycobacterium tuberculosis, Francisella tularensis, Ureaplasma urealyticum, Venezuelan equine encephalitis virus, Vibrio colerae, Guanarito virus, West Nile virus, Trichosporon beigelii, Yersinia pseudotuberculosis, Yersinia enterocolitica, Yellow fever virus, Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthora-mycosis), Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Edwardsiella tarda, Yersinia pestis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospira icterohemorrhagiae, Pneumocystis carinii, Brucella abortus, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsia prowazeki, Rickettsia tsutsugumushi, Clamydia spp.; pathogenic fungi (Aspergillus fumigatus, Candida albicans, Histoplasma capsulatum); protozoa (Entomoeba histolytica, Trichomonas tenas, Trichomonas hominis, Tryoanosoma gambiense, Trypanosoma rhodesiense, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria); or Helminiths (Schistosoma japonicum, Schistosoma mansoni, Schistosoma haematobium, and hookworms).

Other antibodies as cell binding ligands used in this invention for treatment of viral disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxyiridae, Herpesviridae, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, Oncovirus [such as, HBV (Hepatocellular carcinoma), HPV (Cervical cancer, Anal cancer), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), Epstein-Barr virus (Nasopharyngeal carcinoma, Burkitt's lymphoma, Primary central nervous system lymphoma), MCPyV (Merkel cell cancer), SV40 (Simian virus 40), HCV (Hepatocellular carcinoma), HTLV-I (Adult T-cell leukemia/lymphoma)], Immune disorders caused virus: [such as Human Immunodeficiency Virus (AIDS)]; Central nervous system virus: [such as, JCV (Progressive multifocal leukoencephalopathy), MeV (Subacute sclerosing panencephalitis), LCV (Lymphocytic choriomeningitis), Arbovirus encephalitis, Orthomyxoviridae (probable) (Encephalitis lethargica), RV (Rabies), Chandipura virus, Herpesviral meningitis, Ramsay Hunt syndrome type II; Poliovirus (Poliomyelitis, Post-polio syndrome), HTLV-I (Tropical spastic paraparesis)]; Cytomegalovirus

(Cytomegalovirus retinitis, HSV (Herpetic keratitis)); Cardiovascular virus [such as CBV (Pericarditis, Myocarditis)]; Respiratory system/acute viral nasopharyngitis/viral pneumonia: [Epstein-Barr virus (EBV infection/Infectious mononucleosis), Cytomegalovirus; SARS coronavirus (Severe acute respiratory syndrome) Orthomyxoviridae: Influenzavirus A/B/C (Influenza/Avian influenza), Paramyxovirus: Human parainfluenza viruses (Parainfluenza), RSV (Human respiratory syncytialvirus), hMPV]; Digestive system virus [MuV (Mumps), Cytomegalovirus (Cytomegalovirus esophagitis); Adenovirus (Adenovirus infection); Rotavirus, Norovirus, Astrovirus, Coronavirus; HBV (Hepatitis B virus), CBV, HAV (Hepatitis A virus), HCV (Hepatitis C virus), HDV (Hepatitis D virus), HEV (Hepatitis E virus), HGV (Hepatitis G virus)]; Urogenital virus [such as, BK virus, MuV (Mumps)].

According to a further object, the present invention also concerns pharmaceutical compositions comprising the conjugate of the invention together with a pharmaceutically acceptable carrier, diluent, or excipient for treatment of cancers, infections or autoimmune disorders. The method for treatment of cancers, infections and autoimmune disorders can be practiced in vitro, in vivo, or ex vivo. Examples of in vitro uses include treatments of cell cultures in order to kill all cells except for desired variants that do not express the target antigen; or to kill variants that express undesired antigen. Examples of ex vivo uses include treatments of hematopoietic stem cells (HSC) prior to the performance of the transplantation (HSCT) into the same patient in order to kill diseased or malignant cells. For instance, clinical ex vivo treatment to remove tumour cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from allogeneic bone marrow or tissue prior to transplant in order to prevent graft-versus-host disease, can be carried out as follows. Bone marrow is harvested from the patient or other individual and then incubated in medium containing serum to which is added the conjugate of the invention, concentrations range from about 1 pM to 0.1 mM, for about 30 minutes to about 48 hours at about 37 ℃. The exact conditions of concentration and time of incubation (=dose) are readily determined by the skilled clinicians. After incubation, the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or total-body irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

FORMULATION AND APPLICATION

The conjugates of the patent application are formulated to liquid, or suitable to be lyophilized and subsequently be reconstituted to a liquid formulation. The conjugate in a liquid formula or in the

formulated lyophilized powder may take up 0.01%-99% by weight as major gradient in the formulation. In general, a liquid formulation comprising 0.1 g/L ~300 g/L of concentration of the conjugate active ingredient for delivery to a patient without high levels of antibody aggregation may include one or more polyols (e.g. sugars), a buffering agent with pH 4.5 to 7.5, a surfactant (e.g. polysorbate 20 or 80), an antioxidant (e.g. ascorbic acid and/or methionine), a tonicity agent (e.g. mannitol, sorbitol or NaCl), chelating agents such as EDTA; metal complexes (e.g. Zn-protein complexes); biodegradable polymers such as polyesters; a preservative (e.g. benzyl alcohol) and/or a free amino acid.

Suitable buffering agents for use in the formulations include, but are not limited to, organic acid salts such as sodium, potassium, ammounium, or trihydroxyethylamino salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid or phtalic acid; Tris, tromethamine hydrochloride, sulfate or phosphate buffer. In addition, amino acid cationic components can also be used as buffering agent. Such amino acid component includes without limitation arginine, glycine, glycylglycine, and histidine. The arginine buffers include arginine acetate, arginine chloride, arginine phosphate, arginine sulfate, arginine succinate, etc. In one embodiment, the arginine buffer is arginine acetate. Examples of histidine buffers include histidine chloride-arginine chloride, histidine acetate-arginine acetate, histidine phosphate-arginine phosphate, histidine sulfate-arginine sulfate, histidine succinate-argine succinate, etc. The formulations of the buffers have a pH of 4.5 to pH 7.5, preferably from about 4.5 to about 6.5, more preferably from about 5.0 to about 6.2. In some embodiments, the concentration of the organic acid salts in the buffer is from about 10 mM to about 500 mM.

A “polyol” that may optionally be included in the formulation is a substance with multiple hydroxyl groups. Polyols can be used as stabilizing excipients and/or isotonicity agents in both liquid and lyophilized formulations. Polyols can protect biopharmaceuticals from both physical and chemical degradation pathways. Preferentially excluded co-solvents increase the effective surface tension of solvent at the protein interface whereby the most energetically favorable structural conformations are those with the smallest surface areas. Polyols include sugars (reducing and nonreducing sugars), sugar alcohols and sugar acids. A “reducing sugar” is one which contains a hemiacetal group that can reduce metal ions or react covalently with lysine and other amino groups in proteins and a “nonreducing sugar” is one which does not have these properties of a reducing sugar. Examples of reducing sugars are fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose and glucose. Nonreducing sugars include sucrose, trehalose, sorbose, melezitose and raffinose. Sugar alcohols are selected from mannitol, xylitol, erythritol, maltitol, lactitol, erythritol, threitol, sorbitol and glycerol. Sugar acids include L-gluconate and metallic salts thereof.

The polyol in the liquid formula or in the formulated lyophilized solid can be 0.0% -20% by weight. Preferably, a nonreducing sugar, sucrose or trehalose at a concentration of about from 0.1% to 15% is chosen in the formulation, wherein trehalose being preferred over sucrose, because of the solution stability of trehalose.

A surfactant optionally in the formulations is selected from polysorbate (polysorbate 20, polysorbate 40, polysorbate 65, polysorbate 80, polysorbate 81, polysorbate 85 and the like); poloxamer (e.g. poloxamer 188, poly(ethylene oxide)-poly(propylene oxide), poloxamer 407 or polyethylene-polypropylene glycol and the like); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamido-propyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamido-propyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; dodecyl betaine, dodecyl dimethylamine oxide, cocamidopropyl betaine and coco ampho glycinate; and the MONAQUAT^{TM} series (e.g. isostearyl ethylimidonium ethosulfate); polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g. Pluronics, PF68 etc); etc. Preferred surfactants are polyoxyethylene sorbitan fatty acid esters e.g. polysorbate 20, 40, 60 or 80 (Tween 20, 40, 60 or 80). The concentration of a surfactant in the formulation is range from 0.0% to about 2.0% by weight. In certain embodiments, the surfactant concentration is from about 0.01% to about 0.2%. In one embodiment, the surfactant concentration is about 0.02%.

A “preservative” optionally in the formulations is a compound that essentially reduces bacterial action therein. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenoxyl, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. The preservative in the liquid formula or in the formulated lyophilized powder can be 0.0% -5.0% by weight. In one embodiment, the preservative herein is benzyl alcohol.

Suitable free amino acids as a bulky material, or tonicity agent, or osmotic pressure adjustment in the formulation, is selected from, but are not limited to, one or more of arginine, cystine, glycine, lysine, histidine, ornithine, isoleucine, leucine, alanine, glycine glutamic acid or aspartic acid. The inclusion of a basic amino acid is preferred i.e. arginine, lysine and/or histidine. If a composition includes histidine then this may act both as a buffering agent and a free amino acid, but when a

histidine buffer is used it is typical to include a non-histidine free amino acid e.g. to include histidine buffer and lysine. An amino acid may be present in its D- and/or L-form, but the L-form is typical. The amino acid may be present as any suitable salt e.g. a hydrochloride salt, such as arginine-HCl. The amino acid in the liquid formula or in the formulated lyophilized powder can be 0.0% -30% by weight.

The formulations can optionally comprise methionine, glutathione, cysteine, cystine or ascorbic acid as an antioxidant at a concentration of about up to 5 mg/ml in the liquid formula or 0.0%-5.0% by weight in the formulated lyophilized powder; The formulations can optionally comprise metal chelating agent, e.g., EDTA, EGTA, etc., at a concentration of about up to 2 mM in the liquid formula or 0.0%-0.3% by weight in the formulated lyophilized powder.

The final formulation can be adjusted to the preferred pH with a buffer adjusting agent (e.g. an acid, such as HCl, H_2SO_4, acetic acid, H_3PO_4, citric acid, etc, or a base, such as NaOH, KOH, NH_4OH, ethanolamine, diethanolamine or triethanol amine, sodium phosphate, potassium phosphate, trisodium citrate, tromethamine, etc) and the formulation should be controlled "isotonic" which is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example. The isotonic agent is selected from mannitol, sorbitol, sodium acetate, potassium chloride, sodium phosphate, potassium phosphate, trisodium citrate, or NaCl. In general, both the buffer salts and the isotonic agent may take up to 30% by weight in the formulation.

Other excipients which may be useful in either a liquid or lyophilized formulation of the patent application include, for example, fucose, cellobiose, maltotriose, melibiose, octulose, ribose, xylitol, arginine, histidine, glycine, alanine, methionine, glutamic acid, lysine, imidazole, glycylglycine, mannosylglycerate, Triton X-100, Pluoronic F-127, cellulose, cyclodextrin, (2-Hydroxypropyl)-β-cyclodextrin, dextran (10, 40 and/or 70 kD), polydextrose, maltodextrin, ficoll, gelatin, hydroxypropylmeth, sodium phosphate, potassium phosphate, $ZnCl_2$, zinc, zinc oxide, sodium citrate, trisodium citrate, tromethamine, copper, fibronectin, heparin, human serum albumin, protamine, glycerin, glycerol, EDTA, metacresol, benzyl alcohol, phenoxyl, polyhydric alcohols, or polyalcohols, hydrogenated forms of carbohydrate having a carbonyl group reduced to a primary or secondary hydroxyl group.

Other contemplated excipients, which may be utilized in the aqueous pharmaceutical compositions of the patent application include, for example, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, lipids such as phospholipids or fatty acids, steroids such as cholesterol, protein excipients such as serum albumin (human serum albumin), recombinant

human albumin, gelatin, casein, salt-forming counterions such sodium and the like. These and additional known pharmaceutical excipients and/or additives suitable for use in the formulations of the invention are known in the art, e.g., as listed in "The Handbook of Pharmaceutical Excipients, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); and Remington: the Science and Practice of Pharmacy, 21th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2005).

A pharmaceutical container or vessel is used to hold the pharmaceutical formulation of any of conjugates of the patent application. The vessel is a vial, bottle, pre-filled syringe, pre-filled or auto-injector syringe. The liquid formula can be freeze-dried or drum-dryed to a form of cake or powder in a borosilicate vial or soda lime glass vial. The solid powder can also be prepared by efficient spray drying, and then packed to a vial or a pharmaceutical container for storage and distribution.

In a further embodiment, the invention provides a method for preparing a formulation comprising the steps of: (a) lyophilizing the formulation comprising the conjugates, excipients, and a buffer system; and (b) reconstituting the lyophilized mixture of step (a) in a reconstitution medium such that the reconstituted formulation is stable. The formulation of step (a) may further comprise a stabilizer and one or more excipients selected from a group comprising bulking agent, salt, surfactant and preservative as hereinabove described. As reconstitution media, several diluted organic acids or water, i.e. sterile water, bacteriostatic water for injection (BWFI) or may be used. The reconstitution medium may be selected from water, i.e. sterile water, bacteriostatic water for injection (BWFI) or the group consisting of acetic acid, propionic acid, succinic acid, sodium chloride, magnesium chloride, acidic solution of sodium chloride, acidic solution of magnesium chloride and acidic solution of arginine, in an amount from about 10 to about 250 mM.

A liquid pharmaceutical formulation of the conjugates of the patent application should exhibit a variety of pre-defined characteristics. One of the major concerns in liquid drug products is stability, as proteins/antibodies tend to form soluble and insoluble aggregates during manufacturing and storage. In addition, various chemical reactions can occur in solution (deamidation, oxidation, clipping, isomerization etc.) leading to an increase in degradation product levels and/or loss of bioactivity. Preferably, a conjugate in either liquid or loyphilizate formulation should exhibit a shelf life of more than 6 months at 25°C. More preferred a conjugate in either liquid or loyphilizate formulation should exhibit a shelf life of more than 12 months at 25°C. Most preferred liquid formulation should exhibit a shelf life of about 24 to 36 months at 2-8° C and the loyphilizate formulation should exhibit a shelf life of about preferably up to 60 months at 2-8° C. Both liquid and loyphilizate formulations should exhibit a shelf life for at least two years at -20° C, or -70° C.

In certain embodiments, the formulation is stable following freezing (e. g., -20°C, or -70° C.) and thawing of the formulation, for example following 1, 2 or 3 cycles of freezing and thawing.

Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of drug/antibody(protein) ratio and aggregate formation (for example using UV, size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography, image capillary isoelectric focusing (icIEF) or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis, or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), or HPLC-MS/MS; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS--C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (e.g. Asn deamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomeriation), clipping/hydrolysis/fragmentation (e.g. hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc.

A stable conjugate should also "retains its biological activity" in a pharmaceutical formulation, if the biological activity of the conjugate at a given time, e. g. 24 or 36 month, within about 20%, preferably about 10% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared as determined in an antigen binding assay, and/or in vitro, cytotoxic assay, for example.

For clinical in vivo use, the conjugate of the invention will be supplied as solutions or as a lyophilized solid that can be redissolved in sterile water for injection. Examples of suitable protocols of conjugate administration are as follows. Conjugates are given dayly, weekly, biweekly, triweekly, once every four weeks or monthly for 8~54 weeks as an i.v. bolus. Bolus doses are given in 50 to 1000 ml of normal saline to which human serum albumin (e.g. 0.5 to 1 mL of a concentrated solution of human serum albumin, 100 mg/mL) can optionally be added. Dosages will be about 50 μg to 20 mg/kg of body weight per week, i.v. (range of 10 μg to 200 mg/kg per injection). 4~54 weeks after treatment, the patient may receive a second course of treatment. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by the skilled clinicians.

Examples of medical conditions that can be treated according to the *in vivo* or *ex vivo* methods of killing selected cell populations include malignancy of any types of cancer, autoimmune diseases, graft rejections, and infections (viral, bacterial or parasite).

The amount of a conjugate which is required to achieve the desired biological effect, will vary depending upon a number of factors, including the chemical characteristics, the potency, and the bioavailability of the conjugates, the type of disease, the species to which the patient belongs, the

diseased state of the patient, the route of administration, all factors which dictate the required dose amounts, delivery and regimen to be administered.

In general terms, the conjugates of this invention may be provided in an aqueous physiological buffer solution containing 0.1 to 10% w/v conjugates for parenteral administration. Typical dose ranges are from 1 μg/kg to 0.1 g/kg of body weight daily; weekly, biweekly, triweekly, or monthly, a preferred dose range is from 0.01 mg/kg to 25 mg/kg of body weight weekly, biweekly, triweekly, or monthly, an equivalent dose in a human. The preferred dosage of drug to be administered is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound, the route of administration (intravenous, intramuscular, or other), the pharmacokinetic properties of the conjugates by the chosen delivery route, and the speed (bolus or continuous infusion) and schedule of administrations (number of repetitions in a given period of time).

The conjugates of the present invention are also capable of being administered in unit dose forms, wherein the term "unit dose" means a single dose which is capable of being administered to a patient, and which can be readily handled and packaged, remaining as a physically and chemically stable unit dose comprising either the active conjugate itself, or as a pharmaceutically acceptable composition, as described hereinafter. As such, typical total daily/weekly/biweekly/ triweekly/monthly dose ranges are from 0.01 to 100 mg/kg of body weight. By way of general guidance, unit doses for humans range from 1 mg to 3000 mg per day, or per week, per two weeks (biweekly), triweekly, or per month. Preferrably the unit dose range is from 1 to 500 mg administered one to four times a month and even more preferably from 1 mg to 100 mg, once a week, or once a biweek, or once a triweek. Conjugatess provided herein can be formulated into pharmaceutical compositions by admixture with one or more pharmaceutically acceptable excipients. Such unit dose compositions may be prepared for use by oral administration, particularly in the form of tablets, simple capsules or soft gel capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols; or dermally, for example, topically in ointments, creams, lotions, gels or sprays, or via trans-dermal patches. The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical art, for example, as described in *Remington: The Science and Practice of Pharmacy*, 21th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2005.

The formulations include pharmaceutical compositions in which a compound of the present invention is formulated for oral or parenteral administration. For oral administration, tablets, pills, powders, capsules, troches and the like can contain one or more of any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, or gum tragacanth; a diluent

such as starch or lactose; a disintegrant such as starch and cellulose derivatives; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, or methyl salicylate. Capsules can be in the form of a hard capsule or soft capsule, which are generally made from gelatin blends optionally blended with plasticizers, as well as a starch capsule. In addition, dosage unit forms can contain various other materials that modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or enteric agents. Other oral dosage forms syrup or elixir may contain sweetening agents, preservatives, dyes, colorings, and flavorings. In addition, the active compounds may be incorporated into fast dissolve, modified-release or sustained-release preparations and formulations, and wherein such sustained-release formulations are preferably bi-modal. Preferred tablets contain lactose, cornstarch, magnesium silicate, croscarmellose sodium, povidone, magnesium stearate, or talc in any combination.

Liquid preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The liquid compositions may also include binders, buffers, preservatives, chelating agents, sweetening, flavoring and coloring agents, and the like. Non-aqueous solvents include alcohols, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and organic esters such as ethyl oleate. Aqueous carriers include mixtures of alcohols and water, buffered media, and saline. In particular, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control the release of the active compounds. Intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Other potentially useful parenteral delivery systems for these active compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Alternative modes of administration include formulations for inhalation, which include such means as dry powder, aerosol, or drops. They may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for buccal administration include, for example, lozenges or pastilles and may also include a flavored base, such as sucrose or acacia, and other excipients such as glycocholate. Formulations suitable for rectal administration are preferably presented as unit-dose suppositories, with a solid based carrier, such as cocoa butter, and may include a salicylate. Formulations for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly, lanolin, polyethylene glycols, alcohols, or their combinations. Formulations suitable for transdermal administration can be presented as discrete patches and can be lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive.

In yet another embodiment, a pharmaceutical composition comprising a therapeuticcally effective amount of the conjugate of Formula (VI), (VII), (VIII), (IX) or any conjugates described through the present patent can be administered concurrently with the other therapeutic agents such as the chemotherapeutic agent, the radiation therapy, immunotherapy agents, autoimmune disorder agents, anti-infectious agents or the other conjugates for synergistically effective treatment or prevention of a cancer, or an autoimmune disease, or an infectious disease. The synergistic drugs or radiation therapy can be administered prior or subsequent to administration of a conjugate, in one aspect at least an hour, 12 hours, a day, a week, biweeks, triweeks, a month, in further aspects several months, prior or subsequent to administration of a conjugate of the invention.

The synergistic agents are preferably selected from one or several of the following drugs: The synergistic agents are selected from one or several of the following drugs: Abatacept, Abiraterone acetate, Abraxane, Acetaminophen/hydrocodone, Acalabrutinib, aducanumab, Adalimumab, ADXS31-142, ADXS-HER2, Afatinib dimaleate, Aldesleukin, Alectinib, Alemtuzumab, Alitretinoin, ado-trastuzumab emtansine, Amphetamine/ dextroamphetamine, Anastrozole, Aripiprazole, anthracyclines, Aripiprazole, Atazanavir, Atezolizumab, Atorvastatin, Avelumab, Axicabtagene ciloleucel, Axitinib, Belinostat, BCG Live, Bevacizumab, Bexarotene, Blinatumomab, Bortezomib, Bosutinib, Brentuximab vedotin, Brigatinib, Budesonide, Budesonide/formoterol, Buprenorphine, Cabazitaxel, Cabozantinib, Capmatinib, Capecitabine, Carfilzomib, chimeric antigen receptor-engineered T (CAR-T) cells, Celecoxib, Ceritinib, Cetuximab, Chidamide, Ciclosporin, Cinacalcet, Crizotinib, Cobimetinib, Cosentyx, Crizotinib, CTL019, Dabigatran, Dabrafenib, Dacarbazine, Daclizumab, Dacomotinib, Daptomycin, Daratumumab, Darbepoetin alfa, Darunavir, Dasatinib, Denileukin diftitox, Denosumab, Depakote, Dexlansoprazole, Dexmethylphenidate, Dexamethasone, Dinutuximab, Doxycycline, Duloxetine, Duvelisib, Durvalumab, Elotuzumab, Emtricitabine/ Rilpivirine/Tenofovir, Disoproxil fumarate, Emtricitbine/tenofovir/efavirenz, Enoxaparin, Ensartinib, Enzalutamide, Epoetin alfa, erlotinib, Esomeprazole, Eszopiclone, Etanercept, Everolimus, Exemestane, Everolimus, Exenatide ER, Ezetimibe, Ezetimibe/simvastatin, Fenofibrate, Filgrastim, Fingolimod, Fluticasone propionate, Fluticasone/salmeterol, Fulvestrant, Gazyva, Gefitinib, Glatiramer, Goserelin acetate, Icotinib, Imatinib, Ibritumomab tiuxetan, Ibrutinib, Idelalisib, Ifosfamide, Infliximab, Imiquimod, ImmuCyst, Immuno BCG, Iniparib, Insulin aspart, Insulin detemir, Insulin glargine, Insulin lispro, Interferon alfa, Interferon alfa-1b, Interferon alfa-2a, Interferon alfa-2b, Interferon beta, Interferon beta 1a, Interferon beta 1b, Interferon gamma-1a, Iapatinib, Ipilimumab, Ipratropium bromide/salbutamol, Ixazomib, Kanuma, Lanreotide acetate, Lenalidomide, Lenaliomide, Lenvatinib mesylate, Letrozole, Levothyroxine, Levothyroxine, Lidocaine, Linezolid, Liraglutide, Lisdexamfetamine, LN-144, Lorlatinib, Memantine,

Methylphenidate, Metoprolol, Mekinist, Mericitabine/Rilpivirine/ Tenofovir, Modafinil, Mometasone, Mycidac-C, Necitumumab, neratinib, Nilotinib, Niraparib, Nivolumab, Ofatumumab, Obinutuzumab, Olaparib, Olmesartan, Olmesartan/ hydrochlorothiazide, Omalizumab, Omega-3 fatty acid ethyl esters, Oncorine, Oseltamivir, Osimertinib, Oxycodone, Palbociclib, Palivizumab, Panitumumab, Panobinostat, Pazopanib, Pembrolizumab, PD-1 antibody, PD-L1 antibody, Pemetrexed, Pertuzumab, Pneumococcal conjugate vaccine, Pomalidomide, Poziotinib, Pregabalin, ProscaVax, Propranolol, Quetiapine, Rabeprazole, Radium 223 chloride, Raloxifene, Raltegravir, Ramucirumab, Ranibizumab, Regorafenib, Rituximab, Rivaroxaban, Romidepsin, Rosuvastatin, Ruxolitinib phosphate, Salbutamol, Savolitinib, Semaglutide, Sevelamer, Sildenafil, Siltuximab, Sipuleucel-T, Sitagliptin, Sitagliptin/metformin, Solifenacin, Solanezumab, Sonidegib, Sorafenib, Sunitinib, Tacrolimus, Tacrimus, Tadalafil, Tamoxifen, Tafinlar, Talimogene laherparepvec, Talazoparib, Telaprevir, Talazoparib, Temozolomide, Temsirolimus, Tenofovir/emtricitabine, Tenofovir disoproxil fumarate, Testosterone gel, Thalidomide, TICE BCG, Tiotropium bromide, Tisagenlecleucel, Toremifene, Trametinib, Trastuzumab, Trastuzumab deruxtecan, Trabectedin (ecteinascidin 743), Trametinib, Tremelimumab, Trifluridine/tipiracil, Tretinoin, Uro-BCG, Ustekinumab, Valsartan, Veliparib, Vandetanib, Vemurafenib, Venetoclax, Vorinostat, Ziv-aflibercept, Zostavax, and their analogs, derivatives, pharmaceutically acceptable salts, carriers, diluents or excipients thereof or a combination above thereof.

The drugs/ cytotoxic agents used for conjugation of the present patent can be any analogues and/or derivatives of drugs/molecules described in the present patent. One skilled in the art of drugs/cytotoxic agents will readily understand that each of the drugs/cytotoxic agents described herein can be modified in such a manner that the resulting compound still retains the specificity and/or activity of the starting compound. The skilled artisan will also understand that many of these compounds can be used in place of the drugs/cytotoxic agents described herein. Thus, the drugs/cytotoxic agents of the present invention include analogues and derivatives of the compounds described herein.

According to a still further object, the conjugate and process of the present invention may be prepared in a number of ways well known to those skilled in the art. The Cytotoxic compounds used in the conjugates can be synthesized, for example, by application or adaptation of the methods described below, or variations thereon as appreciated by the skilled artisan. The appropriate modifications and substitutions will be readily apparent and well known or readily obtainable from the scientific literature to those skilled in the art. In particular, such methods can be found in R.C. Larock, *Comprehensive Organic Transformations*, 2nd Edition, Wiley-VCH Publishers, 1999.

In the reactions described hereinafter, it may be necessary to protect reactive functional groups, for

example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice, for examples see P. G. Wuts and T.W. Greene, Greene's Protective Groups in Organic Synthesis, Wiley-Interscience; 4th edition (2006). Some reactions may be carried out in the presence of a base, or an acid or in a suitable solvent. There is no particular restriction on the nature of the base, acid and solvent to be used in this reaction, and any base, acid or solvent conventionally used in reactions of this type may equally be used here, provided that it has no adverse effect on other parts of the molecule. The reactions can take place over a wide range of temperatures. In general, we find it convenient to carry out the reaction at a temperature of from -80°C to 150°C (more preferably from about room temperature to 100°C). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 3 hours to 20 hours will usually suffice.

The work-up of the reaction can be carried out by conventional means. For example, the reaction products may be recovered by distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water followed by extraction with a water-immiscible organic solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well known techniques, such as recrystallization, reprecipitation or the various chromatography techniques, notably column chromatography or preparative thin layer chromatography.

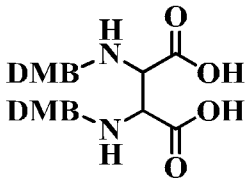
The process of the invention is further illustrated but not restricted by the description in the following examples. All references cited herein and in the examples that follow are expressly incorporated by reference in their entireties.

EXAMPLES

The invention is further described in the following examples, which are not intended to limit the scope of the invention. Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ), or The Shanghai Cell Culture Institute of Chinese Acadmy of Science, unless otherwise specified. Cell culture reagents were obtained from Invitrogen Corp., unless otherwise specified. All anhydrous solvents were commercially obtained and stored in Sure-seal bottles under nitrogen. PEG compounds were purchased from Biomatrik Inc, Jiaxing, China. Some chemical compounds, when were not referred synthesis from, were provided by CROs (e. g. Wuxi Apptec, Haoyuan Chemexpress, Raybow Pharma) in China. Experimental animals were purchased from National Resource Center of Model

Mice via GemPharmatech. Co., Ltd, Najing, China and Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai, China; T-DM1 was purchased from Roche via a pharmacy in Hong Kong, China. All other reagents and solvents were purchased as the highest grade available and used without further purification. The preparative HPLC separations were performed with Varain PreStar HPLC. HPLC analysis was conducted on Agilent 1260. The mass spectral data were acquired on a Waters Xevo QTOF mass spectrum equipped with Waters Acquity UPLC separations module and Acquity TUV detector. NMR spectra were recorded on Zhongke-niujin WNMR-I 400 MHz instrument at the Department of Chemistry of Zhejiang Sci-Tech University. Chemical shifts (δ) are reported in parts per million (ppm) referenced to tetramethylsilane at 0.00 and coupling constants (J) are reported in Hz. The elemental analysis of C, H, and/or N was provided by the Department of Chemistry of Zhejiang Sci-Tech University and conducted on Elementar UNICUBE. Quantitative analysis of metal atoms was performed on Agilent ICPOES 730 ICP-MS.

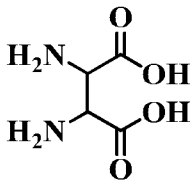
Example 1. Synthesis of meso-2,3-bis((2,4-dimethoxybenzyl)amino)succinic acid (**2**).



2

To a solution of meso-2,3-dibromosuccinic acid (500 g, 1.80 mol) in ethanol (3.6 L) was added trimethylamine (729 g, 7.20 mol), followed by 2,4-dimethoxybenzylamine (903 g, 5.4 mol). After completion of addition, the mixture was heated to 90 °C and stirred under reflux overnight. The mixture was cooled to r.t. and the formed solid was filtered, rinsed with ethanol and dried to give meso-2,3-bis(2,4-dimethoxybenzylamino) succinic acid (600 g, 72% yield).

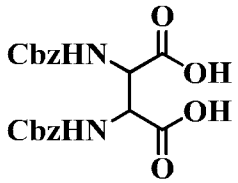
Example 2. Synthesis of meso-2,3-diaminosuccinic acid (**3**).



3

A solution of meso-2,3-bis(2,4-dimethoxybenzylamino)succinic acid (800 g, 1.78 mol) in dichloromethane (100 mL) was treated with trifluoroacetic acid (2035 g, 17.8 mol) at r.t. overnight. The mixture was concentrated and then 1N NaOH (6 L) was added slowly and stirred for 30 min. The precipitate was filtered off and the solution was adjusted to pH 5-6 using aq. HCl. The resulting white precipitate was collected by filtration, rinsed with water and dried to give meso-2,3-diaminosuccinic acid (217 g, 78% yield).

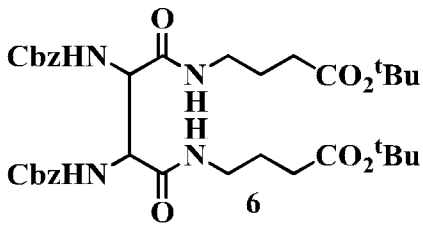
Example 3. Synthesis of meso-2,3-bis(((benzyloxy)carbonyl)amino)succinic acid (**4**).



4

Meso-2,3-diaminosuccinic acid (386 g, 2.6 mol) was dissolved in 2 N NaOH (6.5 L) and mixed with 1,4-dioxane (2.1 L). The solution was cooled to 0 °C and benzyl chloroformate (1333 g, 7.8 mol) was added in a rate to maintain the internal temperature of below 5 °C. After completion of the addition, the mixture was stirred for 3 h, warmed to r.t. and stirred overnight. The reaction was diluted with water (2 L) and washed with ethyl acetate (2 × 1 L). The aqueous layer was acidified with con. HCl until pH 3 was reached, and then extracted with ethyl acetate (3 × 2 L). The organic phase was combined and washed with water (1 L), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was triturated with dichloromethane/ petroleum ether (1:1), filtered to give meso-2,3-bis(((benzyloxy)carbonyl) amino) succinic acid (900 g, 83%).

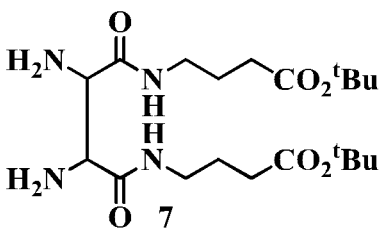
Example 4. Synthesis of di-tert-butyl 4,4'-((2,3-bis(((benzyloxy)carbonyl)amino)succinyl) bis(azanediyl))dibutyrate (**6**)



6

To a solution of compound **4** (10 g, 28.7 mmol) and tert-butyl aminobutyrate hydrochloride (11.2 g, 57.4 mol) in tetrahydrofuran (200 mL) were added HATU (32.8 g, 86.3 mmol) and diisopropylethylamine (19 mL, 115 mmol), and the reaction was stirred at r.t. overnight, diluted with water (400 mL), stirred for 10 minutes, and filtered, dried in an oven to give a white solid (17.7 g, > 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{51}N_4O_{10}$, 699.35; found, 699.35.

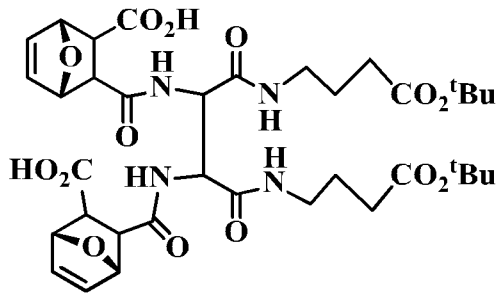
Example 5. Synthesis of di-tert-butyl 4,4'-((2,3-diaminosuccinyl)bis(azanediyl)) dibutyrate (**7**)



7

Compound **6** (16 g, 22.9 mmol) was dissolved in methanol (200 mL), and then Pd/C (2.0 g) was added. The reaction flask was evacuated and back-filled with hydrogen, heated to 60 °C, and stirred until completion of the reaction. Filtration and concentration gave product 7 (9.8 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{39}N_4O_6$, 431.28; found, 431.28.

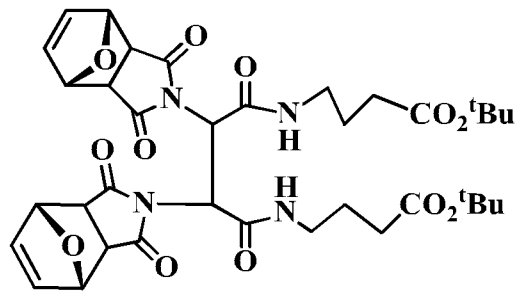
Example 6. Synthesis of compound **9**.



9

Compound **7** (9.8 g, 22.8 mmol) was dissolved in dichloromethane (200 mL), and exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (7.5 g, 45.5 mmol) and triethylamine (6.3 ml, 45.5 mmol) were added. The reaction was stirred at r.t. until completion, an then concentrated to dryness to give a white foamy solid (17.3 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{51}N_4O_{14}$, 763.33; found, 763.33.

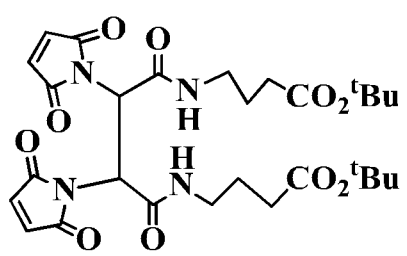
Example 7. Synthesis of di-tert-butyl 4,4'-((2,3-bis((4R,7S)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)succinyl)bis(azanediyl))dibutyrate (**10**).



10

To a solution of compound **9** (17.3 g, 22.7 mmol) dissolved in DMF (300 mL), were added EDC (13 g, 68.2 mmol), HOBt (9.2 g, 68.2 mmol), and DBU (10.4 g, 68.2 mmol) slowly. The mixture was heated to 60 °C and stirred for 5 hours, cooled to r.t. and poured into water (1 L), extracted with dichloromethane (3 × 200 mL). The combined organic phases were washed with 2 N HCl (100 mL), brine (100 mL), dried over sodium sulfate, filtered and concentrated. The residue was triturated with petroleum ether/ethyl acetate (200 mL/500 mL), and the white solid was filtered off. The filtrate was concentrated and purified by a silica gel column to give a white foamy solid (8.2 g, 49% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{47}N_4O_{12}$, 727.31; found, 727.31.

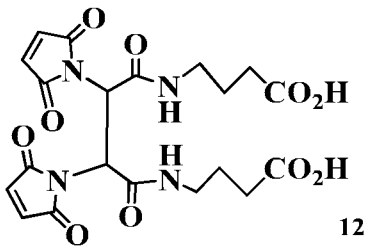
Example 8. Synthesis of di-tert-butyl 4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1 -yl)succinyl)bis(azanediyl))dibutyrate (**11**).



11

Compound **10** (8.2 g, 11.2 mmol) was dissolved in a mixture of toluene (80 mL) and DMF (80 mL), heated to 120°C and stirred under reflux for 4 hours. The reaction was then cooled to r.t. and stirred for more than 30 minutes, and a white solid precipitated, which was then collected by filtration and dried to give the desired product (3.0 g, 45% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{28}H_{39}N_4O_{10}$, 591.26; found, 591.26.

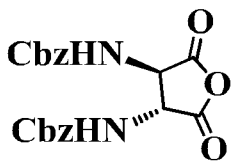
Example 9. Synthesis of 4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)succinyl) bis(azanediyl))dibutyric acid (**12**).



12

Compound **11** (1.6 g, 2.7 mmol) was dissolved in dichloromethane (10 mL) and trifluoroacetic acid (10 mL), and stirred at r.t. for 2 hours. The reaction was concentrated and then co-evaporated with dichloromethane twice, the residue was triturated with dichloromethane and ethyl acetate (10 mL/10 mL), to afford a white solid (1.3 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{23}N_4O_{10}$, 479.13; found, 479.13.

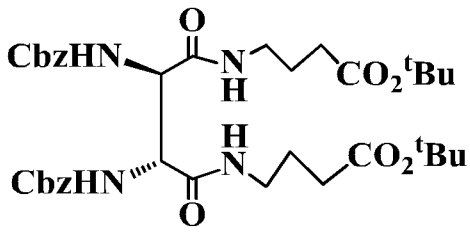
Example 10. Synthesis of dibenzyl ((3R,4S)-2,5-dioxotetrahydrofuran-3,4-diyl)- dicarbamate (**13**).



racemic, 13

The solution of meso-2,3-bis(((benzyloxy)carbonyl)amino)succinic acid (100 g, 0.24 mol) in Ac_2O (1 L) was heated at 90 °C for 6 h, cooled and concentrated to dryness. The residue was co-evaporated with tolune and then triturated with a mixture of acetone (200 mL) and petroleum ether (400 mL). A white solid (80 g diastereomeric mixture) was collected and stirred with dichloromethane (300 mL) overnight. The racemic mixture (61 g) as a white solid was collected, and a racemic/meso mixture was also recovered from the mother liquid, which could be re-processed to afford a clean meso compound (2 g) and other racemic/meso mixture (15 g).

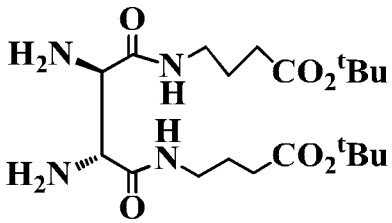
Example 11. Synthesis of di-tert-butyl 4,4'-(((2R,3R)-2,3-bis(((benzyloxy)carbonyl)amino) succinyl)bis(azanediyl))dibutyrate (**14**).



14

To a solution of compound **13** (60 g, 0.151 mol) dissolved in tetrahydrofuran (1 L) was added tert-butyl aminobutyrate hydrochloride (31 g, 0.151 mol). The solution was cooled to 0 °C and triethylamine (42 mL, 0.302 mmol) was added. The reaction was warmed to r.t. and stirred for 30 minutes. Another portion of tert-butyl aminobutyrate (31 g, 0.151 mol), HATU (86.12 g, 0.226 mol) and triethylamine (42 mL, 0.302 mmol) were added, and the reaction was stirred at r.t. overnight, diluted with water (2 L), stirred for 10 minutes, and filtered to give a white solid (93 g, 88.13% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{51}N_4O_{10}$, 699.35; found, 699.35.

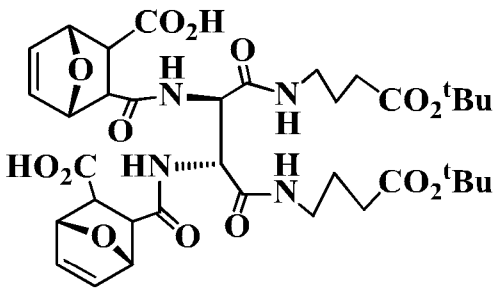
Example 12. Synthesis of di-tert-butyl 4,4'-(((2R,3R)-2,3-diaminosuccinyl)bis(azanediyl)) dibutyrate (**15**).



15

Compound **14** (93 g, 0.133 mol) was dissolved in methanol (2 L), and then Pd/C (10 g) was added. The reaction flask was evacuated and back-filled with hydrogen, heated to 60 °C, and stirred for 6 h. Filtration and concentration gave product 7 (57 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{39}N_4O_6$, 431.28, found, 431.28.

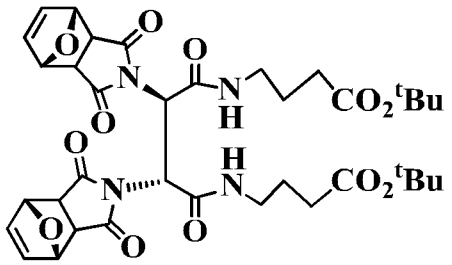
Example 13. Synthesis of compound **16**.



16

Compound **15** (57 g, 0.13 mol) was dissolved in dichloromethane (600 mL), and exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (46 g, 0.27 mol) and triethylamine (36 ml, 0.27 mol) were added. The reaction was stirred at r.t. for 3 hours, concentrated to dryness, and co-evaporated with dichloromethane to give a white foamy solid (100 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{51}N_4O_{14}$, 763.33; found, 763.33.

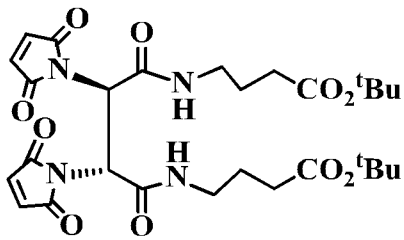
Example 14. Synthesis of di-tert-butyl 4,4'-(((2R,3R)-2,3-bis((4R,7S)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)succinyl)bis(azanediyl))dibutyrate (**17**).



17

To a solution of compound **16** (100 g, 0.13 mol) dissolved in DMF (1 L), were added EDC (75 g, 0.39 mol), HOBt (53 g, 0.39 mol), and DBU (59 g, 0.39 mol) slowly. The mixture was heated to 60 °C and stirred for 5 hours, cooled to r.t. and poured into water (2 L), extracted with dichloromethane (3 × 500 mL). The combined organic phases were washed with 2 N HCl (300 mL), brine (300 mL), dried over sodium sulfate, filtered and concentrated. The residue was triturated with petroleum ether/ethyl acetate (200 mL/500 mL), and the white solid was filtered off. The filtrate was concentrated and purified by a silica gel column to give a white foamy solid (67 g, 70% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{36}H_{47}N_4O_{12}$, 727.31; found, 727.31.

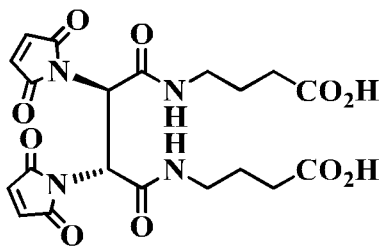
Example 15. Synthesis of di-tert-butyl 4,4'-(((2R,3R)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)succinyl)bis(azanediyl))dibutyrate (**18**).



18

Compound **17** (67 g, 92 mmol) was dissolved in a mixture of toluene (600 mL) and DMF (60 mL), heated to 120°C and stirred under reflux for 4 hours. The reaction was then cooled to r.t. and stirred for more than 30 minutes, and a white solid precipitated, which was then collected by filtration and dried to give the desired product (41 g, 75% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{28}H_{39}N_4O_{10}$, 591.26; found, 591.26.

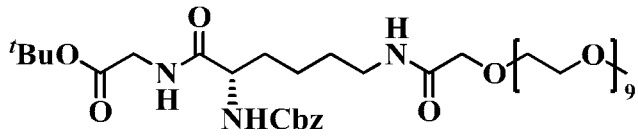
Example 16. Synthesis of 4,4'-(((2R,3R)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) succinyl)bis(azanediyl))dibutyric acid (**19**).



19

Compound **18** (41 g, 69 mmol) was dissolved in dichloromethane (200 mL) and trifluoroacetic acid (200 mL), and stirred at r.t. for 2 hours. The reaction was concentrated and then co-evaporated with dichloromethane twice, the residue was triturated with dichloromethane and ethyl acetate (200 mL/200 mL), to afford a white solid (33 g, 99% yield).

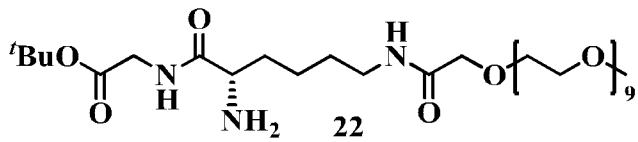
Example 17. Synthesis of tert-butyl (S)-(37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycinate (**21**).



21

To a solution of (S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20, 23,26,29-decaoxa-32-azaoctatriacontan-38-oic acid (**20**, 4.0 g, 5.34 mmol) and H-Gly-OtBu·HCl (0.9 g, 5.34 mmol) in THF (40 mL), HATU (3.05 g, 8.01 mmol) and diisopropylethylamine (1.5 mL, 10.68 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was poured into water (100 mL), extracted with dichloromethane (3×50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **21** (4.1 g, 89% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{41}H_{72}N_3O_{16}$, 862.48; found, 862.48.

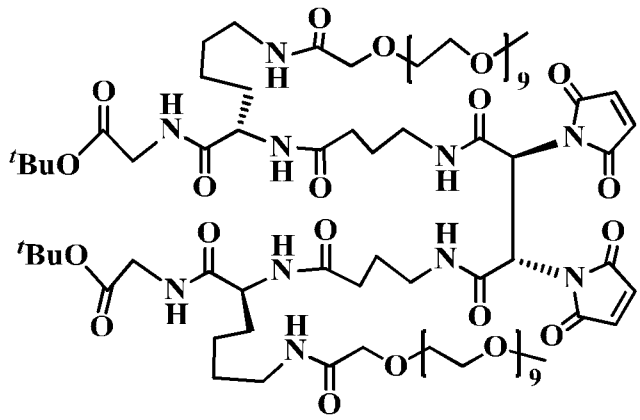
Example 18. Synthesis of tert-butyl (S)-(37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29 - decaoxa-32-azaoctatriacontan-38-oyl)glycinate (**22**).



22

Compound **21** (2.9 g, 3.3 mmol) was dissolved in THF (50 mL), 10% palladium on carbon (0.3 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound 2 (2.1 g, 84% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{33}H_{66}N_3O_{14}$, 728.45; found 728.45.

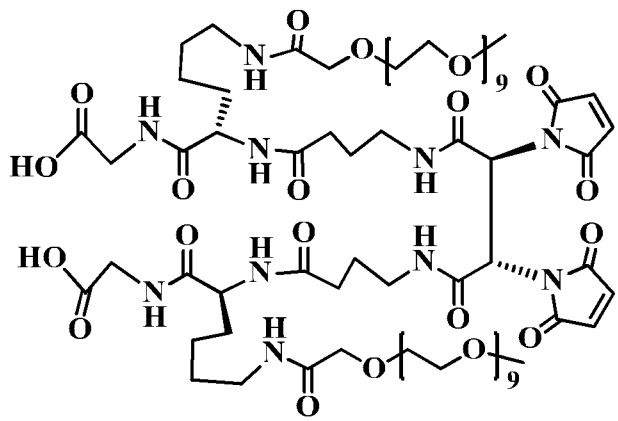
Example 19. Synthesis of di-tert-butyl (5S,13S,14S,22S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H - pyrrol-1-yl)-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioate (**23**).



23

To a solution of compound **19** (120 mg, 0.251 mmol) and compound **22** (365 mg, 0.502 mmol) in mixed solvents of THF (10 mL) and DMF (5 mL), HATU (286 mg, 0.75 mmol) and diisopropylethylamine (82 μL, 0.5 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was dissolved in dichloromethane (100 mL), washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **23** (0.3 g, 62% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{86}H_{149}N_{10}O_{36}$, 1898.01; found, 1898.01.

Example 20. Synthesis of (5S,13S,14S,22S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H -pyrrol-1-yl)-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioic acid (**24**).

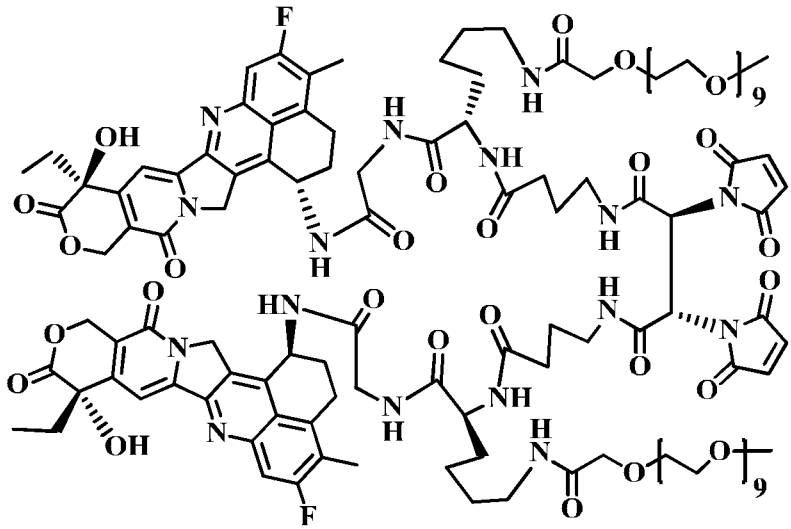


24

Compound **23** (0.3 g, 0.158 mmol) was dissolved in formic acid (10 mL) and dichloromethane (5 mL), and then heated to 60 °C. The reaction was stirred until completion, as indicated by LC-MS and then concentrated to give the title compound (280 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{78}H_{133}N_{10}O_{36}$, 1785.88; found 1785.88.

Example 21. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)- N1,N4-bis((S)-37-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-

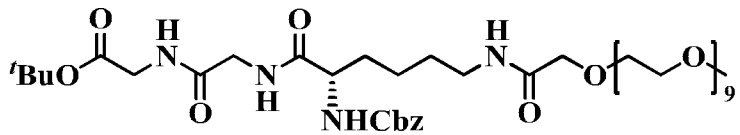
octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**25**).



25

Compound **24** (200 mg, 0.102 mmol) and exatecan mesylate (108 mg 0.204 mmol) were dissolved in DMF (5 mL), HATU (116 mg, 0.306 mmol) and diisopropylethylamine (71 μL, 0.408 mmol) were added. The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give product **25** (120 mg, 46% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{126}H_{173}F_2N_{16}O_{42}$, 2620.18; found, 2620.18.

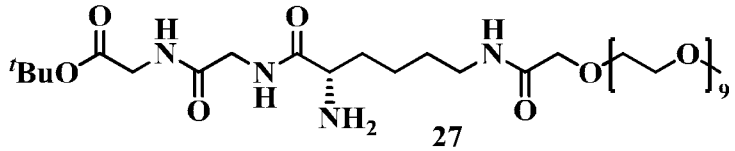
Example 22. Synthesis of tert-butyl (S)-(S)-(37-(((benzyloxy)carbonyl)amino)-31 -oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycinate (**26**).



26

To a solution of compound **20** (5 g, 6.78 mmol) and H-Gly-Gly-O^{t}Bu·HCl (1.5 g, 6.78 mmol) in THF (50 mL), HATU (3.81 g, 10.01 mmol) and diisopropylethylamine (1.8 ml, 13.35 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was poured into water (100 mL), extracted with dichloromethane (3×50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **26** (3.7 g, 60% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{43}H_{75}N_4O_{17}$, 919.50; found, 919.50.

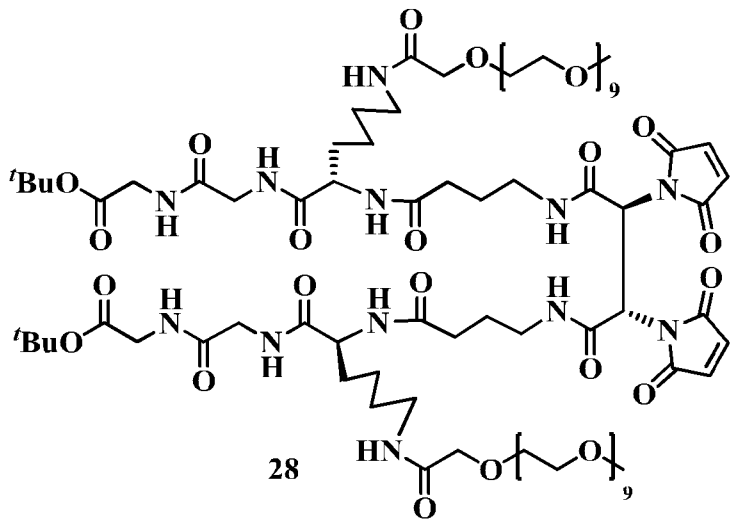
Example 23. Synthesis of tert-butyl (S)-(S)-(37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29 -decaoxa-32-azaoctatriacontan-38-oyl)glycylglycinate (**27**).



27

Compound **26** (819 mg, 0.89 mmol) was dissolved in THF (20 mL), 10% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **27** (700 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{35}H_{69}N_4O_{15}$, 785.47; found, 785.47.

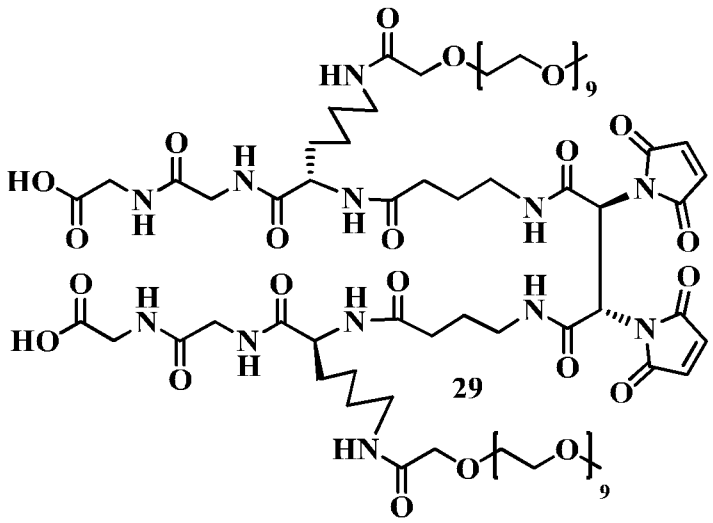
Example 24. Synthesis of di-tert-butyl (8S,16S,17S,25S)-16,17-bis(2,5-dioxo-2,5-dihydro- 1H-pyrrol-1-yl)-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioate (**28**).



28

To a solution of compound **19** (180 mg, 0.376 mmol) and compound **27** (649 mg, 0.828 mmol) in mixed solvents of THF (10 mL) and DMF (5 mL), HATU (429 mg, 1.13 mmol) and diisopropylethylamine (186 μL, 1.13 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was dissolved in dichloromethane (100 mL), washed with water (30 mL), saturated sodium bicarbonate (30 mL), 2 N HCl (30 mL), and brine (30 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound (0.45 g, 59% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{90}H_{155}N_{12}O_{38}$, 2012.05; found, 2012.05.

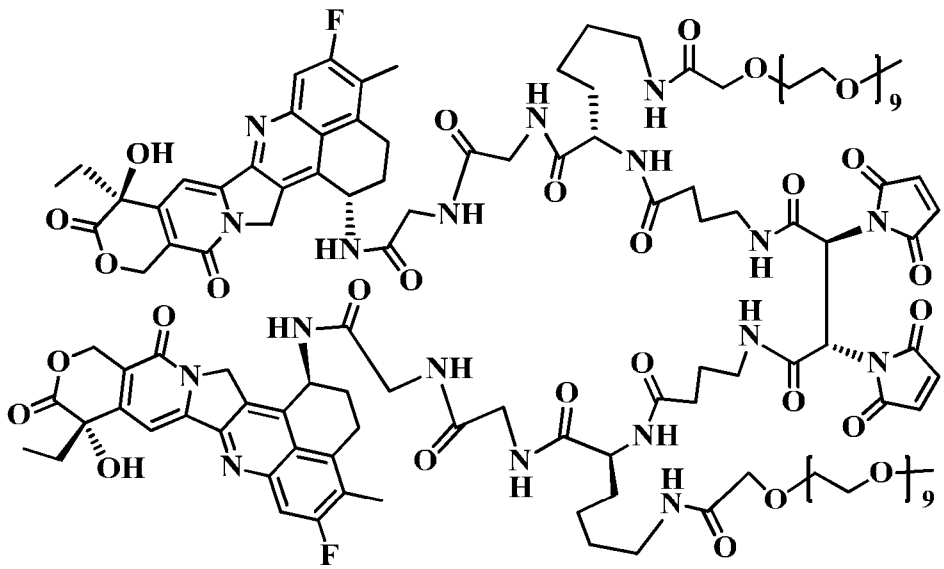
Example 25. Synthesis of (8S,16S,17S,25S)-16,17-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1 -yl)-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioic acid (**29**).



29

Compound **28** (0.30 g, 0.194 mmol) was dissolved in formic acid (10 mL) and dichloromethane (5 mL), and then heated to 60 °C. The reaction was stirred until completion, as indicated by LC-MS and then concentrated to give the title compound (280 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{82}H_{139}N_{12}O_{38}$, 1899.92; found, 1899.92.

Example 26. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4 -bis((S)-37-((2-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**30**).

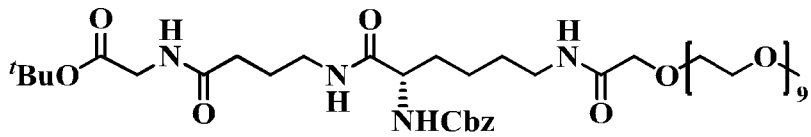


30

Compound **29** (142 mg, 0.075 mmol) and exatecan mesylate (65 mg, 0.15 mmol) were dissolved in DMF (5 mL), HATU (85 mg, 0.225 mmol) and diisopropylethylamine (37 μL, 0.225 mmol) were added. The reaction was stirred at r.t. until complete conversion, and then concentrated

and purified by preparative HPLC to give the title compound (60 mg, 28% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{130}H_{179}F_2N_{18}O_4$, 2734.22; found, 2734.22.

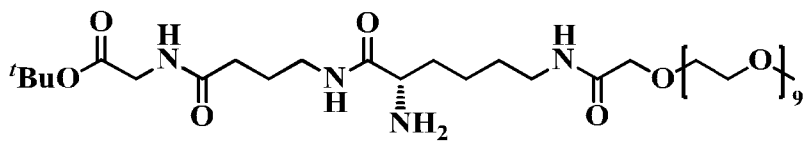
Example 27. Synthesis of tert-butyl (S)-(37-(((benzyloxy)carbonyl)amino)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontan-43-oyl)glycinate (**32**).



32

To a solution of compound **20** (5.00 g, 6.78 mmol) and tert-butyl (4-aminobutanoyl)glycinate hydrochloride (**31**, 1.71 g, 6.78 mmol) in THF (50 mL), HATU (3.81 g, 10.01 mmol) and diisopropylethylamine (1.8 mL, 13.35 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was poured into water (100 mL), extracted with dichloromethane (3×50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **32** (5.4 g, 85% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{45}H_{78}N_4O_{17}$, 947.54; found, 947.57.

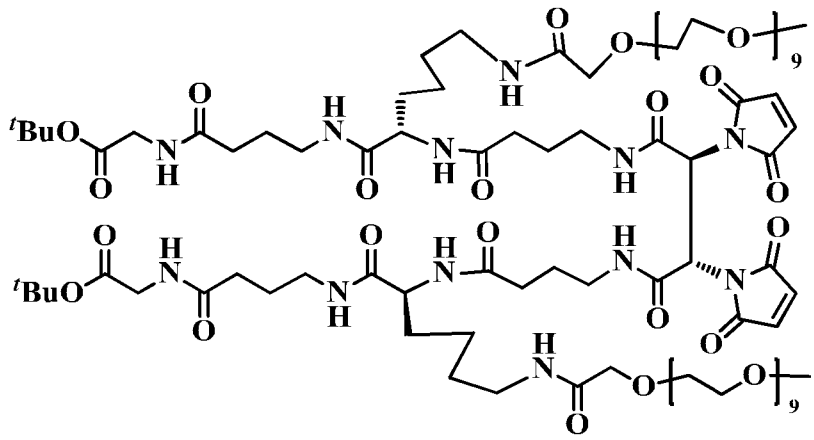
Example 28. Synthesis of tert-butyl (S)-(37-amino-31,38-dioxo-2,5,8,11,14,17,20,23,26,29 -decaoxa-32,39-diazatritetracontan-43-oyl)glycinate (**33**).



33

Compound **32** (819 mg, 0.86 mmol) was dissolved in THF (20 mL), 10% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (700 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{37}H_{73}N_4O_{15}$, 813.50; found, 813.50.

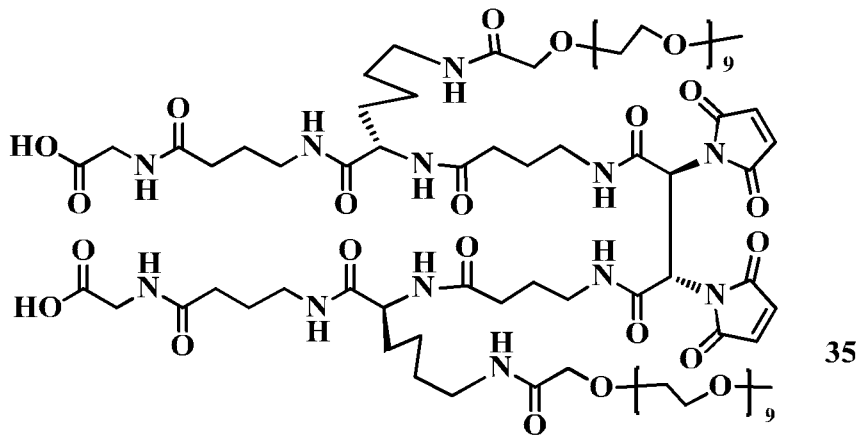
Example 29. Synthesis of di-tert-butyl (10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontanedioate (**34**).



34

To a solution of compound **19** (246 mg, 0.514 mmol) and compound **33** (1.0 g, 1.286 mmol) in mixed solvents of THF (10 mL) and DMF (5 mL), HATU (586 mg, 1.54 mmol) and diisopropylethylamine (245 μL, 1.54 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was dissolved in dichloromethane (100 mL), washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give the title compound (0.54 g, 51% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{94}H_{163}N_{12}O_{38}$, 2068.11; found, 2068.11.

Example 30. Synthesis of (10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H -pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontanedioic acid (**35**).

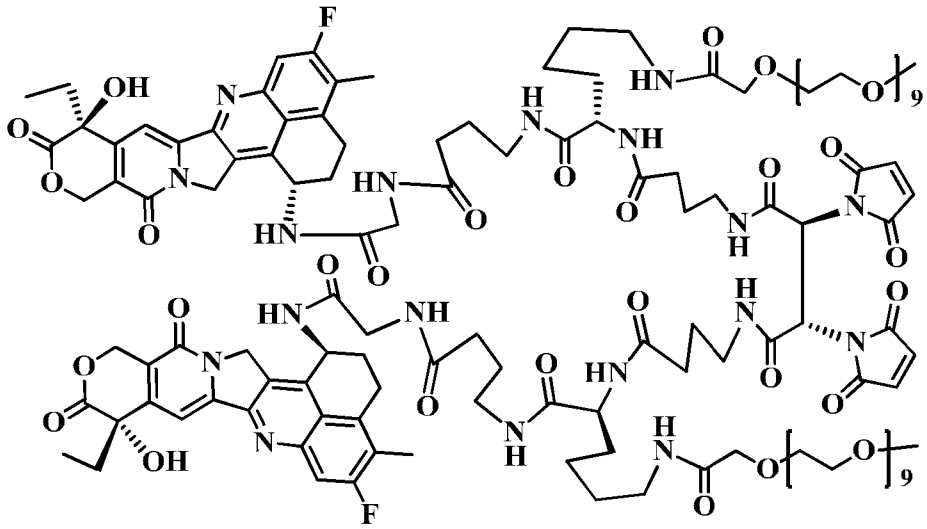


35

Compound **34** (0.50 g, 0.242 mmol) was dissolved in formic acid (10 mL) and dichloromethane (5 mL), and then heated to 60 °C. The reaction was stirred until completion, as indicated by LC-MS and then concentrated to give the title compound (470 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{86}H_{147}N_{12}O_{38}$, 1955.99; found, 1955.99.

Example 31. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4 -bis((S)-37-((4-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-4-

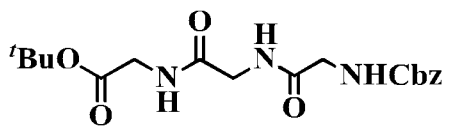
oxobutyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**36**).



36

Compound **35** (200 mg, 0.102 mmol) and exatecan mesylate (108 mg, 0.204 mmol) were dissolved in DMF (5 mL), HATU (116 mg, 0.306 mmol) and diisopropylethylamine (53 μL, 0.306 mmol) were added. The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give the title compound (120 mg, 42% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{130}H_{179}F_2N_{18}O_4$, 2734.22; found, 2734.22.

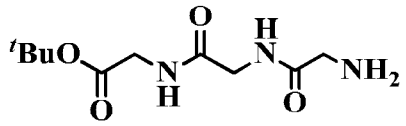
Example 32. Synthesis of tert-butyl ((benzyloxy)carbonyl)glycylglycylglycinate (**38**).



38

Cbz-Gly-Gly-OH (20.3 g, 76.2 mmol) and H-Gly-O^{t}Bu (10.0 g, 76.2 mmol) were dissolved in dichloromethane (300 mL) and cooled to 0°C in an ice-water bath. After addition of HATU (34.8 g, 91.5 mmol) and triethylamine (32 mL, 228.7 mmol), the reaction was warmed to r.t. and stirred overnight. The reaction solution was washed with brine, dried, concentrated, and purified by column chromatography (dichloromethane: MeOH = 20:1) to give 25 g of the desired product with a yield of 86%. MS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{25}N_3O_6$, 380.17; found, 379.9.

Example 33. Synthesis of tert-butyl glycylglycylglycinate (**39**).

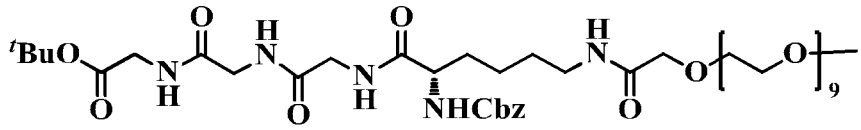


39

Compound **38** (16.5 g, 43.5 mmol) was dissolved in THF (300 mL), 10% palladium on carbon (2.0 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 4 hours, the reaction mixture was filtered and the filtrate was concentrated to give

an off-white solid (10.2 g, 95% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{10}H_{19}N_3O_4$, 246.14; found, 246.14.

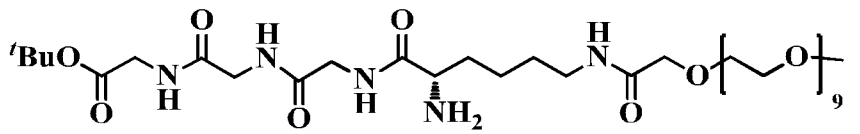
Example 34. Synthesis of tert-butyl (S)-(S)-(S)-(37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycylglycinate (**40**).



40

To a solution of compound **20** (5.0 g, 6.78 mmol) and compound **39** (1.67 g, 6.78 mmol) in THF (50 mL), HATU (3.81 g, 10.01 mmol) and diisopropylethylamine (1.8 ml, 13.35 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was poured into water (100 mL), extracted with dichloromethane (3×50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **40** (4.3 g, 65% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{47}H_{77}N_5O_{18}$, 976.53; found 976.53.

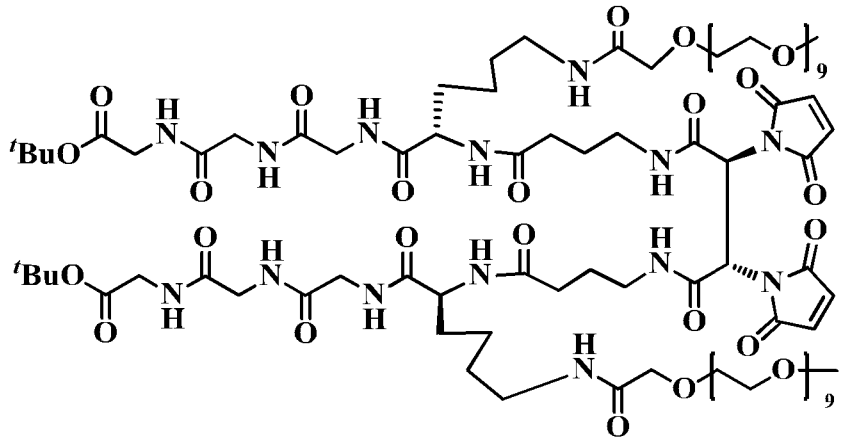
Example 35. Synthesis of tert-butyl (S)-(S)-(S)-(37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29 -decaoxa-32-azaoctatriacontan-38-oyl)glycylglycylglycinate (**41**).



41

Compound **40** (1.5 g, 1.53 mmol) was dissolved in THF (20 mL), 10% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **41** (1.3 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{37}H_{71}N_5O_{16}$, 842.49; found, 842.49.

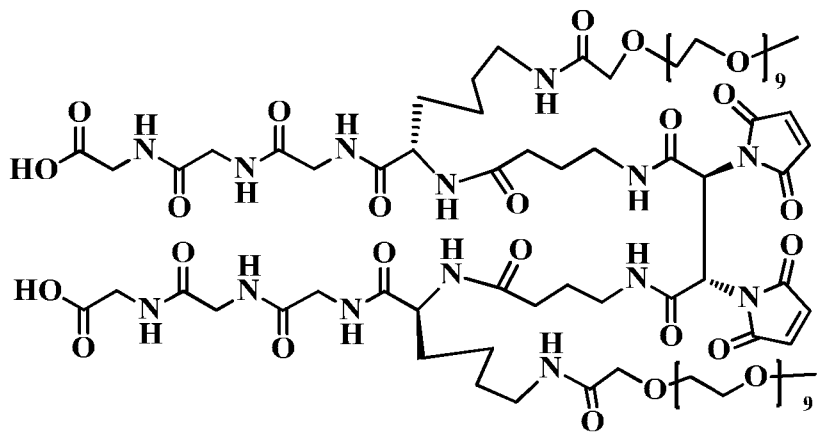
Example 36. Synthesis of di-tert-butyl (11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioate (**42**).



42

Compound **20** (911 mg, 1.08 mmol) and compound **41** (225 mg, 0.470 mmol) were dissolved in DMF (5 mL), HATU (536 mg, 1.411 mmol) and diisopropylethylamine (0.233 mL, 1.411 mmol) were added. The reaction mixture was stirred for 30 minutes, and then poured into 50 mL of water, extracted with 40 mL of dichloromethane for 3 times. The organic phase was washed with 20 mL of brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column to give compound **42** as an oil (671 mg, 0.316 mmol, 67%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{94}H_{160}N_{14}O_{40}$, 2127.37; found, 2128.16.

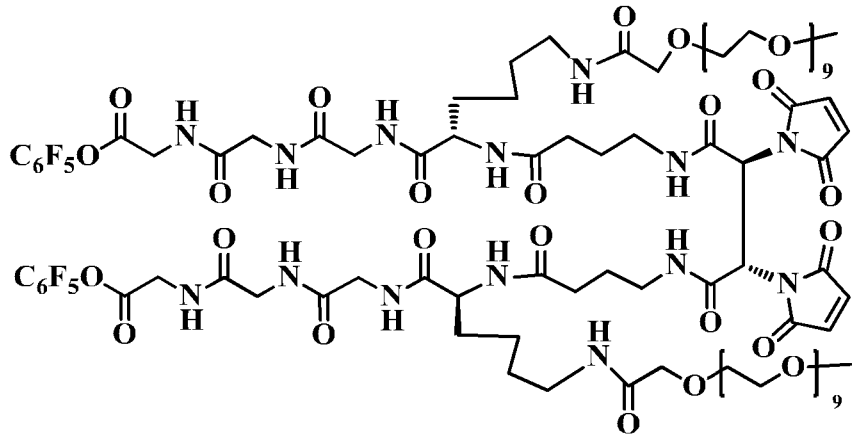
Example 37. Synthesis of (11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol -1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioic acid (**43**).



43

Compound **42** (671 mg, 0.316 mmol) was dissolved in dichloromethane (4 mL) and treated with trifluoroacetic acid (2 mL). After stirring for 8 hours, the reaction solution was concentrated, to give compound **43** as an oil (456 mg, 0.226 mmol, 71%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{86}H_{144}N_{14}O_{40}$, 2015.16; found, 2016.09.

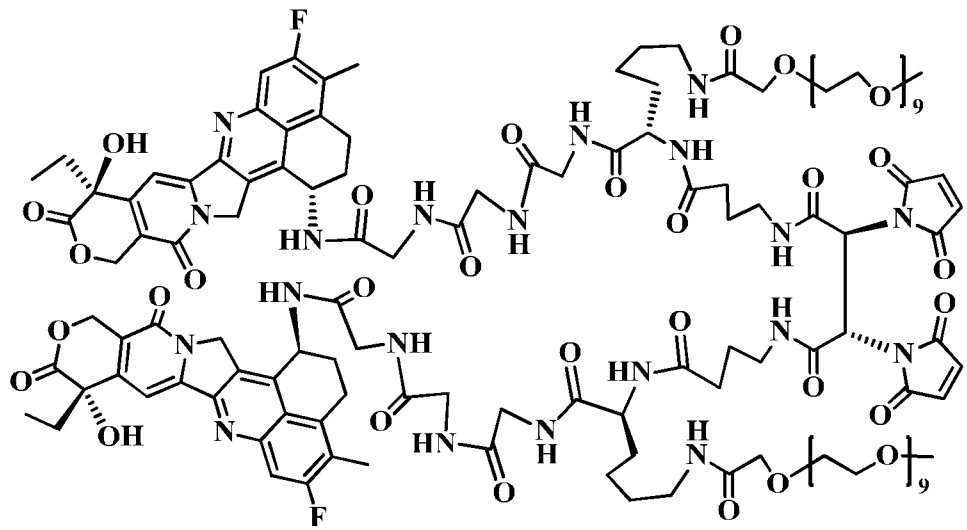
Example 38. Synthesis of bis(perfluorophenyl) (11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioate (**44**).



44

Compound **43** (323 mg, 0.160 mmol) was dissolved in dichloromethane (10 mL), and pentafluorophenol (73.8 mg, 0.401 mmol) and EDCI (76.8 mg, 0.401 mmol) were added. After stirring for 3 hours, the reaction solution was washed with 10 mL of brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **44** as an oil (376 mg, 0.160 mmol, 99%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{98}H_{142}F_{10}N_{14}O_{40}$, 2347.26; found, 2348.26.

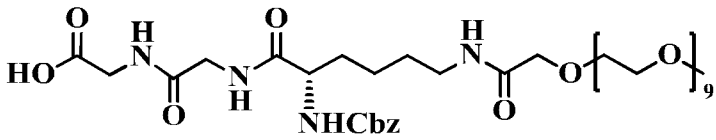
Example 39. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-((2-((2-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**45**).



45

Compound **44** (188 mg, 0.080 mmol) and exatecan mesylate (87 mg, 0.200 mmol) were dissolved in DMF (2 mL), and diisopropylethylamine (0.053 mL, 0.321 mmol) was added. The reaction was stirred for 1 hour, and purified by preparative HPLC (acetonitrile/water) to give compound **45** as a solid (48 mg, 0.017 mmol, 21%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{134}H_{184}F_2N_{20}O_{46}$, 2850.04; found, 1426.00.

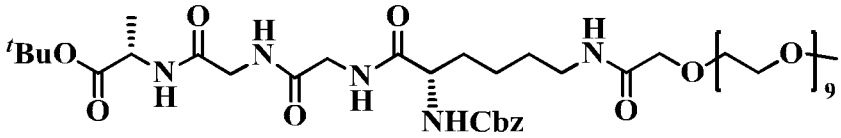
Example 40. Synthesis of (S)-(S)-(37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycine (**46**).



46

Compound **26** (12.0 g, 13.06 mmol) was dissolved in dichloromethane (20 mL) and formic acid (40 mL). After stirring for 3 hours, the reaction solution was concentrated, diluted with dichloromethane, washed with water twice, and brine once. The solution was dried over anhydrous sodium sulfate, filtered and concentrated to give compound **46** (10.4 g, 92% yield).

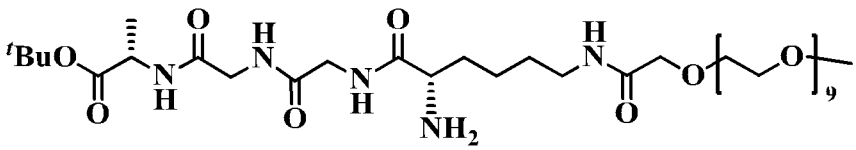
Example 41. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycyl-L-alaninate (**47**).



47

To a solution of compound **46** (2.10 g, 2.43 mmol) in 30 mL of dichloromethane, H-Ala-O^{t}Bu (0.53 g, 2.92 mmol), HATU (1.41 g, 3.65 mmol) and diisopropylethylamine (0.63 g, 4.87 mmol) were added in sequence, and the reaction was carried out at r.t. for 30 min. Water was added to the reaction solution and the layers were separated. The organic phase was washed with 0.5 N HCl, 0.5 N $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated. The residue was purified by preparative HPLC (water/ acetonitrile), and the proper fractions were concentrated to give the title compound (1.4 g, 58% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{46}H_{79}N_5O_{18}$, 991.54; found, 991.20.

Example 42. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycyl-L-alaninate (**48**).

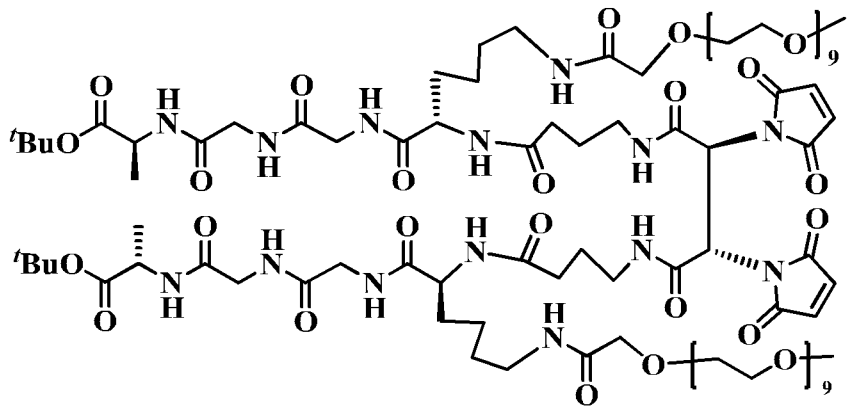


48

Compound **47** (1.4 g, 1.40 mmol) was dissolved in THF (20 mL), 10% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **48** (1.2 g, 99% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{38}H_{73}N_5O_{16}$, 856.51; found, 857.00.

Example 43. Synthesis of di-tert-butyl (2S,11S,19S,20S,28S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,37-dimethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-

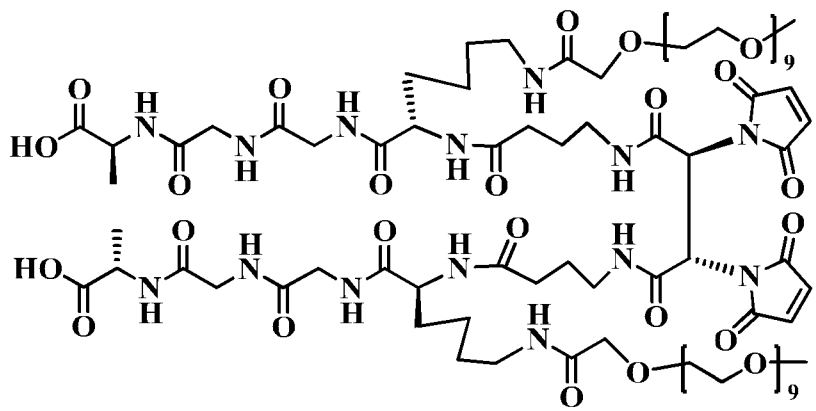
2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioate (**49**).



49

To a solution of compound **19** (0.31g, 0.64 mmol) in 5mL DMF was added compound **48** (1.2 g, 1.4 mmol) in 5 mL of DMF, followed by HATU (0.75 g, 1.93 mmol) and diisopropylethylamine (0.35 g, 2.57 mmol), and the reaction was stirred for 1 h, concentrated, dissolved in dichloromethane, washed with water, 0.5 N HCl, 0.5 $NaHCO_3$, and brine, dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated to yield the title compound (1.1 g, 79% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{96}H_{164}N_{14}O_{40}$, 2154.12; found, 2155.40.

Example 44. Synthesis of (2S,11S,19S,20S,28S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro -1H-pyrrol-1-yl)-2,37-dimethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioic acid (**50**).

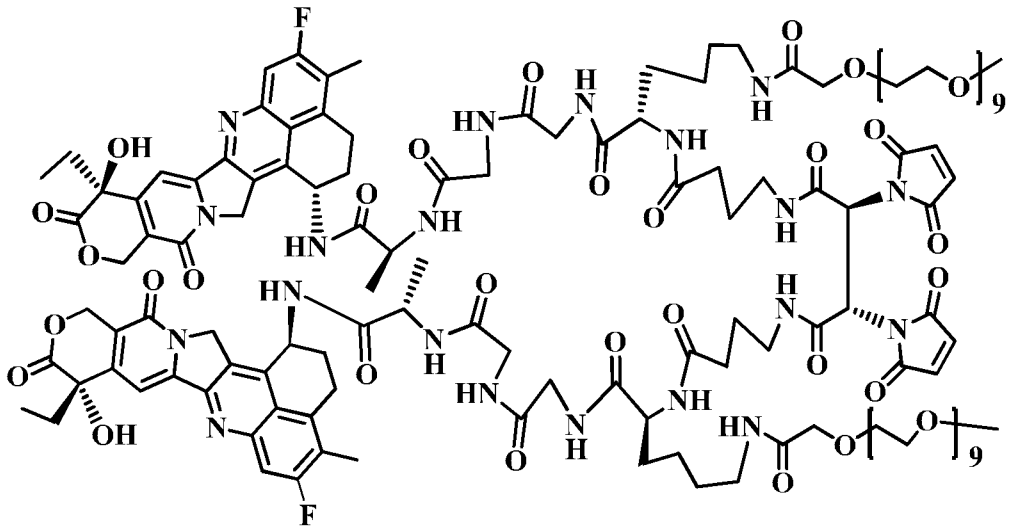


50

Compound **49** (1.1 g, 0.51 mmol) was dissolved in dichloromethane (10 mL) and formic acid (20 mL). After stirring at 50 °C for 2 hours, the reaction solution was concentrated, and the residue was purified by preparative HPLC (water/acetonitrile) to give compound **50** (300 mg, 30% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{88}H_{148}N_{14}O_{40}$, 2042.00; found, 2043.2。

Example 45. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-((2-((2-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-

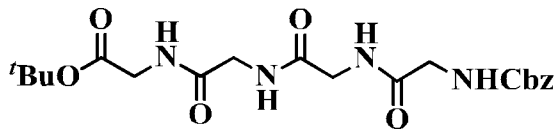
oxopropan-2-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**51**).



51

To a solution of compound **50** (70.0 mg, 0.034 mmol) in 1 mL of DMF, were added exatecan mesylate (32.0 mg, 0.075 mmol) and HATU (39.0 mg, 0.103 mmol), followed by diisopropylethylamine (18 mg, 0.137 mmol). The reaction was stirred at r.t. for 30 min, concentrated, and purified by preparative HPLC (water/acetonitrile). The fraction pool was combined, concentrated and lyophilized to yield the title compound (57.3 mg, yield 58%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{136}H_{188}F_2N_{20}O_{46}$, 2876.30; found, 2878.10.

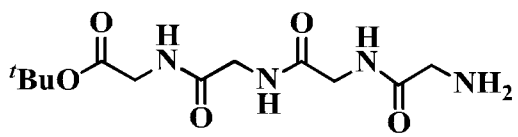
Example 46. Synthesis of tert-butyl ((benzyloxy)carbonyl)glycylglycylglycylglycinate (**52**).



52

To a solution of compound **39** (1.92 g, 7.83 mmol) in 50 mL of dichloromethane, H-Ala-O^{t}Bu (1.96 g, 9.41 mmol), HATU (3.56 g, 9.41 mmol) and diisopropylethylamine (1.22 g, 9.41 mmol) were added in sequence, and the reaction was stirred at r.t. for 30 min. and then quenched with water. After phase separation, the organic phase was washed with 0.5 N HCl, 0.5 N $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated. The residue was purified by column chromatography to give the title compound (2.74 g, 80% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{20}H_{28}N_4O_7$, 437.20; found 437.20.

Example 47. Synthesis of tert-butyl glycylglycylglycylglycinate (**53**).

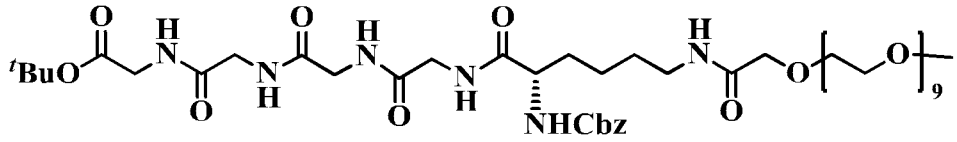


53

Compound **52** (2.74 g, 6.28 mmol) was dissolved in THF (20 mL), 10% palladium on carbon (0.10 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three

times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **53** (1.90 g, 99% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{12}H_{22}N_4O_5$, 303.16; found, 303.18.

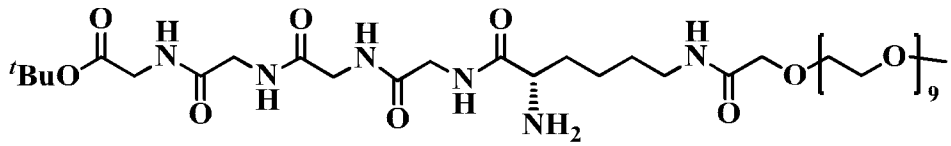
Example 48. Synthesis of tert-butyl (S)-(S)-(S)-(S)-(37-(((benzyloxy)carbonyl)amino)-31 -oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycylglycylglycinate (**54**).



54

To a solution of compound **20** (4.5 g, 6.01 mmol) and H-Gly-Gly-Gly-Gly-OtBu (1.9 g, 6.28 mmol) in THF (20 mL) and DMF (20 mL), HATU (3.43 g, 9.01 mmol) and diisopropylethylamine (1.9 mL, 12.0 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was poured into water (100 mL), extracted with dichloromethane (3×50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give compound **54** (4.9 g, 79% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{47}H_{81}N_6O_{19}$, 1033.55; found, 1033.55.

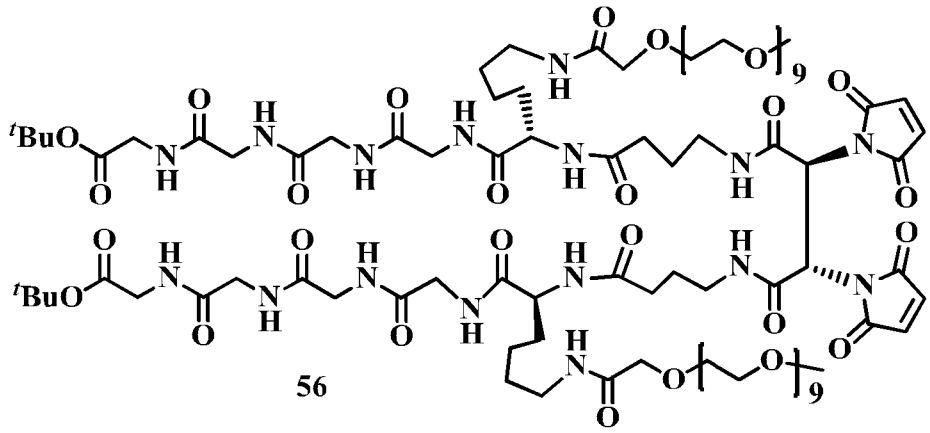
Example 49. Synthesis of tert-butyl (S)-(S)-(S)-(S)-(37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycylglycylglycinate (**55**).



55

Compound **54** (4.9 g, 4.7 mmol) was dissolved in THF (80 mL), 10% palladium on carbon (0.5 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (700 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{39}H_{75}N_6O_{17}$, 899.51; found, 899.51.

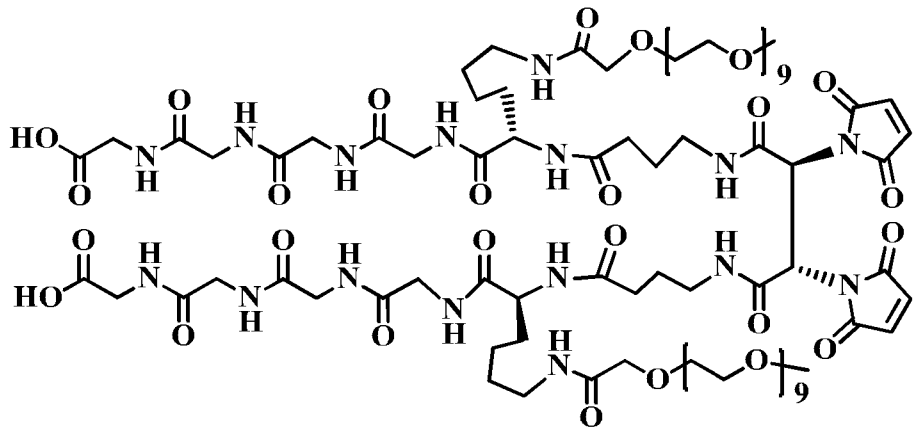
Example 50. Synthesis of di-tert-butyl (14S,22S,23S,31S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioate (**56**).



56

To a solution of compound **19** (1.0 g, 2.09 mmol) and compound **55** (3.7 g, 4.1 mmol) in a mixed solvent of THF (30 mL) and DMF (15 mL), HATU (2.38 mg, 6.27 mmol) and diisopropylethylamine (1.4 mL, 8.36 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was dissolved in dichloromethane (300 mL), washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated, purified by silica gel column (dichloromethane/MeOH=100/0 to 20/1 to 10/1), to give the title compound (3.2 g, 68% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{98}H_{167}N_{16}O_{42}$, 2240.13; found, 2240.13.

Example 51. Synthesis of (14S,22S,23S,31S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H- pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioic acid (**57**).

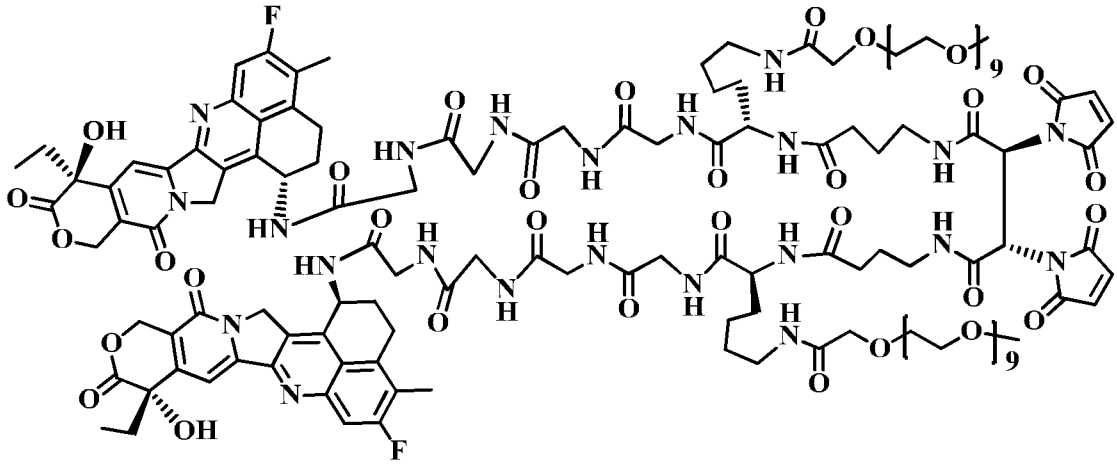


57

Compound **56** (3.2 g, 1.4 mmol) was dissolved in formic acid (20 mL) and dichloromethane (10 mL), and then heated to 60 °C. The reaction was stirred until completion, as indicated by LC-MS and then concentrated to give the title compound (690 mg, 22% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{90}H_{151}N_{16}O_{42}$, 2128.01; found, 2128.01.

Example 52. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4-bis((S)-37-((2-((2-((2-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-

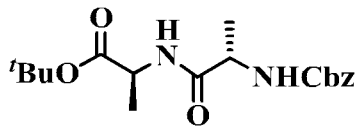
1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**58a**).



58a

Compound **57** (420 mg, 0.197 mmol) and exatecan mesylate (209 mg 0.395 mmol) were dissolved in DMF (5 mL), HATU (255 mg, 0.592 mmol) and diisopropylethylamine (130 μL, 0.789 mmol) were added. The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give the title compound (274 mg, 47% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{138}H_{191}F_2N_{22}O_{48}$, 2962.31; found, 2962.31.

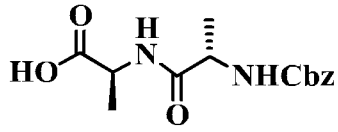
Example 53. Synthesis of tert-butyl ((benzyloxy)carbonyl)-L-alanyl-L-alaninate (**59**).



59

Cbz-Ala-OH (15.0 g, 67.1 mmol) and H-Ala-O^{t}Bu HCl (12.3 g, 67.7 mmol) were dissolved in dichloromethane (100 mL), cooled to 0 °C and EDC (25.7 g, 134 mmol) was added, followed by diisopropylethylamine (18.0 g, 134 mmol) dropwise. After stirring for about 30 min, the reaction was washed with 100 mL of water, 100 mL of brine, dried over anhydrous sodium sulfate, filtered, concentrated, purified by a silica gel column, eluted with petroleum ether and ethyl acetate to give a colorless liquid (19.2 g, 81% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{18}H_{27}N_2O_5$, 351.18; found, 351.18

Example 54. Synthesis of ((benzyloxy)carbonyl)-L-alanyl-L-alanine (**60**).

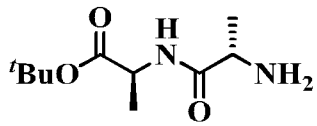


60

Compound **59** (5.0 g, 14.0 mmol) was dissolved in dichloromethane (20 mL) and treated with 20 mL of trifluoroacetic acid at r.t. for 4 h, concentrated to dryness, co-evaporated with 50 mL of dichloromethane, concentrated to dryness, crystallized with ethyl acetate/petroleum ether, filtered,

and dried to give a white solid (3.6 g, 84% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{14}H_{19}N_2O_5$, 295.12; found, 295.12.

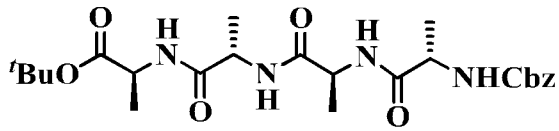
Example 55. Synthesis of tert-butyl L-alanyl-L-alaninate (**61**).



61

Compound **59** (10.0 g, 29.0 mmol) was dissolved in THF (80 mL), and 10% Pd/C (1.1 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 4 h, and at 45-50 °C for 2 h, then filtered and concentrated to dryness. 2M HCl in ethyl acetate was added and the solution was evaporated to dryness, to give compound **61** as a white solid (5.8 g, 80% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{10}H_{21}N_2O_3$, 217.15; found, 217.15.

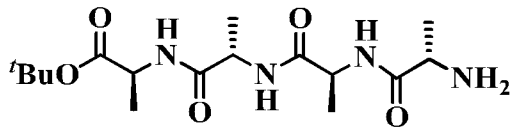
Example 56. Synthesis of tert-butyl ((benzyloxy)carbonyl)-L-alanyl-L-alanyl-L-alanyl-L-alaninate (**62**).



62

Compound **60** (3.0 g, 10.2 mmol) and compound **61** (3.0 g, 13.8 mmol) were dissolved in dichloromethane (50 mL), to which EDC (3.91 g, 20.4 mmol) and diisopropylethylamine (2.67 g, 20.4 mmol) were added, the reaction was stirred at 0°C for 1 h, filtered, and concentrated to give a gray solid (5.0 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{24}H_{37}N_4O_7$, 493.26; found, 493.26.

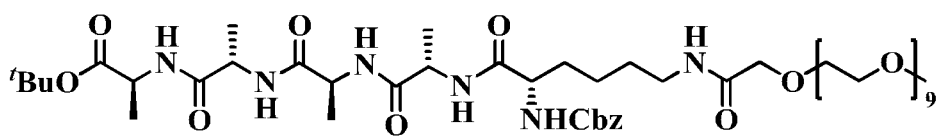
Example 57. Synthesis of tert-butyl L-alanyl-L-alanyl-L-alanyl-L-alaninate (**63**).



63

The crude compound **62** (5.0 g, 10.0 mmol) was dissolved in methanol (160 mL), and 10% Pd/C (1.1 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 1 h, filtered, and concentrated to dryness to give an oil (3.0 g, 82% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{16}H_{31}N_4O_5$, 359.22; found, 359.22.

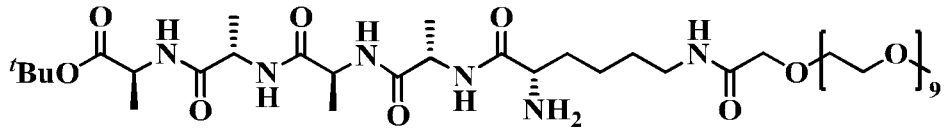
Example 58. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl-L-alanyl-L-alanyl-L-alaninate (**64**).



64

Compound **63** (1.8 g, 2.404 mmol) and compound **20** (1.0 g, 2.874 mmol) were dissolved in THF (30 mL), HATU (1.36 g, 3.592 mmol) and diisopropylethylamine (0.7 g, 4.789 mmol) were added. The reaction was stirred at r.t. for 1h, concentrated, diluted with 20 mL of water and 25 mL of dichloromethane, the separated organic phase was washed with 5% Na_2CO_3, 1M HCl, dried over anhydrous sodium sulfate, concentrated to dryness and purified by preparative HPLC to give a colorless liquid (1.5g, 57% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{51}H_{89}N_6O_{19}$, 1089.61; found, 1089.61.

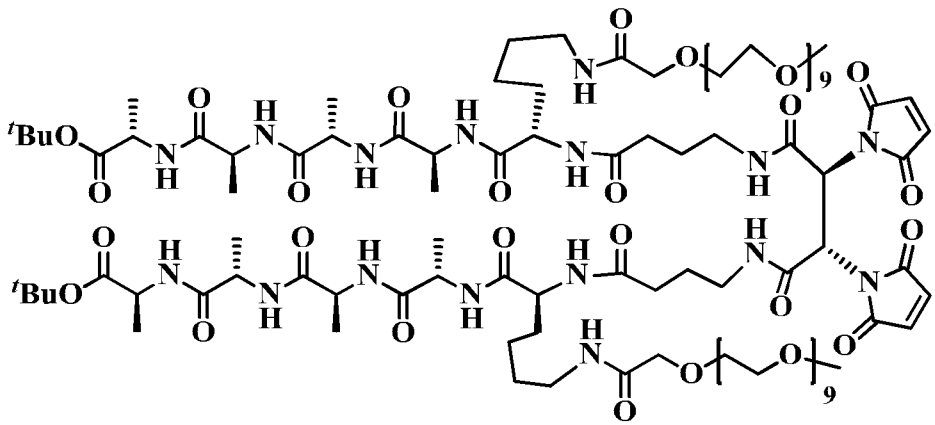
Example 59. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl-L-alanyl-L-alanyl-L-alaninate (**65**).



65

Compound **64** (1.0 g, 0.918 mmol) was dissolved in methanol (100 mL), 10% Pd/C (0.23 g) was added and the reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 2 h, filtered, and concentrated to dryness to give an oil 10 (0.9 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{43}H_{83}N_6O_{17}$, 955.57; found, 955.57.

Example 60. Synthesis of di-tert-butyl (2S,5S,8S,11S,14S,22S,23S,31S,34S,37S,40S,43S) - 22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,11,34,37,40,43-octamethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioate (**66**).

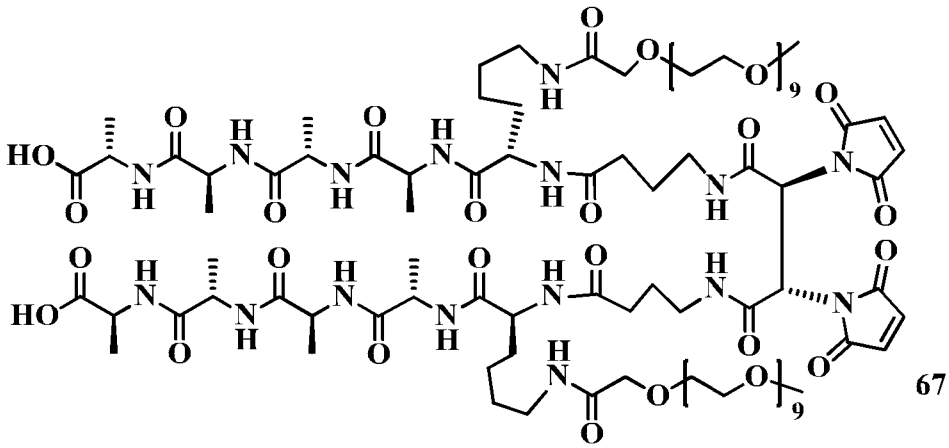


66

Compound **19** (204.9 mg, 0.428 mmol) and compound **65** (880 mg, 0.921 mmol) were dissolved in THF (10 mL) and DMF (10 mL), HATU (485 mg, 1.27 mmol) and diisopropylethylamine (216.5 mg, 1.27 mmol) were added, the reaction was stirred at r.t. for about 30 min, concentrated, diluted with dichloromethane (40 mL) and washed with 30 mL of brine. The aqueous phase was extracted twice with 100 mL of dichloromethane, the organic phases were combined, dried over anhydrous sodium sulfate, filtered, concentrated to dryness, to give a colorless

oil. (985 mg, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{106}H_{183}N_{16}O_{42}$, 2352.26; found, 2352.26.

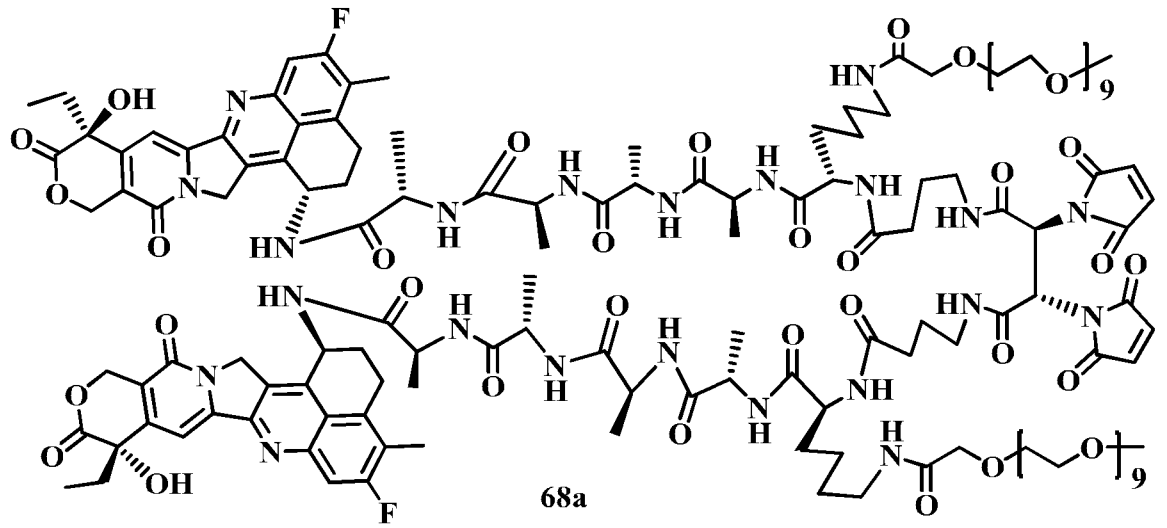
Example 61. Synthesis of (2S,5S,8S,11S,14S,22S,23S,31S,34S,37S,40S,43S)-22,23 -bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,11,34,37,40,43-octamethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioic acid (**67**).



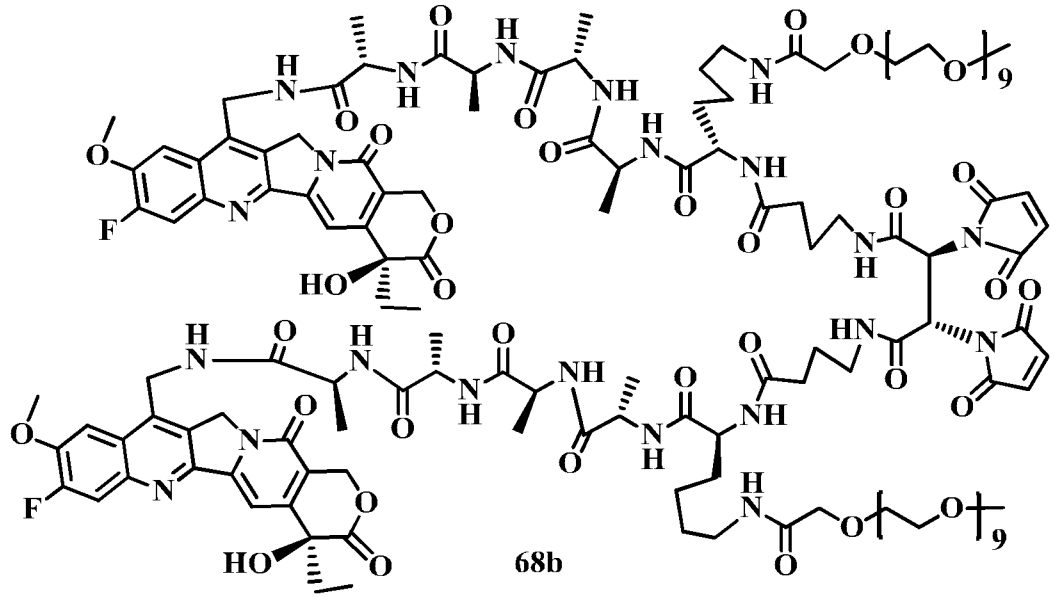
67

Compound **66** (985 mg, 0.419 mmol) was dissolved in dichloromethane (10 mL) and 20 mL of formic acid, reacted at 55-60 °C for 3 h, concentrated to dryness, and purified by preparative HPLC to give a colorless oil (0.6 g, 64% yield). MS-ESI (m/z): [M + H]+ calcd for $C_{98}H_{167}N_{16}O_{42}$, 2240.13; found, 2240.13.

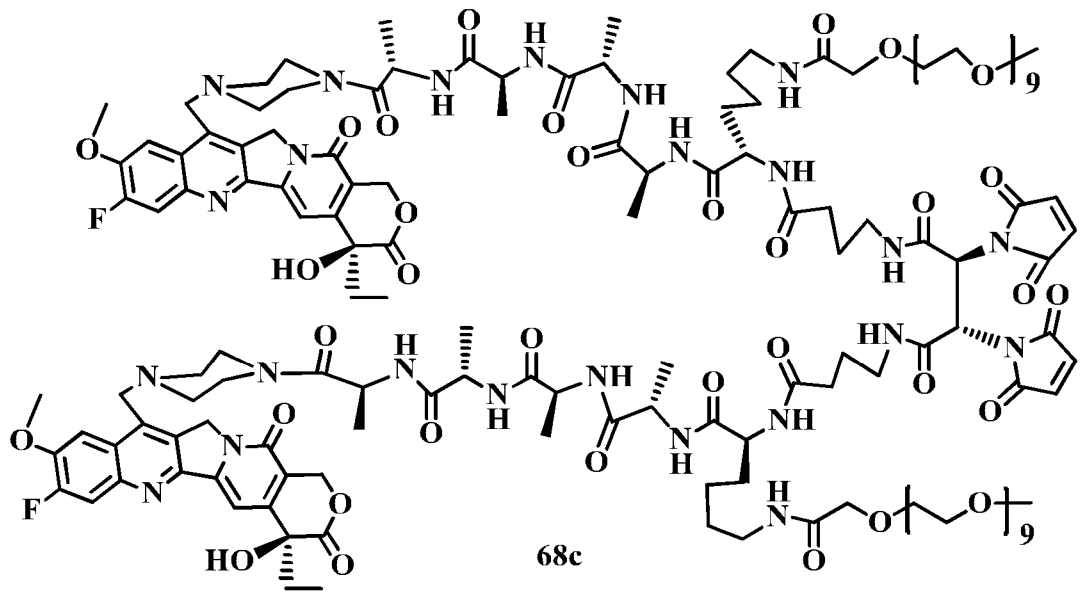
Example 62. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)- N1,N4-bis((S)-37-(((S)-1-(((S)-1-(((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**68a**); (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4-bis((S)-37- (((4S,7S,10S,13S)-1-((S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)-4,7,10-trimethyl-3,6,9,12-tetraoxo-2,5,8,11-tetraazatetradecan-13-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**68b**);. and (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-(((S)-1-(((S)-1-(((S)-1-(((S)-1-(4-(((S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl)piperazin-1-yl)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**68c**).



68a ,



68b ,

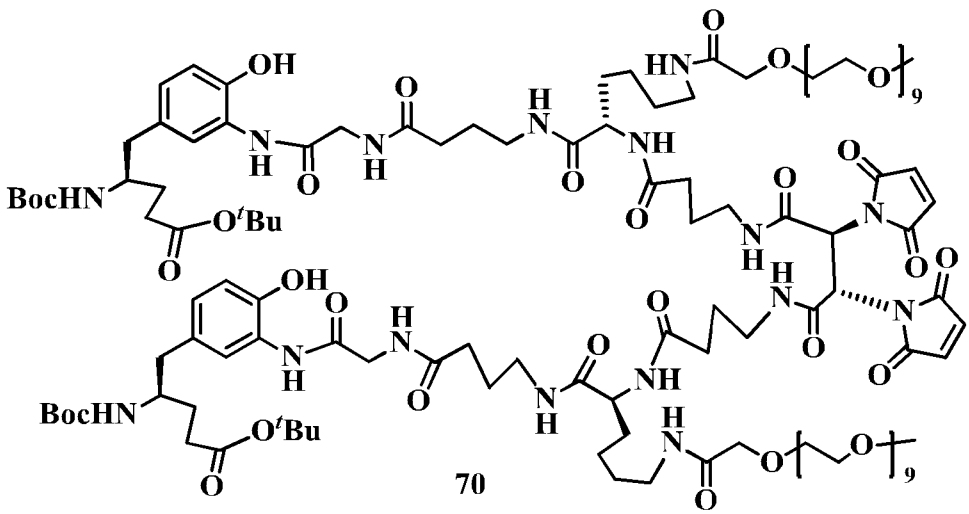


68c ,

To a solution of compound **67** (202.2 mg, 0.090 mmol) HATU (110.3 mg, 0.290 mmol) and diisopropylethylamine (46.5 mg, 0.360 mmol) in DMF (10 mL) were added respectively exatecan

mesylate (98.5 mg, 0.185 mmol), (S)-11-(aminomethyl)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy- 1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione, HCl salt (86.2 mg, 0.187 mmol) or (S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-11-(piperazin-1-ylmethyl)-1H-pyrano [3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione, HCl salt (98.8 mg, 0.186 mmol). The reactions were stirred at r.t. for about 3 h, then concentrated, and purified by preparative HPLC, and lyophilized to give respectively compound **68a** as a light yellow solid (189.1 mg, 67% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{146}H_{207}F_2N_{22}O_{48}$, 3074.73; found, 3074.73; **68b** as a light yellow solid (192.2 mg, 70% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{142}H_{203}F_2N_{22}O_{50}$, 3054.40; found, 3054.90; or **68c** as a light yellow solid (205.8 mg, 71% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{150}H_{217}F_2N_{24}O_{50}$, 3192.51; found, 3192.95;.

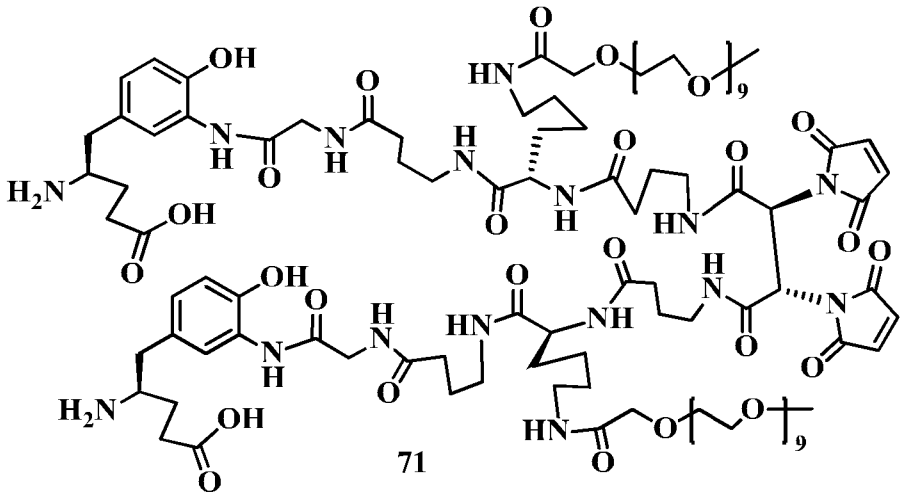
Example 63. Synthesis of di-tert-butyl 5,5'-((((10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**70**).



Compound **35** (720 mg, 0.368 mmol) and tert-butyl (R)-5-(3-amino-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**69**, 350 mg, 0.920 mmol) were dissolved in dichloromethane (20 mL), to which EDCI (211 mg, 1.10 mmol) was added, and the reaction was stirred for 0.5 hours, and then concentrated to dryness. The crude product was purified by preparative HPLC (57% MeCN in H2O) to give compound **70** as an oil (200 mg, 0.075 mmol, 20.27%). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{126}H_{206}N_{16}O_{46}$, 1342.05; found, 1341.91.

Example 64. Synthesis of (4R,4'R)-5,5'-((((10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-

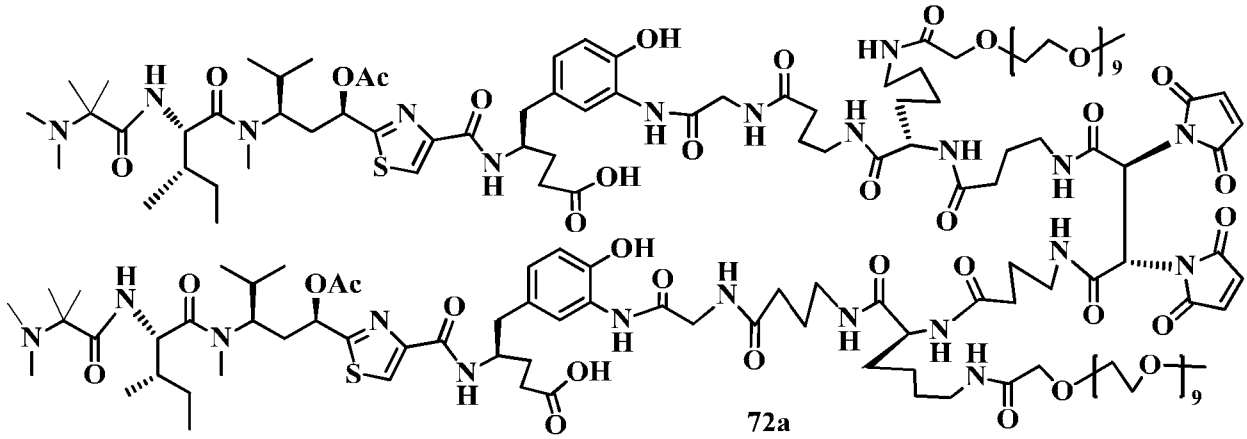
octaazahexatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**71**).



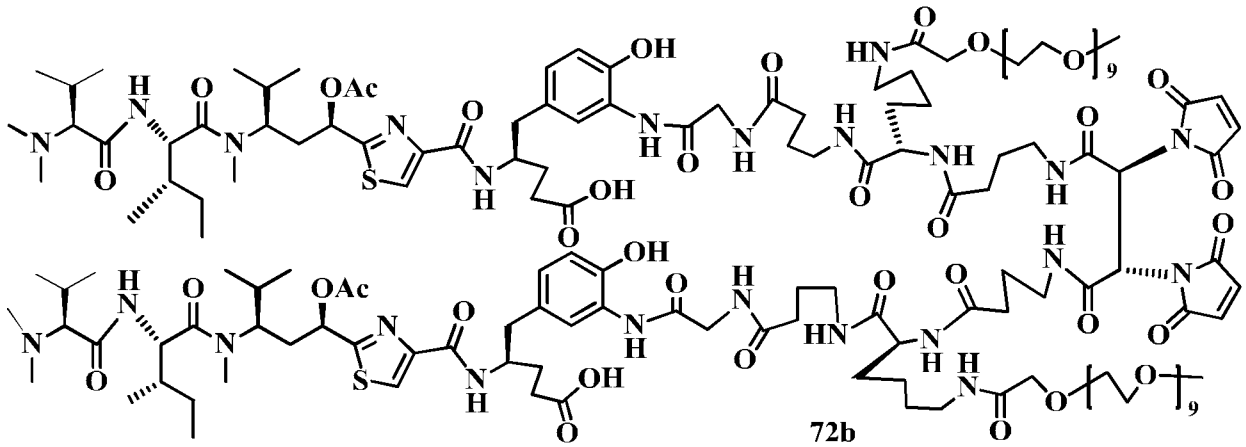
71

Compound **70** (200 mg, 0.075 mmol) was dissolved in dichloromethane (4 mL), trifluoroacetic acid (1 mL) was added, and the reaction was stirred overnight, and then concentrated, co-evaporated with dichloromethane twice, dried on an oil pump to give compound **71** as an oil (176.69 mg, 0.075 mmol, 100.00%) MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{108}H_{174}N_{16}O_{42}$, 1184.60; found, 1185.17.

Example 65. Synthesis of (4R,4'R)-5,5'-((((10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**72a**); and (R,R,S,S,S,4R,4'R)-5,5'-((((10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro -1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontane-1,36-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**72b**).



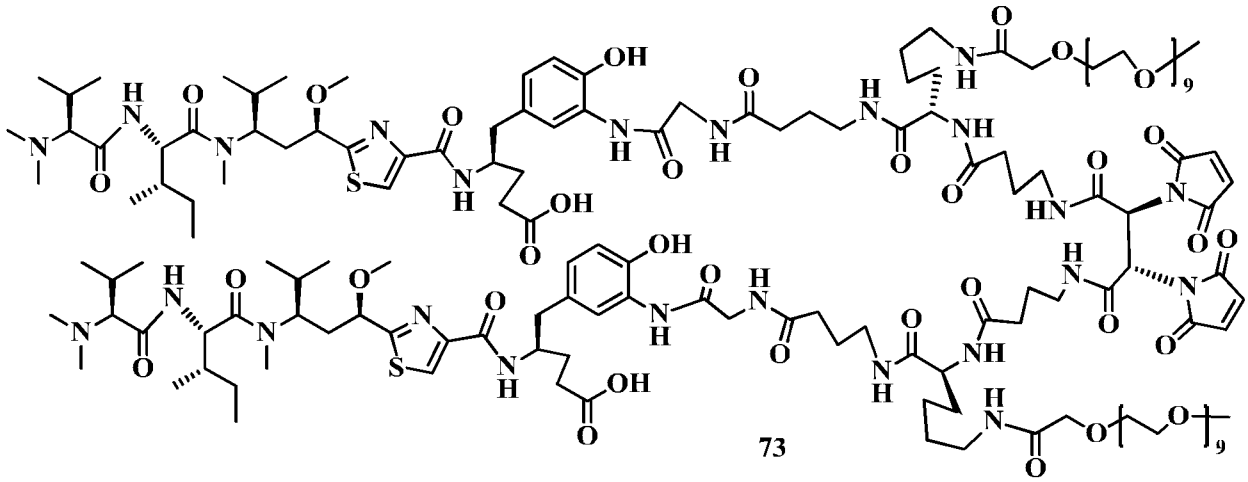
72a



72b

Compound **71** (88 mg, 0.037 mmol) in DMF (1 mL) and diisopropylethylamine (0.025 mL, 0.149 mmol) were added perfluorophenyl 2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl- 2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxylate (**Tub-1**, 64 mg, 0.093 mmol) or perfluorophenyl 2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8- dimethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxylate (**Tub-3**, 67 mg, 0.095 mmol) respectively. The reactions were stirred for 4 hours and purified by preparative HPLC to give compound **72a** as a solid (72 mg, 0.021 mmol, 57%). MS-ESI (m/z): $[M + 3H]^{3+}$ calcd for $C_{158}H_{254}N_{24}O_{52}S_2$, 1128.91; found, 1129.72, or compound **72b** as a solid (69 mg, 0.020 mmol, 54%). MS-ESI (m/z): $[M + 3H]^{3+}$ calcd for $C_{160}H_{261}N_{24}O_{52}S_2$, 1138.26; found, 1139.15.

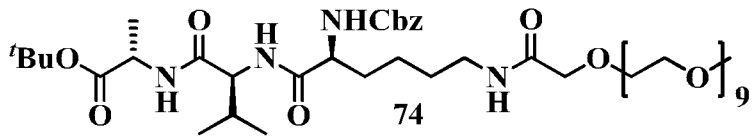
Example 66. Synthesis of (4R,4'R)-5,5'-((((10S,18S,19S,27S)-18,19-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,9,12,17,20,25,28,33-octaoxo-10,27-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,8,11,16,21,26,29,34-octaazahexatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7-dioxo-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**73**).



73

Compound **71** (83.75 mg, 0.035 mmol) and perfluorophenyl 2-((3S,6S,9R,11R)-6-((S) -sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7-dioxo-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxylate (**Tub-2**, 60 mg, 0.088 mmol) were dissolved in DMF (1.5 mL) and diisopropylethylamine (0.023 mL, 0.141 mmol) was added. The reaction was stirred for 2 hours and purified by preparative HPLC to give a solid compound **73** (54 mg, 0.016 mmol, 45%). MS-ESI (m/z): $[M + 3H]^{3+}$ calcd for $C_{158}H_{258}N_{24}O_{50}S_2$, 1120.68; found, 1120.48.

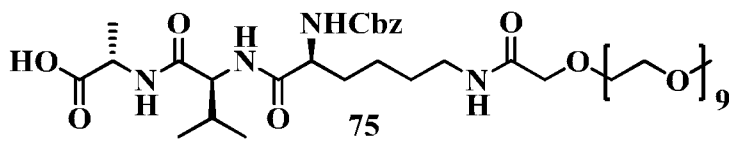
Example 67. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valyl-L-alaninate (**74**).



74

Compound **20** (8.0 g, 10.7 mmol) and H-Val-Ala-O^{t}Bu (2.7 g, 10.7 mmol) were dissolved in dichloromethane (150 mL), HATU (5.4g, 13.9mmol) and diisopropylethylamine (2.8g, 21.4mmol) were added and stirred at r.t. for 3 hours. The reaction solution was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated, and the residue was purified by silica gel column (dichloromethane: methanol = 20:1) to give 9.4 g of the title compound with a yield of 90%. MS m/z: 976.1 $([M + H]^+)$.

Example 68. Synthesis of ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valyl-L-alanine (**75**).

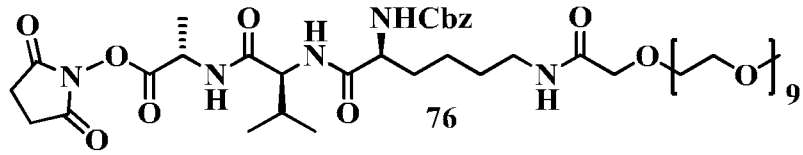


75

Compound **74** (9.4 g, 9.6 mmol) was dissolved in dichloromethane (50 mL), and treated with trifluoroacetic acid (50 mL) at r.t. for 1 hour. The reaction solution was concentrated to remove most of the trifluoroacetic acid, and diluted with 200 mL of dichloromethane, the solution was washed

with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give 8.1 g of the title compound with a yield of 82%. MS m/z: 919.9 ($[M + H]^+$).

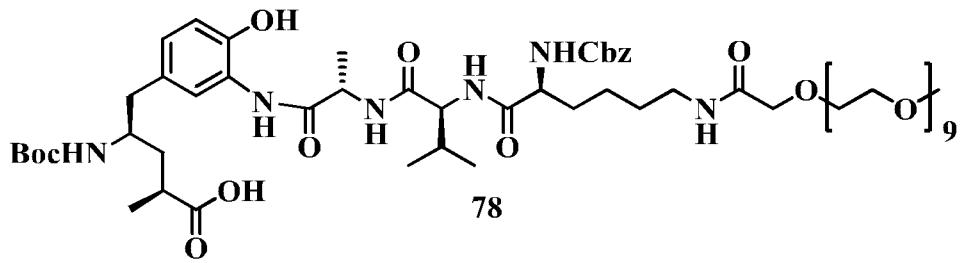
Example 69. Synthesis of 2,5-dioxopyrrolidin-1-yl ((S)-37-(((benzyloxy)carbonyl)amino)-31 -oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valyl-L-alaninate (**76**).



76

Compound **75** (8.1 g, 8.8 mmol) was dissolved in dichloromethane (120 mL), EDCI (6.1 g, 32 mmol) and NHS (2.5 g, 21.3 mmol) were added at 0°C, and the reaction was stirred at 0° for 1 hour, and then washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give 8.1 g of the title compound (91% yield). MS m/z: 1017.1($[M + H]^+$).

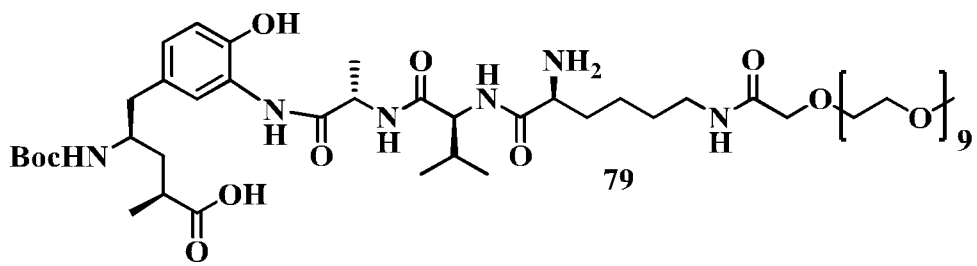
Example 70. Synthesis of (2S,4R)-5-(3-((37S,40S,43S)-37-(((benzyloxy) carbonyl)amino)-40-isopropyl-43-methyl-31,38,41-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**78**).



78

Compound **76** (8.1 g, 7.9 mmol) and (2S,4R)-5-(3-amino-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**77**, 2.7 g. 7.9 mmol) were dissolved in THF (150 mL), heated to 50°C and stirred overnight. The reaction was concentrated and the residue was purified by silica gel column (dichloromethane: methanol = 20:1) to give 8.0 g of the title compound (82% yield). MS m/z: 1240.1($[M + H]^+$).

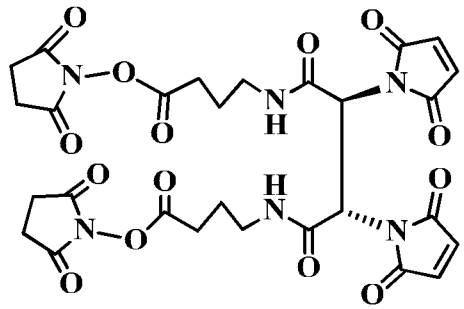
Example 71. Synthesis of (2S,4R)-5-(3-((37S,40S,43S)-37-amino-40-isopropyl-43-methyl-31,38,41-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**79**).



79

Compound **78** (8.0 g, 6.4 mmol) was dissolved in isopropanol (100 mL), palladium on carbon (10 wt%, 2 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times, heated at 50° C for 4 hours. The reaction mixture was filtered, and the filtrate was concentrated to give 6.8 g of the title compound (96% yield). MS m/z: 1106.1([M + H]$^+$).

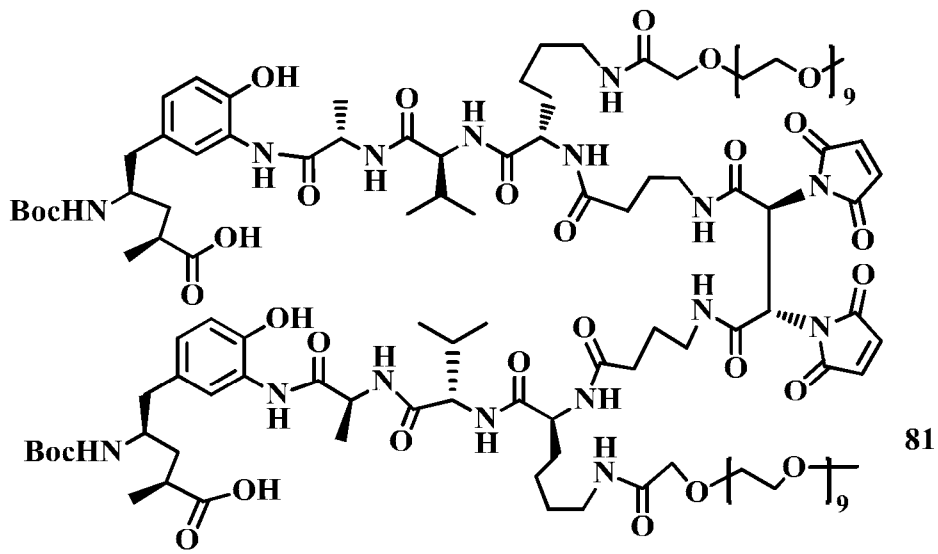
Example 72. Synthesis of bis(2,5-dioxopyrrolidin-1-yl) 4,4'-(((2S,3S)-2,3-bis(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)succinyl)bis(azanediyl))dibutyrate (**80**).



80

Compound **19** (2.40 g, 5.0 mmol) was dissolved in DMF (100 mL), EDCI (2.86 g, 15 mmol) and NHS (1.41 g, 12 mmol) were added at 0°C, and the reaction was stirred at 0° for 1 hour, and then concentrated. The residue was diluted with dichloromethane, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give 3.19 g of the title compound (95% yield).

Example 73. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)-16,17-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis(azanediyl)) bis(4-hydroxy-3,1-phenylene))bis(4-((tert- butoxycarbonyl)amino)-2-methylpentanoic acid) (**81**).



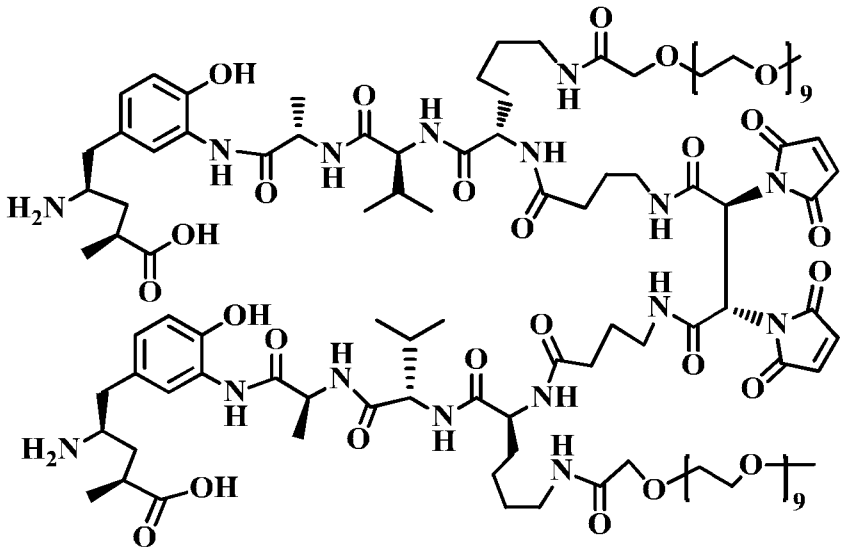
81

Compound **80** (400 mg, 0.6 mmol) and compound **79** (1.3 g, 1.2 mmol) were dissolved in THF (15 mL) and heated to 50°C, stirred overnight. The reaction solution was concentrated and the

residue was purified by preparative HPLC, and 670 mg of the title compound was obtained after lyophilization (42% yield).

MS: m/z=1328.0[1/2M+H+]

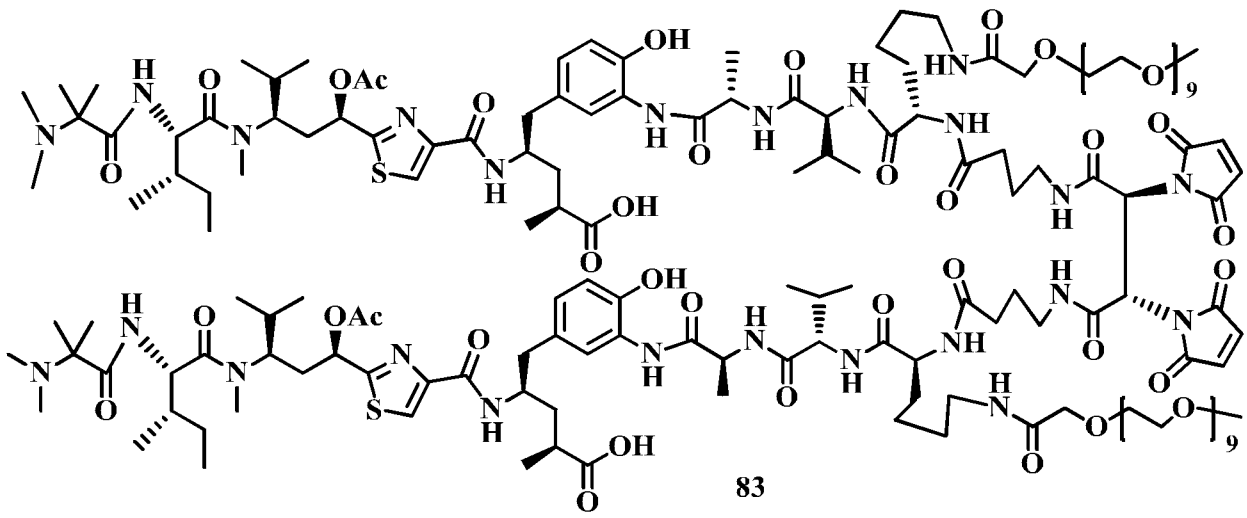
Example 74. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)- 16,17-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis(azanediyl))bis (4-hydroxy-3,1-phenylene))bis(4-amino-2-methylpentanoic acid) (**82**).



82

Compound **81** (670 mg, 0.25 mmol) was dissolved in dichloromethane (8 mL), and stirred with trifluoroacetic acid (4 mL) at r.t. for 1 hour. The reaction was concentrated, the residue was purified by preparative HPLC, and 500 mg of the title compound was obtained after lyophilization (80% yield). MS m/z: 1227.1 ($[M + 2H]^{2+}$).

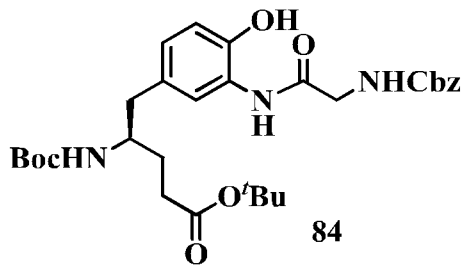
Example 75. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S) -16,17-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis(azanediyl)) bis(4-hydroxy-3,1-phenylene))bis (4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoic acid) (**83**).



83

To a solution of compound **82** (200 mg, 0.08 mmol) and **Tub-1** (120 mg, 0.16 mmol) in DMF (2 mL), diisopropylethylamine (30 mg, 0.24 mmol) was added, and the reaction was stirred at r.t. for 1 hour. The reaction solution was then purified by preparative HPLC, and 50 mg of product was obtained after lyophilization (18% yield). MS m/z: 1736.1([M + 2H]$^{2+}$).

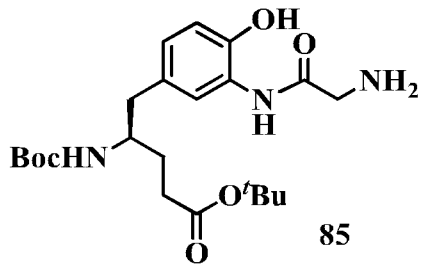
Example 76. Synthesis of tert-butyl (R)-5-(3-(2-(((benzyloxy)carbonyl)amino)acetamido) -4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**84**).



84

Cbz-Gly-OH (2.09 g, 10.0 mmol) was dissolved in dichloromethane (150 mL), compound **69** (4.19 g, 11.0 mmol) and EDCI (3.83 g, 20.0 mmol) were added. The reaction mixture was stirred at r.t. for 3 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated, the crude product was purified by silica gel column (20%-50% EA/PE) to give compound **84** (4.88 g, 85% yield).

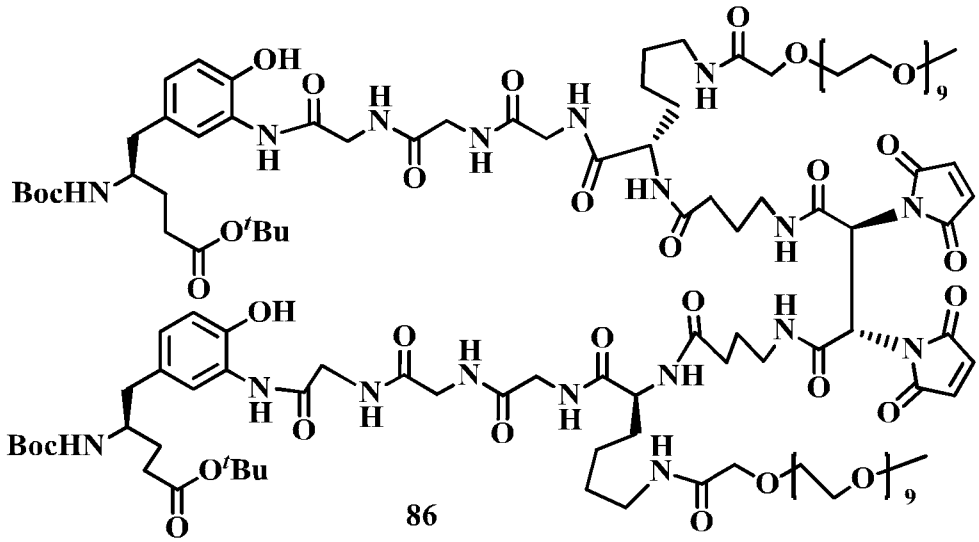
Example 77. Synthesis of tert-butyl (R)-5-(3-(2-aminoacetamido)-4-hydroxyphenyl)-4- ((tert-butoxycarbonyl)amino)pentanoate (**85**).



85

Compound **84** (1.44 g, 2.52 mmol) was dissolved in methanol (40 mL) and Pd/C (10% wet, 0.3 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon for 6 hours, filtered, concentrated, and co-evaporated with 50 mL of dichloromethane, dried on an oil pump to give compound **85** (0.9 g, 82% yield).

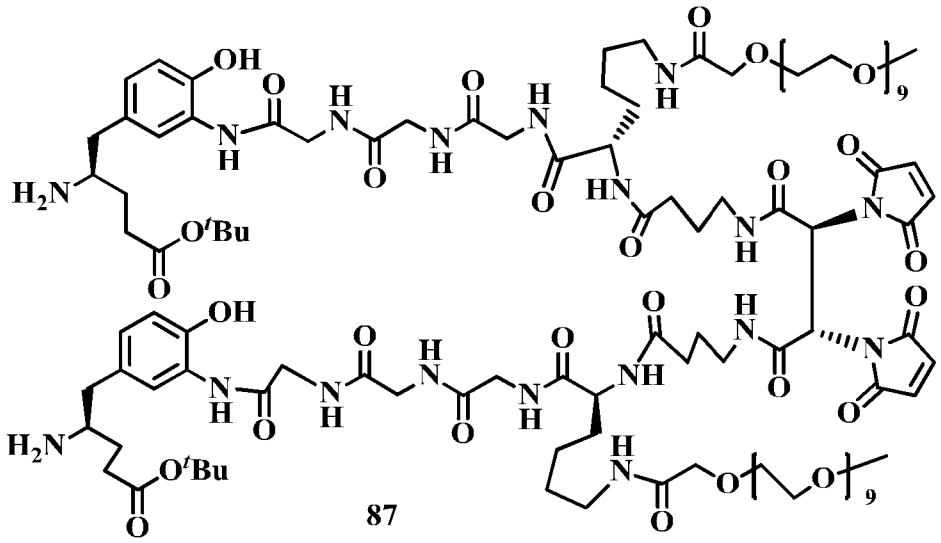
Example 78. Synthesis of di-tert-butyl 5,5'-((((11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**86**).



86

In a 100 mL single-necked reaction flask, compound **29** (840 mg, 0.44 mmol) was dissolved in dichloromethane (50 mL) and the mixture was magnetically stirred at r.t., then compound **85** (391 mg, 0.89 mmol) and EDC (362 mg, 1.89 mmol) were added, and the reaction was carried out at r.t. for 1 hour, then washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by preparative HPLC (acetonitrile/water) to give compound **86** (244 mg, 20% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{126}H_{204}N_{18}O_{48}$, 1370.55; found, 1370.56.

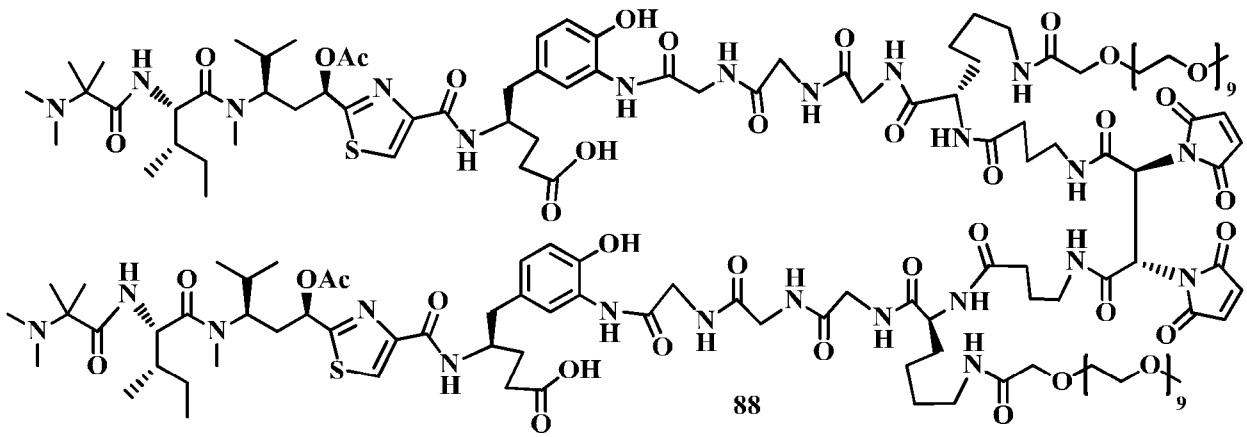
Example 79. Synthesis of (4R,4'R)-5,5'-((((11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**87**).



87

In a 100 mL one-neck reaction flask, compound **86** (244 mg, 0.089 mmol), dichloromethane (8 mL) and trifluoroacetic acid (8 mL) were stirred at r.t. for 1 hour. The reaction mixture was concentrated under reduced pressure, and placed on an oil pump, to give compound **87**, which was used without further purification, assuming 100% yield. MS-ESI (m/z): $[M + H]^+$ calcd for $C_{108}H_{172}N_{18}O_{44}$, 1214.33; found, 1214.02.

Example 80. Synthesis of (4R,4'R)-5,5'-((((11S,19S,20S,28S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**88**).

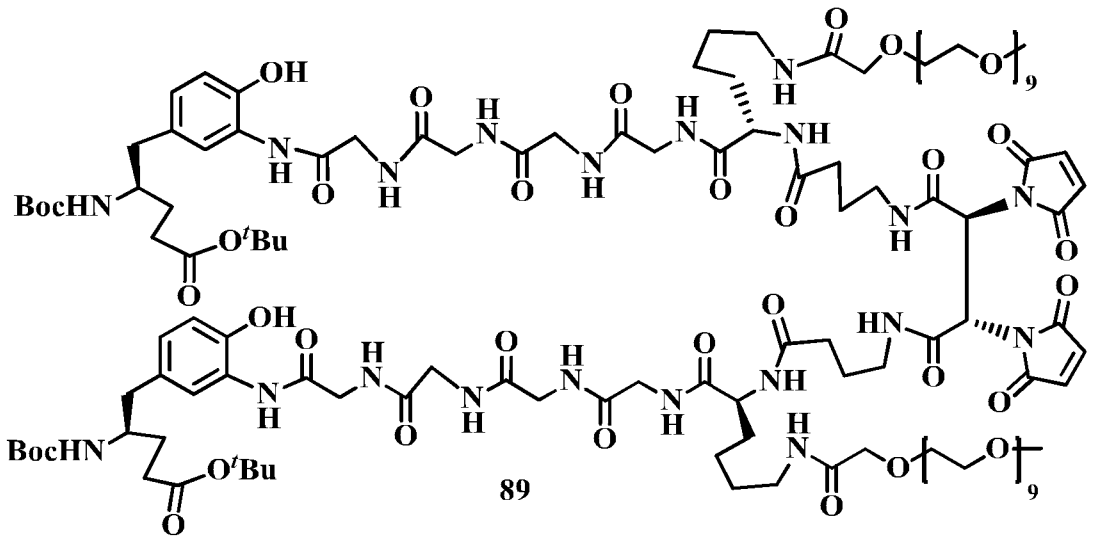


88

In a 100 mL single-necked reaction flask, to a solution of compound **87** (the crude product from previous step, 0.089 mmol) and **Tub-1** (128 mg, 0.185 mmol) in DMF (10 mL), was added dropwise diisopropylethylamine (128 mg, 0.990 mmol). After stirring at r.t. for 5 hours, the reaction was concentrated under reduced pressure. The residue was diluted with 10 mL of dichloromethane, 0.2 mL of formic acid was added dropwise, and the mixture was concentrated, then purified by

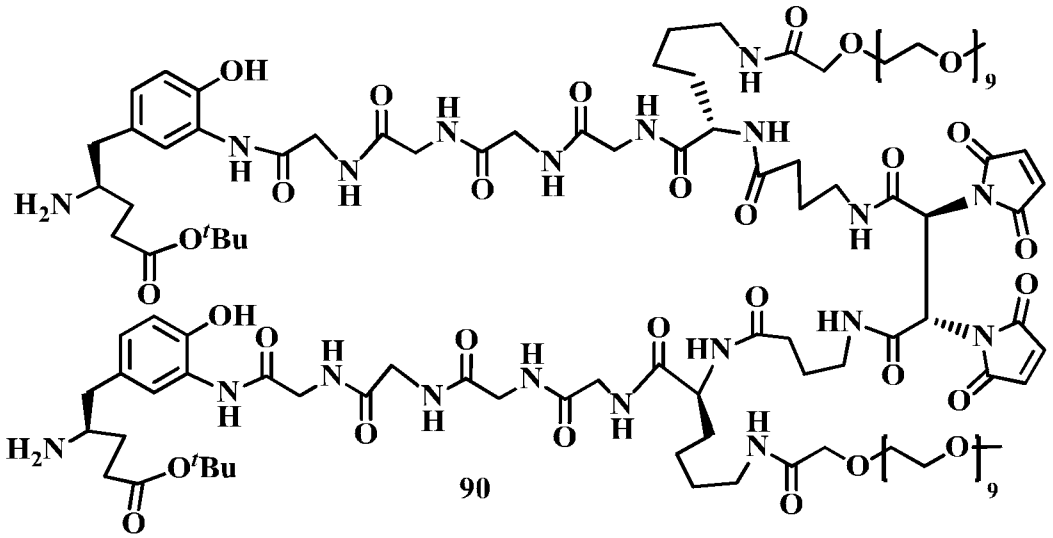
preparative HPLC (acetonitrile/water), to give compound **88** (40 mg, 13% yield). MS-ESI (m/z): [M + 2H]$^{2+}$ calcd for $C_{158}H_{252}N_{26}O_{54}S_2$, 1723.00; found, 1722.84.

Example 81. Synthesis of di-tert-butyl 5,5'-((((14S,22S,23S,31S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**89**).



To a solution of compound **43** (350 mg, 0.17 mmol) and compound **85** (170 mg, 0.38 mmol) in dichloromethane (5 mL), EDCI (100 mg, 0.52 mmol) was added, and the reaction was stirred at r.t. for 2 hours. LCMS as indicated completion of the reaction. And the reaction solution was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% HCOOH) to give 170 mg of the title compound (34% yield).

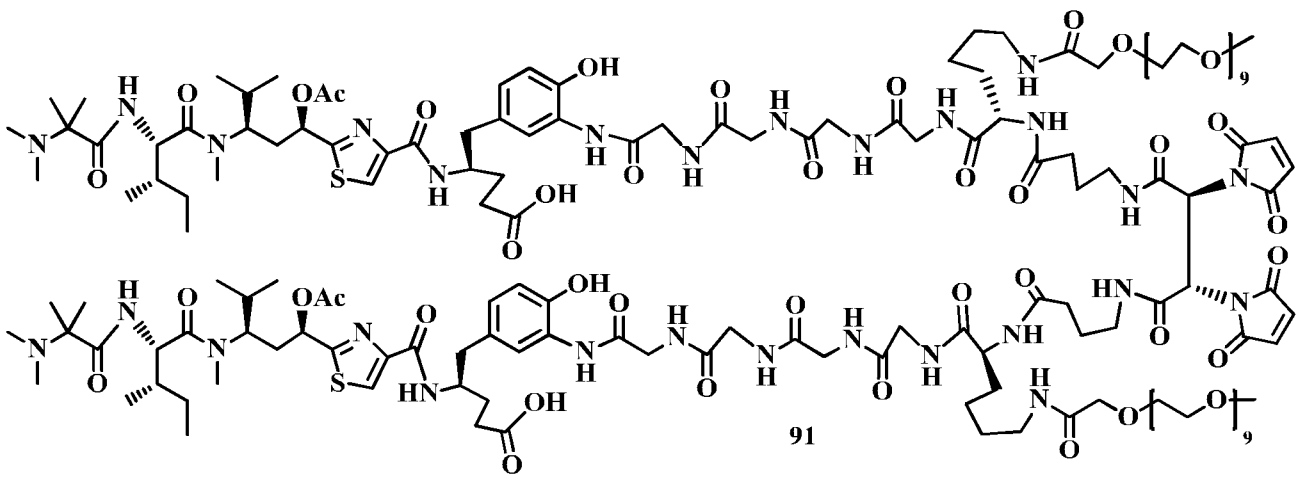
Example 82. Synthesis of (4R,4'R)-5,5'-((((14S,22S,23S,31S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**90**).



90

Compound **89** (170 mg, 0.06 mmol) was dissolved in dichloromethane (4 mL) and reacted with trifluoroacetic acid (2 mL) at r.t. for 2 hours. The reaction mixture was concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% HCOOH) to give 140 mg of the title compound (93% yield). MS m/z: 1271.0 ($[M + 2H]^{2+}$).

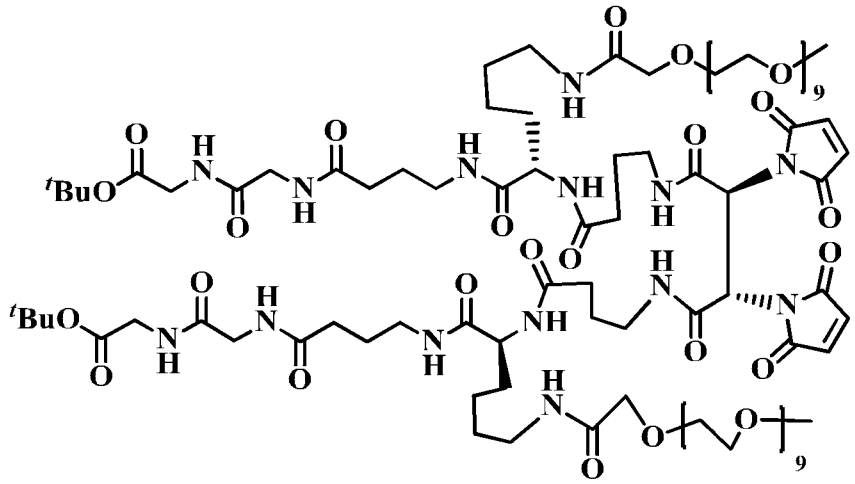
Example 83. Synthesis of (4R,4'R)-5,5'-((((14S,22S,23S,31S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**91**).



91

Compound **90** (140 mg, 0.05 mmol) and **Tub-1** (95 mg, 0.14 mmol) were dissolved in dichloromethane (5 mL), and DIEA (20 mg) was then added to the solution and stirred at r.t. for 1 hour. LCMS as indicated completion of the reaction. And the reaction solution was concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% HCOOH) to give 150 mg of the title compound (75% yield). MS m/z: 1780.0 ($[M + 2H]^{2+}$).

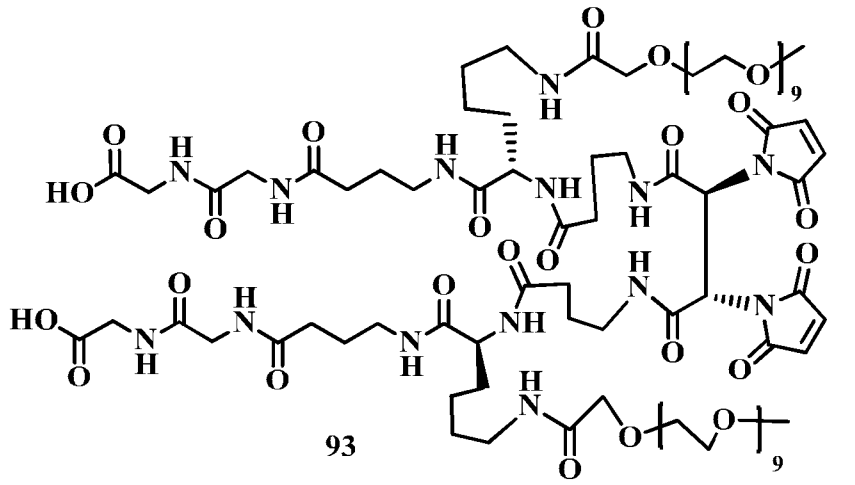
Example 84. Synthesis of di-tert-butyl (13S,21S,22S,30S)-21,22-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,12,15,20,23,28,31,36,39-decaoxo-13,30-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,14,19,24,29,32,37,40-decaazadotetracontanedioate (**92**).



92

To a solution of compound **35** (870 mg, 0.445 mmol) in dichloromethane (30 mL), pentafluorophenol (245 mg, 1.334 mmol) and DIC (224 mg, 1.779 mmol) were added. After stirring for 1 hour, H-Gly-OtBu·HCl (164 mg, 0.978 mmol) and diisopropylethylamine (0.294 mL, 1.779 mmol) were added. The reaction was stirred for 25 minutes, and then washed with 20 mL of brine, dried over anhydrous sodium sulfate and concentrated to give compound **92** as an oil (970 mg, 0.444 mmol, 99%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{98}H_{168}N_{14}O_{40}$, 2183.48; found, 2185.45.

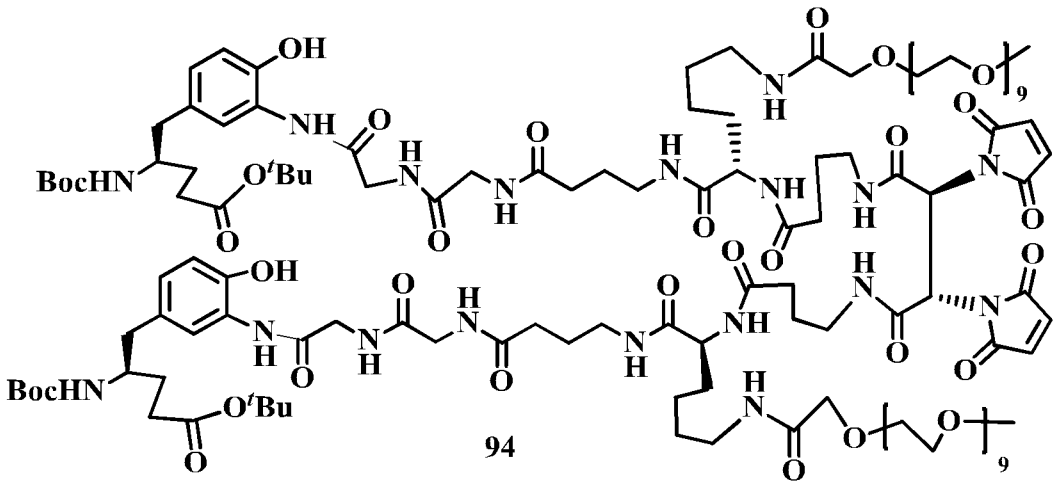
Example 85. Synthesis of (13S,21S,22S,30S)-21,22-bis(2,5-dioxo-2,5-dihydro-1H- pyrrol-1-yl)-4,7,12,15,20,23,28,31,36,39-decaoxo-13,30-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,14,19,24,29,32,37,40-decaazadotetracontanedioic acid (**93**).



93

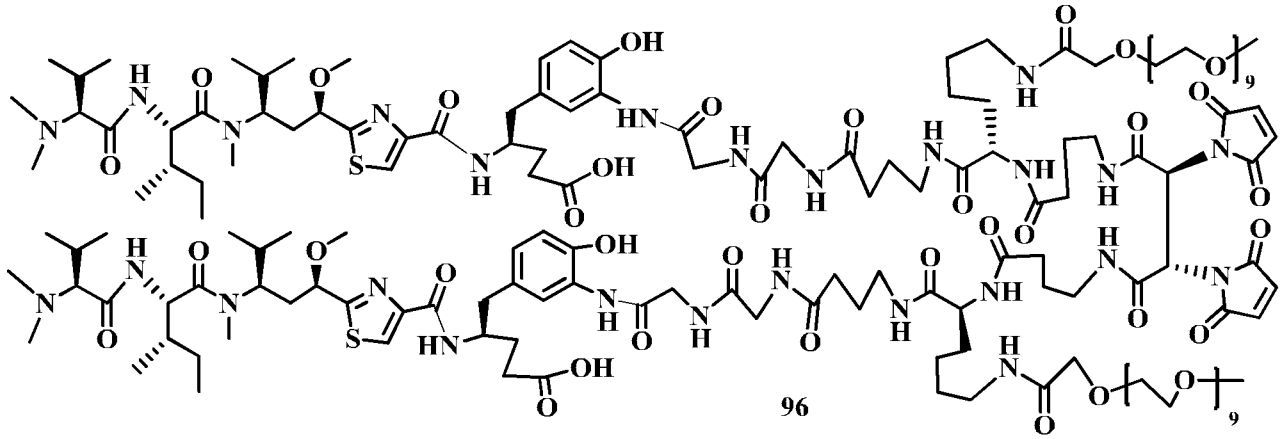
Compound **92** (0.97 g, 0.444 mmol) was dissolved in dichloromethane (10 mL) and treated with trifluoroacetic acid (5 mL). The reaction solution was stirred overnight, concentrated and purified by preparative HPLC to give an oil (636 mg, 0.307 mmol, 69%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{90}H_{152}N_{14}O_{40}$, 2071.26; found, 2071.72.

Example 86. Synthesis of di-tert-butyl 5,5'-((((16S,24S,25S,33S)-24,25-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,15,18,23,26,31,34,39,42,45-dodecaoxo-16,33-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,17,22,27,32,35,40,43,46-dodecaazaoctatetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**94**).


OH
NH
BocHN
O'Bu
O
H
N
O
NH
O
O
O

Compound **94** (263 mg, 0.090 mmol) was dissolved in dichloromethane (12 mL) and treated with trifluoroacetic acid (6 mL). The reaction was stirred for 5 hours, and concentrated to dryness. Compound **95** was obtained as an oil (234 mg, 0.090 mmol, 99%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{116}H_{186}N_{20}O_{46}$, 1299.93; found, 1299.77.

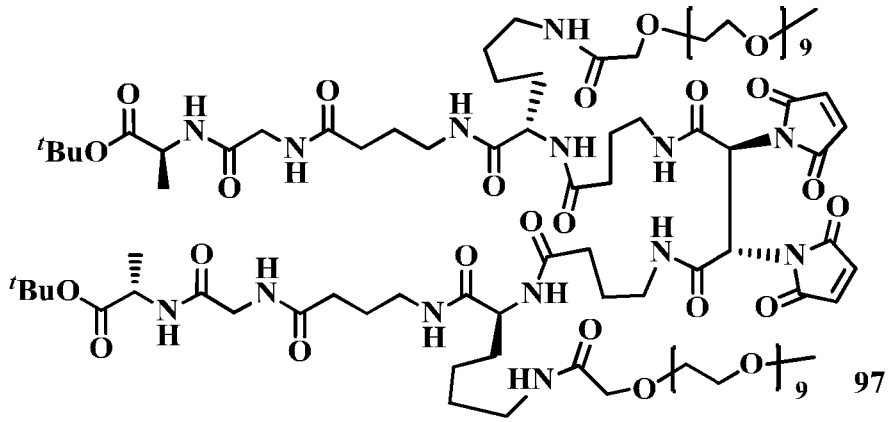
Example 88. Synthesis of (4R,4'R)-5,5'-((((16S,24S,25S,33S)-24,25-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,15,18,23,26,31,34,39,42,45-dodecaoxo-16,33-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,17,22,27,32,35,40,43,46-dodecaazaoctatetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7-dioxo-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**96**).



96

To a solution of **95** (234 mg, 0.090 mmol) and **Tub-2** (152 mg, 0.225 mmol) in DMF (2 mL), diisopropylethylamine (0.060 mL, 0.360 mmol) was added, and the reaction was stirred for 5 hours, and neutralized with formic acid. The mixture was purified by preparative HPLC (acetonitrile/water) to give compound **96** as a solid (100 mg, 0.028 mmol, 31%). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{166}H_{270}N_{28}O_{54}S_2$, 1794.63; found, 1794.83.

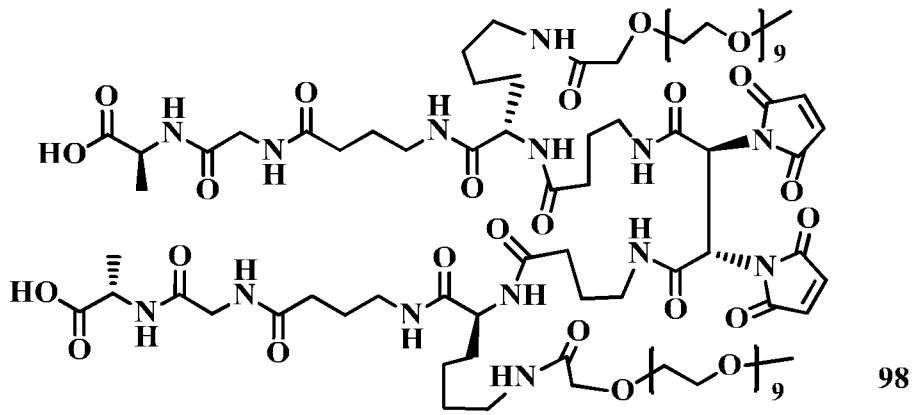
Example 89. Synthesis of di-tert-butyl (2S,13S,21S,22S,30S,41S)-21,22-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,41-dimethyl-4,7,12,15,20,23,28,31,36,39-decaoxo-13,30-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,14,19,24,29,32,37,40-decaazadotetracontanedioate (**97**).



97

To a solution of compound **35** (827 mg, 0.423 mmol) in dichloromethane (30 mL), pentafluorophenol (233.46 mg, 1.268 mmol) and DIC (213.41 mg, 1.691 mmol) were added. After stirring for 1 hour, H-Ala-OtBu·HCl(169 mg, 0.930 mmol) and diisopropylethylamine (0.280 mL, 1.691 mmol) were added. The reaction was stirred for 1 hour and then washed with 20 mL of brine, dried over anhydrous sodium sulfate, filtered and concentrated. Compound **97** was obtained as an oil (934 mg, 0.423 mmol, 99.94%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{100}H_{172}N_{14}O_{40}$, 2211.53; found, 2212.50.

Example 90. Synthesis of (2S,13S,21S,22S,30S,41S)-21,22-bis(2,5-dioxo-2,5-dihydro- 1H-pyrrol-1-yl)-2,41-dimethyl-4,7,12,15,20,23,28,31,36,39-decaoxo-13,30-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,14,19,24,29,32,37,40-decaazadotetracontanedioic acid (**98**).

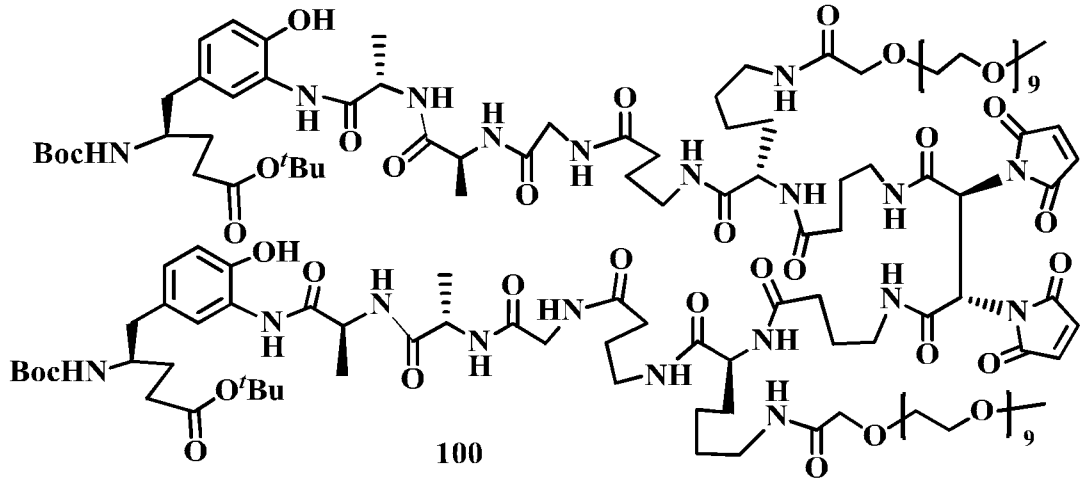


98

Compound **97** (0.93 g, 0.421 mmol) was dissolved in dichloromethane (10 mL) and treated with trifluoroacetic acid (5 mL) overnight. The reaction solution was concentrated and purified by preparative HPLC (acetonitrile/water) to give compound **98** as an oil (695 mg, 0.331 mmol, 79%). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{92}H_{156}N_{14}O_{40}$, 2099.32; found, 2100.87.

Example 91. Synthesis of di-tert-butyl 5,5'-((((2S,5S,16S,24S,25S,33S,44S,47S)-24,25-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,44,47-tetramethyl-4,7,10,15,18,23,26,31,34,39,42,45-dodecaoxo-16,33-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-

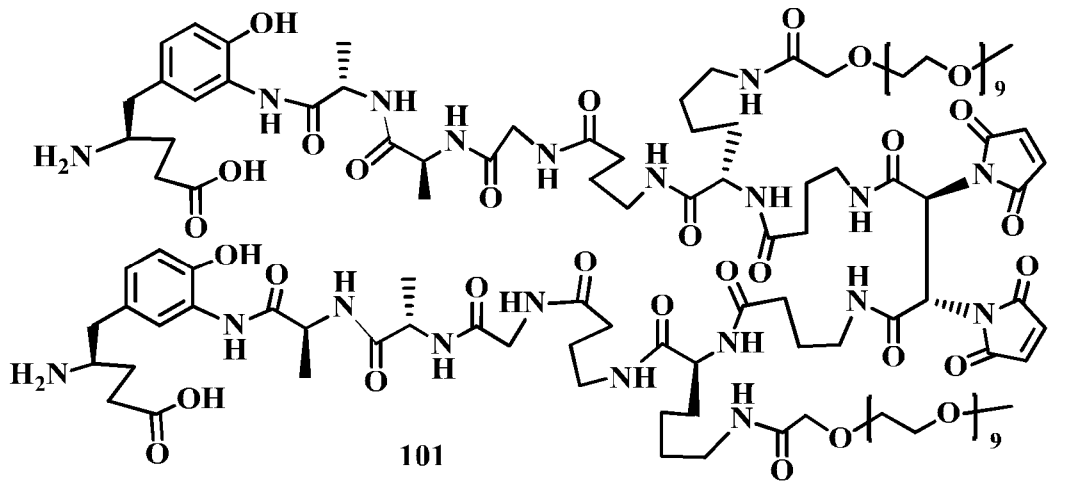
3,6,9,14,17,22,27,32,35,40,43,46-dodecaazaoctatetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**100**).



100

Compound **98** (695 mg, 0.331 mmol) and tert-butyl (R)-5-(3-((S)-2-aminopropanamido) -4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**99**, 329 mg, 0.729 mmol) were dissolved in dichloromethane (30 mL) and EDCI (190 mg, 0.994 mmol) was added. The reaction was stirred for 1 hour, and then washed with 20 mL of brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by preparative HPLC (acetonitrile/water) to give compound **100** as an oil (231 mg, 0.076 mol, 23.52%) MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{138}H_{226}N_{20}O_{50}$, 1484.21; found, 1484.44.

Example 92. Synthesis of (4R,4'R)-5,5'-((((2S,5S,16S,24S,25S,33S,44S,47S)-24,25-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,44,47-tetramethyl-4,7,10,15,18,23,26,31,34,39,42,45-dodecaoxo-16,33-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,17,22,27,32,35,40,43,46-dodecaazaoctatetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**101**).

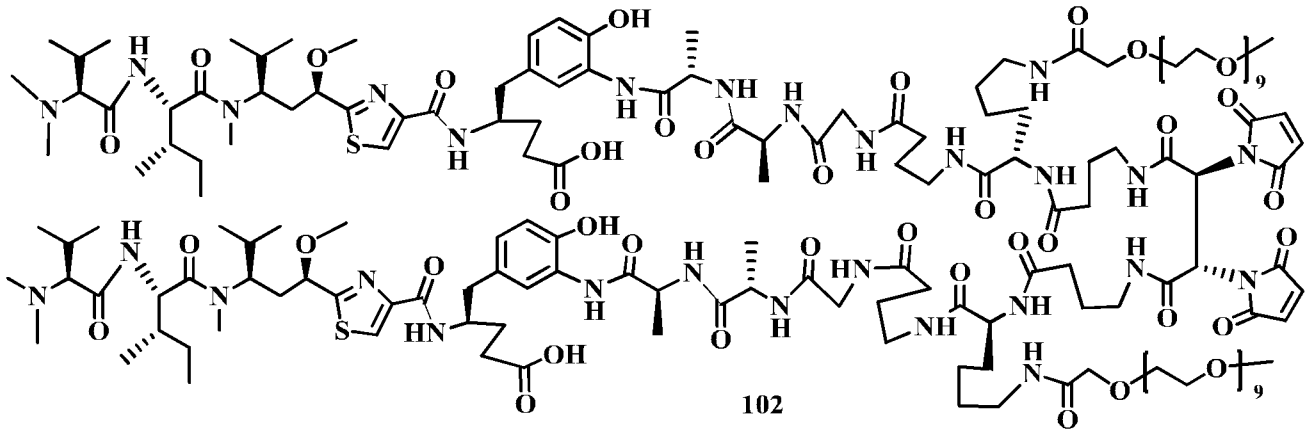


101

Compound **100** (231 mg, 0.078 mmol) was dissolved in dichloromethane (8 mL) and treated with trifluoroacetic acid (4 mL). The reaction was stirred for 3 hours, and then concentrated, co-

evaporated with dichloromethane twice. Compound **101** was obtained as an oil (206 mg, 0.078 mmol, 99%). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{120}H_{194}N_{20}O_{46}$, 1327.98; found, 1327.73.

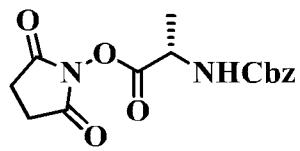
Example 93. Synthesis of (4R,4'R)-5,5'-((((2S,5S,16S,24S,25S,33S,44S,47S)-24,25-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,44,47-tetramethyl-4,7,10,15,18,23,26,31,34,39,42,45-dodecaoxo-16,33-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,17,22,27,32,35,40,43,46-dodecaazaoctatetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7-dioxo-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**102**).



102

Compound **101** (206 mg, 0.078 mmol) and **Tub-2** (131 mg, 0.194 mmol) were dissolved in DMF (2 mL) and then diisopropylethylamine (0.051 mL, 0.311 mmol) was added. The reaction was stirred for 4 hours, neutralized with formic acid, and purified by preparative HPLC (acetonitrile/water, containing 0.1% formic acid) to give compound **102** as a solid (90 mg, 0.02 mmol, 30% yield). MS-ESI (m/z): $[M + 4H]^{4+}$ calcd for $C_{170}H_{278}N_{28}O_{54}S_2$, 911.84; found, 911.67.

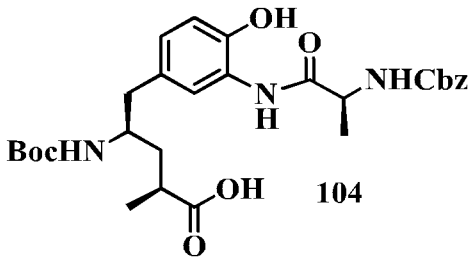
Example 94. Synthesis of 2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-alaninate (**103**).



103

Cbz-Ala-OH (8.93 g, 40 mmol) was dissolved in dichloromethane (300 mL), NHS (9.20 g, 80 mmol) was added, and after stirring for 5 min, EDCI (23.00 g, 120 mmol) was added in portions. After completion of addition, the reaction was stirred at r.t. for 3 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product, which was used in the next step without further purification, assuming 100% yield.

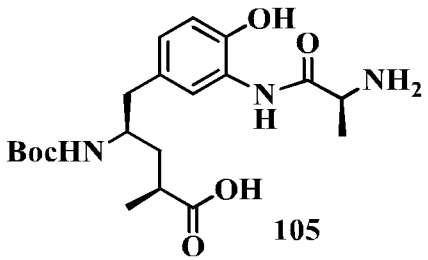
Example 95. Synthesis of (2S,4R)-5-(3-((S)-2-(((benzyloxy)carbonyl)amino) propanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**104**).



104

Compound **103** (crude product from the previous step, 40 mmol) and compound **77** (13.54 g, 40 mmol) were dissolved in tetrahydrofuran (300 mL) and stirred under reflux for 16 hours. The reaction mixture was concentrated under reduced pressure, then diluted with dichloromethane, washed with brine, and dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column (MeOH/dichloromethane) to give compound **104** (13.5g, 62% yield).

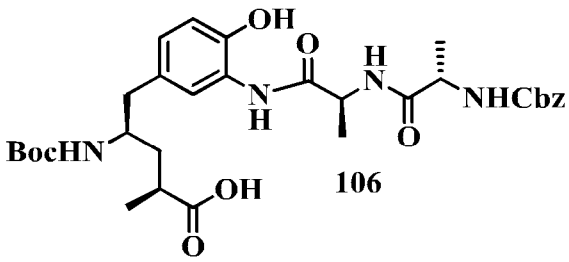
Example 96. Synthesis of (2S,4R)-5-(3-((S)-2-aminopropanamido)-4-hydroxyphenyl) -4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**105**).



105

Compound **104** (3.70 g, 6.80 mmol) was dissolved in methanol (100 mL) and Pd/C (10% wet, 0.7 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon for 6 hours, filtered, concentrated, and co-evaporated with 50 mL of dichloromethane, dried on an oil pump to give compound **105** (2.79 g, 100% yield).

Example 97. Synthesis of (2S,4R)-5-(3-((S)-2-((S)-2-(((benzyloxy)carbonyl)amino) propanamido)propanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**106**).

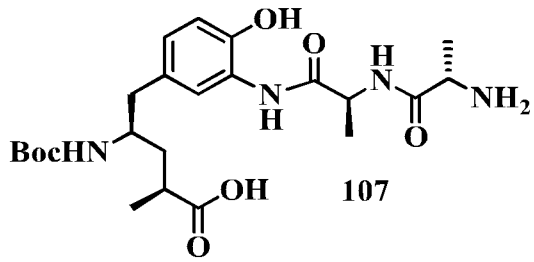


106

Compound **105** (2.79, 6.8 mmol) and compound **103** (2.18 g, 6.8 mmol) were dissolved in tetrahydrofuran (100 mL) and stirred under reflux for 4 hours. The reaction mixture was concentrated under reduced pressure, then diluted with dichloromethane, washed with brine, and dried over

anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column (MeOH/dichloromethane) to give compound **106** (2.2 g, 53% yield).

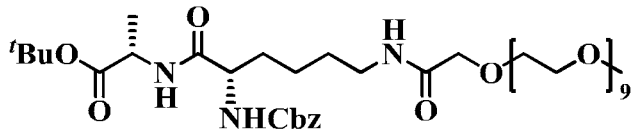
Example 98. Synthesis of (2S,4R)-5-(3-((S)-2-((S)-2-aminopropanamido)propanamido) -4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**107**).



107

Compound **106** (1.39 g, 2.26 mmol) was dissolved in methanol (50 mL) and Pd/C (10% wet, 0.3 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon for 4 hours, filtered, concentrated, and co-evaporated with 50 mL of dichloromethane, dried on an oil pump to give compound **107** (1.01 g, 93% yield).

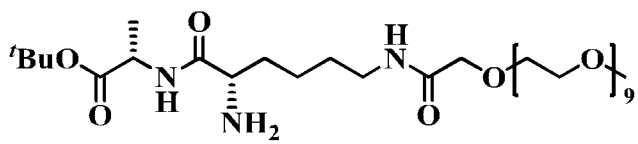
Example 99. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alaninate (**108**).



108

H-Ala-O^{t}Bu HCl (3.63 g, 2.00 mmol) and compound **20** (1.48 g, 2.40 mmol) were dissolved in THF (30 mL), HATU (1.36 g, 2.40 mmol) and triethylamine (0.33 mL, 2.40 mmol) were added. The reaction was stirred at r.t. for 1h, concentrated, diluted with 20 mL of water and 25 mL of dichloromethane, the separated organic phase was washed with 5% Na_2CO_3, 1M HCl, dried over anhydrous sodium sulfate, concentrated to dryness and purified by column chromatography to give a colorless liquid (1.51 g, 86% yield). MS-ESI (m/z): [M + H]+ calcd for $C_{42}H_{73}N_3O_{16}$, 876.50; found, 876.50.

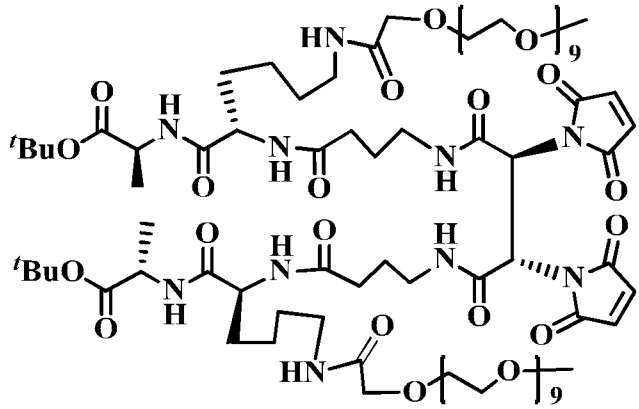
Example 100. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alaninate (**109**).



109

Compound **108** (1.50 g, 1.70 mmol) was dissolved in methanol (80 mL), 10% Pd/C (0.20 g) was added and the reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 2 h, filtered, and concentrated to dryness to give an oil (1.26 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{34}H_{67}N_3O_{14}$, 742.46; found, 742.50.

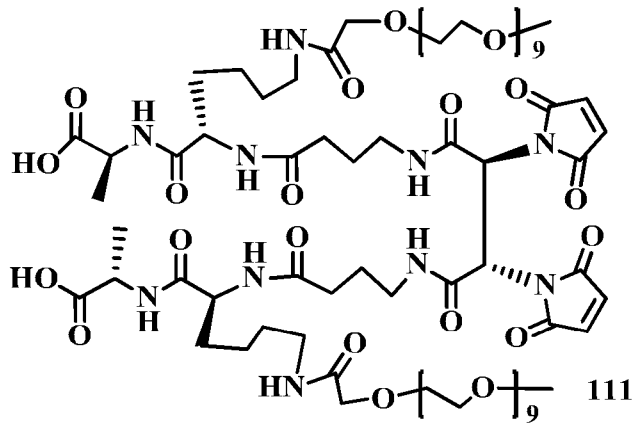
Example 101. Synthesis of di-tert-butyl (2S,5S,13S,14S,22S,25S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioate (**110**).



110

Compound **19** (0.41 g, 0.85 mmol) and compound **109** (1.26 g, 1.70 mmol) were dissolved in THF (10 mL) and DMF (10 mL), HATU (0.78 g, 2.04 mmol) and triethylamine (0.28 mL, 2.04 mmol) were added, the reaction was stirred at r.t. for about 30 min, concentrated, diluted with dichloromethane (40 mL) and washed with 30 mL of brine. The aqueous phase was extracted twice with 100 mL of dichloromethane, the organic phases were combined, dried over anhydrous sodium sulfate, filtered, concentrated to dryness, to give a colorless oil (1.44 g, 88% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{88}H_{152}N_{10}O_{36}$, 1926.04; found, 1926.04.

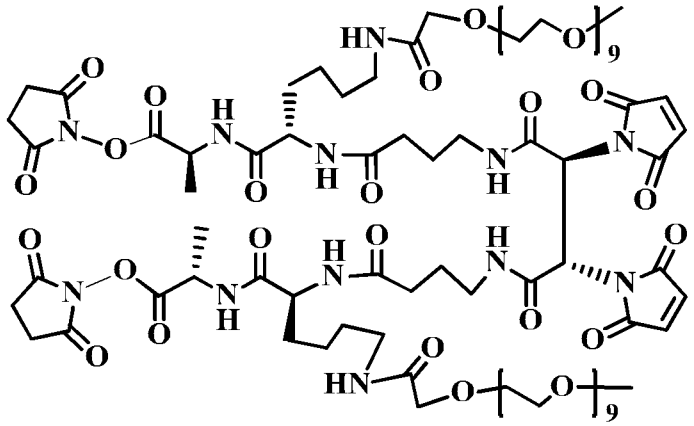
Example 102. Synthesis of (2S,5S,13S,14S,22S,25S)-13,14-bis(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioic acid (**111**).



111

Compound **110** (1.44 g, 0.75 mmol) was dissolved in dichloromethane (10 mL) and formic acid (20 mL), stirred at 55-60 °C for 3 h, concentrated to dryness, and purified by preparative HPLC to give a colorless oil (1.36 g, 100% yield). MS-ESI (m/z): $[M + H]^+$ calcd for $C_{80}H_{136}N_{10}O_{36}$, 1813.91; found, 1813.95.

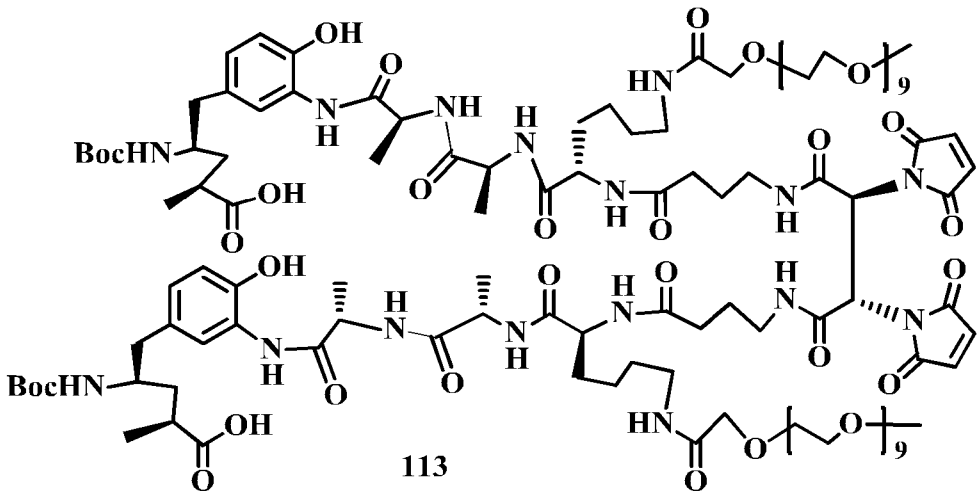
Example 103. Synthesis of bis(2,5-dioxopyrrolidin-1-yl) (2S,5S,13S,14S,22S,25S)-13,14-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioate (**112**).



112

Compound **111** (0.50 g, 0.276 mmol) was dissolved in dichloromethane (50 mL), NHS (0.13 g, 1.103 mmol) was added and stirred for 5 min, and then EDCI (0.32 g, 1.656 mmol) was added under ice-water cooling. The reaction was then warmed to r.t. and stirred for 3 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product, which was used directly without purification, assuming 100% yield. MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{88}H_{142}N_{12}O_{40}$, 1005.08; found, 1004.80.

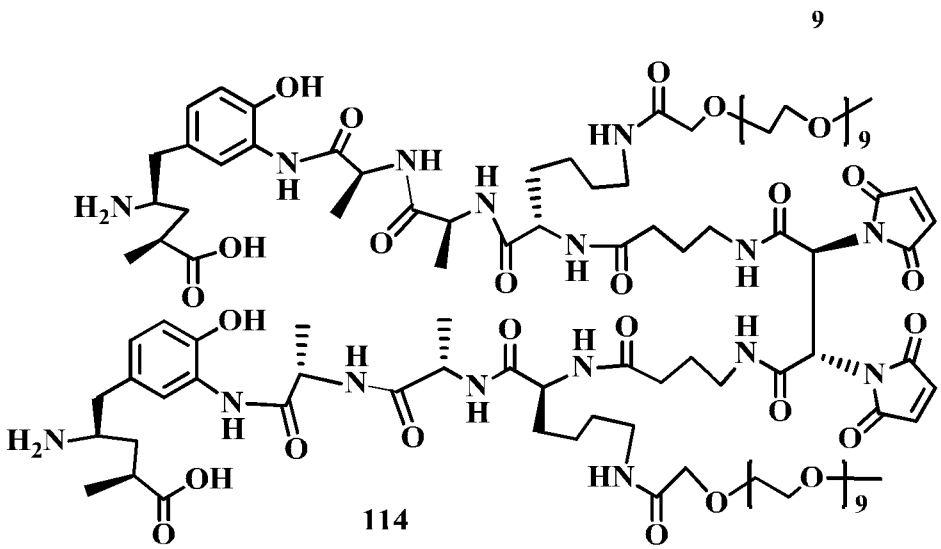
Example 104. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid) (**113**).



113

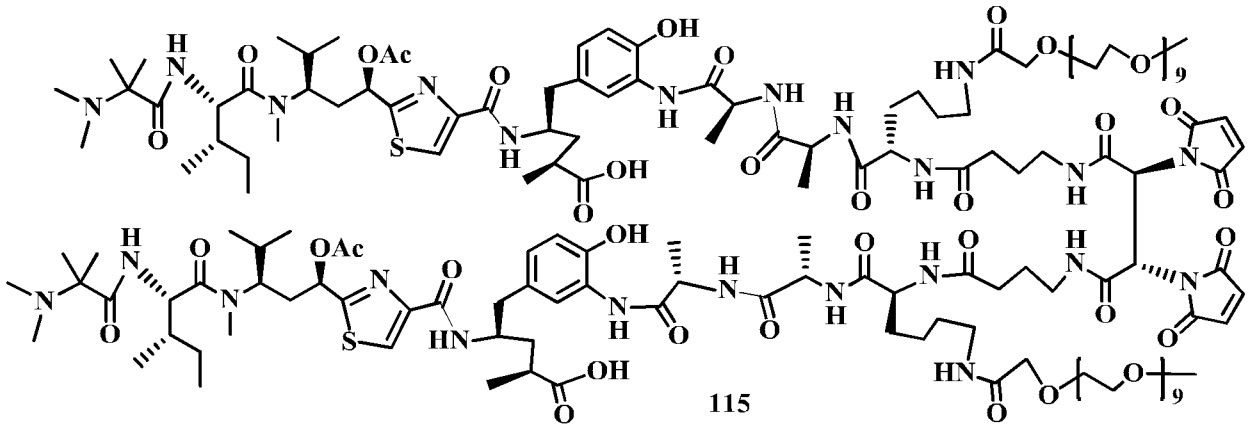
Compound **112** (crude product from the previous step, 0.276 mmol) and compound **107** (0.25 g, 0.521 mmol) were dissolved in tetrahydrofuran (50 mL) and stirred at r.t. for 1 hour. After concentration, the crude product was purified by preparative HPLC (acetonitrile/water) to give compound **113** (268 mg, 35% yield). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{126}H_{204}N_{18}O_{48}$, 1370.55; found, 1370.93.

Example 105. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-amino-2-methylpentanoic acid) (**114**).



In a 100 mL one-neck reaction flask, compound **113** (268 mg, 0.098 mmol), dichloromethane (12 mL) and trifluoroacetic acid (3 mL) were stirred at r.t. for 1 hour. The reaction mixture was concentrated under reduced pressure, and placed on an oil pump, to give compound **114**, which was used without further purification, assuming 100% yield.

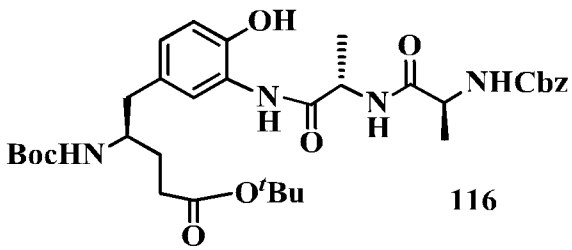
Example 106. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S) -19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoic acid) (**115**).



115

In a 100 mL single-necked reaction flask, to a solution of compound **114** (the crude product from previous step, 0.098 mmol) and **Tub-1** (122 mg, 0.176 mmol) in DMF (10 mL), was added dropwise diisopropylethylamine (101 mg, 0.784 mmol). After stirring at r.t. for 5 hours, the reaction was concentrated under reduced pressure. The residue was diluted with 10 mL of dichloromethane, 0.2 mL of formic acid was added dropwise, and the mixture was concentrated, then purified by preparative HPLC (acetonitrile/water), to give the title compound (106 mg, 30% yield). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd for $C_{166}H_{268}N_{26}O_{54}S_2$, 1779.11; found, 1779.40.

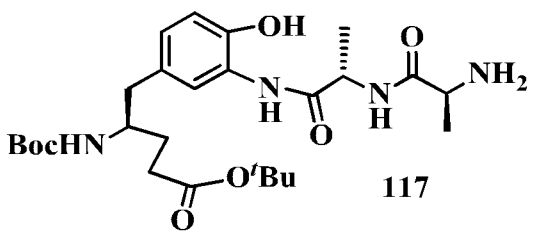
Example 107. Synthesis of tert-butyl (R)-5-(3-((S)-2-((S)-2-(((benzyloxy)carbonyl)amino) propanamido)propanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**116**).



116

To a solution of compound **99** (1.33 g, 2.945 mmol) and Cbz-Ala-OH (0.69 g, 3.093 mmol) in dichloromethane (20 mL), EDCI (1.13 g, 5.891 mmol) was added, the reaction was stirred for 1 hour, washed with brine, dried over anhydrous sodium sulfate, filtered, concentrate and purified by preparative HPLC to give the title compound (1.65 g, 2.512 mmol, 85 %). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{48}N_4O_9$, 657.34; found 657.31.

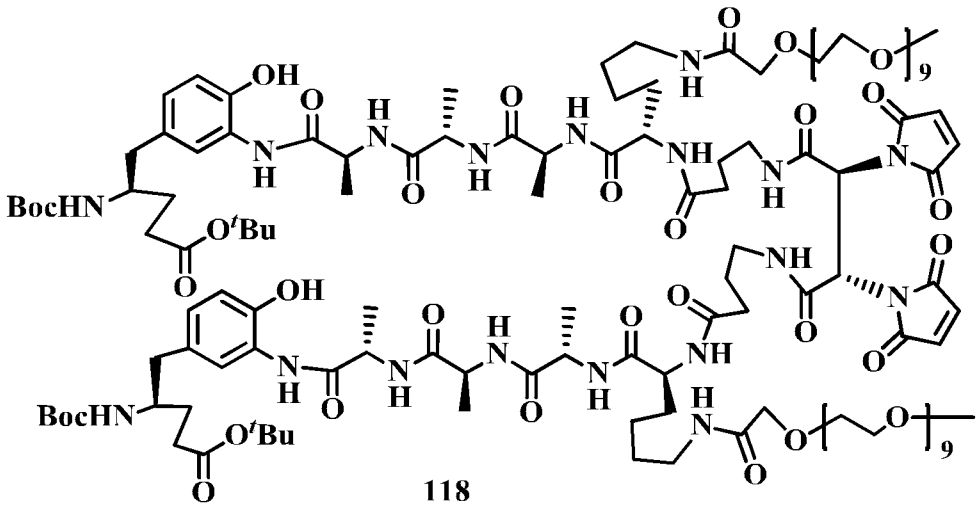
Example 107. Synthesis of tert-butyl (R)-5-(3-((S)-2-((S)-2-aminopropanamido) propanamido) -4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**117**).



117

To a solution of compound **116** (1.65 g, 0.003 mol) in methanol (20 mL), 10% Pd/C (0.27 g, 0.003 mol) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 2 h, filtered, and concentrated to dryness to afford compound **117** (1.24 g, 95% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{42}N_4O_7$, 523.31; found 523.29.

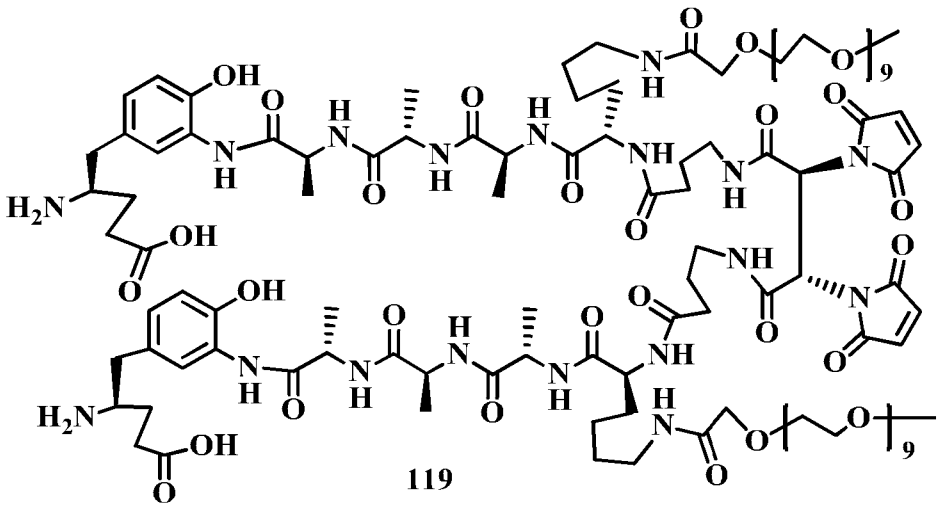
Example 108. Synthesis of di-tert-butyl 5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S) -19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21, 26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22, 27,30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene)) (4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**118**).



118

Compound **112** (0.34 g, 0.647 mmol) and compound **117** (0.65 g, 0.324 mmol) were dissolved in THF (15 mL). The reaction mixture was stirred for 1 hour, concentrated and purified by preparative HPLC to give compound **118** (0.25 g, 27% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{132}H_{216}N_{18}O_{48}$, 1411.75; found 1412.88.

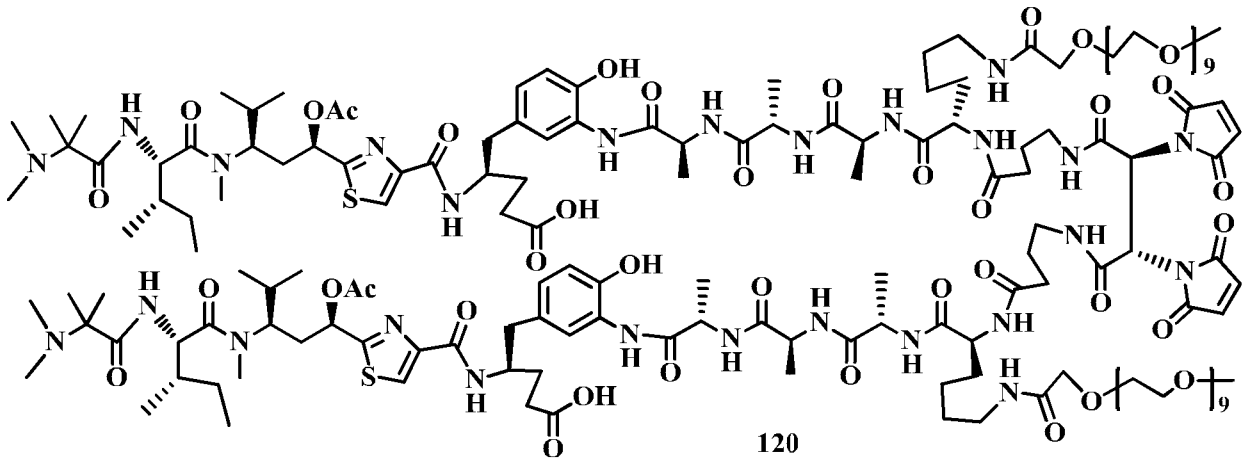
Example 109. Synthesis of (4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S, 31S,34S,37S) -19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl -4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioyl)-bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**119**).



119

Compound **118** (0.25 g, 0.089 mmol) was dissolved in dichloromethane (8 mL) and trifluoroacetic acid (8 mL). After stirring for 1 hour, the reaction solution was concentrated to give compound **119** (0.413 g, >100% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{114}H_{184}N_{18}O_{44}$, 1255.63; found 1256.01.

Example 110. Synthesis of (4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S, 31S,34S,37S)-19,20-bis(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10, 13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27, 30,33,36-decaazaoctatriacontanedioyl)bis(azanediyl))bis(4- hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**120**).

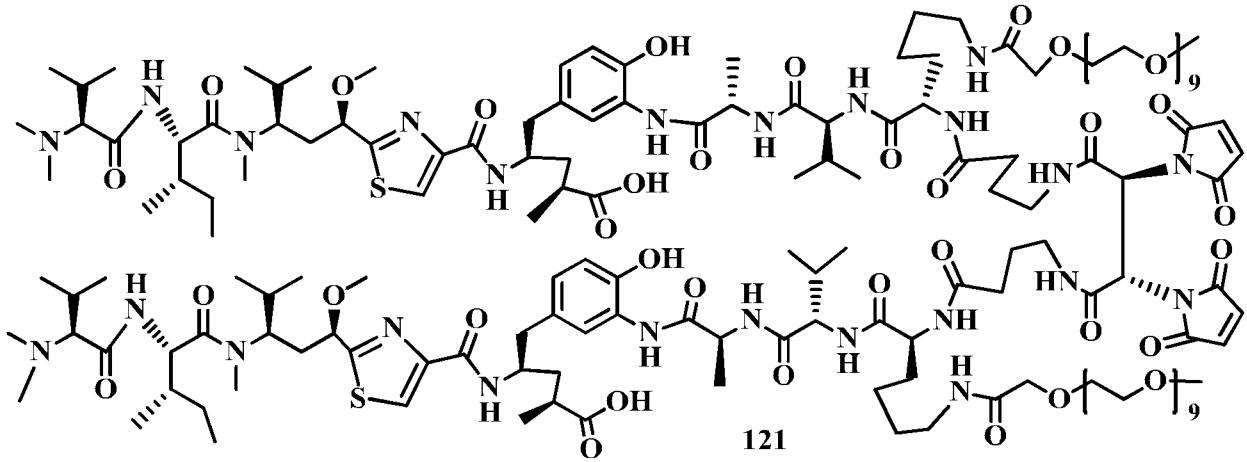


120

Compound **119** (0.12 g, 0.175 mmol) and **Tub-1** (0.22 g, 0.088 mmol) were dissolved in DMF (2 mL), and diisopropylethylamine (0.116 mL, 0.701 mmol) was added dropwise. The reaction was stirred for 2 hours and purified by preparative HPLC to give compound **120** (0.119 g, 38% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{164}H_{264}N_{26}O_{54}S_2$, 1763.91; found 1765.30.

Example 111. Synthesis of (2S,2'S,4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S) -16,17-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-

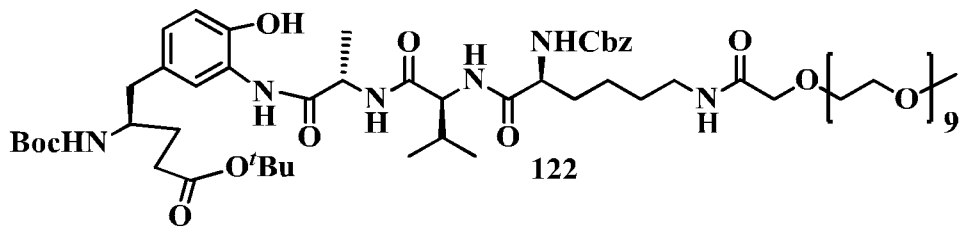
octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis (azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((3S,6S,9R,11R)-6-((S)-sec-butyl)-3,9-diisopropyl-2,8-dimethyl-4,7-dioxo-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoic acid) (**121**).



121

Compound **82** (250 mg, 0.102 mmol) and **Tub-2** (207 mg, 0.306 mmol) were dissolved in DMF (2 mL), and diisopropylethylamine (0.034 mL, 0.204 mmol) was added. The reaction was stirred for 6 hours and purified by preparative HPLC to give compound **121** (180 mg, 51% yield). ESI-MS (m/z): $[M+2H]^{2+}$ calcd for $C_{164}H_{270}N_{24}O_{50}S_2$, 1720.94; found 1721.94.

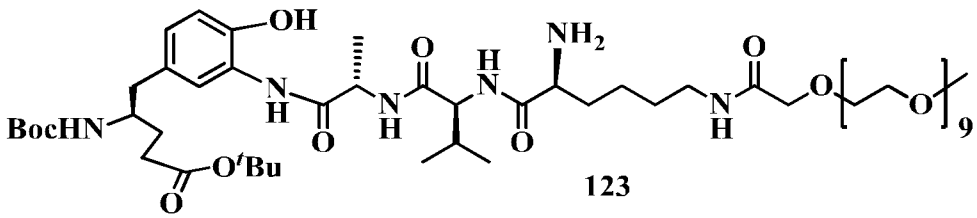
Example 112. Synthesis of tert-butyl (R)-5-(3-((37S,40S,43S)-37-(((benzyloxy)carbonyl)amino)-40-isopropyl-43-methyl-31,38,41-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**122**).



122

Compound **76** (6.0 g, 5.9 mmol) and compound **69** (2.7 g, 7.1 mmol) were dissolved in THF (100 mL) and heated to 60°C for 20 hours. The reaction was concentrated, and the residue was purified by silica gel column (dichloromethane: MeOH=20:1) to afford 7.1 g of the desired product as a gray solid (93% yield).

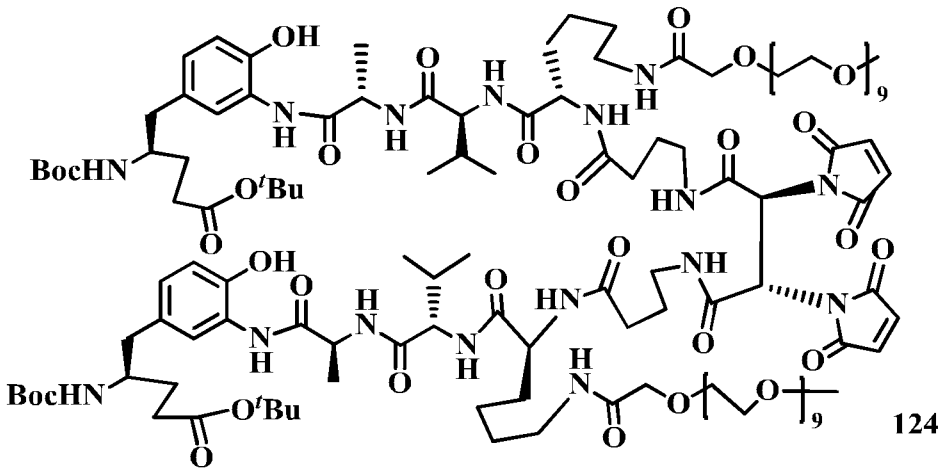
Example 113. Synthesis of tert-butyl (R)-5-(3-((37S,40S,43S)-37-amino-40-isopropyl-43-methyl-31,38,41-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**123**).



123

Compound **122** (7.1 g, 5.7 mmol) was dissolved in isopropanol (80 mL), and Pd/C (10% wet, 0.8 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred at 50 ° C under a hydrogen balloon for 2.5 hours, filtered, concentrated, and dried on an oil pump to give a gray solid (5.9 g, 93% yield).

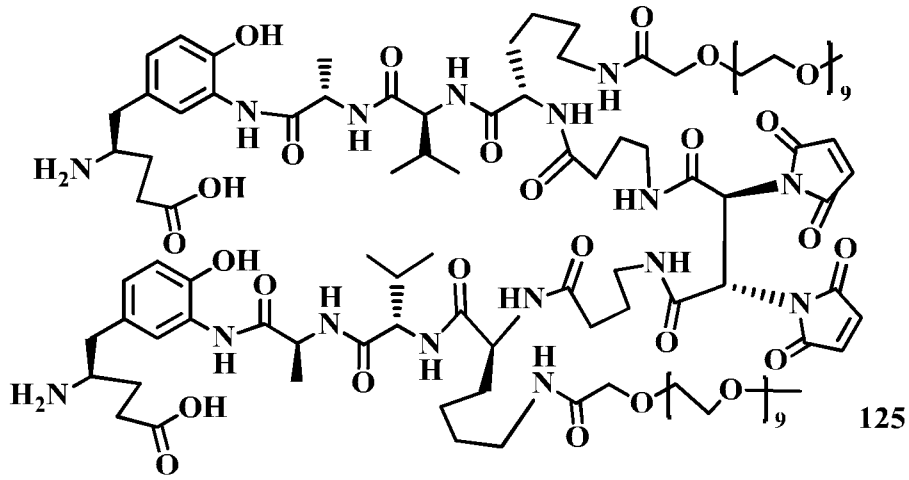
Example 114. Synthesis of di-tert-butyl 5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)-16,17-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis (azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4- ((tert-butoxycarbonyl) amino)pentanoate) (**124**).



124

Compound **80** (510 mg, 0.7 mmol) and compound **123** (1900 mg, 1.7 mmol) were dissolved in DMF (20 mL) and cooled to 0°C. N-methylmorpholine (190 mg, 1.9 mmol) was added, and the reaction was continued at 0°C for 3 hours. The reaction solution was diluted with dichloromethane (50 mL) and washed with brine (4 × 60 mL). The organic phase was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated, purified by silica gel column (dichloromethane: MeOH=20:1~6:1), and 1.2 g of the desired product was obtained as a brown-grey oil (58 % yield). ESI-MS (m/z): $[M+2H]^{2+}$ calcd for $C_{130}H_{214}N_{16}O_{46}$, 1368.75; found 1369.34.

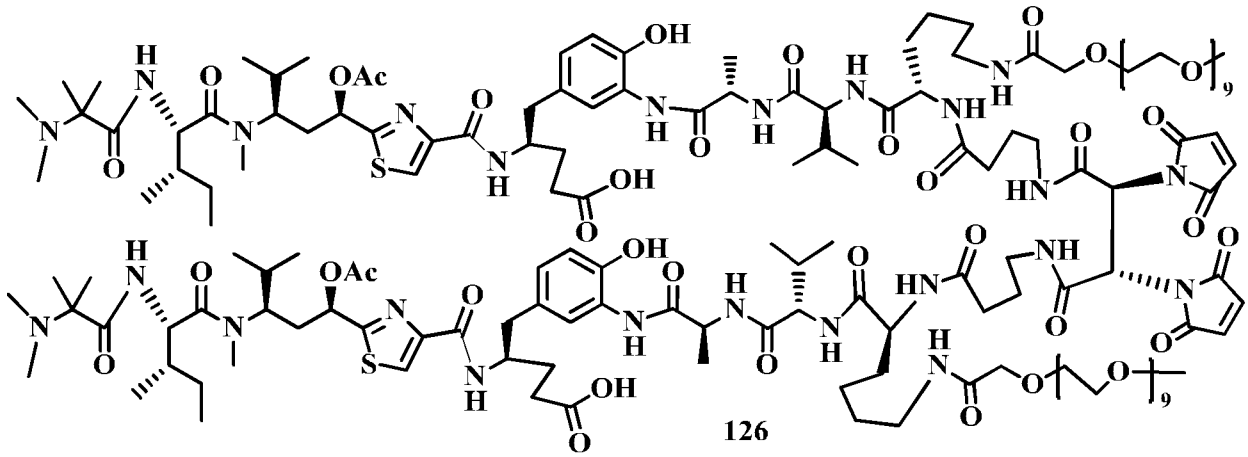
Example 115. Synthesis of (4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)-16,17- bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis (azanediyl))bis(4-hydroxy-3,1-phenylene))bis (4-aminopentanoic acid) (**125**).



125

Compound **124** (500 mg, 0.18 mmol) was dissolved in dichloromethane (5 mL), and treated with trifluoroacetic acid (5 mL) for 2 hours. The reaction solution was concentrated to afford 600 mg of crude product as an orange-red oil. ESI-MS (m/z): $[M+2H]^{2+}$ calcd for $C_{112}H_{182}N_{16}O_{42}$, 1213.63; found 1213.83.

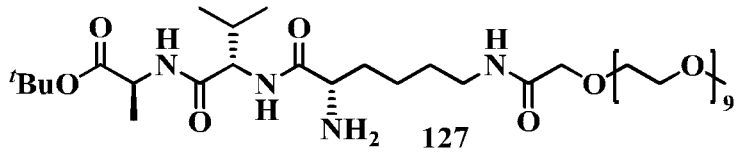
Example 116. Synthesis of (4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)-16,17-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioyl)bis(azanediyl)) bis(4-hydroxy-3,1-phenylene))bis(4-(2- ((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**126**).



126

Compound **125** (440 mg, 0.18 mmol) and **Tub-1** (320 mg, 0.45 mmol) were dissolved in DMF (5 mL), diisopropylethylamine (70 mg, 0.54 mmol) was added at 0 °C and stirred, and the reaction was continued at 0°C for 3 hours. The reaction solution was concentrated and the residue was purified by preparative HPLC to afford 92 mg of the desired product as a white solid (15% yield). ESI-MS (m/z): $[M+2H]^{2+}$ calcd for $C_{162}H_{262}N_{24}O_{52}S$, 1720.90; found 1721.66.

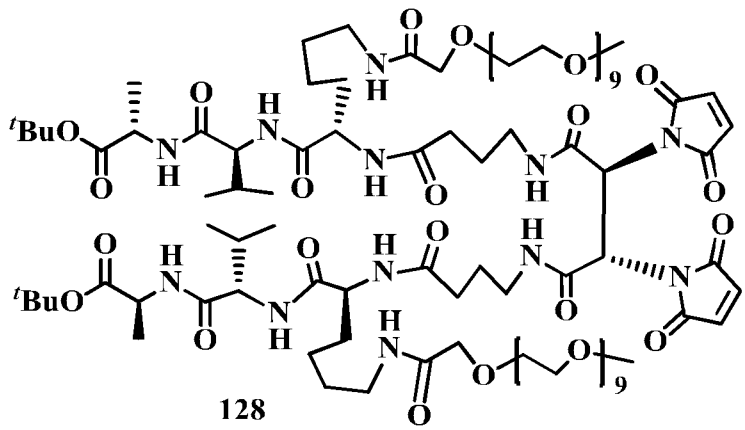
Example 117. Synthesis of tert-butyl ((S)-35-amino-24-methyl-1-(11-oxidaneyl)-29-oxo-3,6,9,12,15,18,21,24l3,27-nonaoxa-30-azahexatriacontan-36-oyl)-L-valyl-L-alaninate (**127**).



127

Compound **74** (15 g, 15.4mmol) was dissolved in isopropyl alcohol (150 mL), palladium on carbon (10 wt%, 2.0 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 days, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **127** (12 g, 92% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{39}H_{77}N_4O_{15}$, 841.53; found 841.53.

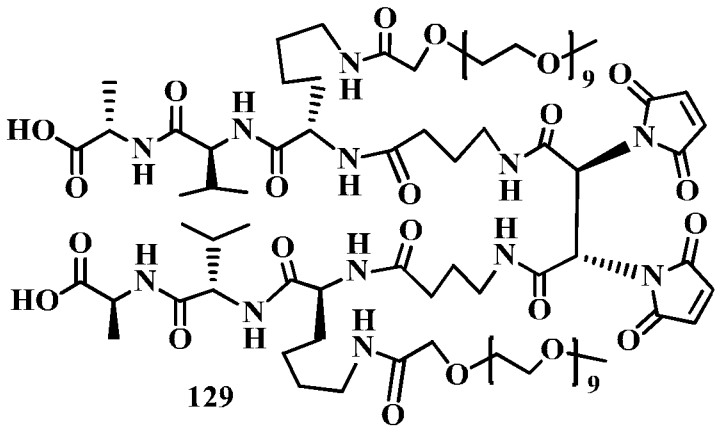
Example 118. Synthesis of di-tert-butyl (2S,5S,8S,16S,17S,25S,28S,31S)-16,17-bis(2,5- dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioate (**128**).



128

To a solution of compound **127** (5.5 g, 6.6 mmol) and compound **19** (1.5 g, 3.1 mmol) in DMF (100 mL), were added HATU (4.8 g, 12.5 mmol) and NMM (1.3 g ,12.5 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated under vacuum and poured into water (200 mL), extracted with dichloromethane (3×100 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give compound **128** (6.5 g, 100% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{98}H_{171}N_{12}O_{38}$, 1063.08; found 1063.78.

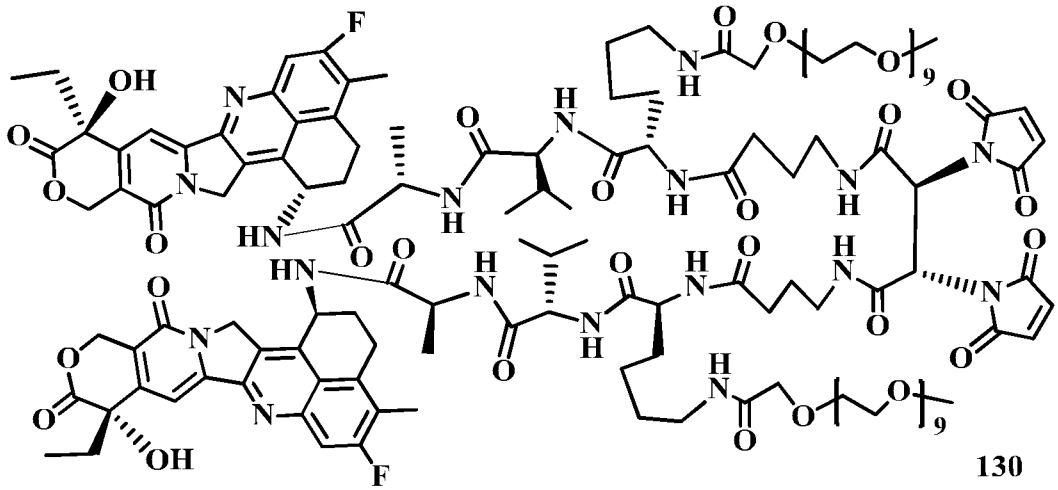
Example 119. Synthesis of (2S,5S,8S,16S,17S,25S,28S,31S)-16,17-bis(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)-5,28-diisopropyl-2,31-dimethyl-4,7,10,15,18,23,26,29-octaoxo-8,25-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,14,19,24,27,30-octaazadotriacontanedioic acid (**129**).



129

Compound **128** (6.5 g, 3.1mmol) was dissolved in dichloromethane (50 mL) and trifluoroacetic acid (50 mL). The mixture was stirred for 2 hours, concentrated under vacuum and purified by preparative HPLC to give product **129** (3.1 g, 50% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{90}H_{155}N_{12}O_{38}$, 1006.03; found 1006.13.

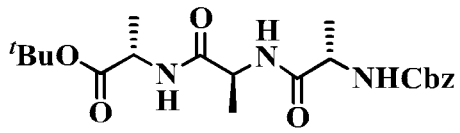
Example 120. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4-bis((S)-37-(((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**130**).



130

To a solution of compound **129** (280 mg, 0.139 mmol) and exatecan mesylate (148 mg, 0.278 mmol) in DMF (5 mL), were added HATU (158 mg, 0.417 mmol) and diisopropylethylamine (92 μL,0.557 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give product **130** (101 mg, 25% yield). MS-ESI (m/z): calcd for $C_{138}H_{195}F_2N_{18}O_{44}$ $[M+2H]^{2+}$: 1424.18, found 1425.00.

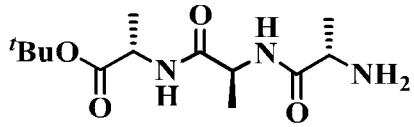
Example 121. Synthesis of tert-butyl ((benzyloxy)carbonyl)-L-alanyl-L-alanyl-L-alaninate (**131**).



131

Compound **61** (4.0 g,18.49 mmol) and Cbz-Ala-OH (4.1 g ,18.49 mmol) was dissolved in dichloromethane (100 mL), to which EDCI (7.0 g, 36.51 mmol) and diisopropylethylamine (4.7 g, 36.36 mmol) were added at 5 °C. After stirring for about 0.5 h, the reaction was washed by water and brine, purified by a silica gel column, eluted with dichloromethane and methanol to give compound **131** as a white solid (1.6 g, 20% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{21}H_{32}N_3O_6$, 422.22; found 422.22.

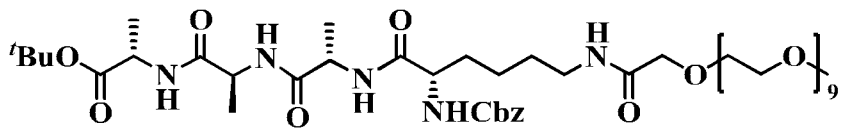
Example 122. Synthesis of tert-butyl L-alanyl-L-alanyl-L-alaninate (**132**).



132

Compound **131** (1.6 g, 3.79 mmol) was dissolved in methanol (100mL), 10% Pd/C (0.16 g) was added, the reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 1 h, filtered, concentrated to give compound **132** as colorless liquid (1.1 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{13}H_{26}N_3O_4$, 288.18; found 288.18.

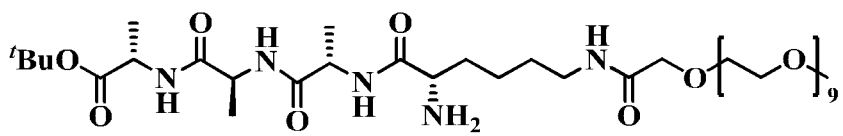
Example 123. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl-L-alanyl-L-alaninate (**133**).



133

Compound **132** (1.1 g, 3.83 mmol) and compound **20** (2.6 g, 3.47 mmol) were dissolved in THF (50 mL), HATU (2.0 g, 5.25 mmol) and diisopropylethylamine (0.9 g, 6.94 mmol) were added at 5 °C. After stirring for about 0.5 h, the reaction was concentrated, diluted with dichloromethane, washed with water (100 mL), 5% Na_2CO_3 (80 mL), 1M HCl (80 mL), filtered and concentrated. The residue was purified by a silica gel column, eluted with dichloromethane and methanol to give compound **133** as white solid (2.9 g, 81% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{48}H_{84}N_5O_{18}$, 1018.57; found 1018.57.

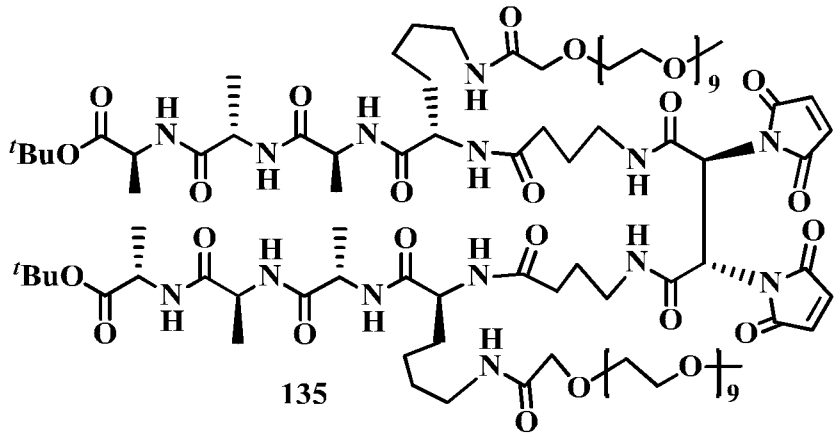
Example 124. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl-L-alanyl-L-alaninate (**134**).



134

Compound **133** (2.9 g, 2.84 mmol) was dissolved in methanol (150 mL), and 10% Pd/C (0.29 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 1 h, filtered, concentrated to give compound **134** as colorless oil (2.5 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{40}H_{78}N_5O_{16}$, 884.54; found 884.54.

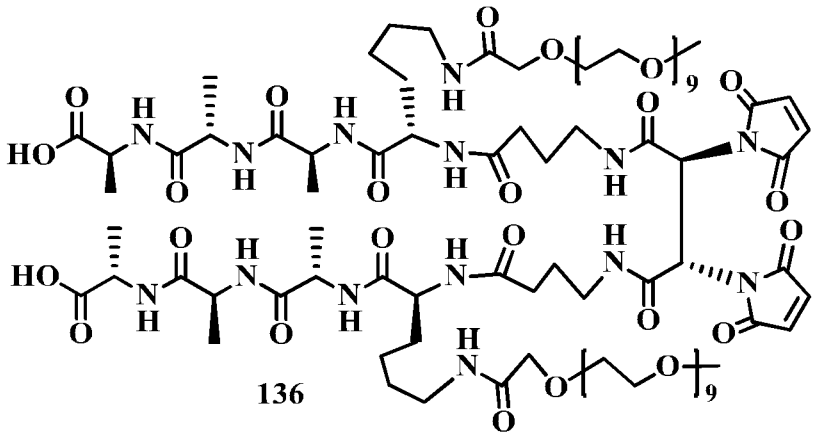
Example 125. Synthesis of di-tert-butyl (2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioate (**135**).



135

Compound **134** (0.83 g, 0.94 mmol) and compound **19** (204.3 mg ,0.43 mmol) were dissolved in mixed solvents of THF (10 mL) and DMF (10 mL). HATU (470 mg, 1.24 mmol) and diisopropylethylamine (216 mg, 1.67mmol) were added. After stirring for 1h at r.t., the reaction was concentrated, diluted with 100 mL of dichloromethane, washed with brine (50 mL), concentrated to give compound **135** as a colorless oil (0.924 g, 100% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{100}H_{173}N_{14}O_{40}$, 1106.04; found 1106.25.

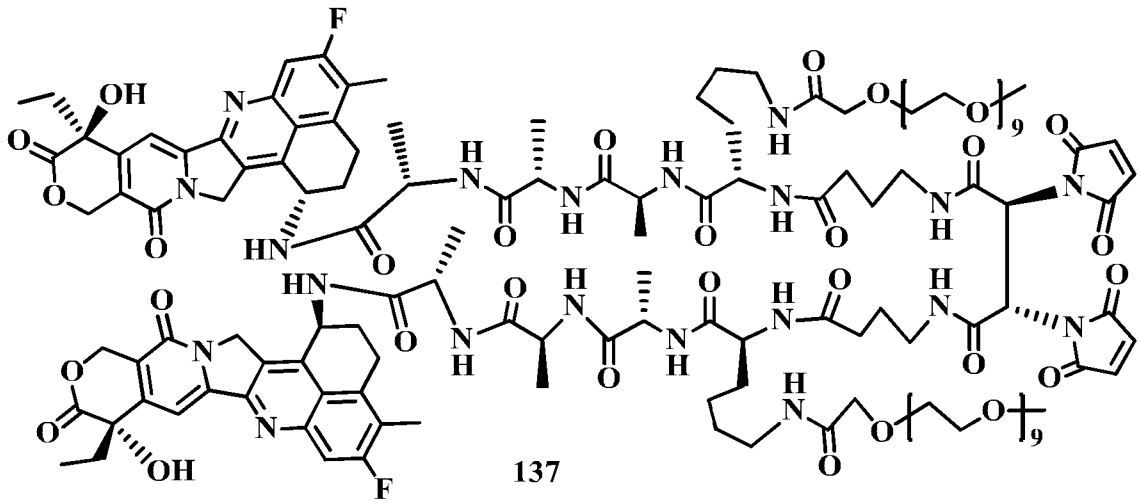
Example 126. Synthesis of (2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20- bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29, 32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioic acid (**136**).



136

Compound **135** (924.1 mg, 0.42 mmol) was dissolved in 10 mL of dichloromethane and 20 mL of formic acid. After stirring for 3 h at 55-60 °C, the reaction was concentrated, and the residue was purified by preparative HPLC to give compound **136** as a white solid (0.42 g, 48% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{92}H_{157}N_{14}O_{40}$, 1050.03; found 1050.03.

Example 127. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-(((S)-1-(((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**137**).

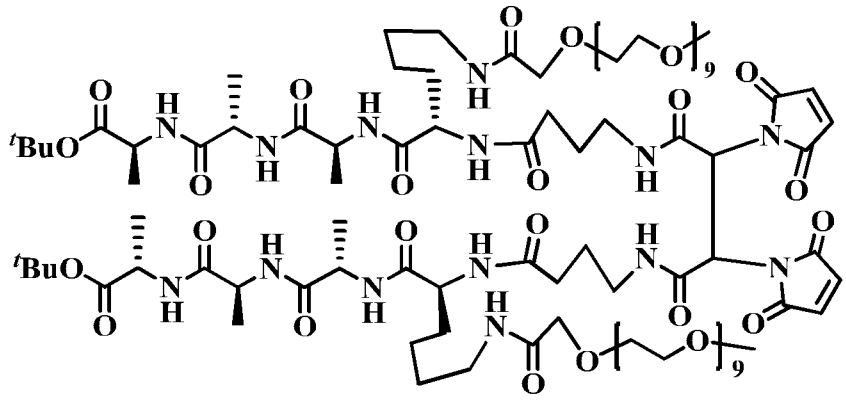


137

Compound **136** (202.5 mg, 0.09 mmol) and exatecan (111.1 mg ,0.21 mmol) were dissolved in 10 mL of DMF, and HATU (110.0 mg,0.29 mmol) and diisopropylethylamine (50.0 mg, 0.38 mmol) were added. The reaction was stirred at r.t. for about 30 min, concentrated, and purified by preparative HPLC to give compound **137** as a white solid (176.0 mg, 62% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{140}H_{197}F_2N_{20}O_{46}$, 1467.18; found 1468.62.

Example 128. Synthesis of di-tert-butyl (2S,5S,8S,11S,28S,31S,34S,37S)-19,20-bis(2,5- dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18, 21,26,29,32,35-decaoxo-

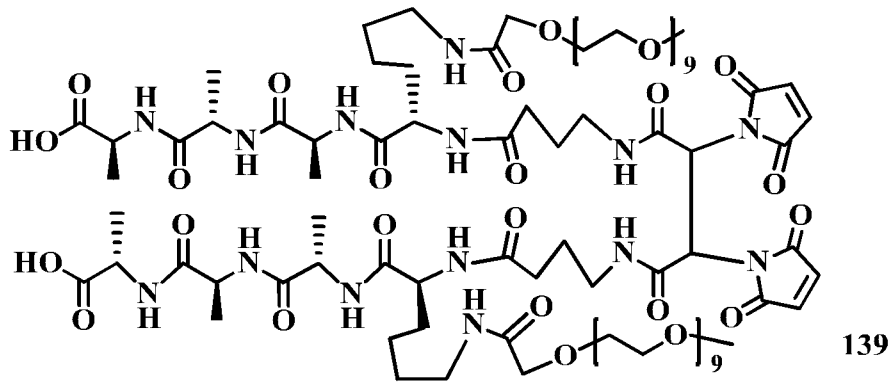
11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioate (**138**).



138

To a solution of compound **134** (4.07 g, 4.60 mmol) and compound **12** (1.00 g, 2.09 mmol) in DMF (40 ml), were added HATU (2.38 g, 6.27 mmol) and diisopropylethylamine (0.69 mL, 4.18 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated under vacuum and diluted with water (200 mL), extracted with dichloromethane (3×100 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give product **138** (4.6 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{100}H_{173}N_{14}O_{40}$, 2210.19; found 2210.19.

Example 129. Synthesis of (2S,5S,8S,11S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontanedioic acid (**139**).

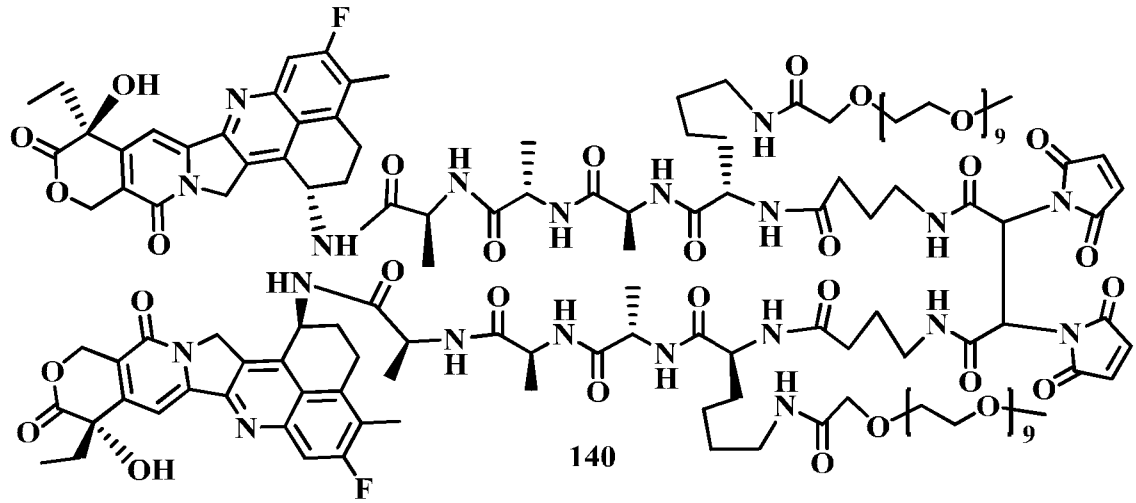


139

Compound **138** (4.6 g, 1.2 mmol) was dissolved in formic acid (40 mL) and dichloromethane (20 mL). The mixture was heated to 60 °C and stirred for 3 hours, and then concentrated under vacuum and purified by preparative HPLC to give product **139** (2.7 g, 61% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{92}H_{158}N_{14}O_{40}$, 2098.06; found 2098.06.

Example 130. Synthesis of 2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1-((S)- 31,39-dioxo-37-(((S)-1-oxo-1-(((S)-1-oxo-1-(((S)-3-oxobutan-2-yl)amino)propan-2-yl)amino)propan-2-

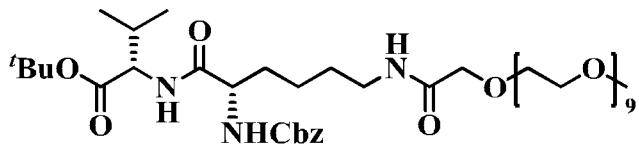
yl)carbamoyl)-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)-N4-((S)-37-(((S)-1-(((S)-1-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**140**).



140

To a solution of compound **139** (300 mg, 0.143 mmol) and exatecan mesylate (152 mg, 0.286 mmol) in DMF (10 mL), were added HATU (163 mg, 0.429 mmol) and diisopropylethylamine (94 μL, 0.572 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give product **140** (263 mg, 62% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{140}H_{197}F_2N_{20}O_{46}$, 2932.36; found 2932.36.

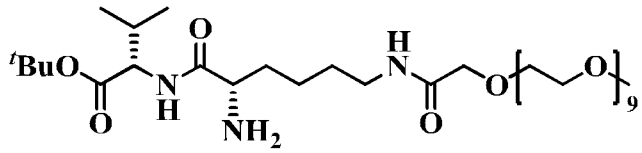
Example 131. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo -2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valinate (**141**).



141

To a solution of compound **20** (10.0 g, 13.4 mmol) and H-Val-O^{t}Bu·HCl (2.40 g, 13.8 mmol) in THF (100 mL), HATU (7.60 g, 20.0 mmol) and diisopropylethylamine (4.4 mL, 26.7 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was diluted with dichloromethane (200 mL), washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give compound **141** (12.0 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{44}H_{78}N_3O_{16}$, 904.53; found 904.53.

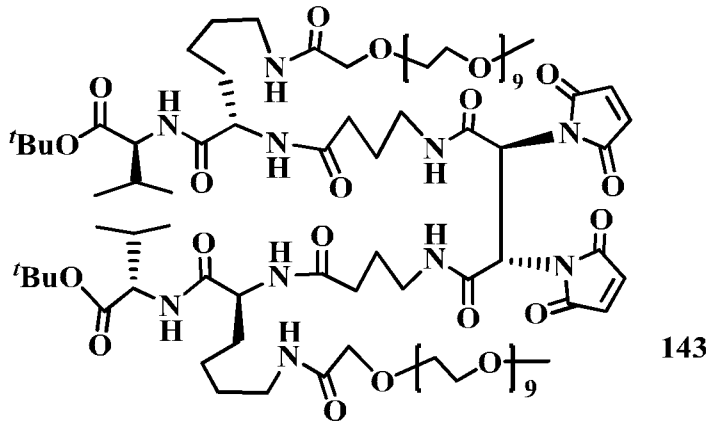
Example 132. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17, 20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valinate (**142**).



142

Compound **141** (10.0 g, 11.1 mmol) was dissolved in THF (100 mL), 10% palladium on carbon (1.0 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **142** (8.6 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{36}H_{72}N_3O_{14}$, 770.49; found 770.49.

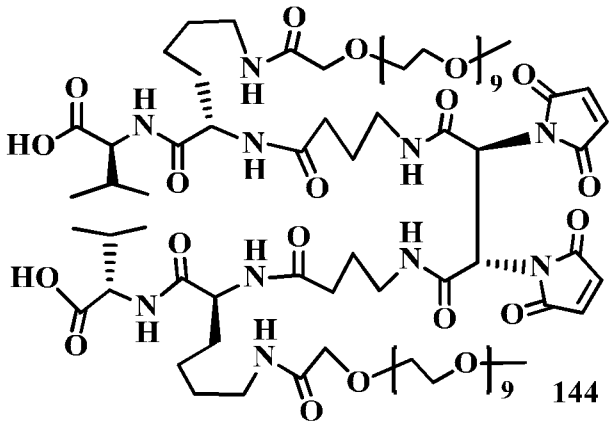
Example 133. Synthesis of di-tert-butyl (2S,5S,13S,14S,22S,25S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,25-diisopropyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioate (**143**).



143

To a solution of compound **19** (1.20 g, 2.5 mmol) and compound **142** (4.10 g, 5.5 mmol) in mixed solvents of THF (50 mL) and DMF (10 mL), HATU (2.80 g, 7.5 mmol) and diisopropylethylamine (1.0 mL, 7.5 mmol) were added. The reaction was stirred at r.t. until completion, as indicated by LC-MS. The solvent was removed and the residue was dissolved in dichloromethane (200 mL), washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (4.8 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{92}H_{161}N_{10}O_{36}$, 1982.10; found 1982.10.

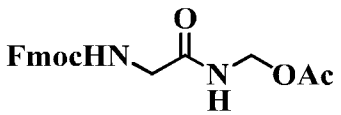
Example 134. Synthesis of (2S,5S,13S,14S,22S,25S)-13,14-bis(2,5-dioxo-2,5-dihydro -1H-pyrrol-1-yl)-2,25-diisopropyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioic acid (**144**).



144

Compound **143** (4.8 g, 2.5 mmol) was dissolved in formic acid (40 mL) and dichloromethane (20 mL), and then heated to 60 °C. The reaction was stirred until completion, as indicated by LC-MS and then concentrated to give the title compound (2.2 g, 48% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{84}H_{145}N_{10}O_{36}$, 1869.97; found 1869.97.

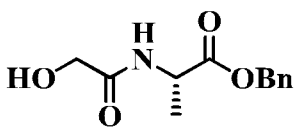
Example 135. Synthesis of (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino) acetamido)methyl acetate (**146**).



146

To a solution of Fmoc-Gly-Gly-OH (7.3 g, 20.6 mmol) in tetrahydrofuran (100 mL) and toluene (30 mL), pyridine (2 mL, 24.8 mmol) was added, followed by lead tetraacetate (11 g, 24.8 mmol) under N_2. The reaction mixture was heated to reflux for 5 hours, cooled to r.t., and then filtered. The filtrate was concentrated, diluted with ethyl acetate (300 mL) and water (50 mL). The separated organic phase was washed with brine (50 mL), dried over sodium sulfate, filtered, concentrated. The residue was purified by silica gel column, eluted with petroleum ether/ethyl acetate to give a white solid (7.1 g, 76% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{20}H_{20}N_2O_5$, 369.14; found 369.14.

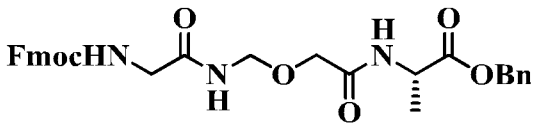
Example 136. Synthesis of benzyl (2-hydroxyacetyl)-L-alaninate (**147**).



147

Glycolic acid (3.3 g, 43.39 mmol) and H-Ala-OBn·HCl (8.5 g 39.44 mmol) were dissolved in dichloromethane (100 mL), to which EDCI (11.3 g, 59.17 mmol) and diisopropylethylamine (13.0 ml, 78.89 mmol) were added. The reaction was stirred at r.t. until complete conversion, and then washed with water (50 ml), brine (50 ml), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column (PE/EA=10/0 to 1/1 to 0/10) to give the title compound (2.9 g, 31% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{12}H_{16}NO_4$, 238.10; found 238.10.

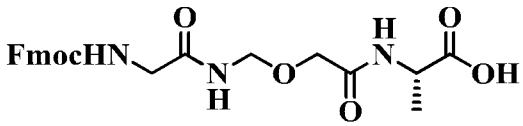
Example 137. Synthesis of benzyl (2-((2-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)acetamido)methoxy)acetyl)-L-alaninate (**148**).



148

A mixture of compound **147** (2.9 g, 12.22 mmol) and compound **146** (4.5 g, 12.22 mmol) in toluene (60 ml), and catalytic amount of PPTS (0.3 g) was heated at 100 °C for 2 hours. The reaction was cooled to r.t. and the resulting white solid was filtered off, the filtrate was concentrated and diluted with 100 mL of ethyl acetate, washed with water (50ml), dried over anhydrous sodium sulfate, filtered and concentrated, purified on silica gel column (PE/EA=10/0 to 1/1 to 1/3 to 0/10) to give the title compound (2.8 g, 42% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{30}H_{32}N_3O_7$, 546.22; found 546.22.

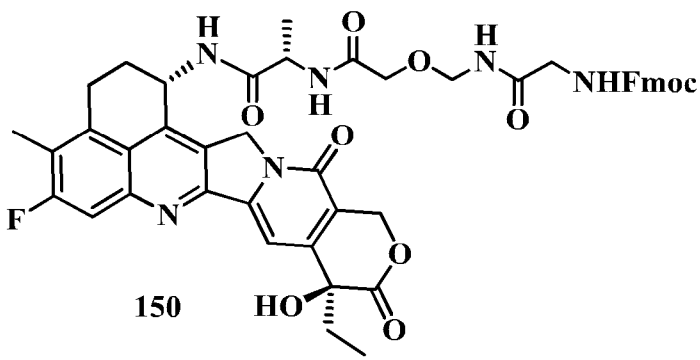
Example 138. Synthesis of (2-((2-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)acetamido)methoxy)acetyl)-L-alanine (**149**).



149

Compound **148** (2.0 g, 3.66 mmol) was dissolved in methanol (40 mL), 10% palladium on carbon (0.2 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **149** (1.45 g, 86% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{23}H_{26}N_3O_7$, 456.17; found 456.17.

Example 139. Synthesis of (9H-fluoren-9-yl)methyl (2-(((2-(((S)-1-(((1S,9S)-9-ethyl-5 -fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)carbamate (**150**).

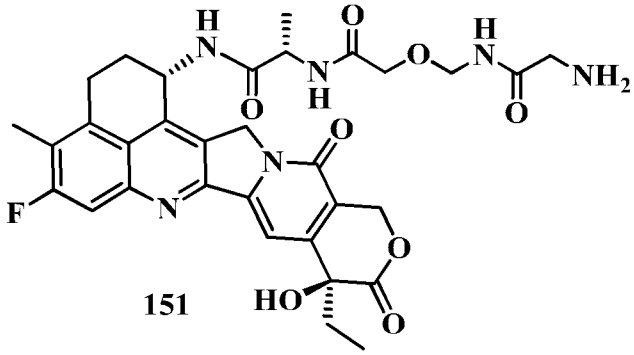


150

Compound **149** (300 mg, 0.659 mmol) and exatecan mesylate (350 mg, 0.659 mmol) were dissolved in DMF (10 mL), HATU (375 mg, 0.988 mmol) and diisopropylethylamine (217 μL, 1.317

mmol) were added. The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give the title compound **150** (400 mg, 70% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{47}H_{45}FN_6O_{10}$, 873.32; found 873.32.

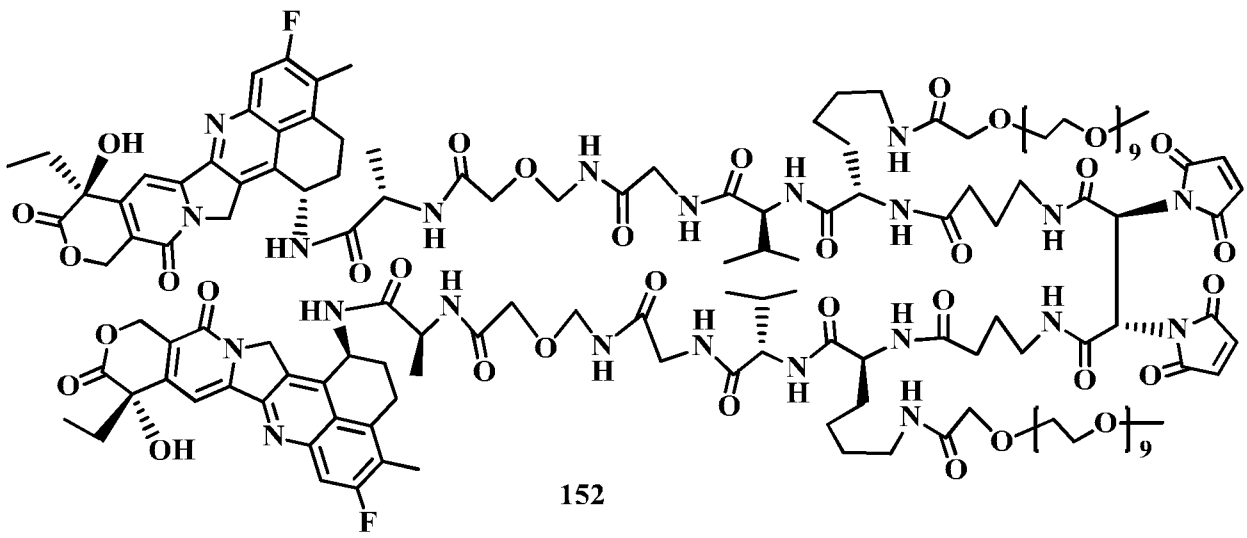
Example 140. Synthesis of (S)-2-(2-((2-aminoacetamido)methoxy)acetamido) -N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)propanamide (**151**).



151

Compound **150** (170 mg, 0.195 mmol) was dissolved in a mixture of DMF (4 mL) and piperidine (0.4 mL), and the reaction was stirred at r.t. until completion. The solvent was removed, and the residue was co-evaporated with DMF (3 mL) to give the title compound, which was directly used in the next step without further purification (0.13g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{32}H_{35}FN_6O_8$, 651.25; found 651.26.

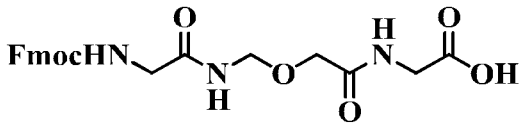
Example 141. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-(((2S,13S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,14-dimethyl-1,4,9,12-tetraoxo-6-oxa-3,8,11-triazapentadecan-13-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**152**).



152

Compound **144** (180 mg, 0.096 mmol) and compound **151** (125 mg, 0.193 mmol) were dissolved in DMF (5 mL) and cooled to about 0 °C. HATU (109 mg, 0.289 mmol) and diisopropylethylamine (31 μL, 0.193 mmol) were added, and the reaction was warmed to r.t. and stirred until completion. The solvent was removed and the residue was purified by preparative HPLC to give compound **152** (101 mg, 34% yield). MS-ESI (m/z): $[M+3H]^{3+}$ calcd for $C_{148}H_{210}F_2N_{22}O_{50}$, 1045.81; found 1046.10.

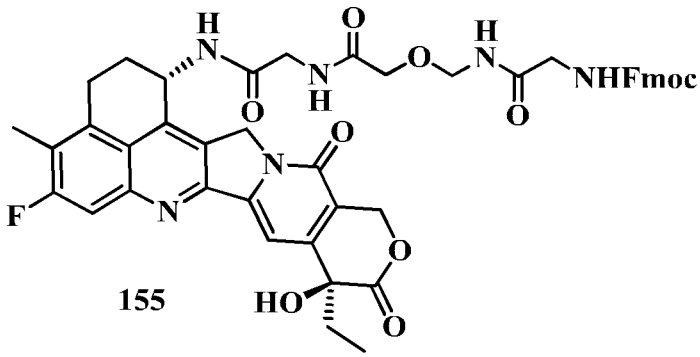
Example 142. Synthesis of (2-((2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino) acetamido)methoxy)acetyl)glycine (**154**).



154

Compound **153** (600.3 mg, 1.1 mmol) was dissolved in 20 mL of MeOH, 10 wt% Pd/C (10.1 mg, 0.1 mmol) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 1 hour, the reaction mixture was filtered and the filtrate was concentrated, and triturated with ethyl acetate. The white solid was collected by filtration (420.2 mg, yield 84%). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{24}N_3O_7$, 442.15; found: 442.15.

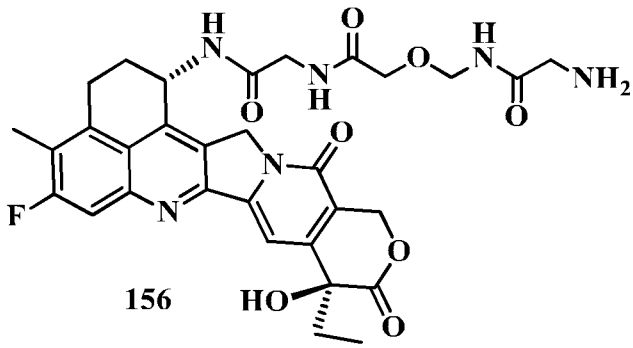
Example 143. Synthesis of (9H-fluoren-9-yl)methyl (2-(((2-((2-(((1S,9S)-9-ethyl-5 -fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-2-oxoethoxy)methyl)amino)-2-oxoethyl)carbamate (**155**).



155

Compound **154** (302.1 mg, 0.68 mmol) was dissolved in 10 mL of DMF, and cooled over an ice-water bath to 0 °C. Exatecan (325.1 mg, 0.61 mmol) and HATU (387.5 mmol, 1.01 mmol) were added, followed by diisopropylethylamine (180.2 μL, 1.36 mmol) dropwise. The reaction was stirred for about 20 min at r.t. and filtered. The filtrate was purified by preparative HPLC to give the title compound (135.2 mg, 23% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{46}H_{44}FN_6O_{10}$, 859.30; found: 859.30.

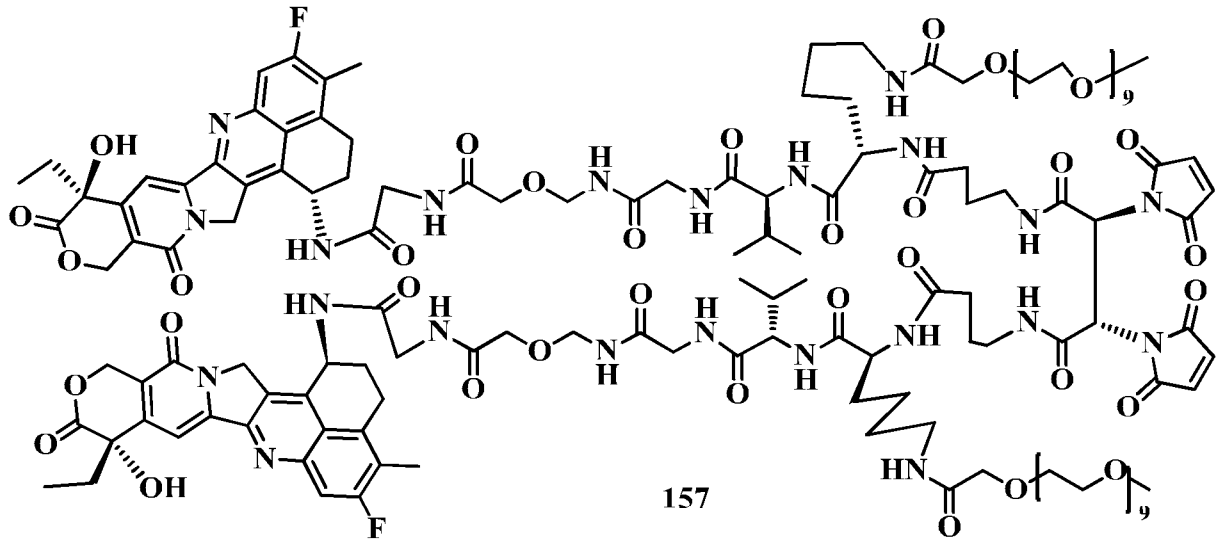
Example 144. Synthesis of 2-amino-N-((2-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy -4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-2-oxoethoxy)methyl)acetamide (**156**).



156

Compound **155** (135 mg, 0.157 mmol) was dissolved in 5 mL of DMF, 0.5 mL of piperidine was added, and the reaction was stirred at r.t. for 20 min, concentrated, re-dissolved in DMF, and concentrated again to give the title compound (100.9 mg, > 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{31}H_{34}FN_6O_8$, 637.23; found: 637.23.

Example 145. Synthesis of (2S,3S)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -N1,N4-bis((S)-37-(((S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-14-methyl-1,4,9,12-tetraoxo-6-oxa-3,8,11-triazapentadecan-13-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**157**).

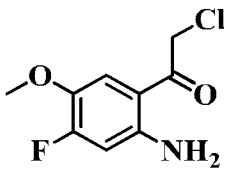


157

Compound **144** (140.2 mg, 0.075 mmol) and compound **156** (100.9 mg, 0.157 mmol) were dissolved in 5 mL of DMF, cooled to 0 °C, and HATU (86.2 mg, 0.224 mmol) was added, followed by diisopropylethylamine (23.6 mg, 0.15 mmol) dropwise. The reaction was warmed to r.t. and after stirring for 20 min, the mixture was purified by preparative HPLC. The fractions were concentrated

and lyophilized to give the title compound (36.6 mg, 16% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{146}H_{207}F_2N_{22}O_{50}$, 3106.42; found: 3106.42.

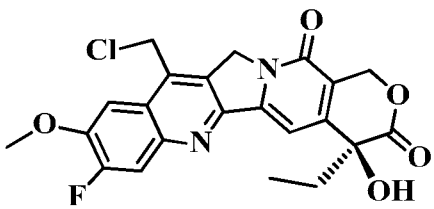
Example 146. Synthesis of 1-(2-amino-4-fluoro-5-methoxyphenyl)-2-chloroethan-1-one (**158**).



158

A solution of 3-fluoro-4-methoxyaniline (5 g, 35.4 mmol) in dichloromethane (20 mL) was added dropwise to an ice-water cooled boron trichloride (1 M in dichloromethane, 38.9 mL) solution. The reaction was stirred for 10 minutes and then chloroacetonitrile (3.2 g, 42.5 mmol) and aluminum trichloride (5.2 g, 38.9 mmol) were added. After the addition was completed, the reaction was warmed to r.t. and then refluxed overnight. The reaction mixture was then cooled to about 0 ° C, quenched with 2 M HCl (80 mL) and stirred at r.t. for 2 hours. Layers were separated and the aqueous phase was extracted with dichloromethane (3 × 80 mL). Combined organic phases were washed with water (100 mL), dried over sodium sulfate, filtered, concentrated, purified on a silica gel column, eluted with petroleum ether/ethyl acetate to give compound **158** (2 g, 26% yield) as a yellow solid. ESI-MS m/z: $[M + H]^+$ calcd for $C_9H_9ClFNO_2$, 218.03; found 218.03.

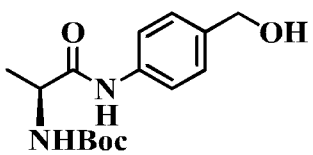
Example 147. Synthesis of (S)-11-(chloromethyl)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy -1,12-dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione (**160**).



160

Compound **158** (0.50 g, 2.29 mmol) and compound **159** (0.57 g, 2.19 mmol) were dissolved in anhydrous toluene (40 mL), and *p*-toluenesulfonic acid (42 mg, 0.219 mmol) was added. The suspension was heated at reflux for 2 days and allowed to cool to r.t. After removal of about two-thirds of toluene, the residue was filtered and the filter cake was washed with dichloromethane, air-dried to give compound **160** (0.7 g, 72% yield) as a gray powdery solid. ESI-MS m/z: $[M + H]^+$ calcd for $C_{22}H_{18}ClFN_2O_5$, 445.09; found 445.09.

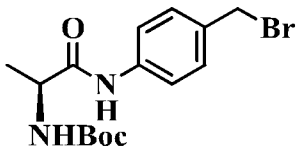
Example 148. Synthesis of tert-butyl (S)-(1-((4-(hydroxymethyl)phenyl)amino)-1 -oxopropan-2-yl)carbamate (**161**).



161

p-Aminobenzyl alcohol (5.0 g, 0.04 mol) and Boc-L-alanine (8.0 g, 0.042 mol) were dissolved in anhydrous THF (100 mL), and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (11 g, 0.044 mol) was added and stirred at r.t. overnight. The reaction mixture was poured into water (300 mL), extracted with ethyl acetate (3 × 100 mL), the combined organic phases were washed with water (100 mL), dried over sodium sulfate, filtered, and concentrated. The crude product was triturated with ethyl acetate / petroleum ether (1: 3) and filtered to yield compound **161** (9.8 g, 84% yield) as a white solid. ESI-MS m/z: $[M + H]^+$ calcd for $C_{15}H_{22}N_2O_4$, 295.16; found 295.16.

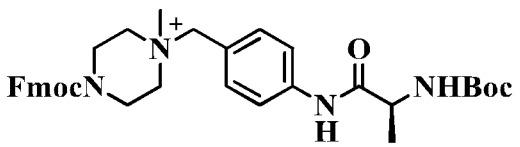
Example 149. Synthesis of tert-butyl (S)-(1-((4-(bromomethyl)phenyl)amino)-1-oxopropan -2-yl)carbamate (**162**).



162

Compound **161**(3.5 g, 11.9 mmol) and carbon tetrabromide (5.9 g, 17.8 mmol) were dissolved in dichloromethane (80 mL), cooled to about 0 ° C, and triphenylphosphine (4.7 g, 17.8 mmol) was added. The reaction was warmed to r.t. and stirred for 30 minutes, and then 20 g of silica gel was added, mixed, and dried on a rotavap, loaded on a silica gel column (100 g of silica gel) and eluted with petroleum ether / ethyl acetate to yield compound **162** (2.6 g, 62% yield). ESI-MS m/z: $[M + H]^+$ calcd for $C_{15}H_{21}BrN_2O_3$, 357.07; found 357.07.

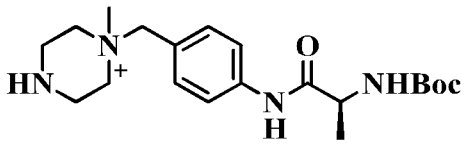
Example 150. Synthesis of (S)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)-1- (4-(2-((tert-butoxycarbonyl)amino)propanamido)benzyl)-1-methylpiperazin-1-ium (**164**).



164

Compound **162** (2.3 g, 6.4 mmol) and (9H-fluoren-9-yl)methyl 4-methylpiperazine-1-carboxylate (**163**, 2.1 g, 6.4 mmol) were dissolved in anhydrous THF (100 mL) and stirred at r.t. overnight. After removal of most THF on a rotavap, ethyl acetate (200 mL) was added to the residue. The resulting slurry was filtered to give a white solid (3.8 g, 87% yield). ESI-MS m/z: M^+ calcd for $C_{35}H_{43}N_4O_5$, 599.32; found 599.32.

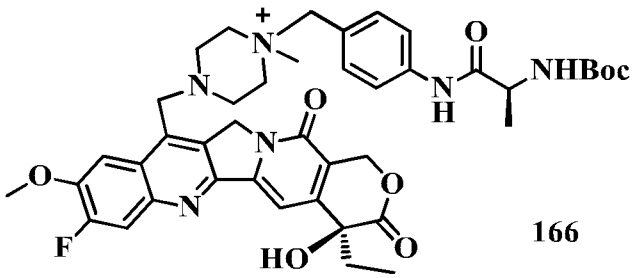
Example 151. Synthesis of (S)-1-(4-(2-((tert-butoxycarbonyl)amino) propanamido)benzyl) -1-methylpiperazin-1-ium (**165**).



165

Compound **164** (3.12 g, 4.6 mmol) was dissolved in DMF (25 mL), and piperidine (3 mL) was added. After stirring at r.t. for 2 hours, 200 mL of ethyl acetate was added and stirred for 10 minutes. The mixture was filtered to give a white solid (1.54 g, 77% yield). ESI-MS m/z: M^+ calcd for $C_{20}H_{33}N_4O_3$, 377.26; found 377.26.

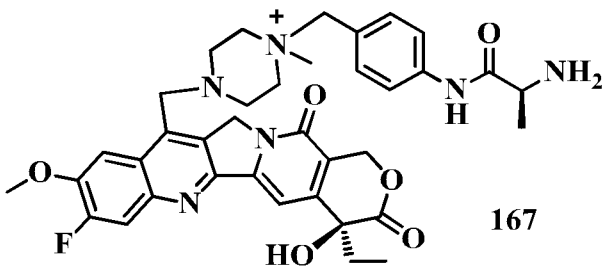
Example 152. Synthesis of 1-(4-((S)-2-((tert-butoxycarbonyl)amino) propanamido)benzyl) -4-(((S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl)-1-methylpiperazin-1-ium (**166**).



166

A mixture of compound **165** (0.30 g, 0.66 mmol), compound **160** (0.25 g, 0.56 mmol) in DMF (10 mL) was stirred at 0 ° C for 30 minutes, then N, N-diisopropylethylamine (49 μL, 0.28 mmol) was added and the reaction was warmed to r.t. and stirred overnight, concentrated and purification by preparative HPLC (acetonitrile/water containing formic acid) to give compound **166** (0.40 g, 80% yield). ESI-MS m/z: M^+ calcd for $C_{42}H_{50}FN_6O_8$, 785.37; found 785.37.

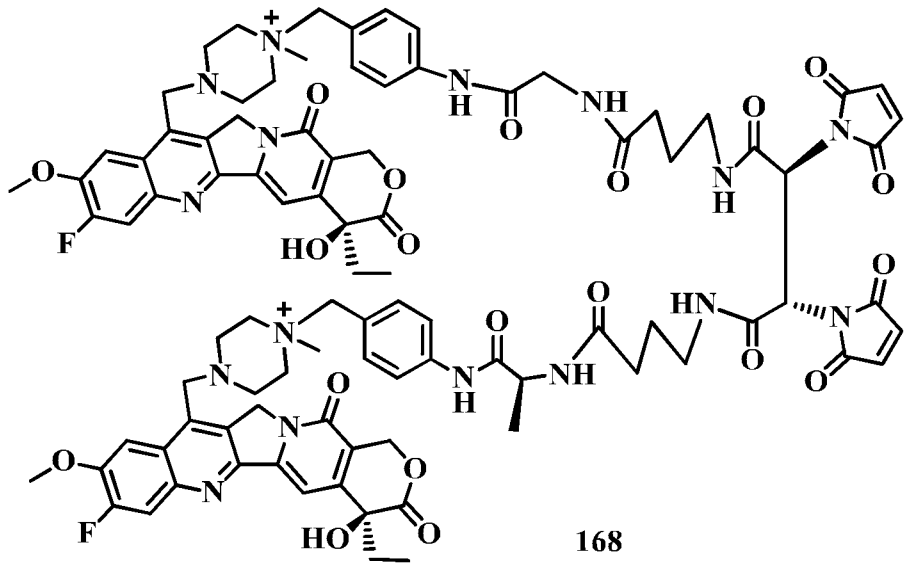
Example 153. Synthesis of 1-(4-((S)-2-aminopropanamido)benzyl)-4-(((S)-4-ethyl-8 -fluoro-4-hydroxy-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7] indolizino[1,2-b]quinolin-11-yl)methyl)-1-methylpiperazin-1-ium (**167**).



167

Compound **166** (0.30 g, 0.35 mmol) was dissolved in a mixture of dichloromethane and trifluoroacetic acid (3 mL/ 3 mL), and stirred at r.t. for 30 minutes. The mixture was then concentrated and dried on a vacuum pump to give compound **167** (0.27 g, 100% yield) as a yellow solid. ESI-MS m/z: M^+ calcd for $C_{37}H_{42}FN_6O_6$, 685.31; found 685.31.

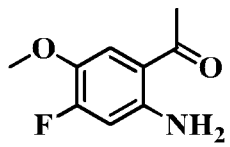
Example 154. Synthesis of compound **168**.



168

Compound **20** (50 mg, 0.1 mmol) and compound **167** (160 mg, 0.23 mmol) were dissolved in DMF (3 mL), HATU (120 mg, 0.3 mmol) and NMM (65 mg, 0.6 mmol) were added and stirred at r.t. for 2 hours. The reaction solution was directly purified by preparative HPLC (acetonitrile/water containing 0.1% HCOOH) to give 86 mg of compound **168** in 46% yield. ESI-MS m/z: M^{2+} calcd for $C_{94}H_{102}F_2N_{16}O_{20}$, 906.4; found 907.1.

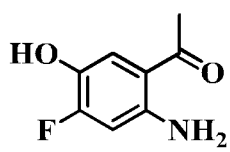
Example 155. Synthesis of 1-(2-amino-4-fluoro-5-methoxyphenyl)ethan-1-one (**169**).



169

To a solution of 3-fluoro-4-methoxyaniline (5.0 g, 35.4 mmol) in dichloromethane (20 mL), was added BCl_3 (1 M, 39.0 mL) in dichloromethane at 0 °C, followed by CH_3CN (2.2 mL, 42.5 mmol) and $AlCl_3$ (5.2 g, 38.9 mmol). The mixture was heated to reflux and stirred under reflux overnight, cooled to 0 °C. 2N HCl (80 mL) was added and stirred for 2 hours, then extracted with ethyl acetate (3 × 50 mL). The organic phase was combined and washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by a silica gel column to give a yellow solid (0.7 g, 11% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_9H_{11}FNO_2$, 184.07; found 184.07.

Example 156. Synthesis of 1-(2-amino-4-fluoro-5-hydroxyphenyl)ethan-1-one (**170**).

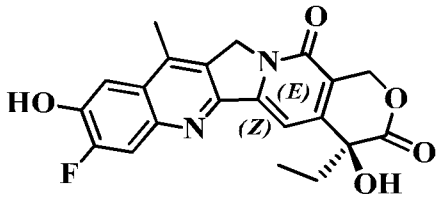


170

To a solution of compound **169** (0.71 g, 3.87 mmol) in dichloromethane (10 mL) was added BBr_3 (1 M, 7.7 mL). The solution was warmed to r.t. and stirred for 48 hours, cooled to 0 °C and quenched with water (100 mL), extracted with ethyl acetate (3 × 50 mL). The organic phases were

combined and washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacumm. The residue was purified by a silica gel column to give a yellow solid (0.4 g, 60% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_8H_9FNO_2$, 170.05; found 170.05.

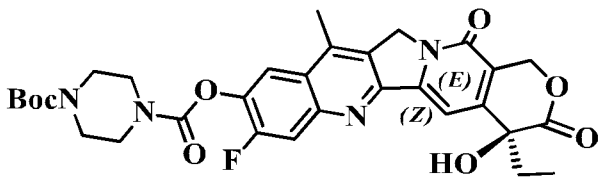
Example 157. Synthesis of (S)-4-ethyl-8-fluoro-4,9-dihydroxy-11-methyl-1,12-dihydro -14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione (**171**).



171

To a solution of compound **170** (2.65 g, 10.05 mmol) and (S)-4-ethyl-4-hydroxy-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,6,10(4H)-trione (2.65 g, 10.05 mmol) in toluene (80 mL) was added p-toluenesulfonic acid (0.1 g), and the mixture was heated to reflux, stirred for 24 hours, and cooled to r.t. The resulting solid was filtered, rinsed with dichloromethane and dried to give compound **171** (1.3 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{21}H_{18}FN_2O_5$, 397.11; found 397.11.

Example 158. Synthesis of (S)-1-(tert-butyl) 4-(4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14 -dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) piperazine-1,4-dicarboxylate (**172**).

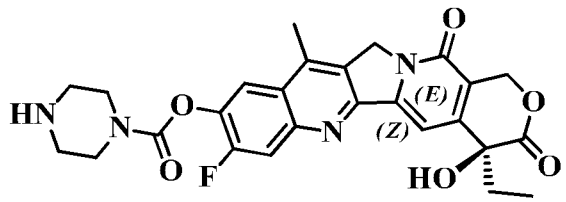


172

To a solution of tert-butyl 1-piperazinecarboxylate (1.0 g, 5.36 mmol) and pyridinium (0.65 ml, 8.05 mmol) in dichloromethane (5 mL) was added triphosgene (1.9 g, 6.44 mmol) at 0 °C. The mixture was warmed to r.t. and stirred for 1 hours, and then diluted with dichloromethane (50 mL) and washed with 1N HCl (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacumm to give a yellow solid (1.3 g, 100% yield).

To a solution of compound **171** (400 mg, 1.01 mmol) and diisopropylethylamine (0.33 mL, 2.02 mol) in DMF (5 mL) was added the above compound (326 mg, 1.31 mmol) at 0 °C. The mixture was warmed to r.t. and stirred for 4 hours, then concentrated under vacuum and purified by a silica gel column to give a brown solid (430 mg, 70% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{31}H_{33}FN_4O_8$, 609.23; found 609.23.

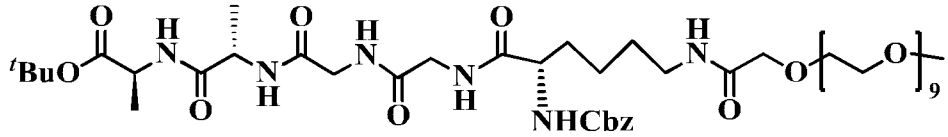
Example 159. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl piperazine-1-carboxylate (**173**).



173

Compound **172** (100 mg, 0.164 mmol) was dissolved in dichloromethane (6 mL) and trifluoroacetic acid (2 mL), and the reaction was stirred for 30 min, and then concentrated, co-evaporated with dichloromethane twice, dried on an oil pump to give compound **173** as a brown solid (83 mg, 100.00%). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{26}FN_4O_6$, 509.18; found 509.18.

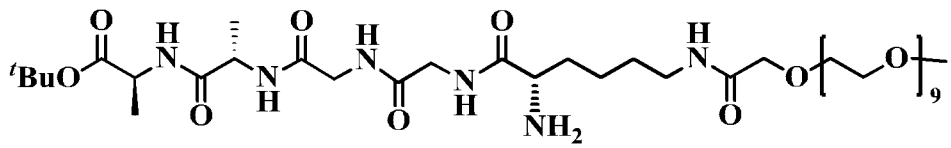
Example 160. Synthesis of tert-butyl ((S)-37-(((benzyloxy)carbonyl)amino)-31-oxo-2,5,8, 11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycyl-L-alanyl-L-alaninate (**174**).



174

To a solution of compound **46** (3.02 g, 3.5 mmol) and H-Ala-Ala-O^{t}Bu (0.9 g, 3.56 mmol) in THF (60 mL), were added HATU (1.99 g, 5.23 mmol) and diisopropylethylamine (1.1 mL, 6.95 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated under vacuum and poured into water (100 mL), extracted with dichloromethane (3 × 50 mL). The combined organic phases were washed with water (50 mL), saturated sodium bicarbonate (50 mL), 2 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give compound **174** (3.6 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{49}H_{84}N_6O_{19}$, 1061.58; found 1061.58.

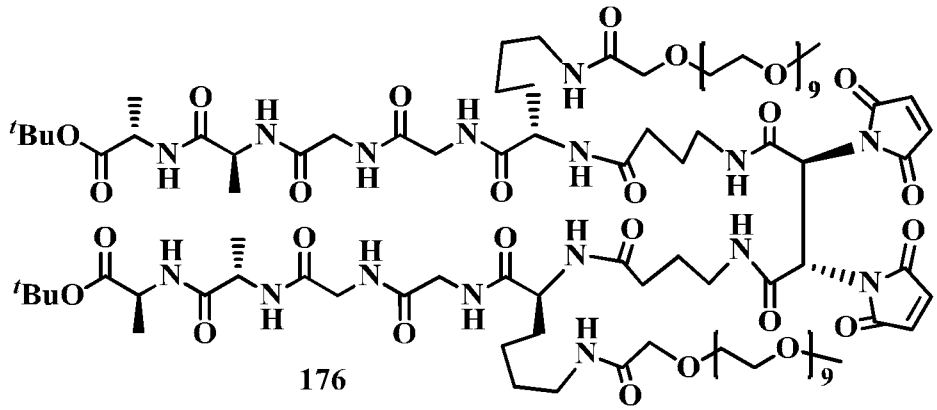
Example 161. Synthesis of tert-butyl ((S)-37-amino-31-oxo-2,5,8,11,14,17,20,23, 26,29-decaoxa-32-azaoctatriacontan-38-oyl)glycylglycyl-L-alanyl-L-alaninate (**175**).



175

Compound **174** (3.6 g, 3.88 mmol) was dissolved in THF (60 mL), palladium on carbon (10 wt%, 0.4 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound **175** (2.6 g, 74% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{41}H_{79}N_6O_{17}$, 927.54; found 927.54.

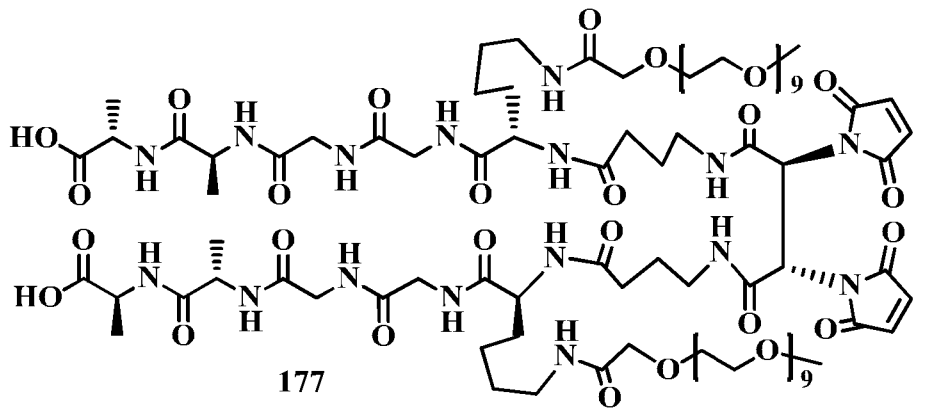
Example 162. Synthesis of di-tert-butyl (2S,5S,14S,22S,23S,31S,40S,43S)-22,23-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,40,43-tetramethyl-4,7,10,13,16,21,24,29, 32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioate (**176**).



176

To a solution of compound **175** (2.6 g, 2.8 mmol) and compound **19** (0.6 g, 1.25 mmol) in THF/DMF (20 mL/4 mL), were added HATU (1.43 g, 3.76 mmol) and diisopropylethylamine (0.62 mL, 3.79 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated under vacuum and poured into water (200 mL), extracted with dichloromethane (3×100 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated to give compound **176** (2.8 g, 100% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{102}H_{175}N_{16}O_{42}$, 2296.20; found 2296.20.

Example 163. Synthesis of (2S,5S,14S,22S,23S,31S,40S,43S)-22,23-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,40,43-tetramethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioic acid (**177**).

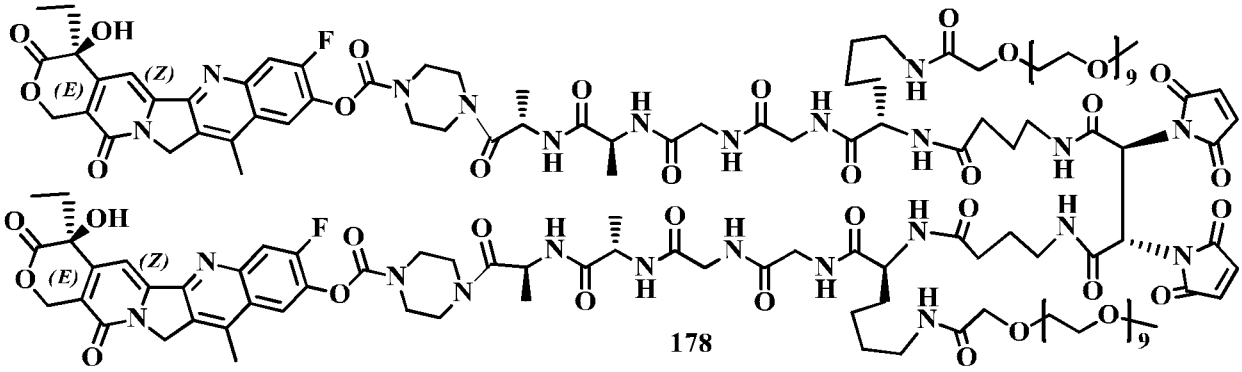


177

Compound **176** (2.8 g, 1.2mmol) was dissolved in formic acid (40 mL) and dichloromethane (20 mL). The mixture was heated to 50 °C and stirred overnight, and then concentrated under vacuum, purified by preparative HPLC to give compound **177** (1.6 g, 61% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{94}H_{159}N_{16}O_{42}$, 2184.07; found 2184.07.

Example 164. Synthesis of bis((S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo -3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) 4,4'-((2S,5S,14S,22S, 23S,31S,40S,43S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,40,43-tetramethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-

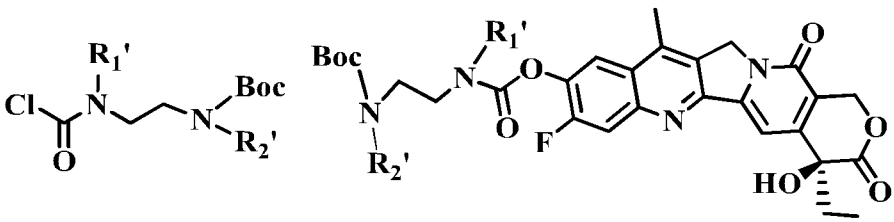
32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30, 33,36,39,42-dodecaazatetratetracontanedioyl)bis (piperazine-1-carboxylate) (**178**).



178

To a solution of compound **177** (125 mg, 0.057 mmol) and compound **173** (58 mg, 0.115 mmol) in DMF (5 mL), were added HATU (65 mg，0.173 mmol) and diisopropylethylamine (38 μL, 0.230 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give compound **178** (67 mg, 37% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{146}H_{205}F_2N_{24}O_{52}$, 3164.40; found 3164.40.

Example 165. Synthesis of (S)-tert-butyl (4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo -3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ethane-1,2-diylbis (methylcarbamate) (**179**).

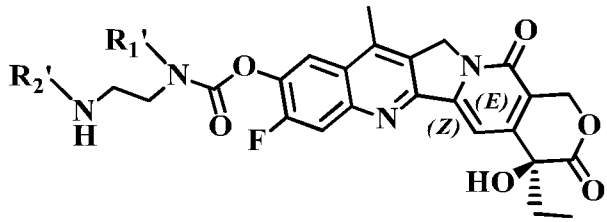


179a, $R_1'=R_2'=CH_3$;
179b, $R_1'=CH_3$, $R_2'=H$;
179c, $R_1'=H$, $R_2'=CH_3$;
179d, $R_1'=R_2'=H$;

To a solution of tert-butyl 2-(methylamino)ethylcarbamate (1.0 g, 5.74 mmol) and pyridinium (0.69 ml, 8.61 mmol) in dichloromethane(20 mL) was added triphosgene (2.0 g, 6.89 mmol) at 0 °C. The mixture was warmed to r.t. and stirred for 1 hour, diluted with dichloromethane (50 mL) and washed with 1N HCl (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacumm to give a yellow oil (1.3 g, 100% yield).

To a solution of compound **171** (712 mg, 1.79 mmol) and diisopropylethylamine (0.59 ml, 3.59 mol) in DMF (10 mL) were added the above compound (710 mg, 3.00 mmol) and DMAP (43 mg, 0.36 mmol) at 0 °C. The reaction was warmed to r.t. and stirred overnight, then concentrated under vacumm and purified by a silica gel column to give a brown solid (700 mg, 65% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{30}H_{34}FN_4O_8$, 597.23; found 597.23.

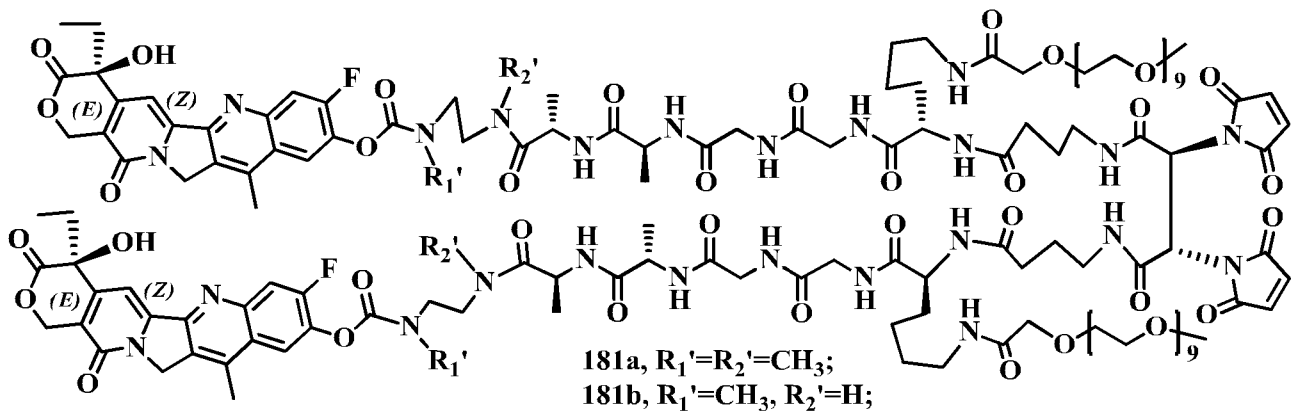
Example 166. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo -3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl methyl(2-(methylamino) ethyl)carbamate (**180**).



180a, $R_1'=R_2'=CH_3$;
180b, $R_1'=CH_3$, $R_2'=H$;
180c, $R_1'=H$, $R_2'=CH_3$;
180d, $R_1'=R_2'=H$;

Compound **179** (55 mg, 0.092 mmol) was dissolved in dichloromethane (3 mL). trifluoroacetic acid (1 mL) was added and the reaction was stirred for 30 min, and then concentrated, co-evaporated with dichloromethane twice, dried on an oil pump to give compound **180** as a brown solid (50 mg, >100.00% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{26}FN_4O_6$, 497.18; found 497.18.

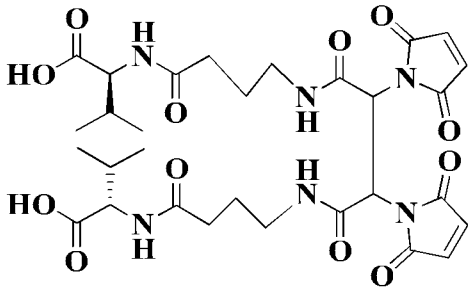
Example 167. Synthesis of bis((S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12, 14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((5S,8S,17S,25S,26S,34S,43S, 46S)-25,26-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,5,8,43,46,49-hexamethyl-4,7,10,13,16,19, 24,27,32,35,38,41,44,47,48-pentadecaoxo-17,34-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,18,23,28,33,36,39,42,45,49-tetradecaazahenpentacontane-1,51-diyl)bis(methylcarbamate) (**181**).



181a, $R_1'=R_2'=CH_3$;
181b, $R_1'=CH_3$, $R_2'=H$;
181c, $R_1'=H$, $R_2'=CH_3$;
181d, $R_1'=R_2'=H$;

To a solution of compound **173** (168 mg, 0.08 mmol) and compound **180** (81 mg, 0.16 mmol) in DMF (5 mL), were added HATU (100 mg, 0.26 mmol) and diisopropylethylamine (52μL, 0.32 mmol). The reaction was stirred at r.t. until complete conversion, and then concentrated and purified by preparative HPLC to give compound **181** (97 mg, 39% yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{144}H_{207}F_2N_{22}O_{50}$, 3081.42, found 3081.42.

Example 168. Synthesis of (4-(4-((4-(((S)-1-carboxy-2-methylpropyl)amino)-4-oxobutyl) amino)-2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4-oxobutanamido) butanoyl)-L-valine (**184**)

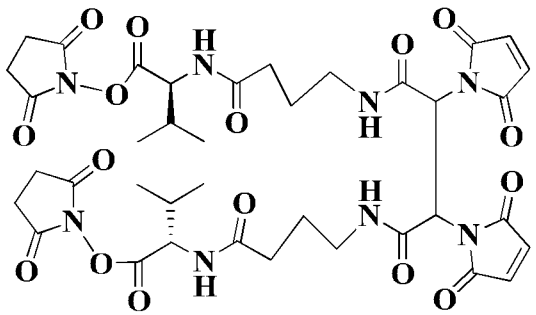


184

To a mixture of compound **12** (2.0 g, 4.18 mmol) in anhydrous acetonitrile (50 mL) were added 3-(ethyliminomethylideneamino)-N,N-dimethylpropan-1-amine hydrochloride (3.2 g, 16.72 mmol) and N-hydroxysuccinimide (1.9 g, 16.72 mmol). The mixture was stirred at r.t. until complete conversion and diluted with DCM (100 mL) and washed with brine (200 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum to give an off-white solid.

The obtained compound was dissolved in DMF (50 mL) and valine (1.5 g, 12.54 mmol) was added. The mixture was stirred at r.t. until complete conversion and then directly purified by preparative HPLC to give compound **184** (2.2 g, 78%yield) as an off-white solid. MS-ESI (m/z): $[M + H]^+$ calcd for $C_{30}H_{40}N_6O_{12}$, 677.27; found, 677.53.

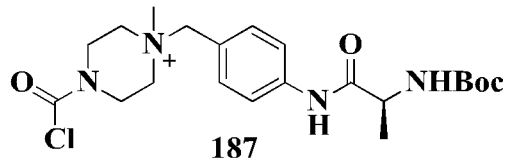
Example 169. Synthesis of 2,5-dioxopyrrolidin-1-yl (4-(2,3-bis(2,5-dioxo-2,5-dihydro- 1H-pyrrol-1-yl)-4-((4-(((R)-1-((2,5-dioxopyrrolidin-1-yl)oxy)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutyl)amino)-4-oxobutanamido)butanoyl)-L-valinate (**185**).



185

To a mixture of compound **184** (1.0 g, 1.48 mmol) in anhydrous acetonitrile (40 mL) were added 3-(ethyliminomethylideneamino)-N,N-dimethylpropan-1-amine hydrochloride (1.2 g, 5.92 mmol) and N-hydroxysuccinimide (685 mg, 5.92 mmol). The mixture was stirred at r.t. until complete conversion, diluted with DCM (50 mL) and washed with brine (150 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum to give compound **185** (1.2 g, 93%yield) as an off-white solid. MS-ESI (m/z): $[M + H]^+$ calcd for $C_{38}H_{46}N_8O_{16}$, 871.30; found, 871.65.

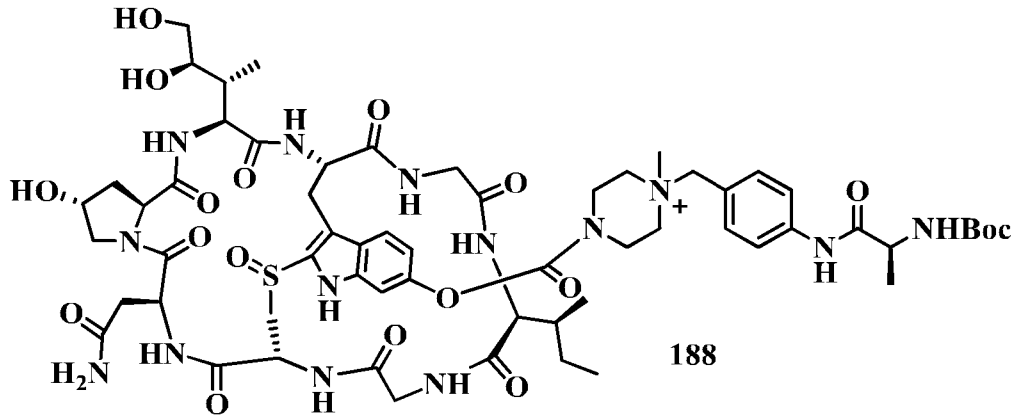
Example 170. Synthesis of (S)-1-(4-(2-((tert-butoxycarbonyl)amino)propanamido) benzyl)-4-(chlorocarbonyl)-1-methylpiperazin-1-ium (**187**).



187

To a solution of compound **186** (300 mg, 0.796 mmol) in DCM (15 mL), was added triphosgene (98 mg, 0.318 mmol) at 0 °C. The reaction was stirred at 0 °C until complete conversion, and then concentrated and purified by preparative HPLC to give compound **187** (190 mg, 54%yield). MS-ESI (m/z): $[M]^+$ calcd for $C_{21}H_{32}ClN_4O_4$, 439.20; found 439.35.

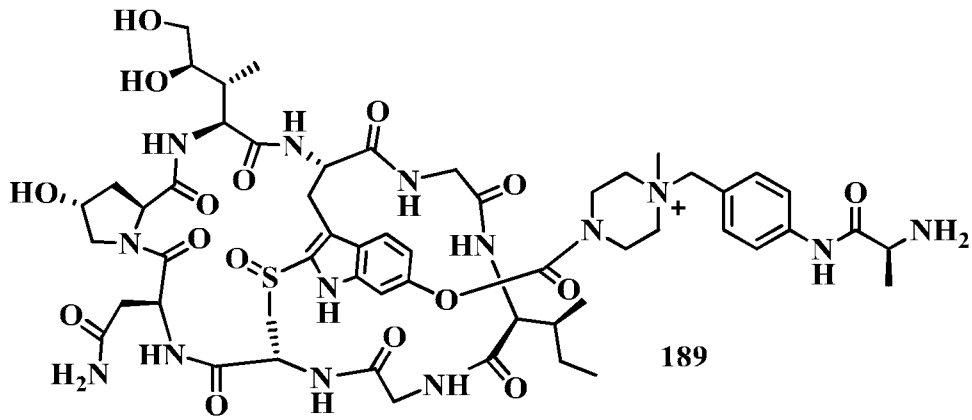
Example 171. Synthesis of compound **188**.



188

To a solution of α-amanitin (30 mg, 0.033 mmol) and compound **187** (42.9 mg, 0.099 mmol) in DMF (1 mL) was added diisopropylethylamine (12.6 mg, 0.099 mmol) at 0 °C. The reaction was stirred at 0 °C until complete conversion and then purified by preparative HPLC to give compound **189** (36 mg, 83%yield). MS-ESI (m/z): $[M]^+$ calcd for $C_{60}H_{85}N_{14}O_{18}S$, 1321.58; found 1321.69.

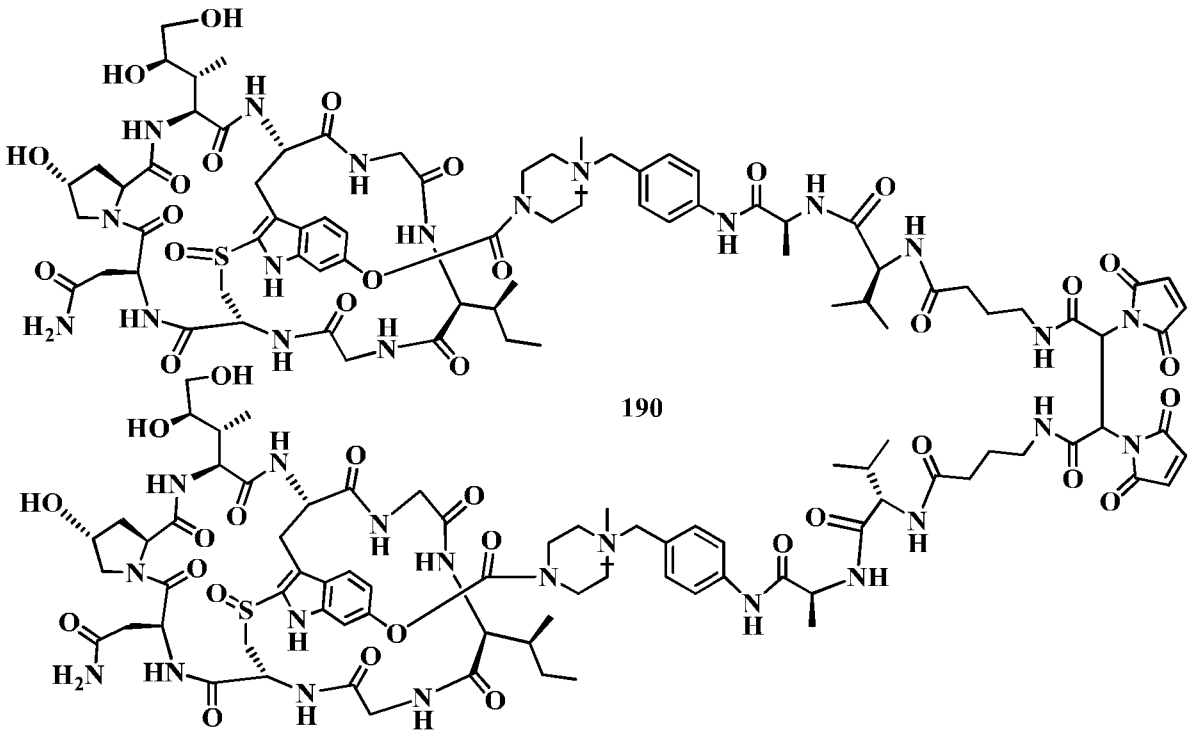
Example 172. Synthesis of compound **189**.



189

A solution of compound **188** (36 mg, 27.2 umol) in TFA/DCM (1/10, 1 mL) was stirred at r.t. until complete conversion. The reaction mixture was concentrated under vacuum to give compound **189** (35 mg, crude product, 100%yield). MS-ESI (m/z): $[M]^+$ calcd for $C_{55}H_{77}N_{14}O_{16}S^+$, 1221.58; found 1221.70.

Example 173. Synthesis of compound **190**.

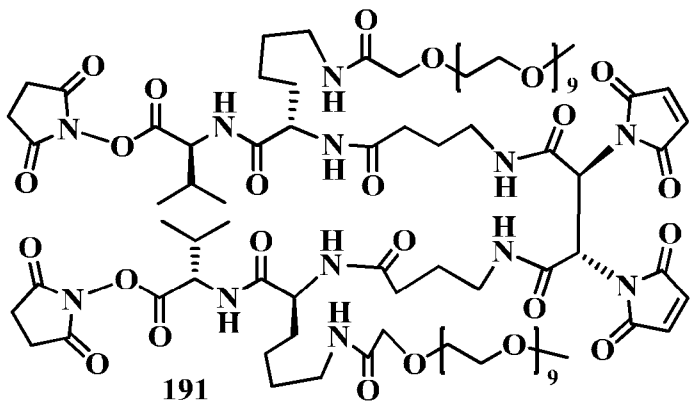


190

To a mixture of compound **185** (4 mg, 4.59 μmol) and **189** (11.8 mg, 9.65 μmol) in anhydrous DMF (2 mL) was added diisopropylethylamine (1.5 mg, 11.47 μmol) at 0 °C.

The mixture was stirred at 0 °C until complete conversion, and directly purified by preparative HPLC to give compound **190** (8.6 mg, 60%yield) as an off-white solid. MS-ESI (m/z): $[M]^+$ calcd for $C_{140}H_{190}N_{34}O_{42}S_2$, 1541.66; found, 1541.69.

Example 174. Synthesis of bis(2,5-dioxopyrrolidin-1-yl) (2S,5S,13S,14S,22S,25S)-13,14-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,25-diisopropyl-4,7,12,15,20,23-hexaoxo-5,22-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,11,16,21,24-hexaazahexacosanedioate (**191**).

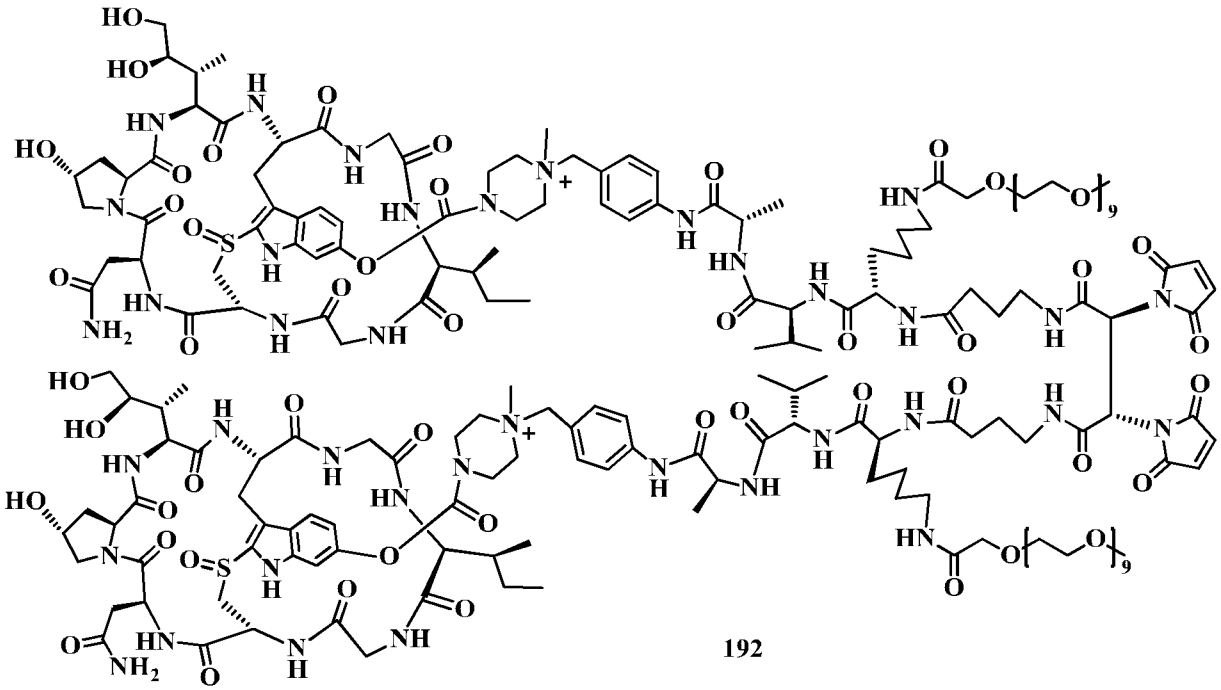


191

To a mixture of compound **144** (100 mg, 53.47 umol) in anhydrous DCM (5 mL) were added 3-(ethyliminomethylideneamino)-N,N-dimethylpropan-1-amine hydrochloride (41 mg, 214 umol) and N-hydroxysuccinimide (25 mg, 214 umol). The mixture was stirred at 0 °C until complete conversion, and then diluted with DCM (10 mL), washed with brine (2 ×10 mL). The organic layer

was dried over Na_2SO_4 and concentrated under vacuum to give compound **191** (100 mg, 91%yield) as an oil. MS-ESI (m/z): $[M + H]^+$ calcd for $C_{92}H_{150}N_{12}O_{40}$, 2064.01; found, 2064.12.

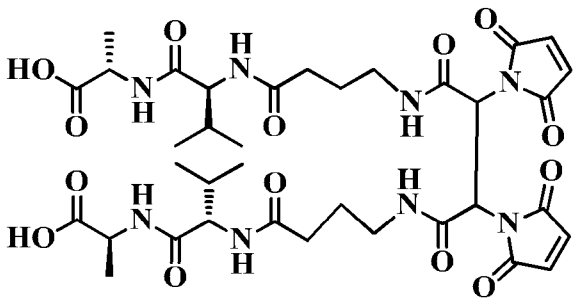
Example 175. Synthesis of compound **192**.



192

To a mixture of compound **191** (8 mg, 3.88 μmol) and **189** (10 mg, 8.14 μmol) in anhydrous DMF (2 mL) were added diisopropylethylamine (1.5 mg, 11.47 umol) at 0 °C. The mixture was stirred at 0 °C until complete conversion and directly purified by preparative HPLC to give compound **192** (10.6 mg, 64%yield) as an off-white solid. MS-ESI (m/z): $[M]^+$ calcd for $C_{194}H_{294}N_{38}O_{66}S_2$, 2138.03; found, 2138.03.

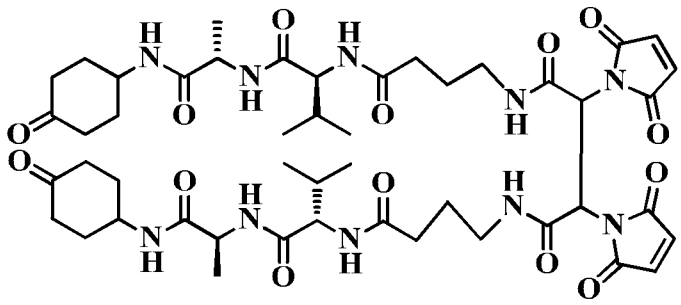
Example 176. Synthesis of (2S,5S,22S,25S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol -1-yl)-5,22-diisopropyl-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-3,6,11,16,21,24-hexaazahexacosanedioic acid (**193**).



193

To a mixture of compound **185** (600 mg, 689 μmol) and L-analine (184 mg, 2.07 mmol) in anhydrous DMF (20 mL) were added diisopropylethylamine (267 mg, 2.07 mmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion and directly purified by preparative HPLC to give compound **193** (510 mg, 91%yield) as an off-white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{36}H_{50}N_8O_{14}$, 819.36; found, 819.53.

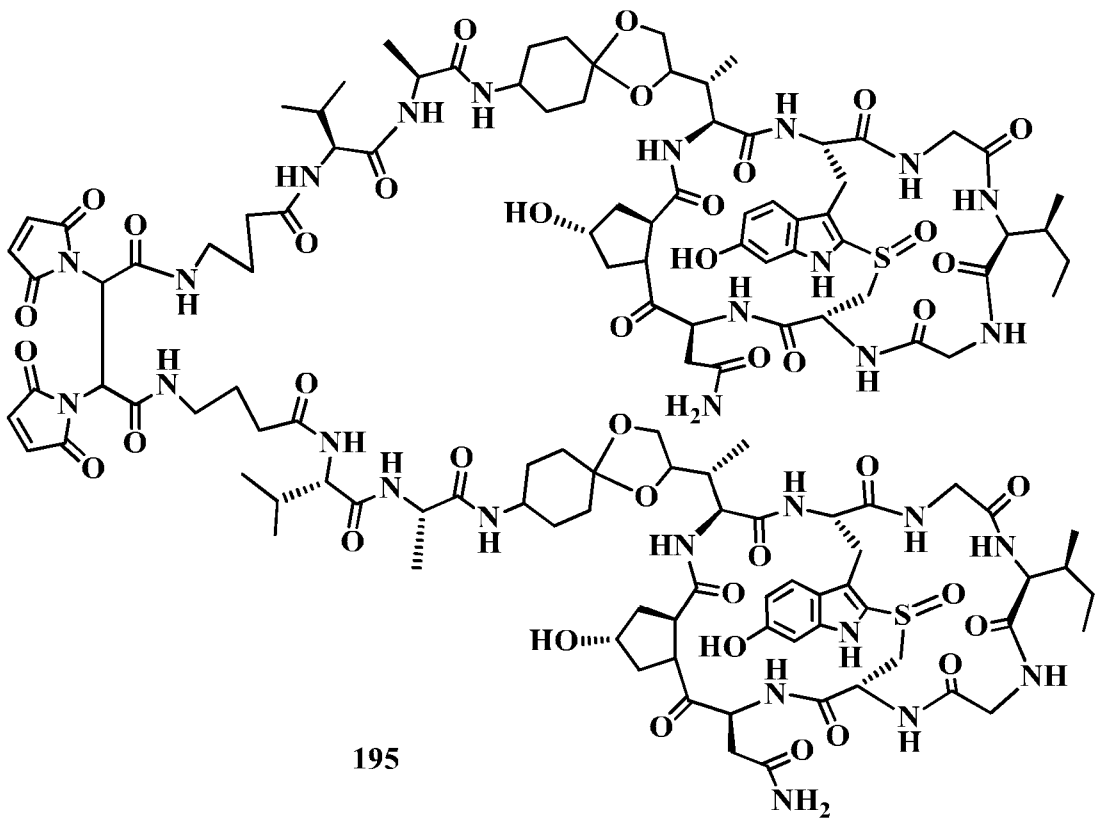
Example 177. Synthesis of 2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4-bis (4-(((S)-3-methyl-1-oxo-1-(((S)-1-oxo-1-((4-oxocyclohexyl)amino)propan-2-yl)amino)butan-2-yl)amino)-4-oxobutyl)succinamide (**194**).



194

To a mixture of compound **193** (100 mg, 122 μmol) and 4-aminocyclohexan-1-one hydrochloride (55 mg, 366 umol) in anhydrous DMF (2 mL) were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMTMM, 135 mg, 488 μmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion, diluted with DCM (10 mL) and washed with brine (3 × 10 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column to give compound **194** (110 mg, 89%yield) as an off-white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{48}H_{68}N_{10}O_{14}$, 1009.49; found, 1009.68.

Example 178. Synthesis of compound **195**.

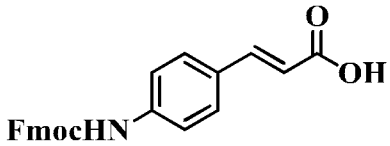


195

A mixture of compound **191** (5 mg, 4.95 μmol), toluene-4-sulfonic acid (1 mg, 5.86 μmol) and α-amantin (**182**, 9.6 mg, 10.41 μmol) in anhydrous THF (5 mL) was stirred at 60 °C until complete

conversion. The mixture was concentrated under vacuum and purified by preparative HPLC to give compound **195** (4.6 mg, 33%yield) as an off-white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{128}H_{174}N_{28}O_{42}S_2$, 2840.18; found, 2840.75.

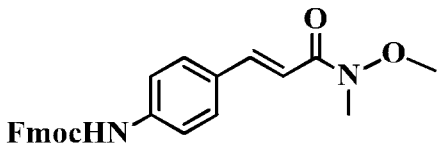
Example 179. Synthesis of 3-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino) phenyl)acrylic acid (**197**).



197

To a mixture of 4-aminocinnamic acid (1.0 g, 6.13 mmol) and N-(9-fluorenylmethoxycarbonyloxy) succinimide (2.3 g, 6.74 mmol) in DCM (20 mL) was added trimethylamine (930 mg, 9.21 mmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion, and then washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column to give compound **197** (2.2 g, 93%yield) as a white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{19}NO_4$, 386.13; found, 386.24.

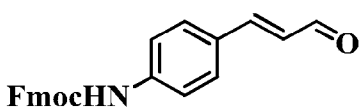
Example 180. Synthesis of (9H-fluoren-9-yl)methyl (E)-(4-(3-(methoxy(methyl) amino)-3-oxoprop-1-en-1-yl)phenyl)carbamate (**198**).



198

To a mixture of compound **197** (2.0 g, 5.19 mmol) and N-methoxymethanamine (610 mg, 6.23 mmol) in DCM (20 mL) were added HATU (3.0 g, 7.80 mmol) and trimethylamine (1.1 g, 10.20 mmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion, and then washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column to give compound **198** (1.8 g, 81%yield) as a white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{24}N_2O_4$,429.17; found, 429.30

Example 181. Synthesis of (9H-fluoren-9-yl)methyl (E)-(4-(3-oxoprop-1-en-1-yl) phenyl)carbamate (**199**).

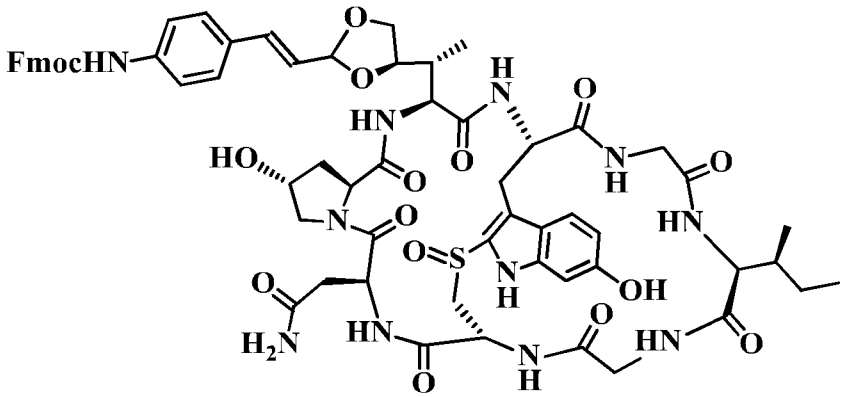


199

A mixture of compound **198** (1.8 g, 4.20 mmol) in DCM (20 mL) was cooled down to -60 °C under N_2. Diisobutylaluminium hydride (12.6 mL, 12.6 mmol, 1.0 M in THF) was added dropwise to the mixture. After additional, the mixture was stirred at -60 °C until complete conversion. The reaction was quenched with 10% NH_4Cl, and then washed brine (20 mL), dried over Na_2SO_4 and

concentrated under vacuum. The residue was purified by flash column to give compound **199** (900 mg, 58%yield) as an off-white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{19}NO_3$,370.17; found, 370.58.

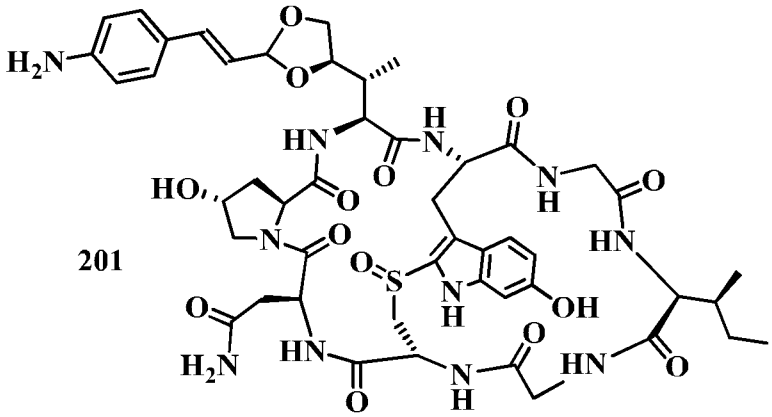
Example 182. Synthesis of compound **200**.



200

A mixture of compound **199** (8.8 mg, 23.94 μmol), toluene-4-sulfonic acid (1 mg, 5.86 μmol) and α-amantin (20.0 mg, 21.76 μmol) in anhydrous THF (5 mL) was stirred at 60 °C until complete conversion and concentrated under vacuum. The residue was purified by flash column to give compound **200** (16 mg, 57.9%yield). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{63}H_{71}N_{11}O_{16}S$, 1270.48; found, 1270.85.

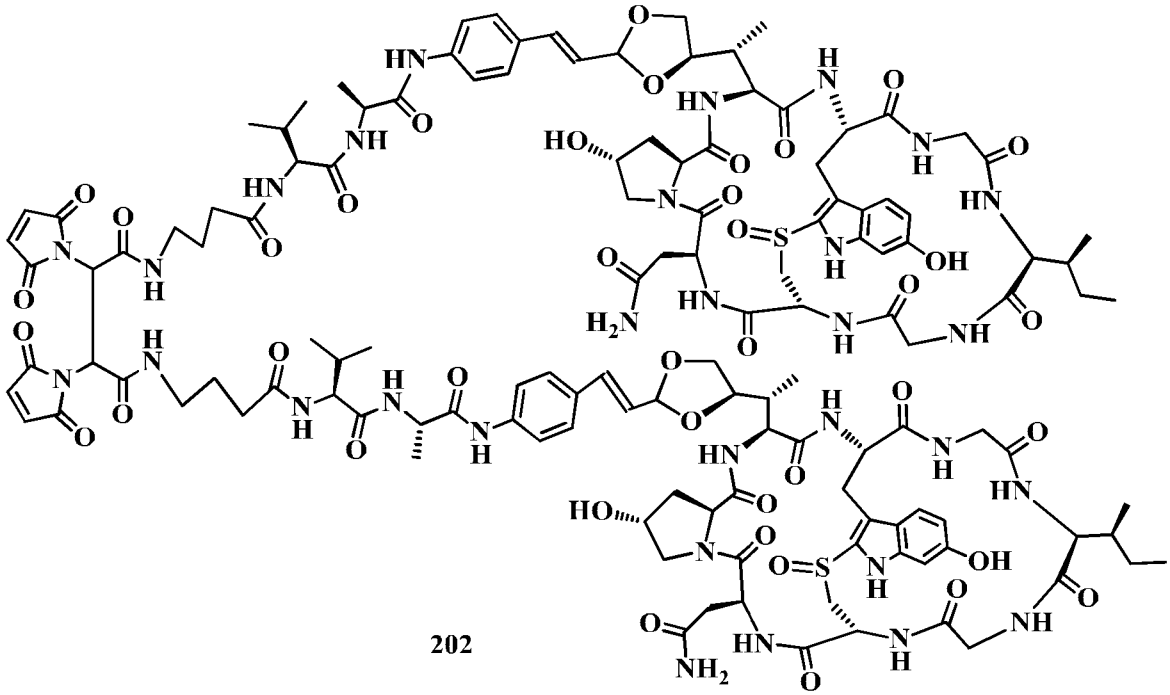
Example 183. Synthesis of compound **201**.



201

A solution of compound **200** (16 mg, 12.60 μmol) in piperidine/DMF (1 mL, 1/10) was stirred at r.t. for 10min and concentrated under vacuum to give compound **201** (16 mg, crude). MS-ESI (m/z): $[M+H]^+$ calcd for $C_{48}H_{61}N_{11}O_{14}S$, 1048.48; found, 1048.85.

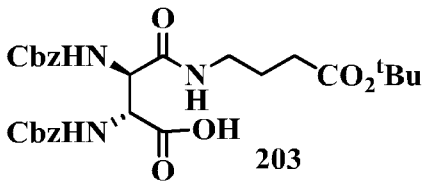
Example 184. Synthesis of compound **202**.



202

To a mixture of compound **193** (5 mg, 6.11 μmol) and compound **201** (13 mg, 12.81 μmol) in anhydrous DMF (1 mL) were added DMTMM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride) (7 mg, 24.44 μmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion and then purified by preparative HPLC to give compound **202** (10 mg, 57%yield) as an off-white solid. MS-ESI (m/z): $[M+H]^+$ calcd for $C_{132}H_{168}N_{30}O_{40}S_2$, 2878.44; found, 2878.92.

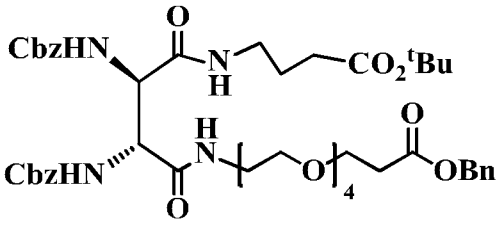
Example 185. Synthesis of (2R,3R)-2,3-bis(((benzyloxy)carbonyl)amino)-4- ((4-(tert-butoxy)-4-oxobutyl)amino)-4-oxobutanoic acid (**203**).



203

To a mixture of compound **13** (4.25 g, 10.68 mmol, 1.0 eq) and DMAP (13 mg, 0.11 mmol, 0.01 eq) in 20 mL of dry DCM was added a solution of *t*-butyl aminobutyrate (1.78 g, 11.21 mmol, 1.05 eq) in 10 mL of anhydrous DCM. After the addition was completed, compound **13** was completely dissolved and the reaction was allowed to stir at r.t. overnight. The crude product was loaded on a silica gel column and eluted with 3-5% MeOH/DCM. Fractions were combined and concentrated, the residue was triturated with PE/DCM (1:1) to afford 3.3 g of a white solid (yield 56%). MS-ESI (m/z): $[M+H]^+$ calcd. for $C_{28}H_{36}N_3O_9$ 558.2; found, 558.2.

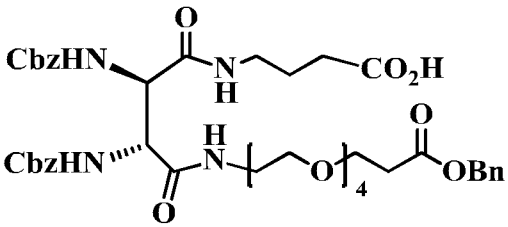
Example 186. Synthesis of 1-benzyl 25-(tert-butyl) (18R,19R)-18,19-bis(((benzyloxy)-carbonyl)amino)-17,20-dioxo-4,7,10,13-tetraoxa-16,21-diazapentacosanedioate (**204**).



204

Benzyl 1-amino-3,6,9,12-tetraoxapentadecan-15-oate (365 mg, 1.03 mmol, 1.0 eq) was dissolved in 10 mL of DMF, cooled over ice water bath. To which DIPEA (0.53 g, 4.12 mmol, 4.0 eq), compound **203** (0.56 g, 1.03 mmol, 1.0 eq) and HATU (1.17 g, 3.09 mmol, 3.0 eq) were added in sequence. After stirring over the ice water bath for 1 hour, 100 mL of water was added, and a solid precipitated out. The solid was collected by filtration and washed with water, dissolved in DCM, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was dissolved in small amount of DCM, loaded on a silica gel column, and eluted 0-10% MeOH/DCM to give 0.60 g of light yellow foam (yield 65%). MS-ESI (m/z): calcd. for $C_{46}H_{62}N_4O_{14}$ $[M+H]^+$ 895.43; found, 895.40.

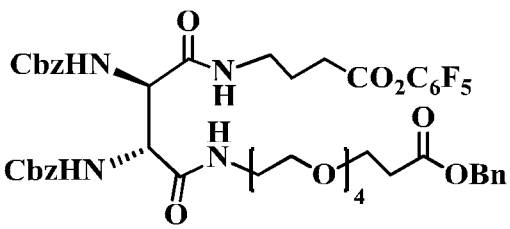
Example 187. Synthesis of (20R,21R)-20,21-bis(((benzyloxy)carbonyl)amino)- 3,19,22-trioxo-1-phenyl-2,6,9,12,15-pentaoxa-18,23-diazaheptacosan-27-oic acid (**205**).



205

Compound **204** (0.60 g, 0.67 mmol, 1.0 eq) was dissolved in 5 mL of DCM, and stirred with 5 mL of TFA at r.t. for 3 h, and completion of the reaction was monitored by LCMS. DCM was removed and the residue was co-evaporated with DCM for three times, placed on high vacuum pump. The crude product was dissolved in a small amount of DCM and loaded on a silica gel column, and then eluted with 15-20% MeOH/DCM. Fractions were combined and concentrated to give 0.34 g of white foam (yield 60%). MS-ESI (m/z): calcd. for $C_{42}H_{54}N_4O_{14}$ $[M+H]^+$ 839.36; found, 839.45.

Example 188. Synthesis of 1-benzyl 25-(perfluorophenyl) (18R,19R)-18,19-bis(((benzyl-oxy) carbonyl)amino)-17,20-dioxo-4,7,10,13-tetraoxa-16,21-diazapentacosanedioate (**206**).

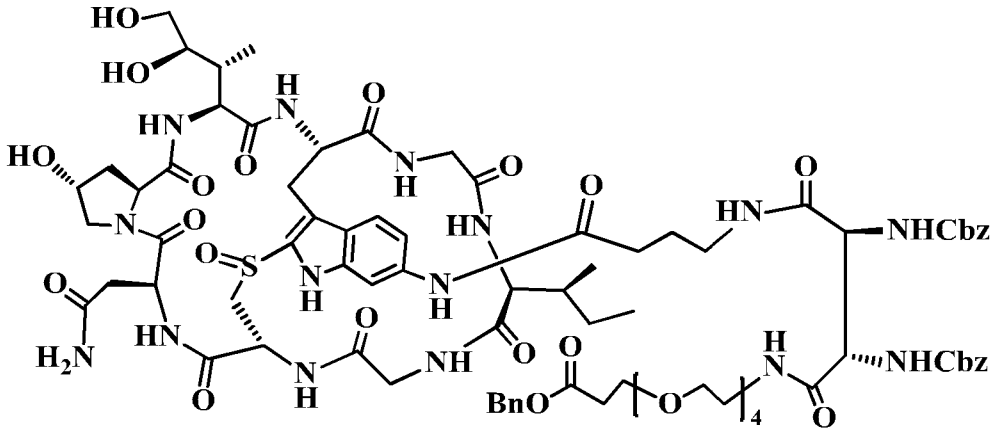


206

Compound **205** (0.34 g, 0.40 mmol, 1.0 eq) was dissolved in 10 mL DCM, to which pentafluorophenol (0.081 g, 0.44 mmol, 1.1 eq) and EDC (0.38 g, 2.0 mmol, 5.0 eq) were added. The reaction was stirred at r.t. overnight and then washed (2 × 10 mL) and brine (20 mL), dried over

anhydrous sodium sulfate, filtered and concentrated. The crude product was used directly in the next step. MS-ESI (m/z): calcd. for $C_{48}H_{53}F_5N_4O_{14}$ $[M+H]^+$ 1561.6; found, 1561.6.

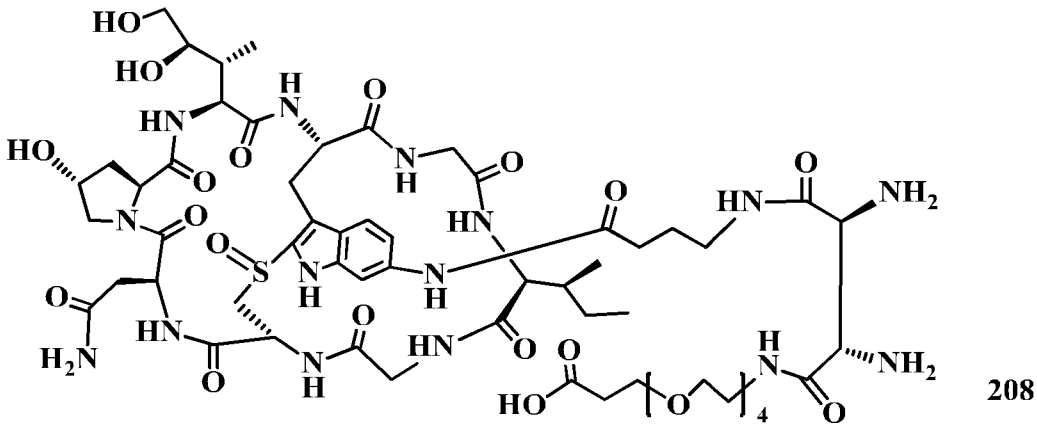
Example 189. Synthesis of compound **207**.



207

The crude product from the previous step (0.40 mmol, 1.0 eq) was dissolved in 10 mL DMF, cooled over ice water bath. To which compound **183** (0.39 g,0.4 mmol,1.0 eq) and DIPEA (0.15 g, 1.2 mmol, 3.0 eq) were added in sequence. After stirring over the ice water bath for 1 hour, the reaction was concentrated, and re-dissolved in a small amount of DCM, loaded on a silica gel column and eluted with 0-20% MeOH/DCM to give a colorless oil (0.40 g, 58% yield). MS-ESI (m/z): calcd. for $C_{81}H_{107}N_{15}O_{26}S$ $[M+2H]^{2+}$: 869.86; found, 869.96.

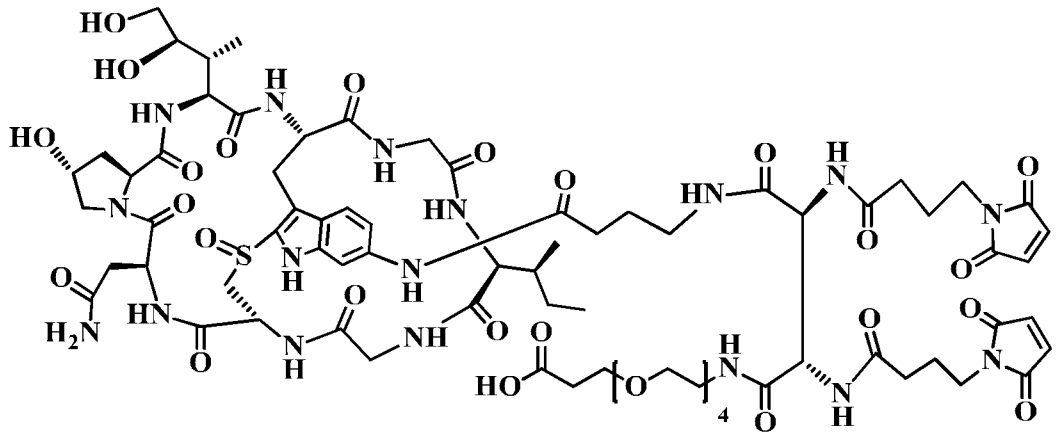
Example 190. Synthesis of compound **208**.



208

Compound **207** (0.40 g, 0.23 mmol, 1.0 eq) was dissolved in 5 mL methanol, dry palladium carbon (0.1 g, 10% wt) was added and the reaction flask was evacuated and back-filled with H_2 for three times. The reaction mixture was stirred under H_2 overnight, filtered and filtrate concentrated to give 0.32 g of crude material, which was directly used for the next reaction. MS-ESI (m/z): calcd. for $C_{58}H_{89}N_{15}O_{22}S$ $[M+2H]^{2+}$ 690.80; found, 690.85.

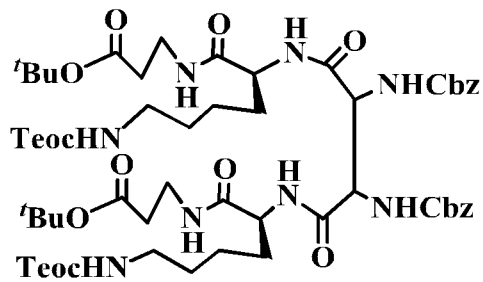
Example 191. Synthesis of compound **209**.



209

The crude product from the previous step (0.32 g, 0.23 mmol, 1.0 eq) was dissolved in 2 mL of ethanol, 0.2 mL of 0.1M NaH_2PO_4. N-(4-maleimidobutyryloxy) succinimide (0.19 g, 0.69 mmol, 3.0 eq) was added and the reaction was stirred at r.t. overnight, and then concentrated and re-dissolved in DCM, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was dissolved in a small amount of DCM, and loaded on silica gel column, eluted with 0-20% MeOH/DCM to give a colorless oil (0.13 g, 33% yield). MS-ESI (m/z): calcd. for $C_{74}H_{103}N_{17}O_{28}S$ $[M+2H]^{2+}$ 855.84; found, 855.86.

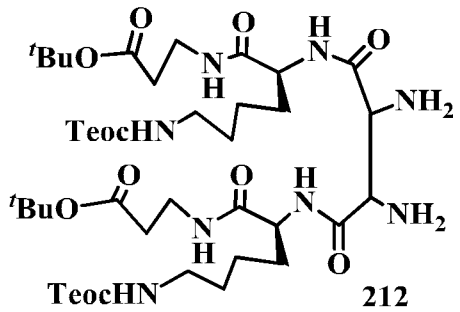
Example 192. Synthesis of di-tert-butyl (6S,13S)-9,10-bis(((benzyloxy)carbonyl) amino)-5,8,11,14-tetraoxo-6,13-bis(4-(((2,2,2-trichloroethoxy)carbonyl)amino)butyl)-4,7,12,15-tetraazaoctadecanedioate (**211**).



211

To a solution of tert-butyl (S)-3-(2-amino-6-(((2,2,2-trichloroethoxy)carbonyl) amino)hexanamido)propanoate (6.88 g, 14.4 mmol) and 2,3-bis(((benzyloxy)carbonyl) amino)succinic acid (5.00 g, 12.0 mmol) in DMA (60 mL), EDC·HCl (2.76 g, 14.4 mmol) and DIPEA (4.7 mL, 26.4 mmol) were added. The reaction mixture was stirred at r.t. overnight, then diluted with 150 mL dichloromethane and poured over 100 mL of water in a separatory funnel. The organic phase was separated, washed with brine (2 × 50 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (10-80% ethyl acetate/petroleum ether) to afford the title compound **211** (13.0 g, 85% yield). MS-ESI (m/z): calcd. for $C_{52}H_{72}Cl_6N_8O_{16}$ $[M+H]^+$ 638.16; found, 638.18.

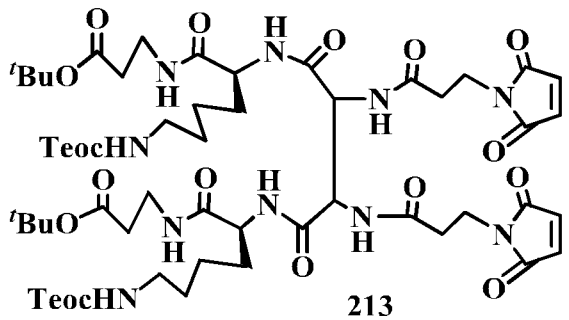
Example 193. Synthesis of di-tert-butyl (6S,13S)-9,10-diamino-5,8,11,14-tetraoxo-6,13-bis(4-(((2,2,2-trichloroethoxy)carbonyl)amino)butyl)-4,7,12,15-tetraazaoctadecanedioate (**212**).



212

To a solution of compound **211** (12.4 g, 9.72 mmol) in methanol (50 mL) was added Pd/C (10 wt%, 0.10 g) in a hydrogenation bottle. After the bottle was evacuated and back-filled with hydrogen three times, the mixture was shaken for 2 h, filtered through Celite (filter aid), and the filtrate was concentrated to afford compound **212** (9.47 g, 97% yield) as a colorless oil. MS-ESI (m/z): calcd. for $C_{36}H_{60}Cl_6N_8O_{12}$ $[M+H]^+$ 1007.25; found, 1007.82.

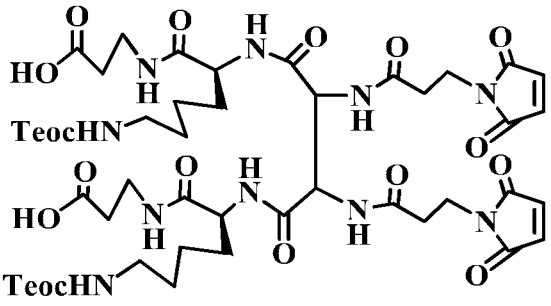
Example 194. Synthesis of di-tert-butyl (6S,13S)-9,10-bis(3-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)propanamido)-5,8,11,14-tetraoxo-6,13-bis(4-(((2,2,2-trichloroethoxy)carbonyl)amino)butyl)-4,7,12,15-tetraazaoctadecanedioate (**213**).



213

To a solution of compound **210** (9.47 g, 9.40 mmol) in dichloromethane (50 mL), 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (1.91 g, 11.3 mmol) and EDC·HCl (2.17 g, 11.3 mmol) were added, followed by DIPEA (4.0 mL, 23.5 mmol). The reaction was stirred at r.t. for 2 h, then diluted with water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column (10-80 % ethyl acetate/petroleum ether) to give a colorless oil (9.49 g, 77% yield). MS-ESI (m/z): calcd. for $C_{50}H_{70}Cl_6N_{10}O_{18}$ $[M+2H]^{2+}$ 655.15; found, 655.10.

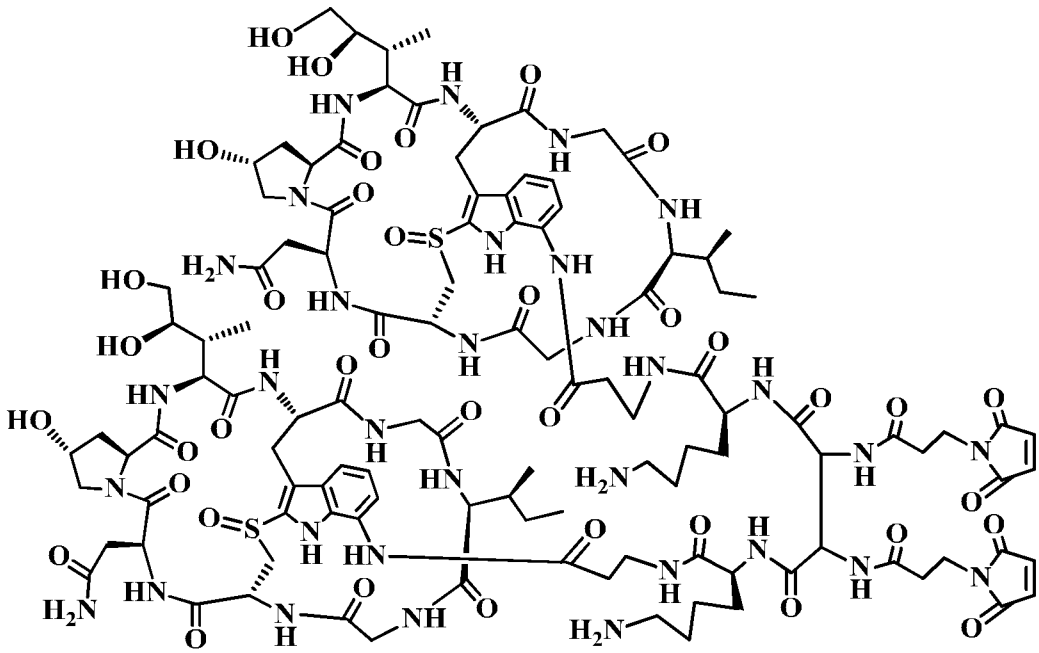
Example 195. Synthesis of (6S,13S)-9,10-bis(3-(2,5-dioxo-2,5-dihydro-1H- pyrrol-1-yl)propanamido)-5,8,11,14-tetraoxo-6,13-bis(4-(((2,2,2-trichloroethoxy)carbonyl) amino)butyl)-4,7,12,15-tetraazaoctadecanedioic acid (**214**).



214

A solution of compound **213** (9.49 g, 7.60 mmol) in THF (15 mL) was treated with 4 N HCl (2 mL) at 0 °C for 30 min then concentrated and loaded on a short silica gel column and eluted with 0-15% methanol/dichloromethane to give a colorless oil (8.50 g, 93% yield). MS-ESI (m/z): calcd. for $C_{42}H_{54}Cl_6N_{10}O_{18}$ $[M+2H]^{2+}$ 599.08; found, 599.10.

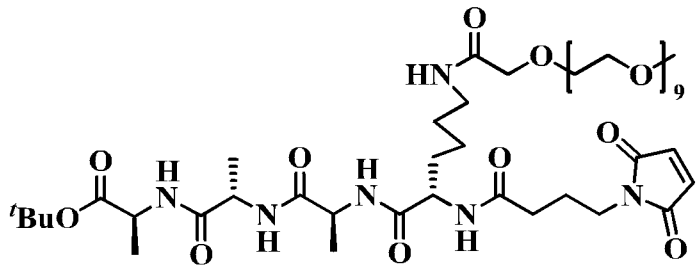
Example 196. Synthesis of compound **215**.



215

To a solution of compound **183** (10.0 mg, 0.0109 mmol) and compound **214** (6.5 mg, 0.00545 mmol) in DMF (1 mL), TBTU (3.50mg, 0.0109 mmol) and DIPEA (2.0 μL, 0.0109 mmol) were added and the mixture was stirred at r.t. for 2 h. After removal of DMF under high vacuum, the residue was purified by prep-HPLC (acetonitrile/water) to give a colorless oil (14.0 mg). This oil was dissolved in THF (1.0 mL) and treated with TBAF (1.0 M in THF, 35 μL) at 0 °C for 30 min, then concentrated and purified by a short silica gel column (0-10% methanol/dichloromethane) to afford a colorless oil (10.0 mg, 34% yield). MS-ESI (m/z): calcd. for $C_{114}H_{158}N_{32}O_{38}S_2$ $[M+3H]^{3+}$ 883.36; found, 883.36.

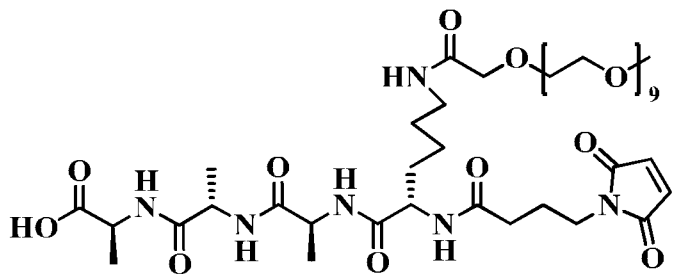
Example 197. Synthesis of tert-butyl ((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31-oxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32-azaoctatriacontan-38-oyl)-L -alanyl-L-alanyl-L-alaninate (**216**).



216

Compound **134** (1.70g, 1.92mmol) and 4-maleimidobutanoic acid (0.35 g, 1.92 mmol) were dissolved in 20 mL of DCM, and EDC·HCl (0.74 g) was added. After stirring at 0 °C for 2 hours the reaction was diluted with 50 mL of DCM, washed with 50 mL of water, 50 mL of brine, concentrated to give a white solid (1.9 g, 94% yield). MS-ESI (m/z): calcd. for $C_{48}H_{85}N_6O_{19}$ $[M+H]^+$1049.58; found, 1049.58.

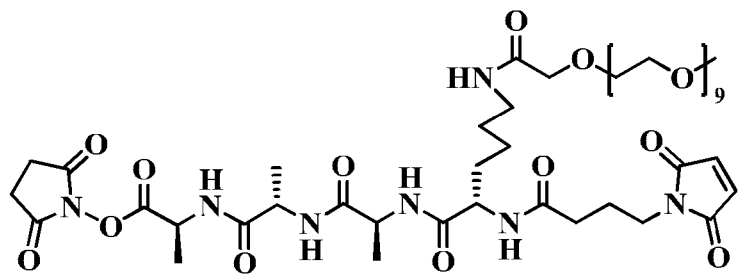
Example 198. Synthesis of ((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -31-oxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl- L-alanyl-L-alanine (**217**).



217

Compound **216** (1.9 g, 1.81 mmol) was dissolved in 15 mL of DCM and 15 mL of TFA. After stirring at r.t. for 1h, the reaction was concentrated, and then purified by preparative HPLC to give a colorless liquid (1.1 g, 63% yield). MS-ESI (m/z): calcd. for $C_{44}H_{77}N_6O_{19}[M+H]^+$ 993.52; found, 993.52.

Example 199. Synthesis of 2, 5-dioxopyrrolidin-1-yl ((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31-oxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32-azaoctatriacontan -38-oyl)-L-alanyl-L-alanyl-L-alaninate (**218**).

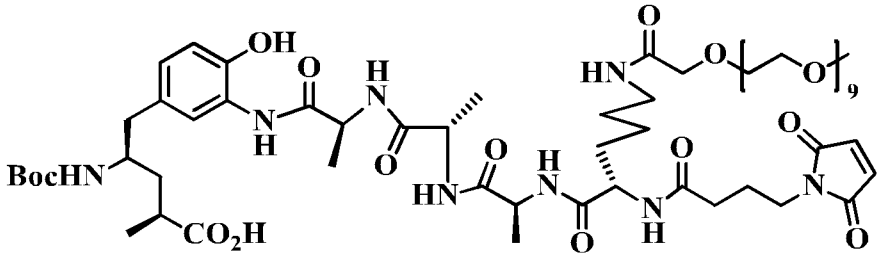


218

Compound **217** (1.1 g, 1.14 mmol) was dissolved in 40 mL of DCM, NHS (0.21 g, 1.82 mmol) and EDC·HCl (0.45 g, 2.35 mmol) were added. After stirring at r.t. for 1 h, the reaction was washed with 50 mL of water, 50 mL of brine, dried over anhydrous sodium sulfate, filtered, concentrated to

give a white solid (1.0 g, 100% yield). MS-ESI (m/z): calcd. for $C_{48}H_{80}N_7O_{21}[M+H]^+$ 1090.53; found, 1090.55.

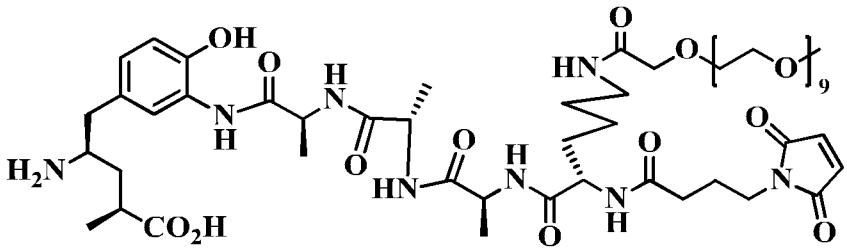
Example 200. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan -47-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**219**).



219

Compound **218** (1.00 g, 0.91 mmol) and compound **77** (0.43 g, 1.27 mmol) were dissolved in 40 mL of THF. After stirring at 50 °C overnight, the reaction was concentrated, and the residue was purified by preparative HPLC to give a colorless liquid (0.50 g, 33% yield). MS-ESI (m/z): calcd. for $C_{61}H_{101}N_8O_{23}[M+H]^+$ 1313.69; found, 1313.69.·

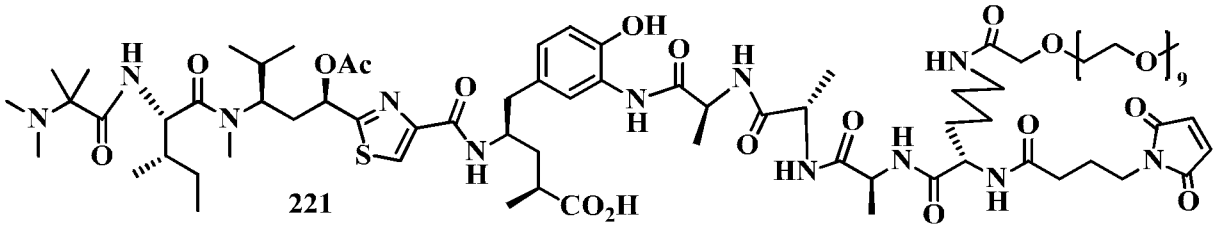
Example 201. Synthesis of (2S, 4R)-4-amino-5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan-47-amido)-4- hydroxyphenyl)-2-methylpentanoic acid (**220**).



220

Compound **219** (286 mg, 0.22 mmol) was dissolved in 10 mL of DCM and 10 mL of TFA. After stirring at r.t. for 1h, the reaction was concentrated to give a light yellow liquid (563 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{56}H_{93}N_8O_{21}[M+H]^+$ 1213.64; found, 1213.64.

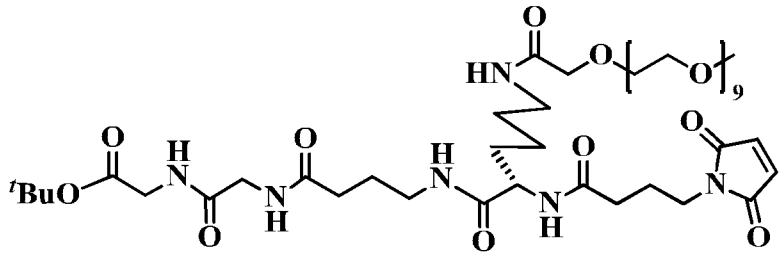
Example 202. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan-47-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**221**).



221

Compound **220** (260.0 mg, 0.21 mmol) and **Tub-1** (178 mg, 0.26 mmol) were dissolved in 10 mL of DMF, N,N-diisopropylethylamine (499mg, 3.86mmol) was added until pH ~ 9. After stirring at 0 °C for 0.5 h, the reaction was concentrated, purified by preparative HPLC to give a light yellow liquid (407 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{81}H_{133}N_{12}O_{26}S$ $[M+H]^+$ 1721.91; found, 1721.91.

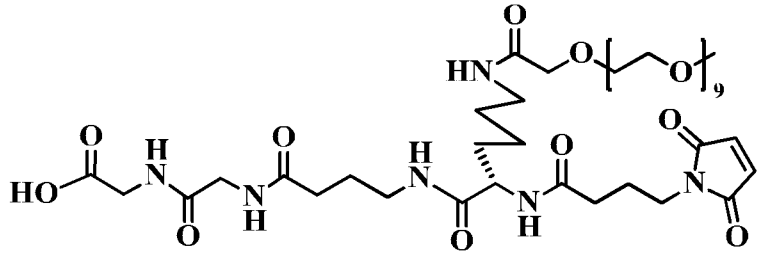
Example 203. Synthesis of tert-butyl (S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan -43-oyl)glycylglycinate (**222**).



222

Compound **27** (6.10 g, 0.007 mol) was dissolved in DCM (80 mL), pentafluorophenol (1.83 g, 0.010 mol) and N, N′-diisopropylcarbodiimide (1.67 g, 0.013 mol) were added. After stirring for 1 h, 4-maleimidobutanoic acid (1.22 g, 0.007 mol) and N,N-diisopropylethylamine (2.2 mL, 0.013 mol) were added. The reaction was stirred for 1 h, washed with 100 mL of water, brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column (DCM/MeOH=100/12), to give compound **222** (6.85 g, 100% yield). MS-ESI (m/z): calcd. for $C_{47}H_{82}N_6O_{19}$ $[M+H]^+$ 1035.56; found, 1035.61 .

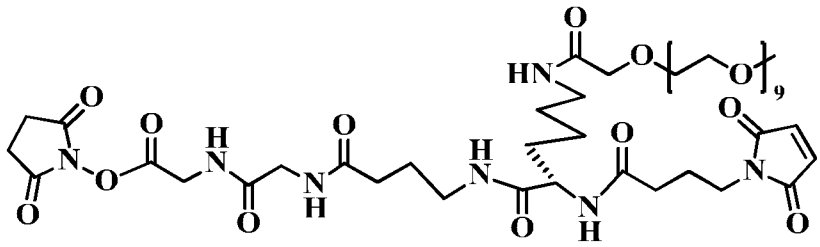
Example 204. Synthesis of (S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan -43-oyl)glycylglycine (**223**).



223

Compound **222** (6.85 g, 0.007 mol) was dissolved in DCM (30 mL) and TFA (15 mL). The reaction was stirred overnight, concentrated, and purified by preparative HPLC to give compound **223** (6.03 g, 93% yield). MS-ESI (m/z): calcd. for $C_{43}H_{74}N_6O_{19}$ $[M+H]^+$ 979.50; found, 980.21 .

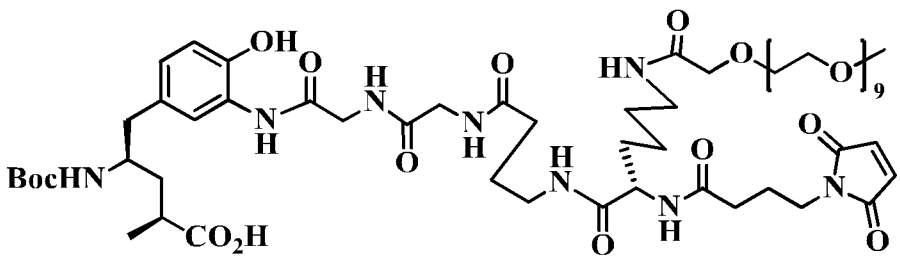
Example 205. Synthesis of 2, 5-dioxopyrrolidin-1-yl (S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycylglycinate (**224**).



224

Compound **223** (3.52 g, 0.004 mol) was dissolved in DCM (40 mL), N-hydroxysuccinimide (0.54 g, 0.005 mol) and EDC·HCl (1.24 g, 0.006 mol) were added. The reaction was stirred for 1.5 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **224** (3.20 g, 83% yield). MS-ESI (m/z): calcd. for $C_{47}H_{77}N_7O_{21}$ $[M+H]^+$ 1076.52; found, 1076.69.

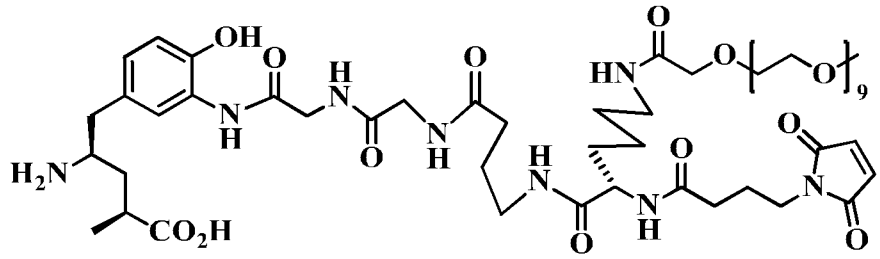
Example 206. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47-tetraazanonatetracontan-49-amido)-4-hydroxyphenyl)- 2-methyl pentanoic acid (**225**).



225

Compound **224** (3.20 g, 0.003 mol) and compound **77** (1.21 g, 0.004 mol) were dissolved in THF (30 mL), stirred at 25 °C for 2 days, concentrated and purified by preparative HPLC to give compound **225** (2.66 g, 69% yield). MS-ESI (m/z): calcd. for $C_{60}H_{98}N_8O_{23}$ $[M+H]^+$ 1299.67; found, 1300.30.

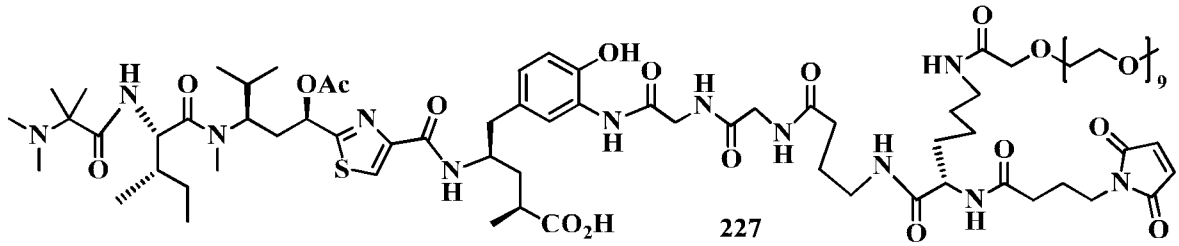
Example 207. Synthesis of (2S, 4R)-4-amino-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro -1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47-tetraazanonatetracontan-49-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**226**).



226

Compound **225** (2.66 g, 0.002 mol) was dissolved in DCM (10 mL) and TFA (20 mL). The reaction was stirred for 2 hours, and then concentrated. The residue was purified by preparative HPLC to give compound **226** (2.46 g, 100% yield). MS-ESI (m/z): calcd. for $C_{55}H_{90}N_8O_{21}$ $[M+H]^+$ 1199.62; found, 1199.92.

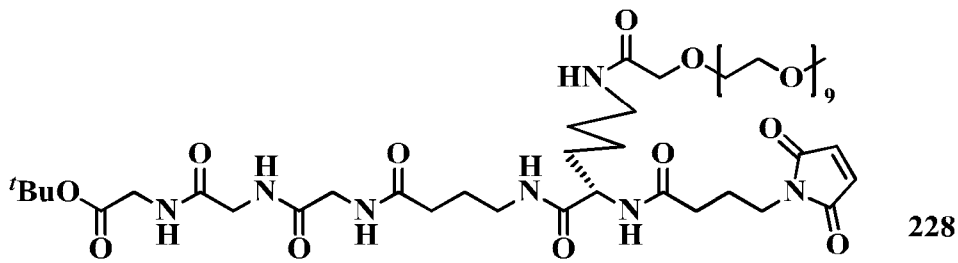
Example 208. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47-tetraazanonatetracontan-49-amido)-4- hydroxyphenyl)-2-methylpentanoic acid (**227**).



227

Compound **226** (2.46 g, 0.002 mol) and **Tub-1** (1.71 g, 0.002 mol) were dissolved in DMF (30 mL) and N,N-diisopropylethylamine (0.339 mL, 0.002 mol) was added. The reaction was stirred for 1 h and concentrated, the residue was purified by preparative HPLC to give compound **227** (1.225 g, 35% yield). MS-ESI (m/z): calcd. for $C_{80}H_{130}N_{12}O_{26}S$ $[M+H]^+$ 1707.89; found, 1708.42.

Example 209. Synthesis of tert-butyl (S)-(S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol -1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycylglycylglycinate (**228**).

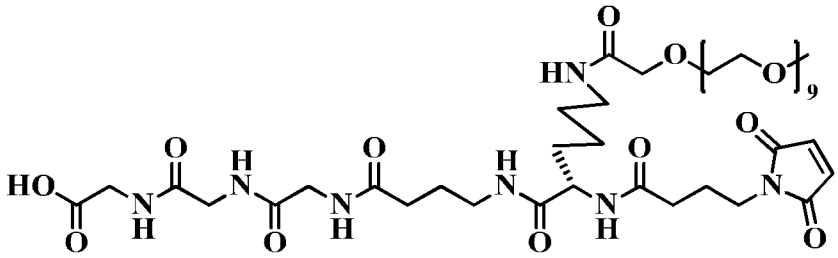


228

Compound **41** (1.85 g, 2.00 mmol) and 4-maleimidobutanoic acid (0.46 g, 2.50 mmol) were dissolved in 20 mL of DCM, and EDC·HCl (0.58 g, 3.00 mmol) was added. After stirring at 0 °C for 2 hours the reaction was diluted with 50 mL of DCM, washed with 50 mL of water, 50 mL of brine,

concentrated to give a white solid (2.10 g, 95% yield). MS-ESI (m/z): calcd. for $C_{49}H_{85}N_7O_{20}$ $[M+H]^+$ 1092.58; found, 1092.58.

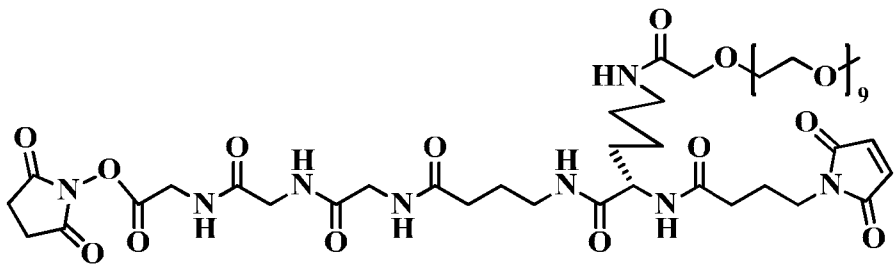
Example 210. Synthesis of (S)-(S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan -43-oyl)glycylglycylglycine (**229**).



229

Compound **228** (2.10 g, 1.92 mmol) was dissolved in DCM (30 mL) and TFA (15 mL). The reaction was stirred overnight, concentrated, and purified by preparative HPLC to give compound **229** (1.83 g, 92% yield). MS-ESI (m/z): calcd. for $C_{45}H_{75}N_7O_{20}$ $[M+H]^+$ 1036.52; found, 1036.55.

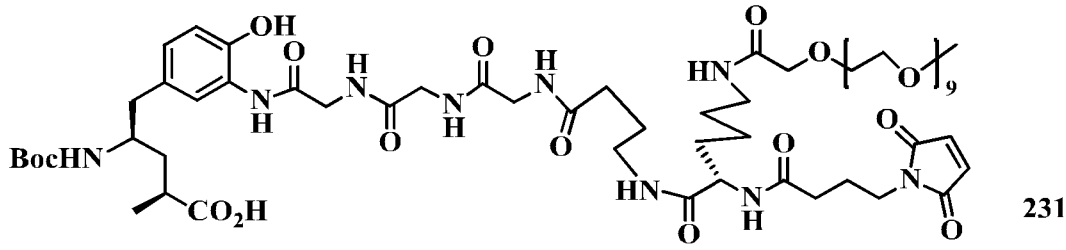
Example 211. Synthesis of 2, 5-dioxopyrrolidin-1-yl (S)-(S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycylglycylglycinate (**230**).



230

Compound **229** (500 mg, 0.483 mmol) was dissolved in DCM (10 mL), N-hydroxysuccinimide (67 mg, 0.579 mmol) and EDC·HCl (139 mg, 0.724 mmol) were added. The reaction was stirred at r.t. for 6 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **230** (546 mg, 99% yield). MS-ESI (m/z): calcd. for $C_{49}H_{80}N_8O_{22}$ $[M+H]^+$ 1133.54; found, 1134.01.

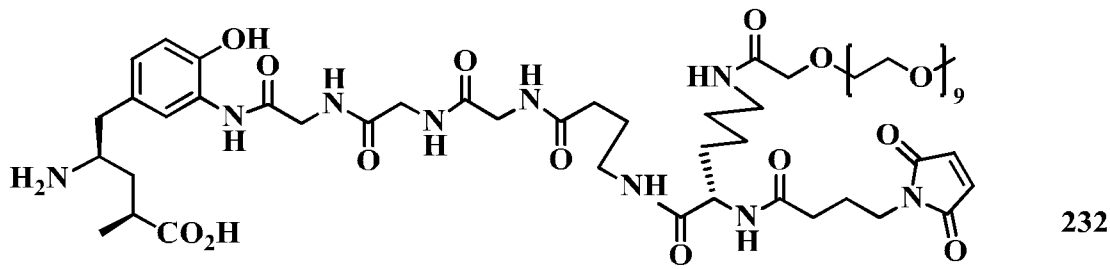
Example 212. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46, 49-pentaoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47, 50-pentaazadopentacontan-52-amido) -4-hydroxyphenyl)-2-methylpentanoic acid (**231**).



231

Compound **230** (360 mg, 0.318 mmol) and compound 77 (129 mg, 0.381 mmol) were dissolved in THF (15 mL) and DCM (10 mL). The reaction was stirred at r.t. overnight, concentrated and purified by preparative HPLC to give compound **231** (200 mg, 0.147 mmol, 46% yield). MS-ESI (m/z): calcd. for $C_{62}H_{101}N_9O_{24}$ $[M+H]^+$ 1356.70; found, 1357.00.

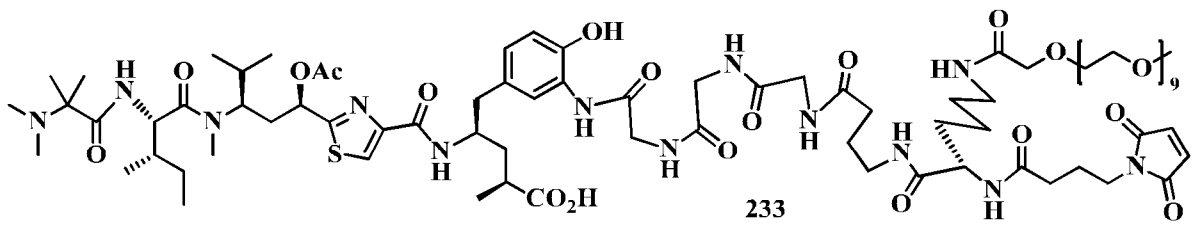
Example 213. Synthesis of (2S, 4R)-4-amino-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46, 49-pentaoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47, 50-pentaazadopentacontan-52-amido)-4-hydroxyphenyl)-2-methyl pentanoic acid (**232**).



232

Compound **231** (200 mg, 0.147 mmol) was dissolved in DCM (4 mL) and TFA (2 mL). After stirring for 2 hours, the reaction solution was concentrated to give **232** (185 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{57}H_{93}N_9O_{22}$ $[M+H]^+$ 1256.64; found, 1257.07.

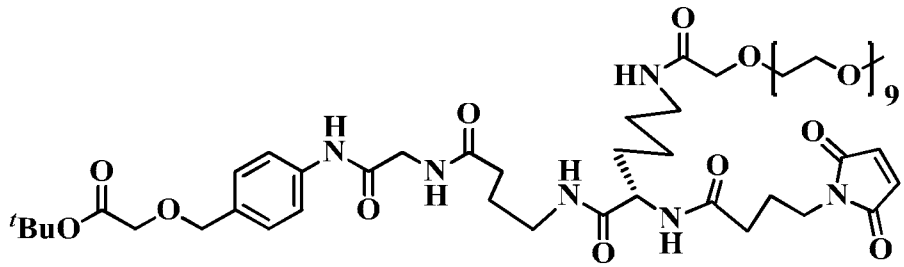
Example 214. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46, 49-pentaoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47, 50-pentaazadopentacontan-52-amido) -4-hydroxyphenyl)-2-methylpentanoic acid (**233**).



233

Compound **232** (185 mg, 0.147 mmol) and **Tub-1** (102 mg, 0.147 mmol) were dissolved in DMF (2 mL) and N,N-diisopropylethylamine (0.097 mL, 0.589 mmol) was added. The reaction was stirred overnight and directly purified by preparative HPLC to give compound **233** (107 mg, 41% yield). MS-ESI (m/z): calcd. for $C_{82}H_{133}N_{13}O_{27}S$ $[M+H]^+$ 1764.92; found, 1765.19.

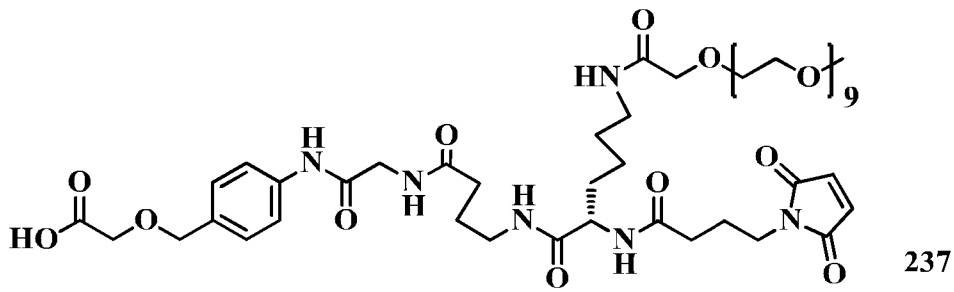
Example 215. Synthesis of tert-butyl (S)-2-((4-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy)acetate (**236**).



236

Compound 234 (8.40 g, 9.11 mmol) and compound 235 (2.59 g, 10.9 mmol) were dissolved in 60 mL of DCM. EDC·HCl (3.48 g, 18.2 mmol) and N,N-diisopropylethylamine (3.49 g, 18.2 mmol) were added. The reaction was stirred at 0 °C for 1h, washed with 100 mL of water, and the aqueous phase was extracted with 50 mL of DCM. The organic phases were combined, dried over anhydrous sodium sulfate, filtered and concentrated. The crude was purified by a silica gel column, eluted with ethyl acetate/petroleum ether and by preparative HPLC, to give compound **236** as brown liquid (3.0 g, 29% yield). MS-ESI (m/z): calcd. for $C_{54}H_{89}N_6O_{20}$ $[M+H]^+$ 1141.61; found, 1141.61.

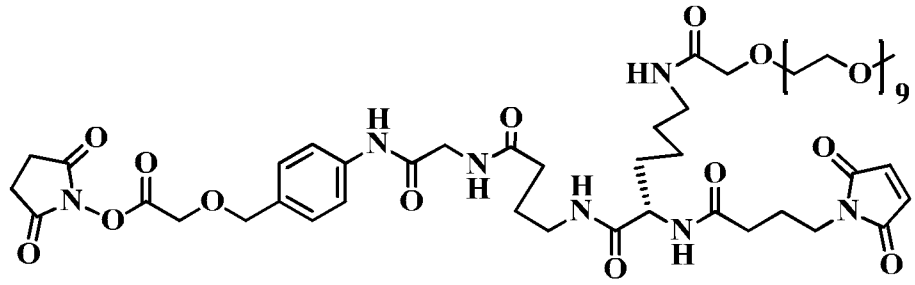
Example 216. Synthesis of (S)-2-((4-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy)acetic acid (**237**).



237

Compound **236** (3.0 g, 2.63 mmol) was dissolved in 30 mL of formic acid and 30 mL of DCM. The reaction was stirred at 40-50 °C for 3h, concentrated and purified by preparative HPLC to give **237** as a colorless liquid (1.2 g, 42% yield). MS-ESI (m/z): calcd. for $C_{50}H_{81}N_6O_{20}$ $[M+H]^+$ 1085.54; found, 1085.54.

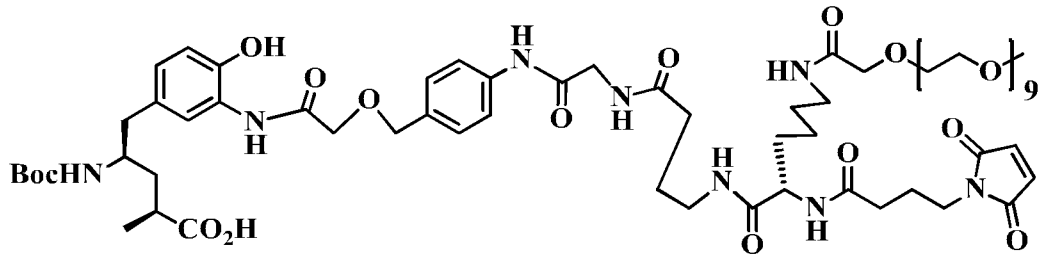
Example 217. Synthesis of 2, 5-dioxopyrrolidin-1-yl (S)-2-((4-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy)acetate (**238**).



238

Compound **237** (450 mg, 0.41 mmol) and N-hydroxysuccinimide (71 mg, 0.617 mmol) were dissolved in 10 mL of DCM, EDC·HCl (126 mg, 0.657 mmol) was then added. After stirring at r.t. for 3 h, the reaction was washed with 30 mL of water, 30 mL of brine, dried over anhydrous sodium sulfate, filtered and concentrated to give **238** as a colorless liquid (0.71 g, 100% yield). MS-ESI (m/z): calcd. for $C_{54}H_{84}N_7O_{22}$ $[M+H]^+$ 1182.56; found, 1182.56.

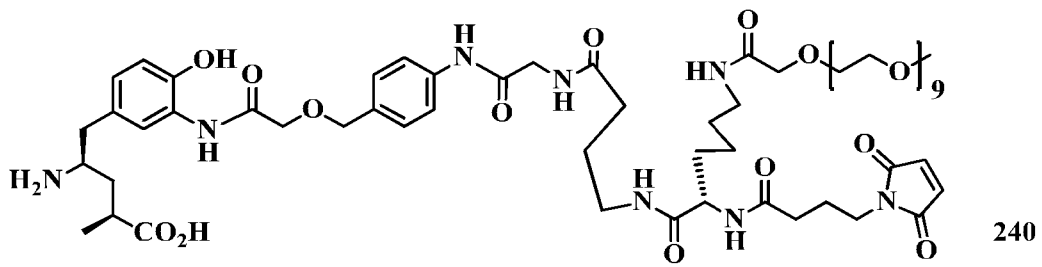
Example 218. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-(2-((4-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy)acetamido)-4-hydroxyphenyl) -2-methylpentanoic acid (**239**).



239

Compound **238** (710 mg, 0.41 mmol) and compound **77** (174 mg, 0.51 mmol) were dissolved in 10 mL of THF and stirred at 50-55 °C overnight. The reaction was then concentrated, diluted with 30 mL of DCM, washed with 30 mL of water, 30 mL of brine, dried over anhydrous sodium sulfate, filtered, concentrated and purified by preparative HPLC to give a colorless liquid (114 mg, 20% yield). MS-ESI (m/z): calcd. for $C_{67}H_{105}N_8O_{24}$ $[M+H]^+$ 1405.72; found, 1405.72.

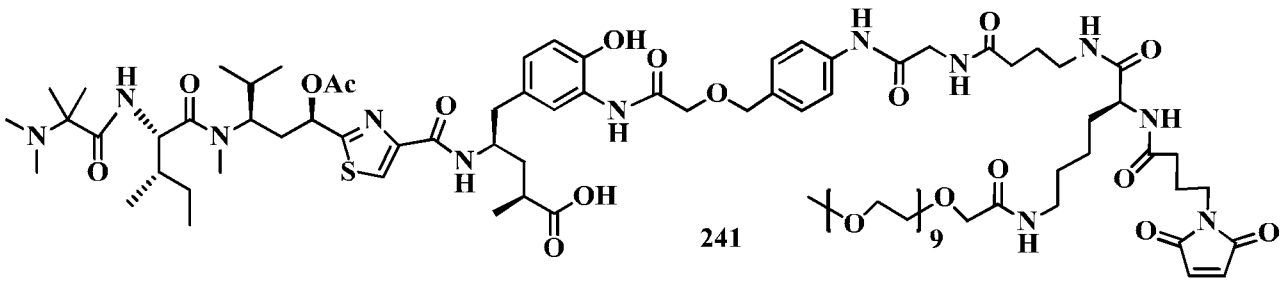
Example 219. Synthesis of (2S, 4R)-4-amino-5-(3-(2-((4-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy)acetamido)-4-hydroxyphenyl) -2-methylpentanoic acid (**240**).



240

Compound **239** (100 mg, 0.07 mmol) was dissolved in 10 mL of DCM and 3 mL of TFA. After stirring at 0 °C for 2 hours, the reaction was concentrated to give a colorless liquid (0.50 g, 100% yield). MS-ESI (m/z): calcd. for $C_{62}H_{97}N_8O_{22}$ $[M+H]^+$ 13.5.66; found, 1305.66.

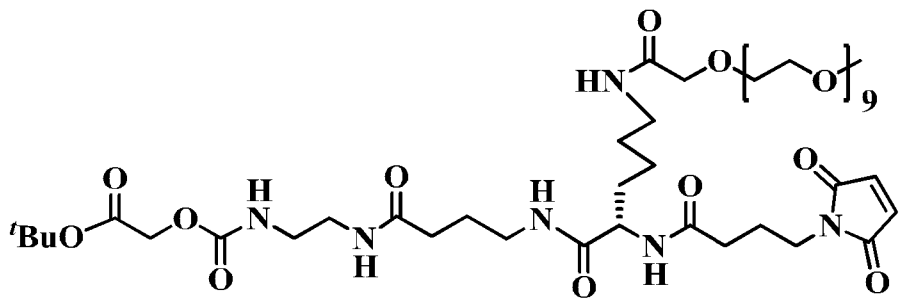
Example 220. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-(2-((4-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)benzyl)oxy) acetamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**241**).



241

Compound **239** (92.8 mg, 0.07 mmol) and **Tub-1** (60.2 mg, 0.08 mmol) were dissolved in 10 mL of DMF, N,N-diisopropylethylamine (445 mg, 3.44 mmol) was added. After stirring at 0 °C for 0.5 h and r.t. for 2 hours, the reaction was concentrated and purified by preparative HPLC, to give a light yellow solid (75 mg, 58% yield). MS-ESI (m/z): calcd. for $C_{87}H_{137}N_{12}O_{27}S$ $[M+H]^+$ 1813.94; found, 1813.94.

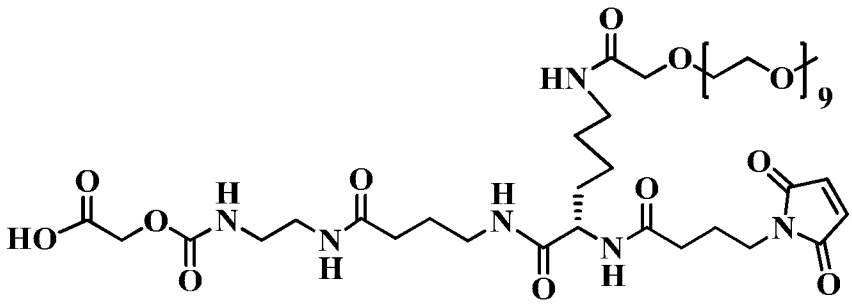
Example 221. Synthesis of tert-butyl (S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-oate (**244**).



244

Compound **243** (2.80 g, 12.8 mmol) and compound 242 (4.44 g, 5.13 mmol) were dissolved in DCM (50 mL), HATU (2.14 g, 5.64 mmol) and N,N-diisopropylethylamine (2.5 mL, 15.4 mmol) were added. The reaction was stirred for 3 hours, and then concentrated, the residue was purified by silica gel column (DCM/MeOH=100/8), to give compound **244** (4.70 g, 86% yield). MS-ESI (m/z): calcd. for $C_{48}H_{84}N_6O_{20}$ $[M+H]^+$ 1065.57; found, 1065.94.

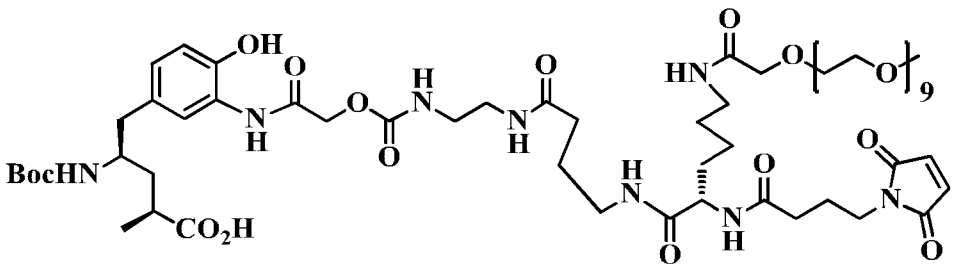
Example 222. Synthesis of (S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-oic acid (**245**).



245

Compound **244** (4.70 g, 0.004 mol) was dissolved in DCM (40 mL) and TFA (20 mL). The reaction was stirred overnight, concentrated, and purified by preparative HPLC to give compound **245** (1.00 g, 22% yield). MS-ESI (m/z): calcd. for $C_{44}H_{76}N_6O_{20}$ $[M+H]^+$ 1009.51; found, 1009.72.

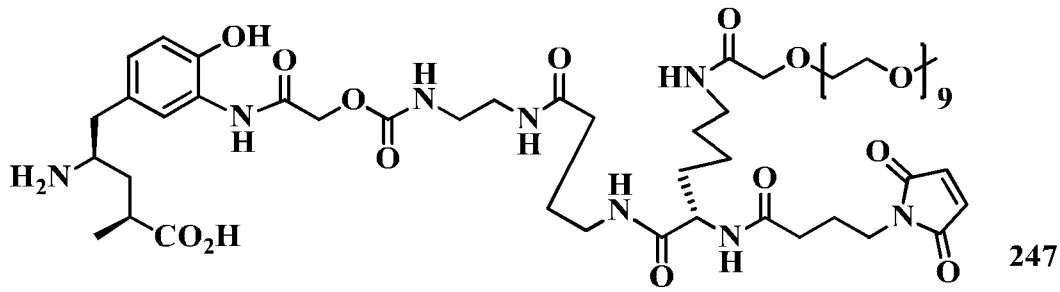
Example 223. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4-hydroxyphenyl) -2-methylpentanoic acid (**246**).



246

Compound **245** (343 mg, 0.340 mmol) was dissolved in DCM (10 mL), and N-hydroxysuccinimide (47 mg, 0.408 mmol) and EDC·HCl (98 mg, 0.510 mmol) were added. The reaction was stirred for 4 hours, washed with brine (5 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was dissolved in 20 mL of anhydrous THF, and compound **77** (138 mg, 0.408 mmol) was added. The reaction was stirred overnight, concentrated and purified by preparative HPLC to give compound **246** (177 mg, 39% yield). MS-ESI (m/z): calcd. for $C_{61}H_{100}N_8O_{24}$ $[M+H]^+$ 1329.69; found, 1329.98.

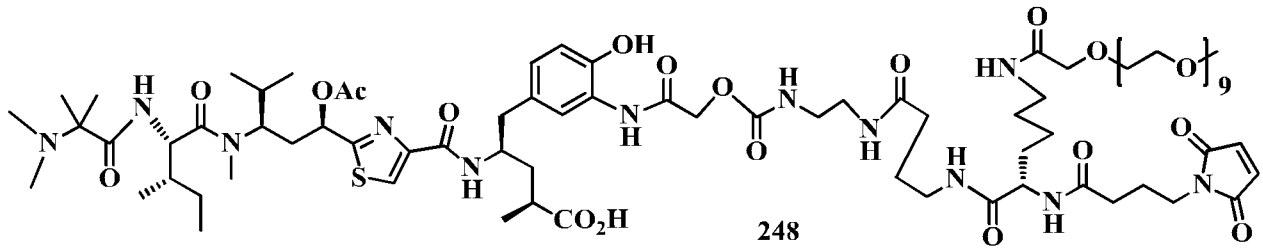
Example 224. Synthesis of (2S, 4R)-4-amino-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4-hydroxyphenyl)-2-methyl pentanoic acid (**247**).



247

Compound **246** (177 mg, 0.133 mol) was dissolved in DCM (4 mL) and TFA (2 mL). The reaction was stirred for 1 h, and concentrated to give compound **247** (0.16 g, 97% yield). MS-ESI (m/z): calcd. for $C_{56}H_{92}N_8O_{22}$ $[M+H]^+$ 1229.63; found, 1231.31.

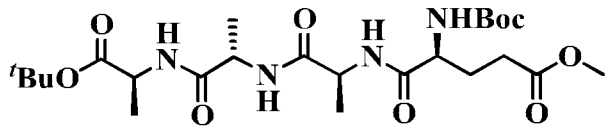
Example 225. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6, 9-diisopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3- ((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4- hydroxyphenyl)-2-methylpentanoic acid (**248**).



248

Compound **247** (160 mg, 0.130 mmol) and **Tub-1** (90 mg, 0.130 mmol) were dissolved in DMF (2 mL), and N,N-diisopropylethylamine (17 mg, 0.130 mmol) was added. The reaction was stirred for 4 hours and directly purified by preparative HPLC to give compound **248** (127 mg, 56% yield). MS-ESI (m/z): calcd. for $C_{81}H_{132}N_{12}O_{27}S$ $[M+H]^+$ 1737.90; found, 1738.58.

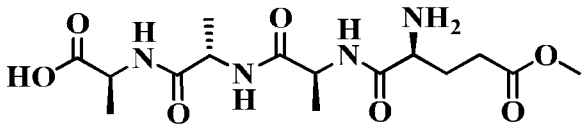
Example 226. Synthesis of tert-butyl (6S, 9S, 12S, 15S)-6-(3-methoxy-3-oxopropyl)-2, 2, 9, 12, 15-pentamethyl-4, 7, 10, 13-tetraoxo-3-oxa-5, 8, 11, 14-tetraazahexadecan-16-oate (**249**).



249

Compound **132** (4.00 g, 0.014 mol) and Boc-Glu(OMe)-OH (3.64 g, 0.014 mol) were dissolved in DCM (100 mL), and HATU (7.94 g, 0.021 mol) and TEA (3.870 mL, 0.028 mol) were added. The reaction was stirred for 1.5 hours, and washed with brine (2 × 150 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give compound **249** (7.39 g, 100% yield), which was directly used in the next step without further purification. MS-ESI (m/z): calcd. for $C_{24}H_{42}N_4O_9$ $[M+H]^+$ 531.30; found, 531.26.

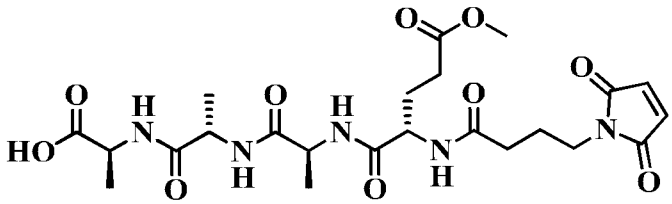
Example 227. Synthesis of ((S)-2-amino-5-methoxy-5-oxopentanoyl)-L-alanyl -L-alanyl-L-alanine (**250**).



250

Compound **249** (7.39 g, 0.014 mol) was dissolved in DCM (20 mL) and TFA (20 mL). The reaction was stirred overnight, and concentrated to give compound **250** (5.21 g, 100% yield), which was directly used in the next step without further purification. MS-ESI (m/z): calcd. for $C_{15}H_{26}N_4O_7$ $[M+H]^+$ 375.18; found, 375.22.

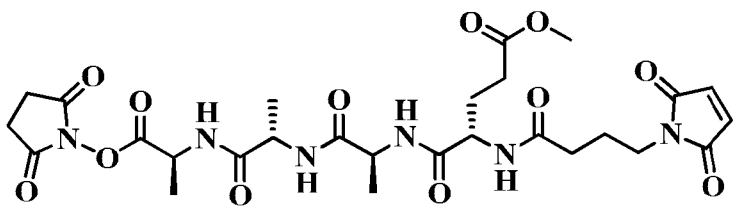
Example 228. Synthesis of ((S)-2-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-5-methoxy-5-oxopentanoyl)-L-alanyl-L-alanyl-L-alanine (**251**).



251

Compound **250** (1.38 g, 0.004 mol) was dissolved in DCM (60 mL) and N,N-diisopropylethylamine (1.2 mL, 0.007 mol) was added, followed by 4-maleimidobutyric acid N-hydroxysuccinimide ester (2.06 g, 0.008 mol). After stirring for 3 hours, the reaction was extracted with water (2 × 100 mL), and the aqueous phase was concentrated to give a white solid (1.40 g, 70% yield). MS-ESI (m/z): calcd. for $C_{23}H_{33}N_5O_{10}$ $[M+H]^+$ 540.22; found, 540.29.

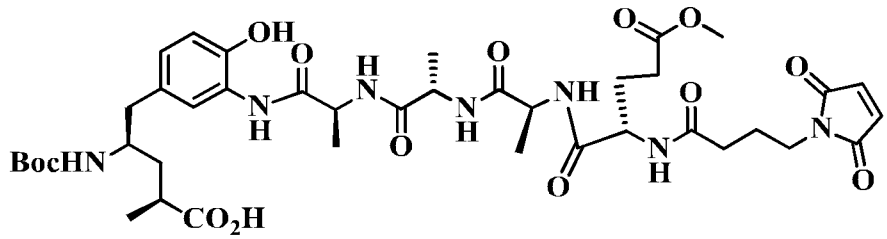
Example 229. Synthesis of 2, 5-dioxopyrrolidin-1-yl (2S, 5S, 8S, 11S)-16-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)-11-(3-methoxy-3-oxopropyl)-2, 5, 8-trimethyl-4, 7, 10, 13-tetraoxo-3, 6, 9, 12-tetraazahexadecanoate (**252**).



252

Compound **251** (1.40 g, 0.003 mol) was dissolved in DCM (100 mL), NHS (0.33 g, 0.003 mol) and EDC·HCl (0.99 g, 0.005 mol) were added. The reaction was stirred for 1 h, washed with 50 mL of brine, and the organic phase was dried over anhydrous sodium sulfate, filtered and concentrated to give compound **252** (1.65 g, 100% yield). MS-ESI (m/z): calcd. for $C_{27}H_{36}N_6O_{12}$ $[M+H]^+$ 637.24; found, 637.36.

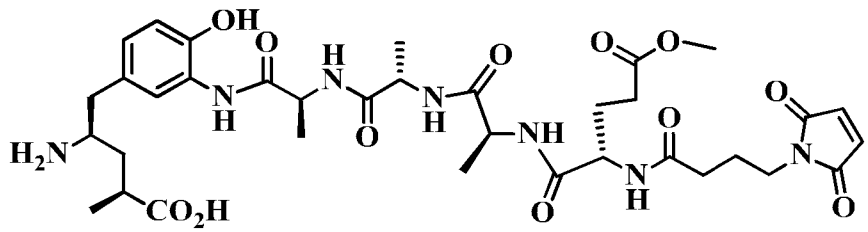
Example 230. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((6S, 9S, 12S, 15S)-6-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-9, 12, 15-trimethyl-3, 7, 10, 13-tetraoxo-2-oxa-8, 11, 14-triazahexadecan-16-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**253**).



253

Compound **252** (1.65 g, 0.003 mol) and compound **77** (0.88 g, 0.003 mol) were dissolved in THF (50 mL) and reacted at 50 °C overnight. Concentration and purification by preparative HPLC gave compound **253** (0.17 g, 7.6% yield). MS-ESI (m/z): calcd. for $C_{40}H_{57}N_7O_{14}$ $[M+H]^+$ 860.40; found, 860.44.

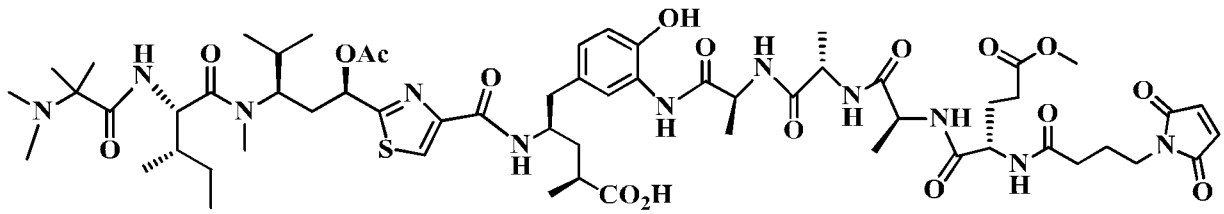
Example 231. Synthesis of (2S, 4R)-4-amino-5-(3-((6S, 9S, 12S, 15S)-6-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-9, 12, 15-trimethyl-3, 7, 10, 13-tetraoxo-2-oxa-8, 11, 14-triazahexadecan-16-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**254**).



254

Compound **253** (0.17 g, 0.198 mmol) was dissolved in DCM (4 mL) and TFA (4 mL). The reaction was stirred for 1 h, concentrated to give compound **254** (0.15 g, 100% yield). MS-ESI (m/z): calcd. for $C_{35}H_{49}N_7O_{12}$ $[M+H]^+$ 760.34; found, 760.42.

Example 232. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((6S, 9S, 12S, 15S)-6-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-9, 12, 15-trimethyl-3, 7, 10, 13-tetraoxo-2-oxa-8, 11, 14-triazahexadecan-16-amido)-4-hydroxyphenyl) -2-methylpentanoic acid (**255**).

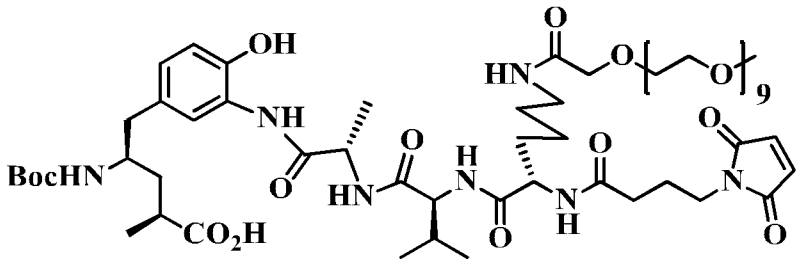


255

Compound **254** (0.14 g, 0.20 mmol) and **Tub-1** (0.15 g, 0.21 mol) were dissolved in DMF (2 mL) and N,N-diisopropylethylamine (0.098 mL, 0.001 mol) was added. The reaction was stirred for

1 h, directly purified by preparative HPLC to give compound **255** (55 mg, 22% yield). MS-ESI (m/z): calcd. for $C_{60}H_{89}N_{11}O_{17}S$ $[M+H]^+$ 1268.62; found, 1268.94.

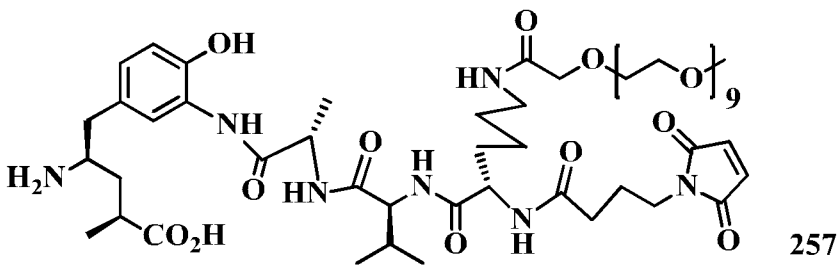
Example 233. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido) -4-hydroxyphenyl)-2-methylpentanoic acid (**256**).



256

To a solution of 4-maleimidobutyric acid N-hydroxysuccinimide ester (2.6 mmol) in DCM (20 mL), compound **79** (1.95 g, 1.7 mmol) and N-methylmorpholine (0.53 g, 5.2 mmol) were added. After stirring at r.t. overnight, the reaction was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by preparative HPLC to give a white soild (1.8 g, 80% yield). MS-ESI (m/z): calcd. for $C_{60}H_{99}N_7O_{22}$ $[M+H]^+$ 1270.68; found,1271.24.

Example 234. Synthesis of (2S, 4R)-4-amino-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl) -2-methylpentanoic acid (**257**).

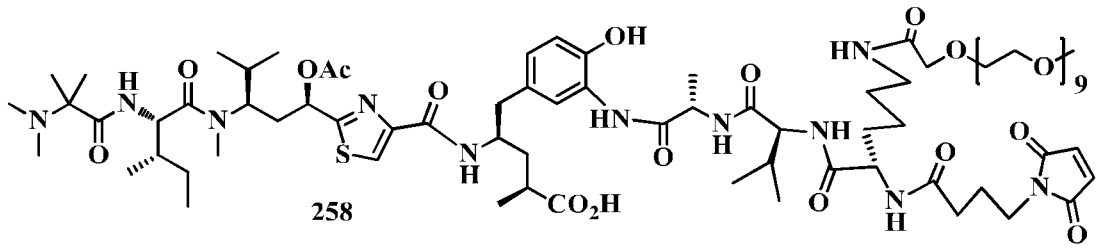


257

Compound **256** (1.8 g, 1.4 mmol) was dissolved in DCM (10 mL) and TFA (10 mL). The reaction was stirred at r.t. for 2 hours, concentrated and purified by preparative HPLC to give compound **257** (1.6 g, 95% yield). MS-ESI (m/z): calcd. for $C_{55}H_{91}N_7O_{20}$ $[M+H]^+$ 1170.63; found,1171.10.

Example 235. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -40-isopropyl-43-

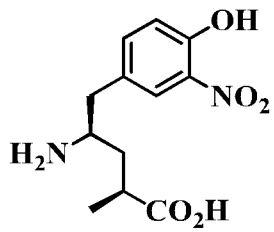
methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**258**).



258

Compound **257** (520 mg, 0.44 mmol) and **Tub-1** (320 mg, 0.44 mmol) were dissolved in 5 mL of DMF, N,N-diisopropylethylamine (90 mg, 0.67 mmol) was added at 0 °C. After stirring at 0 °C for 2 hours, the reaction was concentrated and purified by preparative HPLC, to give an off-white solid (435 mg, 58% yield). MS-ESI (m/z): calcd. for $C_{80}H_{131}N_{11}O_{25}S$ $[M+H]^+$ 1678.90; found,1679.56.

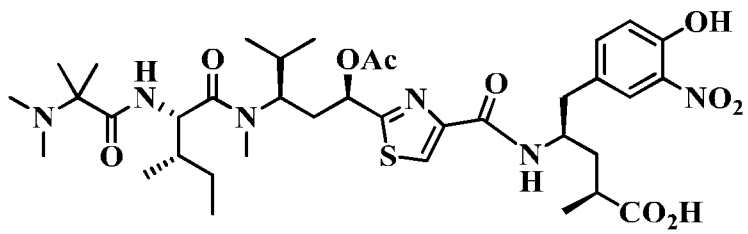
Example 236. Synthesis of (2S, 4R)-4-amino-5-(4-hydroxy-3-nitrophenyl)-2-methylpentanoic acid (**260**).



260

Compound 259 (2.0 g, 5.43 mmol) was dissolved in methanolic HCl (4 M, 20 mL) and stirred for 3 hours, and then concentrated to give a yellow oil (1.66 g, 100% yield). MS-ESI (m/z): calcd. for $C_{12}H_{16}N_2O_5$ $[M+H]^+$ 269.12; found, 269.32.

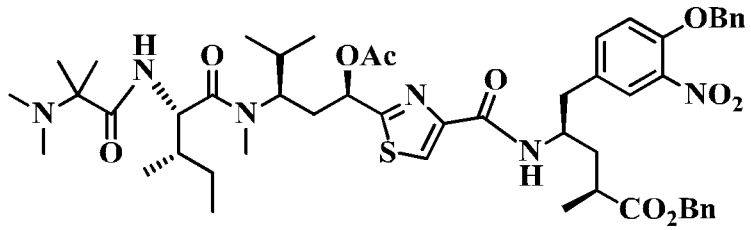
Example 237. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(4-hydroxy-3-nitrophenyl)-2-methylpentanoic acid (**261**).



261

Compound **260** (1.5 g, 5.59 mmol) and **Tub-1** (3.9 g, 5.59 mmol) were dissolved in 30 mL of DMF and cooled to 0 °C, N,N-diisopropylethylamine (1.5 g, 11.2 mmol) was added. After stirring at 0 °C for 2 hours, the reaction was diluted with DCM (50 mL) and washed with brine (4 × 50 mL), dried, filtered and concentrated and purified by preparative HPLC, to give a light yellow solid (3.5 g, 80% yield). MS-ESI (m/z): calcd. for $C_{37}H_{56}N_6O_{10}S$ $[M+H]^+$ 777.38; found, 777.58.

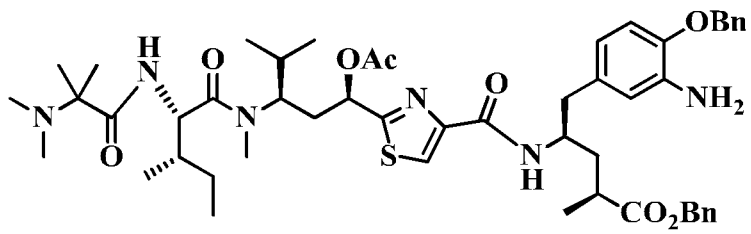
Example 238. Synthesis of benzyl (2S, 4R)-5-(4-(benzyloxy)-3-nitrophenyl)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoate (**262**).



262

Compound **261** (3.5 g, 4.51 mmol) and benzyl bromide (2.3 g, 13.5 mmol) were dissolved in dry DMF (60 mL) and cooled to 0°C in an ice-water bath. Sodium hydrogen (540 mg, 60 wt%) was added in portions, and after the addition, the reaction was warmed to r.t. and stirred overnight. The solution was diluted with DCM (100 mL) and washed with brine (4 × 100 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated and purified by a silica gel column (DCM/ MeOH = 20:1~10:1) to give a yellow solid (2.8 g, 65% yield). MS-ESI (m/z): calcd. for $C_{51}H_{68}N_6O_{10}S$ $[M+H]^+$ 957.47; found, 957.85.

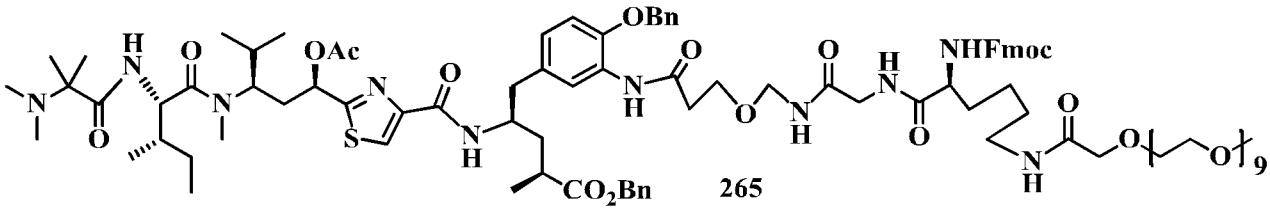
Example 239. Synthesis of benzyl (2S, 4R)-5-(3-amino-4-(benzyloxy)phenyl)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoate (**263**).



263

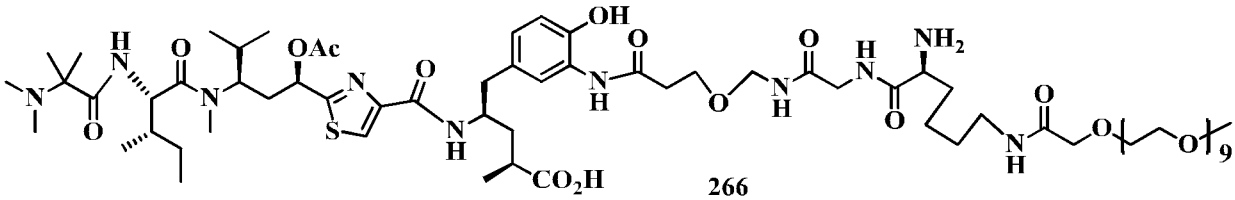
Compound **262** (2.8 g, 2.93 mmol) was dissolved in 95% yield ethanol (50 mL), ammonium chloride (1.6 g, 29.25 mmol) and iron powder (1.6 g, 29.25 mmol) were added, and the reaction was heated under reflux overnight. The solution was diluted with DCM (100 mL) and washed with brine (200 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated, purified by a silica gel column (DCM/MeOH = 1:0~20:1) to give a yellow solid (2.3 g, 85% yield). MS-ESI (m/z): calcd. for $C_{51}H_{70}N_6O_8S$ $[M+H]^+$ 927.51; found, 927.65.

Example 240. Synthesis of benzyl (2S, 4R)-5-(3-((S)-37-((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 44-undecaoxa-32, 39, 42-triazaheptatetracontan-47-amido)-4-(benzyloxy)phenyl)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole -4-carboxamido)-2-methylpentanoate (**265**).



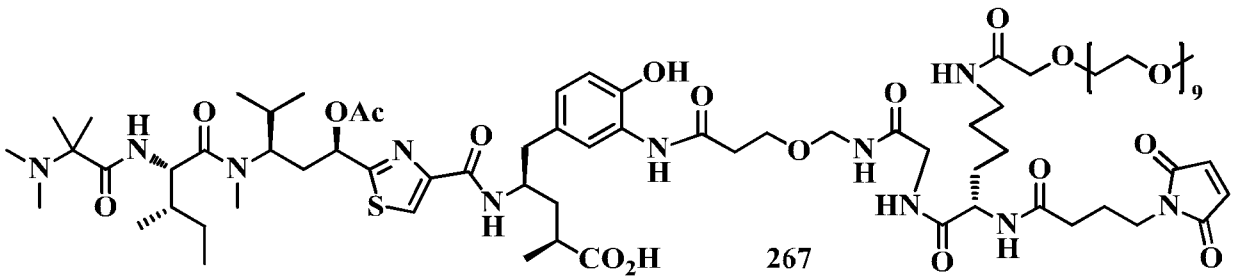
Compound **264** (750 mg, 0.75 mmol) and compound **263** (700 mg, 0.75 mmol) were dissolved in DMC (20 mL), PyBrOP (360 mg, 0.85 mmol) and N,N-diisopropylethylamine (100 mg, 0.75 mmol) were added and stirred overnight at r.t.. The reaction solution was washed with brine, dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated. The residue was purified by a silica gel column (DCM/MeOH = 20:1~10:1) to give a yellow-brown solid (420 mg, 29% yield). MS-ESI (m/z): calcd. for $C_{99}H_{142}N_{10}O_{25}S$ $[M+H]^+$ 1903.99; found, 1904.53.

Example 241. Synthesis of benzyl (2S, 4R)-5-(3-((S)-37-amino-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 44-undecaoxa-32, 39, 42-triazaheptatetracontan-47-amido) -4-(benzyloxy)phenyl)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoate (**266**).



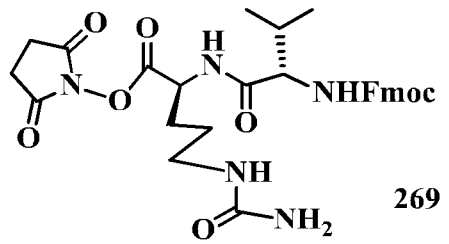
Compound **265** (420 mg, 0.22 mmol) was dissolved in isopropanol (10 mL), palladium on carbon (100 mg, 10 wt%) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times and heated to 50° C and stirred for 3 hours. The reaction mixture was filtered and the filtrate was concentrated, and purified preparative HPLC to a white solid (110 mg, 33% yield). MS-ESI (m/z): calcd. for $C_{70}H_{120}N_{10}O_{23}S$ $[M+H]^+$ 1501.83; found, 1502.12.

Example 242. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 44-undecaoxa-32, 39, 42-triazaheptatetracontan-47-amido) -4-hydroxyphenyl)-2-methylpentanoic acid (**267**).



To a solution of compound **266** (110 mg, 0.07 mmol) and 4-maleimidobutyric acid N-hydroxysuccinimide ester (21 mg, 0.07 mmol) in DCM (5 mL) was added N-methylmorpholine (10 mg, 0.07 mmol). The reaction was stirred at r.t. overnight, concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **267** (15 mg, 12% yield). MS-ESI (m/z): calcd. for $C_{78}H_{127}N_{11}O_{26}S$ $[M+H]^+$ 1666.87; found, 1667.52.

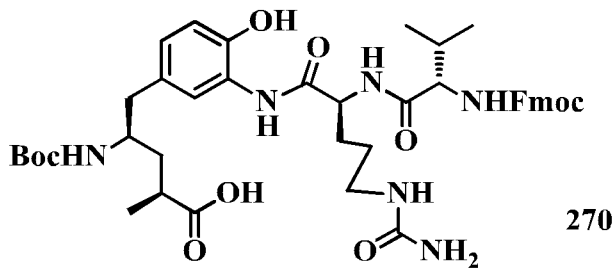
Example 243. Synthesis of 2, 5-dioxopyrrolidin-1-yl (S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy) carbonyl)amino)-3-methylbutanamido)-5-ureidopentanoate (**269**).



269

To a solution of compound **268** (4.00 g, 8.06 mmol) in 200 mL of DCM, NHS (1.39 g, 12.10 mmol) and EDC·HCl (3.09 g, 16.13 mmol) were added. The reaction was stirred for 4 hours, and diluted with 100 mL of water, and filtered to give 4.40 g of white solid (92% yield). MS-ESI (m/z): calcd. for $C_{30}H_{35}N_5O_8$ $[M+H]^+$ 594.25; found, 594.25.

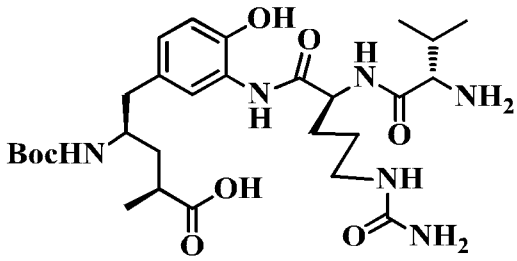
Example 244. Synthesis of (2S, 4R)-5-(3-((S)-2-((S)-2-((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**270**).



270

A solution of compound **269** (2.00 g, 3.37 mmol) and compound 77 (1.25 g, 3.70 mmol) in 150 mL of anhydrous THF was heated at 70 °C overnight. The reaction was concentrated and purified on a silica gel column (11% MeOH/ DCM) to give a red solid (0.79 g, 29% yield). MS-ESI (m/z): calcd. for $C_{43}H_{56}N_6O_{10}$ $[M+H]^+$ 817.41; found, 817.32.

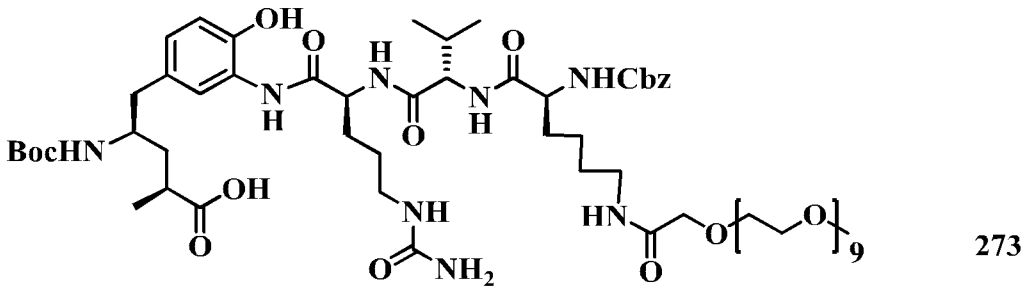
Example 245. Synthesis of (2S, 4R)-5-(3-((S)-2-((S)-2-amino-3-methylbutanamido) -5-ureidopentanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**271**).



271

A solution of compound **270** (0.79 g, 0.97 mmol) in DMF wash stirred with piperidine (0.50 mL) at r.t. for 10 min., and then concentrated, co-evaporated with 5 mL of THF, to give compound **271** (0.87 g, >100% yield). MS-ESI (m/z): calcd. for $C_{28}H_{46}N_6O_8$ $[M+H]^+$ 595.34; found, 595.35.

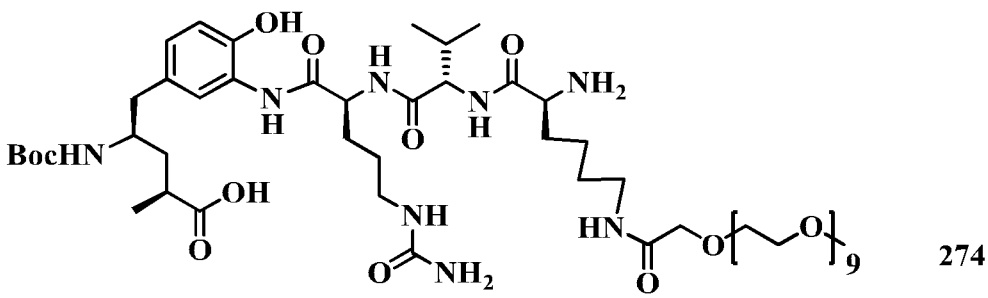
Example 246. Synthesis of (2S, 4R)-5-(3-((37S, 40S, 43S)-37-(((benzyloxy)carbonyl)amino) -40-isopropyl-31, 38, 41-trioxo-43-(3-ureidopropyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**273**).



273

A solution of crude compound **271** (0.97 mmol) and compound **272** (1.34 mmol) in 20 mL of anhydrous THF was heated at 70 °C for 0.5 h. The reaction was concentrated and purified on a silica gel column (13% MeOH/ DCM) to give compound **273** (1.13 g, 88% yield). MS-ESI (m/z): calcd. for $C_{63}H_{104}N_8O_{22}$ $[M+H]^+$ 1325.73; found, 1326.28.

Example 247. Synthesis of (2S, 4R)-5-(3-((37S, 40S, 43S)-37-amino-40-isopropyl-31, 38, 41-trioxo-43-(3-ureidopropyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**274**).

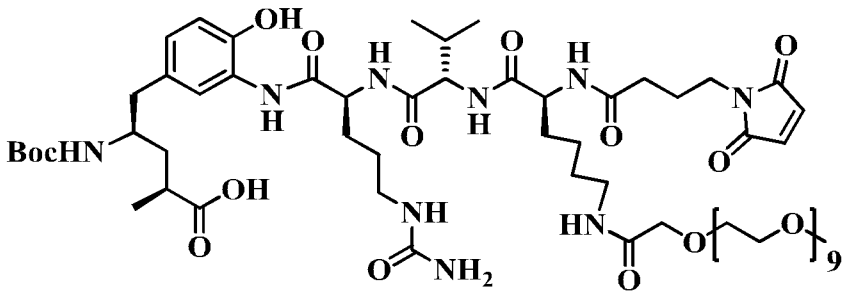


274

Compound **273** (1.13 g, 8.52 mmol) was dissolved in methanol (20 mL), palladium on carbon (310 mg, 10 wt%) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times and stirred at 30° C for 2 hours. The reaction mixture was filtered and the filtrate was

concentrated to give compound **274** (0.77 g, 75% yield). MS-ESI (m/z): calcd. for $C_{55}H_{98}N_8O_{20}$ $[M+H]^+$ 1191.69; found, 1192.10.

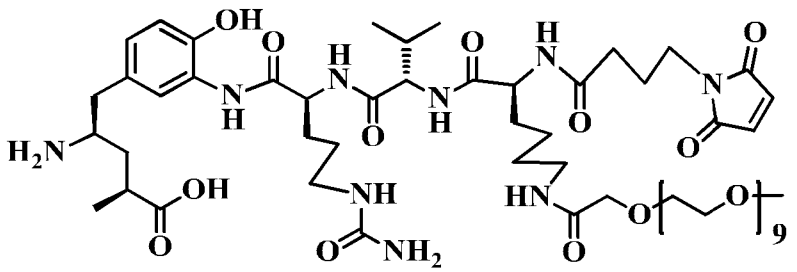
Example 248. Synthesis of (2S, 4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31, 38, 41-trioxo-43-(3-ureidopropyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**275**).



275

A solution of compound **274** (0.77 g, 6.44 mmol) and 4-maleimidobutyric acid N-hydroxysuccinimide ester (0.18 g, 6.55 mmol) in THF (20 mL) was stirred at 30° C for 1 h, concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **275** (0.66 g, 76% yield). MS-ESI (m/z): calcd. for $C_{63}H_{105}N_9O_{23}$ $[M+H]^+$ 1356.73; found, 1356.33.

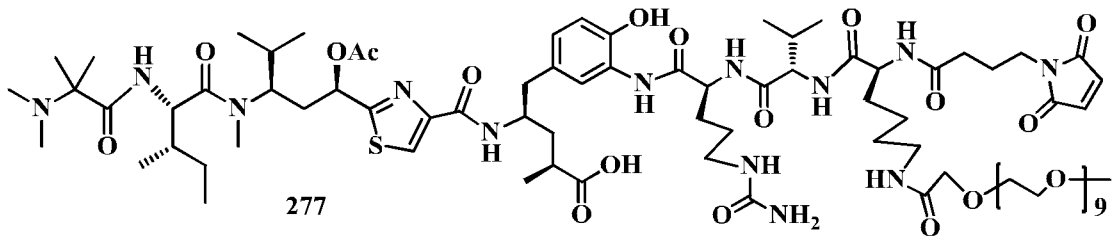
Example 249. Synthesis of (2S, 4R)-4-amino-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31, 38, 41-trioxo-43-(3-ureidopropyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**276**).



276

A solution of compound **276** (0.66 g, 4.86 mmol) in DCM (10 mL) was stirred with TFA (10 mL) for 40 min. and then concentrated to give a crude product (1.02 g, > 100% yield). MS-ESI (m/z): calcd. for $C_{58}H_{97}N_9O_{21}$ $[M+H]^+$ 1256.68; found, 1257.23.

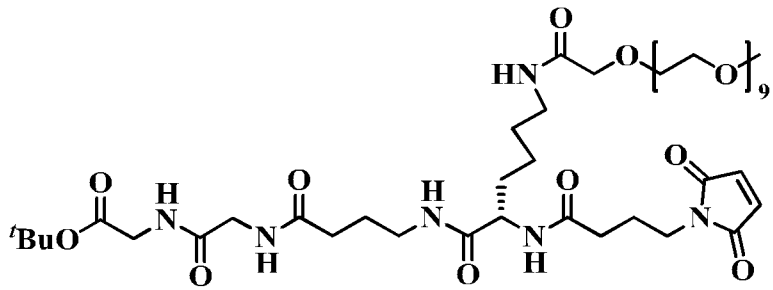
Example 250. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31, 38, 41-trioxo-43-(3-ureidopropyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**277**).



277

Compound **276** (0.31 g, 2.47 mmol) and **Tub-1** (0.17 g, 2.49 mmol) were dissolved in 2 mL of DMF, N,N-diisopropylethylamine (163 μL, 0.99 mmol) was added and stirred for 2 hours. The reaction was concentrated and purified by preparative HPLC, to give compound **277** (343 mg, 79% yield). MS-ESI (m/z): calcd. for $C_{83}H_{137}N_{13}O_{26}S$ $[M+H]^+$ 1764.95; found, 1765.99.

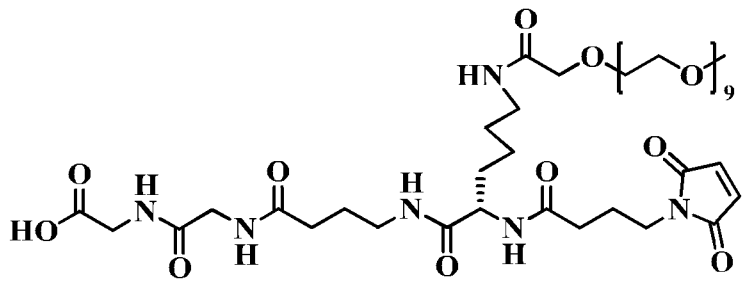
Example 251. Synthesis of tert-butyl (S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycylglycinate (**278**).



278

Compound **234** (1.60 g, 1.73 mmol), pentafluorophenol (0.47 g, 2.55 mmol) and N, N′-diisopropylcarbodiimide (0.43 g, 3.40 mmol) were dissolved in 20 mL of DCM, and stirred for 2 hours. H-Gly-O^{t}Bu (0.32 g, 1.91 mmol) and N,N-diisopropylethylamine (0.45 g, 3.47 mmol) were then added, and the reaction was stirred overnight, washed with 50 mL of water, 50 mL of brine, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The crude was purified by a silica gel column to give compound **278** as a light yellow liquid (1.5 g, 83% yield). MS-ESI (m/z): calcd. for $C_{47}H_{83}N_6O_{19}$ $[M+H]^+$ 1035.56; found, 1035.56.

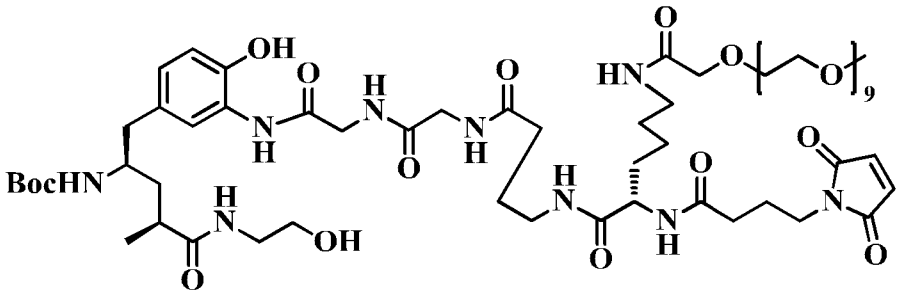
Example 252. Synthesis of (S)-(S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycylglycine (**279**).



279

Compound **278** (1.50 g, 1.45 mmol) was dissolved in DCM (10 mL) and stirred with 20 mL of formic acid. After stirring at 40 °C for 2 hours, the reaction was concentrated to give compound **279** as a colorless liquid (1.50 g, 100% yield). MS-ESI (m/z): calcd. for $C_{43}H_{75}N_6O_{19}$ $[M+H]^+$ 979.50; found, 979.50.

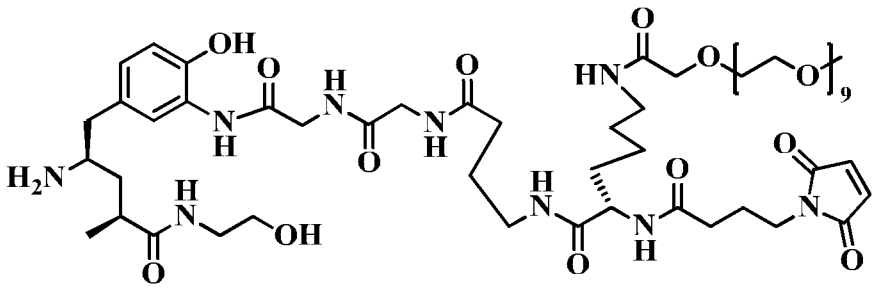
Example 253. Synthesis of tert-butyl ((2R, 4S)-1-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 46-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47-tetraazanonatetracontan-49-amido)-4-hydroxyphenyl)-5- ((2-hydroxyethyl)amino)-4-methyl-5-oxopentan-2-yl)carbamate (**281**).



281

Compound **279** (500 mg, 0.51 mmol) and compound 280 (234 mg, 0.61 mmol) were dissolved in 10 mL of DCM and 3 mL of DMF, and stirred for 1h at 0 °C. The reaction mixture was concentrated and purified by a silica gel column, to give compound **281** as a light yellow liquid (0.22 g, 32% yield). MS-ESI (m/z): calcd. for $C_{62}H_{104}N_9O_{23}$ $[M+H]^+$ 1342.72; found, 1342.72.

Example 254. Synthesis of N-((S)-6-((4-((2-((2-((5-((2R, 4S)-2-amino-5-((2-hydroxyethyl)amino)-4-methyl-5-oxopentyl)-2-hydroxyphenyl)amino)-2-oxoethyl)amino)-2-oxoethyl)amino)-4-oxobutyl)amino)-5-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-6-oxohexyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxahentriacontan-31-amide (**282**).

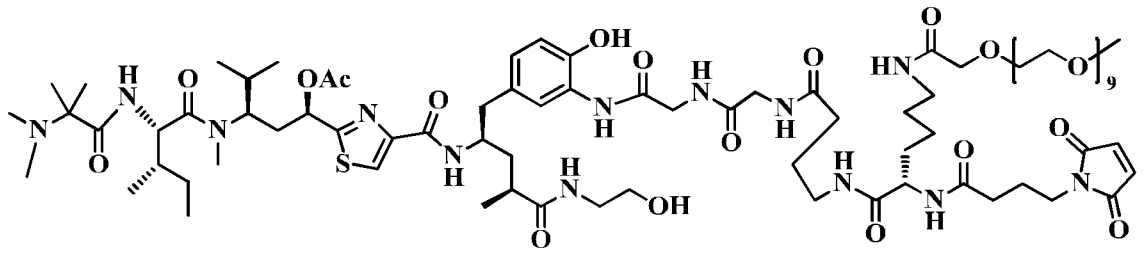


282

Compound **281** (0.11 g, 0.08 mmol) was dissolved in 6 mL of DCM and 2 mL of TFA. The reaction was stirred at 0 °C for 2 hours and then concentrated to give compound **282** as a light yellow liquid (0.10 g, 100% yield). MS-ESI (m/z): calcd. for $C_{57}H_{96}N_9O_{21}$ $[M+H]^+$ 1242.66; found, 1242.66.

Example 255. Synthesis of (1R, 3R)-3-((2S, 3S)-2-(2-(dimethylamino)-2-methylpropanamido) -N, 3-dimethylpentanamido)-1-(4-(((2R, 4S)-1-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H- pyrrol-1-yl)butanamido)-31, 38, 43, 46-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44, 47-

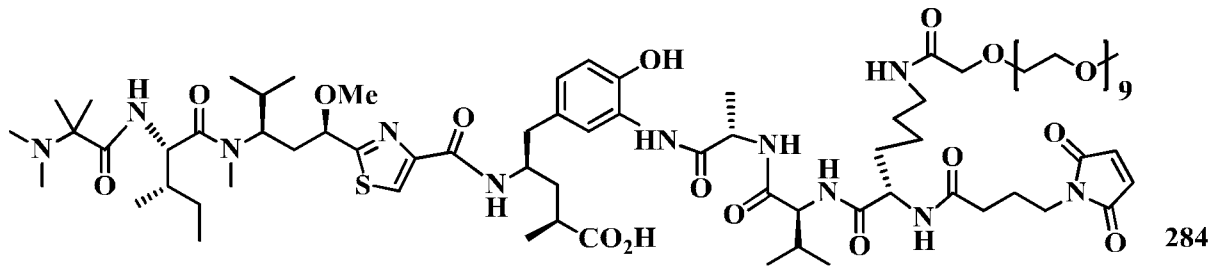
tetraazanonatetracontan-49-amido)-4-hydroxyphenyl)-5-((2-hydroxyethyl)amino)-4-methyl -5-oxopentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentyl acetate (**283**).



283

Compound **282** (0.10 g, 0.08 mmol) and **Tub-1** (61 mg, 0.08 mmol) were dissolved in 5 mL of DMF, and N,N-diisopropylethylamine (125 mg, 0.96 mmol) was added at 0 °C to adjust pH to ~ 9. The reaction was stirred at 0 °C for 2 hours and at r.t. for 1 h, directly purified by preparative HPLC to give compound **283** as a light yellow liquid (45 mg, 31% yield). MS-ESI (m/z): calcd. for $C_{82}H_{136}N_{13}O_{26}S$ $[M+H]^+$ 1750.94; found, 1750.94.

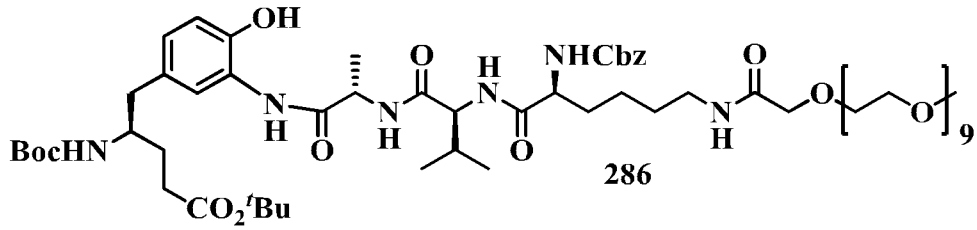
Example 256. Synthesis of (2S, 4R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7-dioxo-12-oxa-2, 5, 8-triazatridecan-11-yl)thiazole-4-carboxamido)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan -44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**284**).



284

To a solution of compound **257** (300 mg, 0.256 mmol) and Tub-3 (175 mg, 0.256 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (66 mg, 0.512 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **284** (60 mg, 14% yield). MS-ESI (m/z): calcd. for $C_{79}H_{131}N11O_{24}S$ $[M+H]^+$ 1650.90; found, 1651.50.

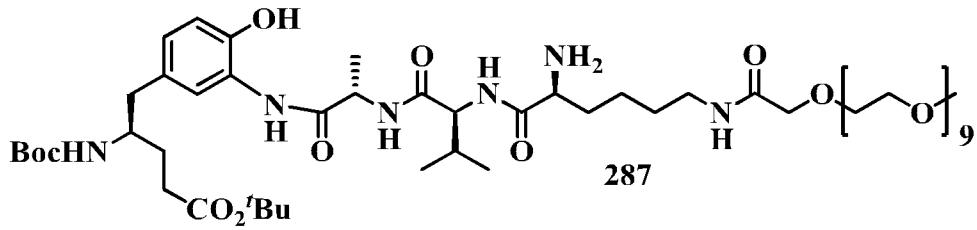
Example 257. Synthesis of tert-butyl (R)-5-(3-((37S, 40S, 43S)-37-(((benzyloxy)carbonyl) amino)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**286**).



286

A solution of compound **76** (6.0 g, 5.9 mmol) and compound **69** (2.7 g, 7.1 mmol) in THF (100 mL) was heated at 60 °C for 20 h and then concentrated. The residue was purified on a silica gel column (DCM/MeOH = 20:1) to give compound **286** as an off-white solid (7.1 g, 93% yield). MS-ESI (m/z): calcd. for $C_{164}H_{266}N_{24}O_{52}S_2$ $[M+H]^+$ 3472.19; found, 3472.95.

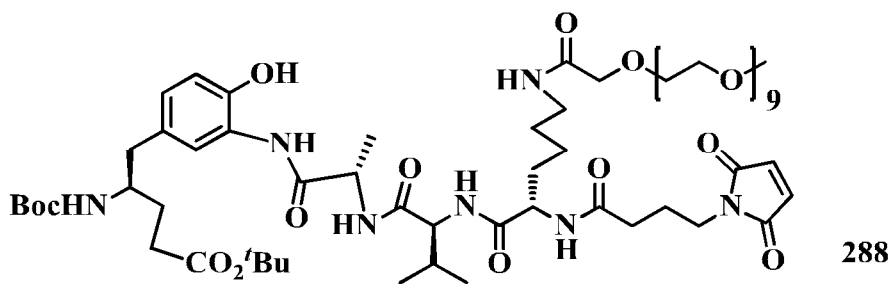
Example 258. Synthesis of tert-butyl (R)-5-(3-((37S, 40S, 43S)-37-amino-40 -isopropyl-43 -methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**287**).



287

To a solution of compound **286** (7.1g, 5.7mmol) in isopropanol (80mL) was added Pd/C (5 wt%, 800 mg). The reaction flask was evacuated and back-filled with hydrogen for 3 times, and then heated to 50 °C and stirred for 2.5 h. The mixture was filtered and the filtrate was concentrated to give an off-white solid (5.9 g, 93% yield). MS-ESI (m/z): calcd. for $C_{55}H_{98}N_6O_{19}$ $[M+H]^+$ 1147.69; found, 1148.12.

Example 259. Synthesis of tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido) -4-hydroxyphenyl)pentanoate (**288**).

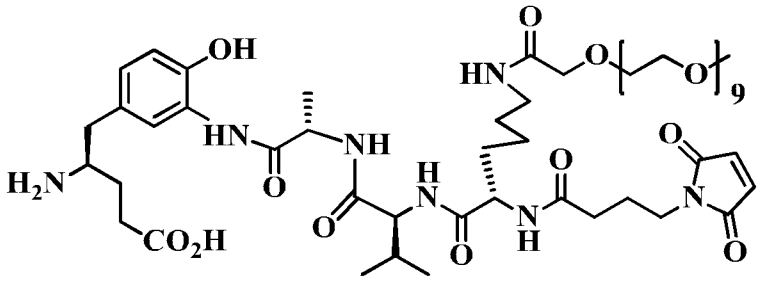


288

A solution of compound **287** (2.0 g, 1.7 mmol), 4-maleimidobutyric acid N-hydroxysuccinimide ester (2.6 mmol) and N-methylmorpholine (0.53 g, 5.2 mmol) in DCM (20 mL) was stirred at r.t. overnight. The mixture was then washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by a silica gel column

(DCM/MeOH = 20:1) to afford compound **288** (2.1 g, 91% yield) as a light brown oil. MS-ESI (m/z): calcd. for $C_{63}H_{105}N_7O_{22}$ $[M+H]^+$ 1312.73; found, 1313.12.

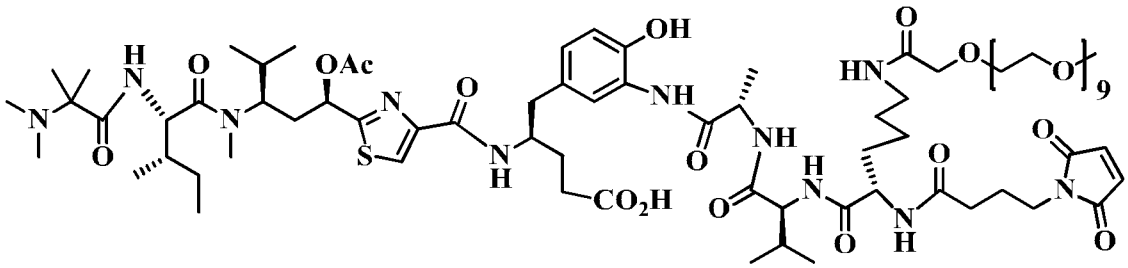
Example 260. Synthesis of (R)-4-amino-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**289**).



289

A solution of compound **288** (2g, 1.5mmol) in DCM (10 mL) was treated with TFA (10 mL) at r.t. for 2 hours. The solution was then concentrated and the residue was purified by preparative HPLC to give a white solid (1.4 g, 78% yield). MS-ESI (m/z): calcd. for $C_{54}H_{89}N_7O_{20}$ $[M+H]^+$ 1156.63; found, 1157.23.

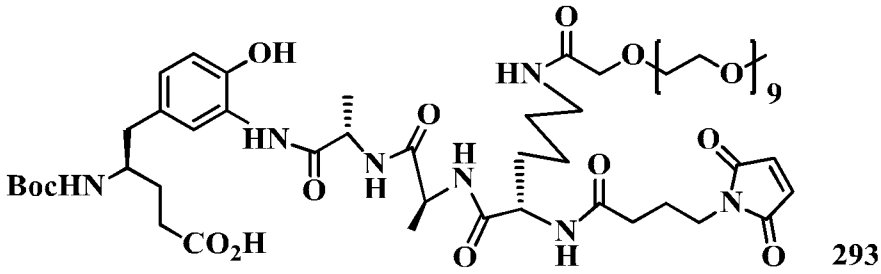
Example 261. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl -43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**290**).



290

To a solution of compound **289** (1.37g, 1.19 mmol), **Tub-1** (0.90g, 1.30 mmol) in DMF (12 mL) was added N,N-diisopropylethylamine (0.31g, 2.37mmol) at 0 °C. The solution was stirred at 0 °C for 2 hours and then concentrated. The residue was purified by preparative HPLC to give a white solid (880 mg, 45% yield). MS-ESI (m/z): calcd. for $C_{79}H_{129}N_{11}O_{25}S$ $[M+H]^+$ 1664.89; found, 1665.63.

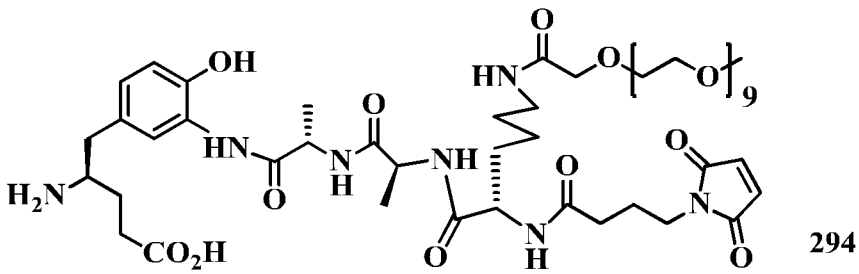
Example 262. Synthesis of (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43-dimethyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**293**).



293

To a solution of compound **291** (470 mg, 0.51 mmol) and compound **292** (250 mg, 0.66 mmol) in DCM (10 mL) was added EDC·HCl (120 mg, 0.63 mmol) at 0 °C. After stirring for 2 hours, the reaction solution was washed with water (50 mL) and brine (50 mL), dried and concentrated. The residue was purified by a silica gel column (DCM/MeOH=10:1) to give compound **293** (390 mg, 56% yield). MS-ESI (m/z): calcd. for $C_{57}H_{93}N_7O_{22}$ $[M+H]^+$ 1229.64; found, 1229.64.

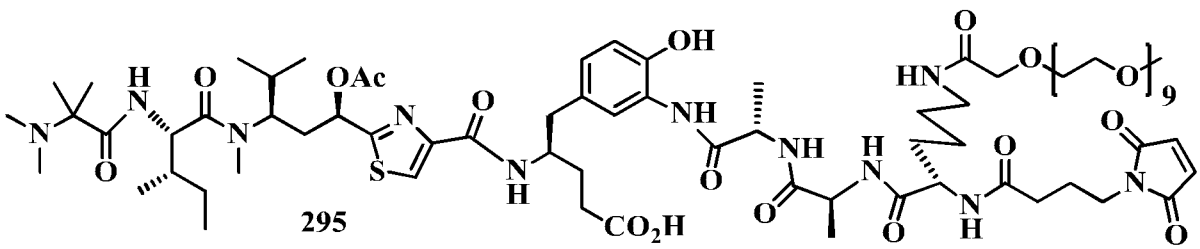
Example 263. Synthesis of (R)-4-amino-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43-dimethyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**294**).



294

A solution of compound **293** (390 mg, 0.30 mmol) in DCM (10 mL) was treated with TFA (5 mL) at r.t. for 1 h, and then concentrated to yield compound **294** (580 mg, >100% yield), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{52}H_{85}N_7O_{20}$ $[M+H]^+$ 1129.64; found, 1129.64.

Example 264. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43-dimethyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**295**).

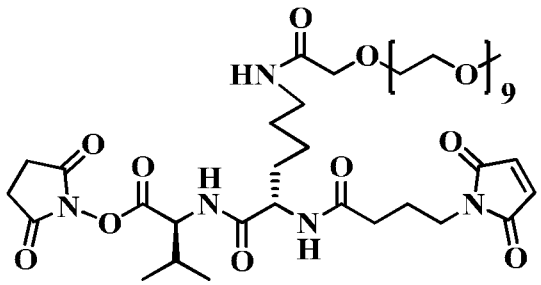


295

To a solution of compound **294** (580 mg, 0.43 mmol), **Tub-1** (210 mg, 0.31 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (0.3 mL) at 0 °C. The solution was stirred at 0 °C for 2

hours and then concentrated. The residue was purified by preparative HPLC to give a white solid (227 mg, 46% yield). MS-ESI (m/z): calcd. for $C_{77}H_{125}N_{11}O_{25}S$ $[M+H]^+$ 1636.86; found, 1636.86.

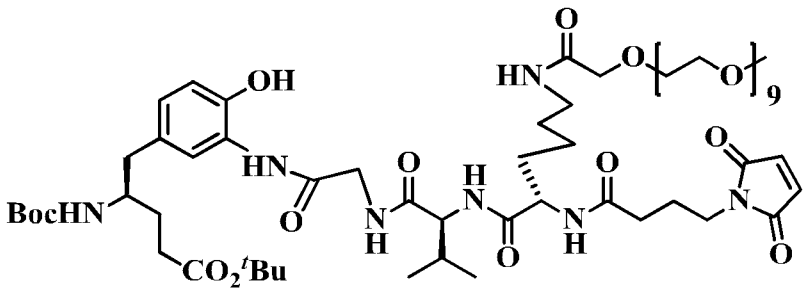
Example 265. Synthesis of 2, 5-dioxopyrrolidin-1-yl ((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31-oxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32-azaoctatriacontan-38-oyl)-L-valinate (**297**).



297

To a solution of compound **296** (879.0 mg, 1.0 mmol) in DCM (30 mL) were added NHS (126.6mg, 1.1 mmol) and EDC·HCl (383.4mg, 2.0 mmol). The reaction was stirred at r.t. for 2 hours and quenched with water. The organic phase was separated and washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give the title compound (1.1 g, >100 yield). MS-ESI (m/z): calcd. for $C_{44}H_{74}N_5O_{19}$ $[M+H]^+$ 976.49; found,: 976.49.

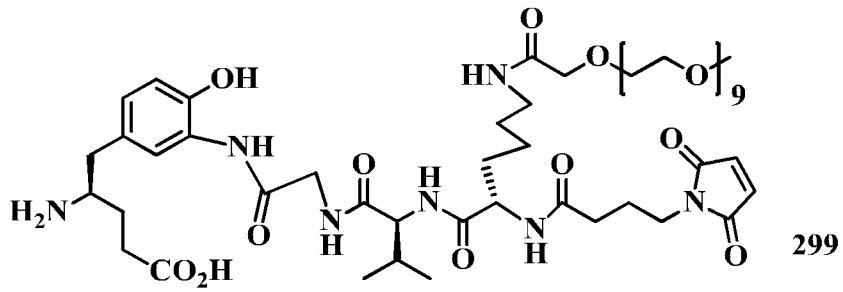
Example 266. Synthesis of tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido) -4-hydroxyphenyl)pentanoate (**298**).



298

Compound **297** from previous step (550 mg, 0.5 mmol) was dissolved in THF (10 mL). Compound **85** (215.0 mg, 0.5 mmol) was added to the solution and stirred until being completely consumed. The reaction mixture was concentrated and purified by preparative HPLC, to give compound **298** (201 mg, 31% yield). MS-ESI (m/z): calcd. for $C_{62}H_{104}N_7O_{22}$ $[M+H]^+$ 1298.72; found, 1298.72.

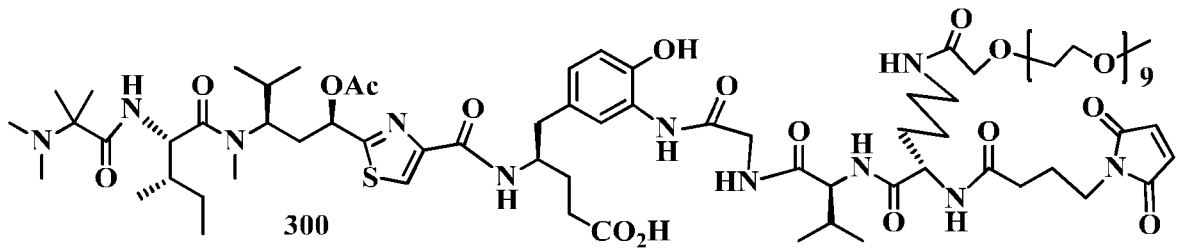
Example 267. Synthesis of (R)-4-amino-5-(3-((37S, 40S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**299**).



299

A solution of compound **298** (101 mg, 0.078 mmol) in DCM (1 mL) was treated with TFA (2 mL) at r.t. for 1 h, and then concentrated to yield compound **299** (128 mg, >100% yield), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{53}H_{88}N_7O_{20}$ $[M+H]^+$ 1142.60; found, 1142.60.

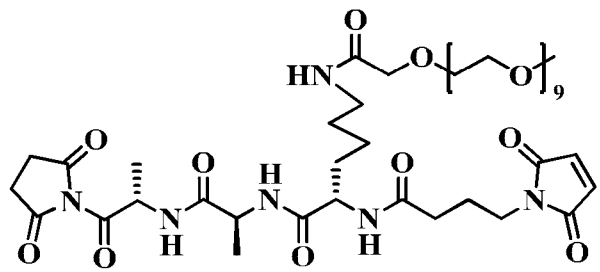
Example 268. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4- carboxamido)-5-(3-((37S, 40S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -40-isopropyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**300**).



300

To a solution of compound **299** (70 mg, 0.06 mmol), **Tub-1** (40 mg, 0.06 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (15 mg, 0.12 mmol) at 0 °C. The solution was stirred at r.t. for 2 hours and then concentrated. The residue was purified by preparative HPLC to give a white solid (38 mg, 38% yield). MS-ESI (m/z): calcd. for $C_{78}H_{128}N_{11}O_{25}S$ $[M+H]^+$ 1650.87; found, 1650.87.

Example 269. Synthesis of N-((S)-5-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido) -6-(((S)-1-(((S)-1-(2, 5-dioxopyrrolidin-1-yl)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino) -6-oxohexyl)-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxahentriacontan-31-amide (**302**).

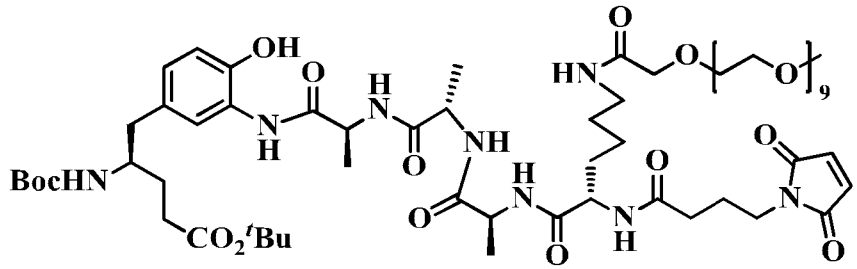


302

A solution of compound **301** (300 mg, 0.325 mmol), NHS (42 mg, 0.36 mmol) and EDC·HCl (95 mg, 0.50 mmol) in DCM (3 mL) was stirred at r.t. for 2 hours. The reaction mixture was diluted

with DCM (20 mL) and washed with water (25 mL) and brine (25 mL), dried and concentrated to give compound **302** (280 mg, 85% yield). MS-ESI (m/z): calcd. for $C_{45}H_{72}N_6O_{20}$ $[M+H]^+$ 1017.48; found, 1017.48.

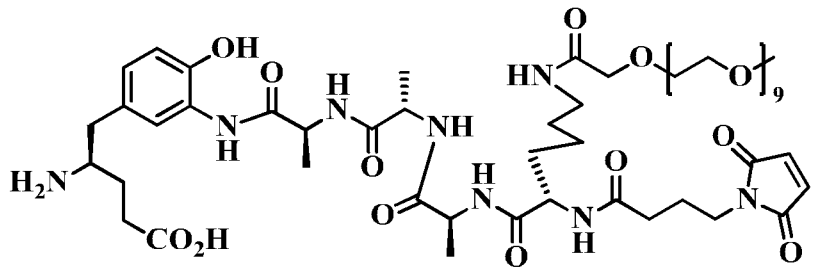
Example 270. Synthesis of tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan- 47-amido)-4-hydroxyphenyl)pentanoate (**304**).



304

A solution of compound **302** (280 mg, 0.28 mmol) and compound **303** (200 mg, 0.44 mmol) in THF (5 mL) was stirred at r.t for 2 hours and then concentrated. The residue was purified by a silica gel column (DCM/ MeOH = 10:1) to give compound **304** (180 mg, 48% yield). MS-ESI (m/z): calcd. for $C_{64}H_{106}N_8O_{23}$ $[M+H]^+$ 1355.74; found, 1355.74.

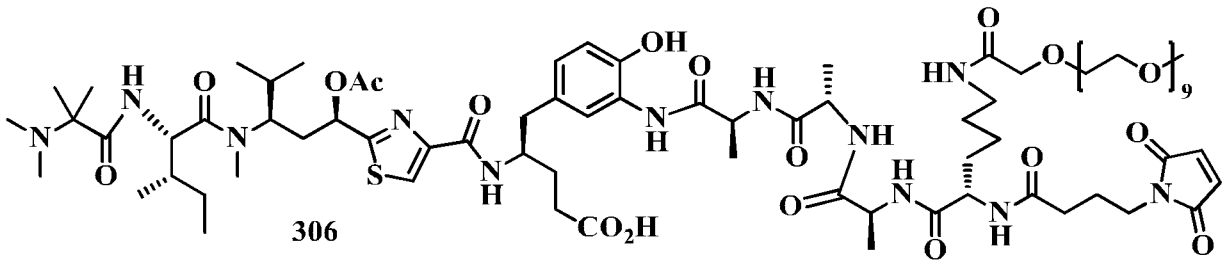
Example 271. Synthesis of (R)-4-amino-5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan-47-amido)-4-hydroxyl phenyl)pentanoic acid (**305**).



305

A solution of compound **307** (180 mg, 0.13 mmol) in DCM (6 mL) was treated with TFA (3 mL) at r.t. for 1 h, and then concentrated to give compound **305** (300 mg, >100% yield). MS-ESI (m/z): calcd. for $C_{55}H_{90}N_8O_{21}$ $[M+H]^+$ 1199.62; found, 1199.62.

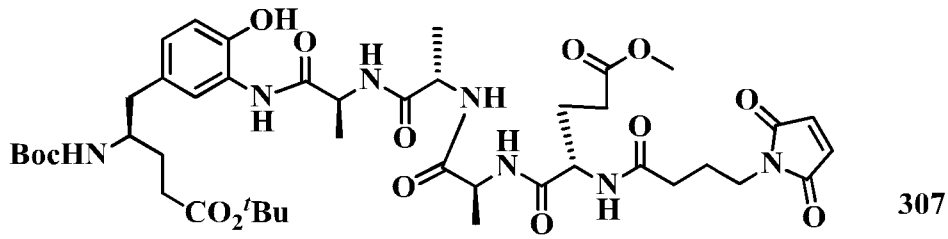
Example 272. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S, 46S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40, 43, 46-trimethyl-31, 38, 41, 44-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42, 45-tetraazaheptatetracontan-47-amido)-4-hydroxyphenyl)pentanoic acid (**306**).



306

To a solution of compound **305** (160 mg, 0.13 mmol), **Tub-1** (100 mg, 0.14 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (0.3 mmol) at 0 °C. The solution was warmed to r.t. and stirred for 1 h and then concentrated. The residue was purified by preparative HPLC to give a white solid (58 mg, 25% yield). MS-ESI (m/z): calcd. for $C_{80}H_{130}N_{12}O_{26}S$ $[M+H]^+$ 1707.89; found, 1707.89.

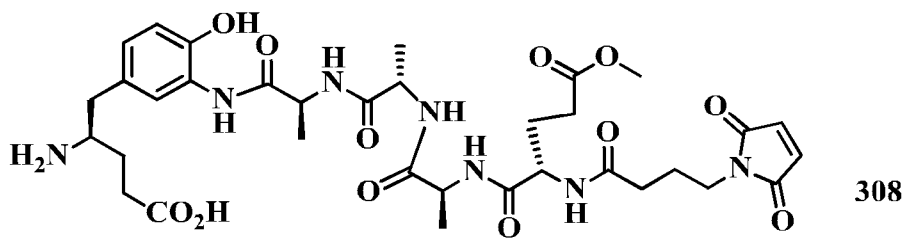
Example 273. Synthesis of methyl (S)-5-(((S)-1-(((S)-1-(((S)-1-((5-((R)-5-(tert-butoxy) -2-((tert-butoxycarbonyl)amino)-5-oxopentyl)-2-hydroxyphenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-5-oxopentanoate (**307**).



307

To a solution of compound **251** (0.28 g, 0.5 mmol) and compound **69** (0.20 g, 0.5 mmol) in DMF (2 mL) were added HATU (0.30g, 0.8 mmol) and N,N-diisopropylethylamine (130 μL, 0.8mmol). The reaction was stirred at r.t. for 10 min and directly purified by preparative HPLC to give a white solid (324 mg, 69% yield). MS-ESI (m/z): calcd. for $C_{43}H_{63}N_7O_{14}$ $[M+H]^+$ 902.44; found, 902.81.

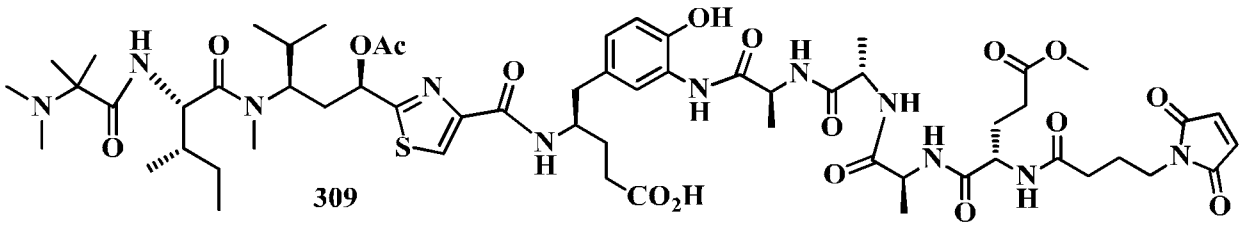
Example 274. Synthesis of (R)-4-amino-5-(3-((6S, 9S, 12S, 15S)-6-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-9, 12, 15-trimethyl-3, 7, 10, 13-tetraoxo-2-oxa-8, 11, 14-triazahexadecan-16-amido)-4-hydroxyphenyl)pentanoic acid (**308**).



308

A solution of compound **307** (0.32g, 0.36mmol) in DCM (10 mL) was treated with TFA (10 mL) at r.t. for 1 h, and then concentrated to give compound **308** (0.27 g, >100 yield). MS-ESI (m/z): calcd. for $C_{34}H_{47}N_7O_{12}$ $[M+H]^+$ 746.33; found, 746.67.

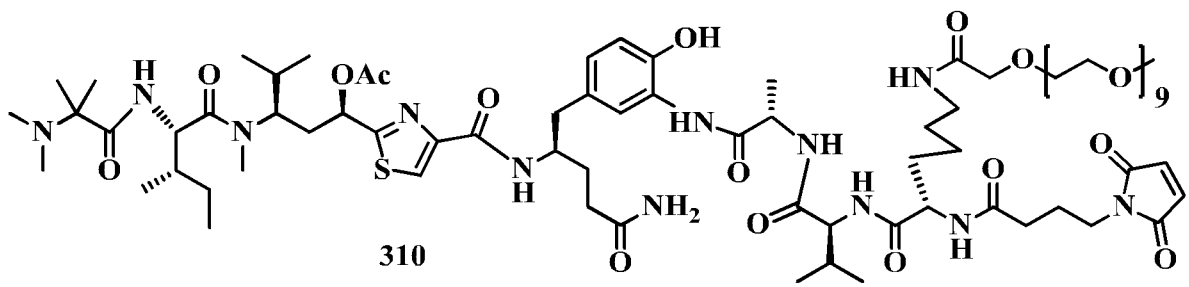
Example 275. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole -4-carboxamido)-5-(3-((6S, 9S, 12S, 15S)-6-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-9, 12, 15-trimethyl-3, 7, 10, 13-tetraoxo-2-oxa-8, 11, 14-triazahexadecan-16- amido)-4-hydroxyphenyl)pentanoic acid (**309**).



309

To a solution of compound **308** (0.27 g, 0.36 mmol), **Tub-1** (0.25 g, 0.36 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (180 μL, 1.09 mmol). The solution was stirred at r.t. for 30 min and then concentrated. The residue was purified by preparative HPLC to give a white solid (367 mg, 59% yield). MS-ESI (m/z): calcd. for $C_{59}H_{87}N_{11}O_{17}S$ $[M+H]^+$ 1254.60; found, 1255.32.

Example 276. Synthesis of (1R, 3R)-1-(4-(((R)-5-amino-1-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan -44-amido)-4-hydroxyphenyl)-5-oxopentan-2-yl)carbamoyl)thiazol-2-yl)-3-((2S, 3S)-2-(2- (dimethylamino)-2-methylpropanamido)-N, 3-dimethylpentanamido)-4-methylpentyl acetate (**310**).

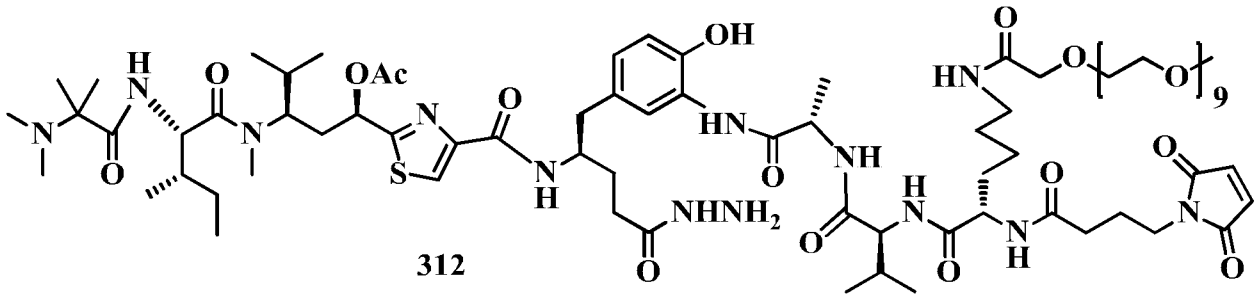


310

To a solution of compound **290** (300 mg, 0.2 mmol) in DCM (10 mL) were added NHS (31 mg, 0.27 mmol) and EDC·HCl (52 mg, 0.27 mmol) at 0 °C. The reaction was stirred at 0 °C for 1 h and ammonium chloride (15 mg, 0.4 mmol) and N-methylmorpholine (55 mg, 0.54 mmol) were added. The reaction mixture was warmed to r.t. and stirred overnight. LC-MS indicated complete conversion of the intermediate and the crude product was then directly purified by preparative HPLC to give compound **310** (140 mg, 47% yield). MS-ESI (m/z): calcd. for $C_{79}H_{130}N_{12}O_{24}S$ $[M+H]^+$1663.90; found, 1664.93.

Example 277. Synthesis of (1R, 3R)-3-((2S, 3S)-2-(2-(dimethylamino)-2-methylpropanamido)-N, 3-dimethylpentanamido)-1-(4-(((R)-1-(3- ((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl- 43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-

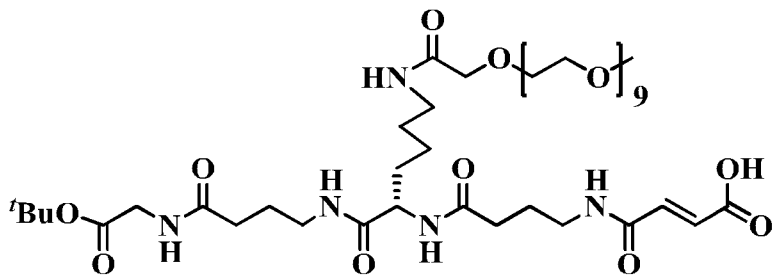
decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-5-hydrazineyl-5-oxopentan-2-yl)carbamoyl)thiazol-2-yl)-4-methylpentyl acetate (**312**).



312

To a solution of compound **290** (300 mg, 0.2 mmol) in DCM (10 mL) were added NHS (42 mg, 0.36 mmol) and EDC·HCl (70 mg, 0.36 mmol) at 0 °C. The reaction was stirred at 0 °C for 1 h and hydrazine (20 mg, 0.36 mmol) and N,N-diisopropylethylamine (70 mg, 0.54 mmol) were added. The reaction mixture was warmed to r.t. and stirred overnight. LC-MS indicated complete conversion of the intermediate and the crude product was then directly purified by preparative HPLC to give compound **312** (100 mg, 33% yield). MS-ESI (m/z): calcd. for $C_{79}H_{131}N_{13}O_{24}S$ $[M+H]^+$1678.92; found, 1678.90.

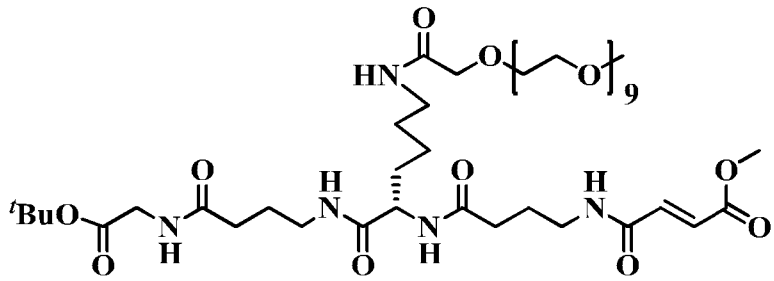
Example 278. Synthesis of (S, E)-37-((4-((2-(tert-butoxy)-2-oxoethyl)amino) -4-oxobutyl)carbamoyl)-31, 39, 44-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 38, 43-triazaheptatetracont-45-en-47-oic acid (**314**).



314

A mixture of compound **313** (1.3 g, 0.001 mol) in acetonitrile (20 mL) and sodium carbonate solution (10 mL) was stirred overnight. The organic solvent was removed and 30 mL of water was added to the solution. After adjusting pH to weak acidic with diluted hydrochloric acid, the solution was extracted with DCM (5 × 30 mL). The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated to give compound **314** (1.30 g, 98% yield). MS-ESI (m/z): calcd. for $C_{45}H_{81}N_5O_{19}$ $[M+H]^+$ 996.55; found, 996.65.

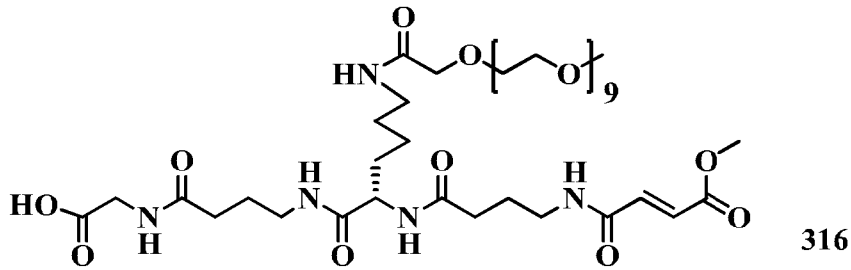
Example 279. Synthesis of 1-(tert-butyl) 20-methyl (S, E)-4, 9, 12, 17-tetraoxo-10-(31-oxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32-azahexatriacontan-36-yl)-3, 8, 11, 16-tetraazaicos -18-enedioate (**315**).



315

To a solution of compound **314** (1.30 g, 0.001 mol) in methanol (15 mL) was added thionyl chloride (0.095 mL, 0.001 mol) dropwise over an ice-water bath. After the bath was removed, the reaction was stirred at r.t. overnight, concentrated and purified by preparative HPLC to give compound **315** (474 mg, 36% yield). MS-ESI (m/z): calcd. for $C_{46}H_{83}N_5O_{19}$ $[M+H]^+$ 1010.57; found, 1010.61.

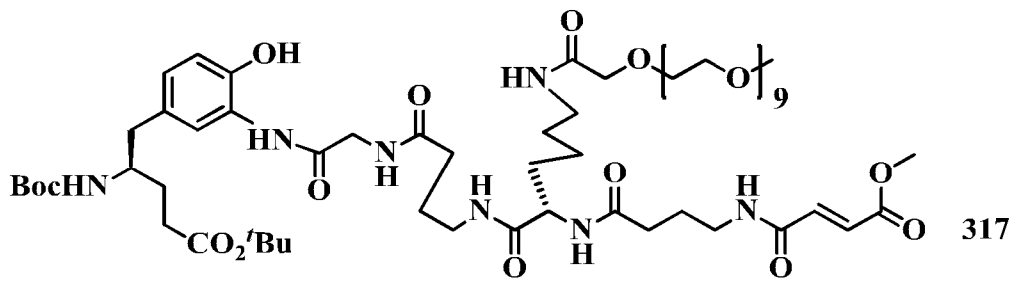
Example 280. Synthesis of (S, E)-(37-(4-(4-methoxy-4-oxobut-2-enamido)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycine (**316**).



316

A solution of compound **315** (474 mg, 0.469 mmol) in DCM (10 mL) was treated with TFA (5 mL) at r.t. for 4 hours, concentrated and purified by preparative HPLC to give compound **316** (213 mg, 47.33% yield). MS-ESI (m/z): calcd. for $C_{42}H_{75}N_5O_{19}$ $[M+H]^+$ 954.51; found, 954.87.

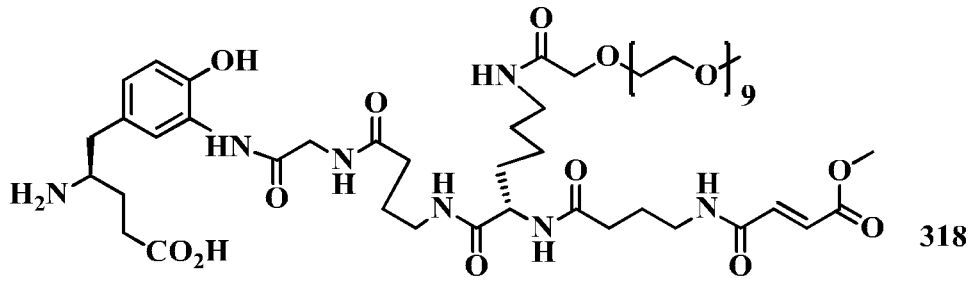
Example 281. Synthesis of methyl (S, E)-37-((4-((2-((5-((R)-5-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-5-oxopentyl)-2-hydroxyphenyl)amino)-2-oxoethyl)amino)-4-oxobutyl)carbamoyl)-31, 39, 44-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 38, 43-triazaheptatetracont-45-en-47-oate (**317**).



317

To a solution of compound **316** (213 mg, 0.223 mmol) and compound **69** (144 mg, 0.378 mmol) in DCM (10 mL), was added EDC·HCl (74 mg, 0.386 mmol). The reaction was stirred at r.t. for 3 hours, and then concentrated and purified by preparative HPLC to give compound **317** (171 mg, 59% yield). MS-ESI (m/z): calcd. for $C_{62}H_{105}N_7O_{23}$ $[M+H]^+$ 1316.73; found, 1317.66.

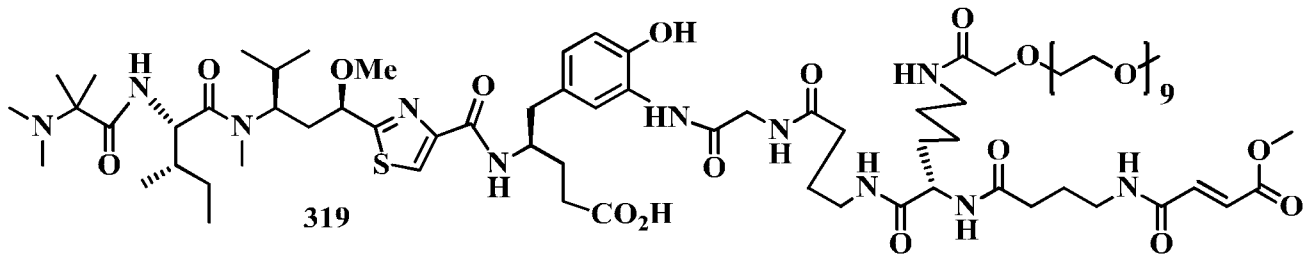
Example 282. Synthesis of (R)-4-amino-5-(4-hydroxy-3-((S)-37-(4-((E)-4- methoxy-4-oxobut-2-enamido)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)phenyl)pentanoic acid (**318**).



318

Compound **317** (171 mg, 0.130 mmol) was dissolved in DCM (3 mL) and TFA (1 mL). The solution was stirred at r.t. for 2 hours and concentrated to give compound **318** (0.15 g, 100% yield). MS-ESI (m/z): calcd. for $C_{53}H_{89}N_7O_{21}$ $[M+H]^+$ 1160.61; found, 1161.26.

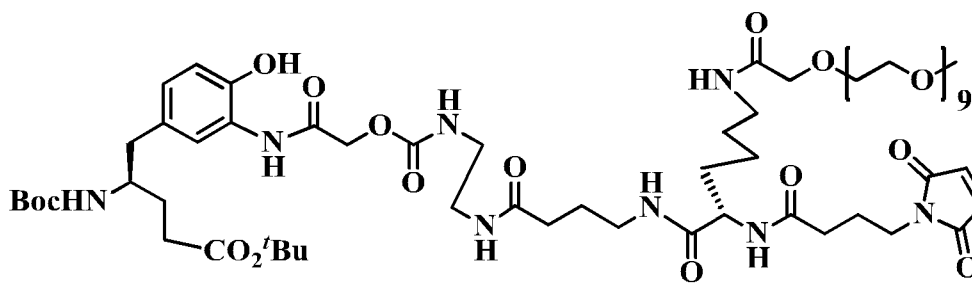
Example 283. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7-dioxo-12-oxa-2, 5, 8-triazatridecan-11-yl)thiazole-4-carboxamido) -5-(4-hydroxy-3-((S)-37-(4-((E)-4-methoxy-4-oxobut-2-enamido)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido) phenyl)pentanoic acid (**319**).



319

To a solution of compound **318** (150 mg, 0.129 mmol) and **Tub-1** (89 mg, 0.129 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (0.021 mL, 0.129 mmol). The reaction was stirred at r.t. for 1.5 hours, and directly purified by preparative HPLC to give compound **319** (17 mg, 8% yield). MS-ESI (m/z): calcd. for $C_{78}H_{129}N_{11}O_{26}S$ $[M+H]^+$ 1668.88; found, 1669.34.

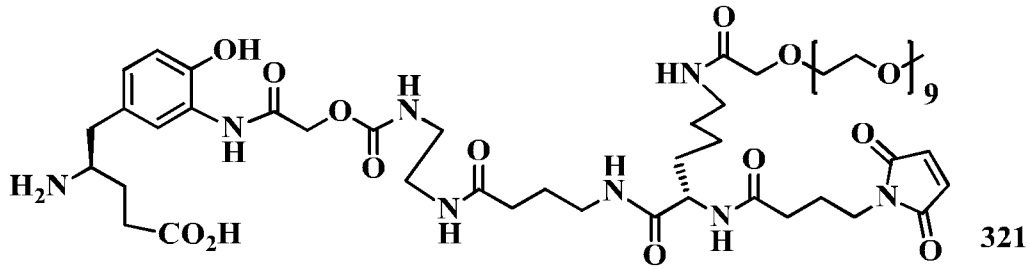
Example 284. Synthesis of tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4-hydroxyphenyl) pentanoate (**320**).



320

Compound **245** (327 mg, 0.324 mmol) and compound **69** (148 mg, 0.389 mmol) were dissolved in DCM (10 mL), EDC·HCl (75 mg, 0.389 mmol) was added, and the reaction was stirred for 1 h, and then directly taken to the next step. MS-ESI (m/z): calcd. for $C_{64}H_{106}N_8O_{24}$ $[M+H]^+$ 1371.73; found, 1372.41.

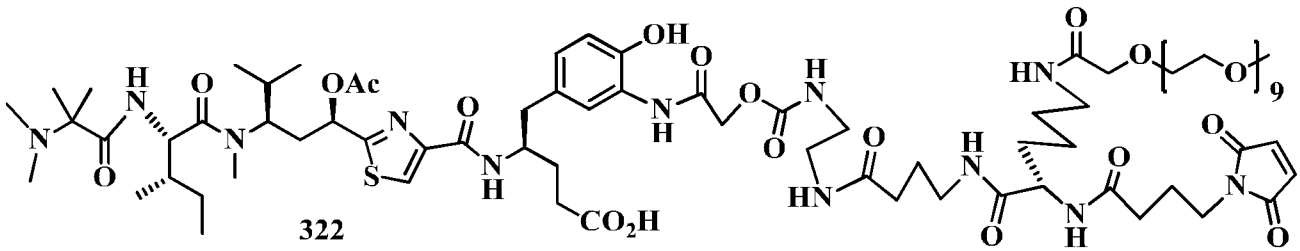
Example 285. Synthesis of (R)-4-amino-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H- pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4-hydroxyphenyl)pentanoic acid (**321**).



321

To the reaction mixture of previous step, 10 mL of TFA was added. The reaction was stirred for 1 h, concentrated and purified by preparative HPLC to give compound **321** (0.20 g, 51% yield). MS-ESI (m/z): calcd. for $C_{55}H_{90}N_8O_{22}$ $[M+H]^+$ 1215.62; found, 1216.25.

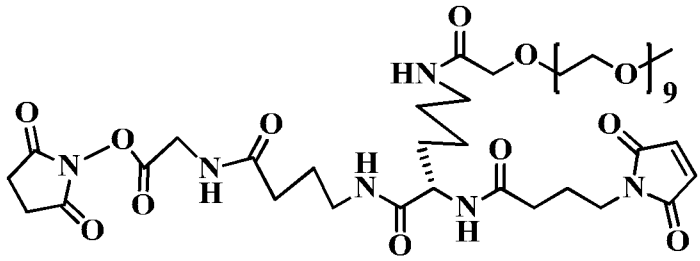
Example 286. Synthesis of (R)-4-(2-((6S, 9R, 11R)-6-((S)-sec-butyl)-9-isopropyl-2, 3, 3, 8-tetramethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4- carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43, 48-tetraoxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 49-undecaoxa-32, 39, 44, 47-tetraazahenpentacontan-51-amido)-4-hydroxyphenyl)pentanoic acid (**322**).



322

Compound **321** (200 mg, 0.165 mmol) and Tub-1 (114 mg, 0.165 mmol) were dissolved in DMF (2 mL), N,N-diisopropylethylamine (0.027 mL, 0.165 mmol) was added and stirred for 4 hours. The reaction was directly purified by preparative HPLC to give compound **322** (94 mg, 33% yield). MS-ESI (m/z): calcd. for $C_{80}H_{130}N_{12}O_{27}S$ $[M+H]^+$ 1723.89; found, 1724.81.

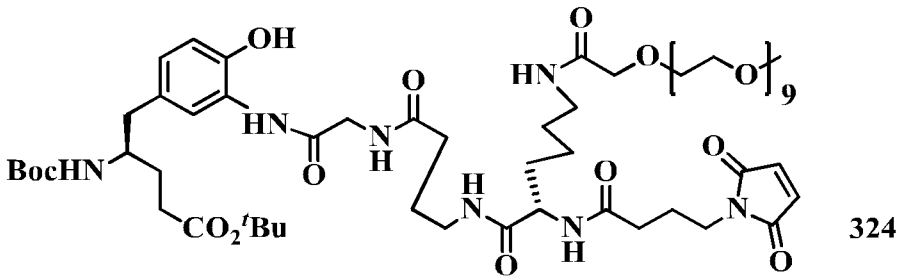
Example 287. Synthesis of 2, 5-dioxopyrrolidin-1-yl (S)-(37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-oyl)glycinate (**323**).



323

To a solution of compound **234** (1.0 g, 1.08 mmol) in DCM (20 mL) were added NHS (0.15 g, 1.30 mmol) and EDC·HCl (0.43 g, 2.17 mmol). The reaction mixture was stirred at r.t. for 2 hours and quenched with water. The layers were separated and the organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **323** (1.1 g, 99% yield). MS-ESI (m/z): calcd. for $C_{45}H_{75}N_6O_{20}$ $[M+H]^+$ 1019.50; found, 1019.50.

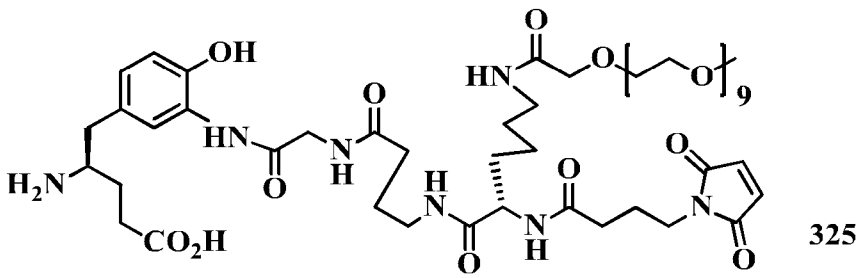
Example 288. Synthesis of tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)-4-hydroxyphenyl)pentanoate (**324**).



324

Compound **323** from previous step (1.1 g, 1.08 mmol) was dissoved in THF (10 mL). Compound 69 (215.0mg, 0.5mmol) was added to the solution and stirred at r.t. overnight. The reaction mixture was concentrated and purified by a silica gel column (6% MeOH/DCM) to give compound **324** (200 mg, 15% yield). MS-ESI (m/z): calcd. for $C_{61}H_{102}N_7O_{22}$ $[M+H]^+$ 1284.70; found, 1284.70.

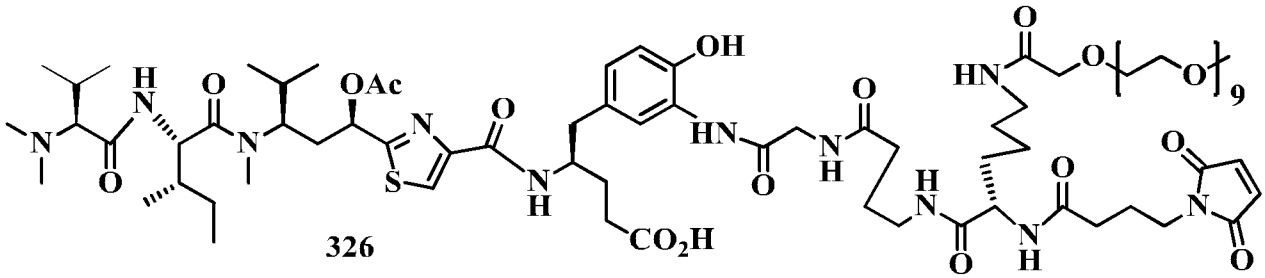
Example 289. Synthesis of (R)-4-amino-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H- pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)-4-hydroxyphenyl)pentanoic acid (**325**).



325

A solution of compound **324** (180 mg, 0.15 mmol) in DCM (4 mL) was treated with TFA (2 mL) at r.t. for 2 hours, concentrated, triturated with MTBE (10 mL) to give compound **325** (174 mg, > 100% yield). MS-ESI (m/z): calcd. for $C_{52}H_{86}N_7O_{20}$ $[M+H]^+$ 1128.58; found, 1128.58.

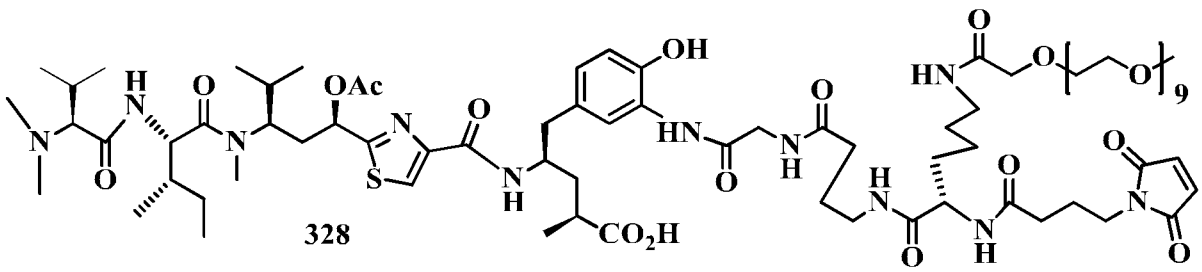
Example 290. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole-4-carboxamido) -5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)-4- hydroxyphenyl)pentanoic acid (**326**).



326

To a solution of compound **325** (174 mg, 0.15 mmol) in DMF (0.5 mL), **Tub-4** (98 mg, 0.13 mmol) in DMF (0.5 mL) was added, followed by N,N-diisopropylethylamine (40 mg, 0.29 mmol). The solution was stirred at r.t. for 30 min and then concentrated. The residue was purified by preparative HPLC to give compound **326** (34 mg, 14% yield). MS-ESI (m/z): calcd. for $C_{78}H_{128}N_{11}O_{25}S$ $[M+H]^+$ 1650.87; found, 1650.87.

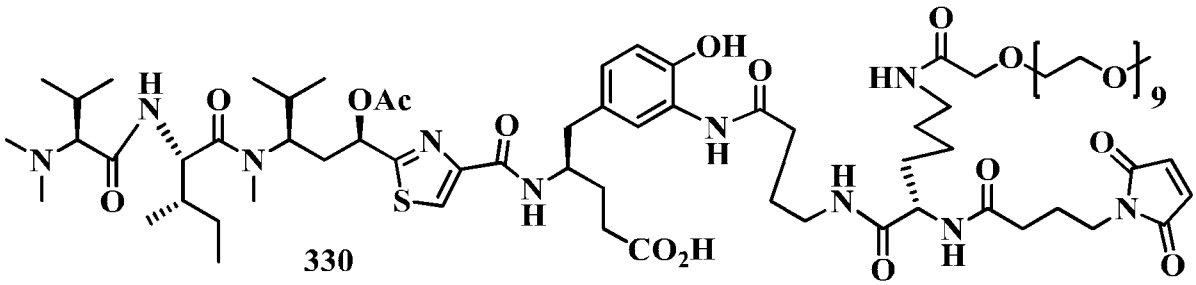
Example 291. Synthesis of (2S, 4R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl) thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 43-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 44-triazahexatetracontan-46-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**328**).



328

To a solution of **Tub-4** (95 mg, 0.13 mmol) and compound **327** (150 mg, 0.13 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (34 mg, 0.36 mmol) at 0 °C. The reaction was warmed to r.t. and stirred for 2 hours, concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **328** (50 mg, 23% yield). MS-ESI (m/z): calcd. for $C_{79}H_{130}N_{11}O_{25}S$ $[M+H]^+$ 1666.01; found, 1666.01.

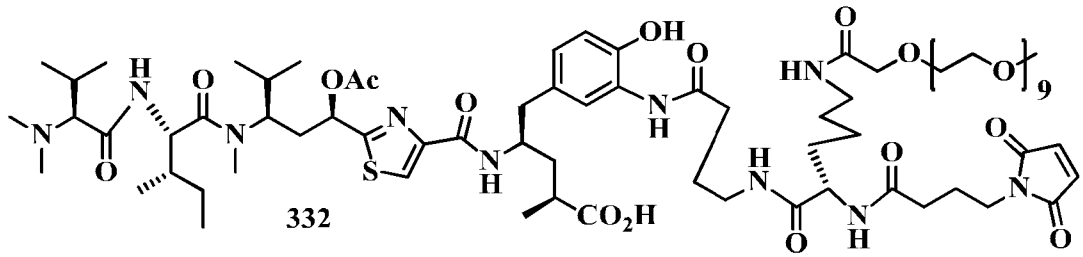
Example 292. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl)thiazole -4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-amido)-4-hydroxyphenyl)pentanoic acid (**330**).



330

To a solution of **Tub-4** (49 mg, 0.070 mmol) and compound **329** (74 mg, 0.070 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (0.036 mL, 0.28 mmol) at r.t. The reaction was stirred for 3 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **330** (51 mg, 46% yield). MS-ESI (m/z): calcd. for $C_{76}H_{124}N_{10}O_{24}S$ $[M+H]^+$ 1593.85; found, 1594.62.

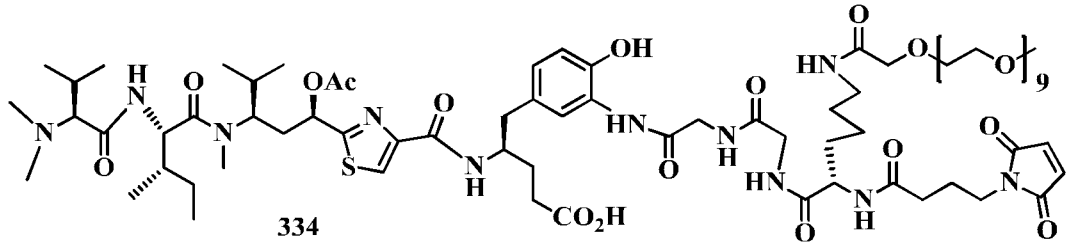
Example 293. Synthesis of (2S, 4R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl) thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38-dioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39-diazatritetracontan-43-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**332**).



332

To a solution of **Tub-4** (100 mg, 0.14 mmol) and compound **331** (146 mg, 0.14 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (0.073 mL, 0.42 mmol) at r.t. The reaction was stirred for 3.5 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **332** (82 mg, 37% yield). MS-ESI (m/z): calcd. for $C_{75}H_{122}N_{10}O_{23}S$ $[M+H]^+$ 1563.84; found, 1564.46.

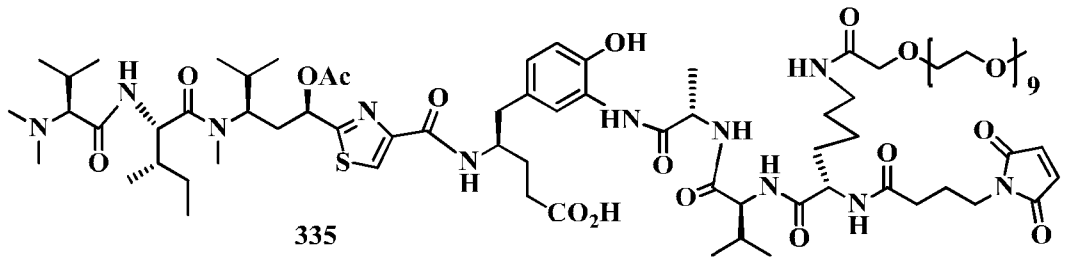
Example 294. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl) thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**334**).



334

To a solution of **Tub-4** (117 mg, 0.17 mmol) and compound **333** (180 mg, 0.17 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (45 mg, 0.33 mmol) at r.t. The reaction was stirred for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **334** (73 mg, 27% yield). MS-ESI (m/z): calcd. for $C_{76}H_{124}N_{11}O_{25}S$ $[M+H]^+$ 1622.84; found, 1622.84.

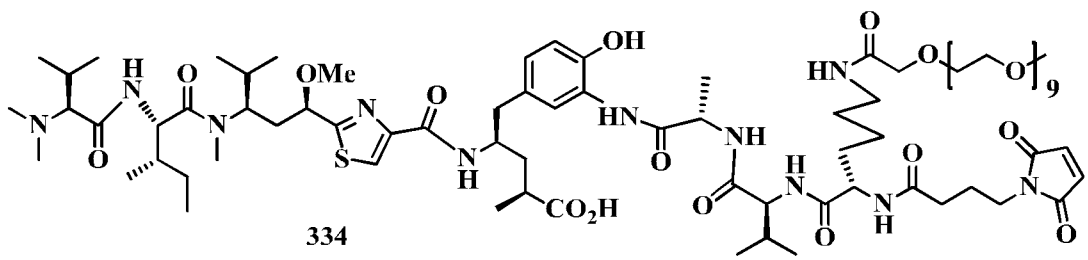
Example 295. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl) thiazole-4- carboxamido)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)pentanoic acid (**335**).



335

To a solution of **Tub-4** (185 mg, 0.256 mmol) and compound **316** (300 mg, 0.256 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (66 mg, 0.512 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **335** (120 mg, 29% yield). MS-ESI (m/z): calcd. for $C_{80}H_{131}N_{11}O_{25}S$ $[M+H]^+$ 1678.90; found, 1679.30.

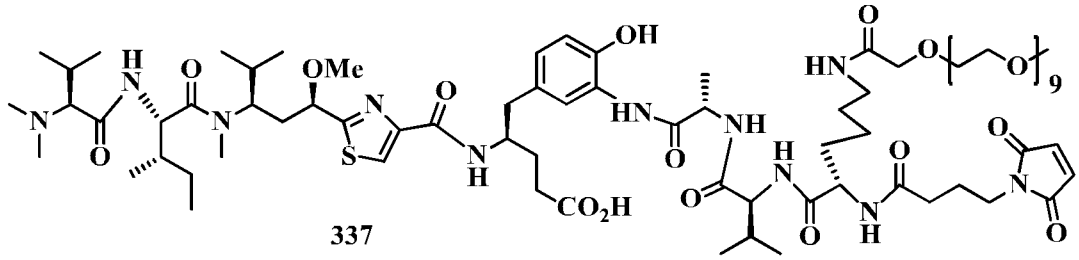
Example 306. Synthesis of (2S, 4R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl -2, 8-dimethyl-4, 7, 13-trioxo-12-oxa-2, 5, 8-triazatetradecan-11-yl) thiazole-4-carboxamido) -5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl) butanamido)-40- isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido)-4-hydroxyphenyl)-2-methylpentanoic acid (**336**).



334

To a solution of **Tub-2** (180 mg, 0.256 mmol) and compound **257** (300 mg, 0.256 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (66 mg, 0.512 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **336** (90 mg, 20% yield). MS-ESI (m/z): calcd. for $C_{80}H_{133}N_{11}O_{24}S$ [M+H$^+$] 1664.92; found, 1665.50.

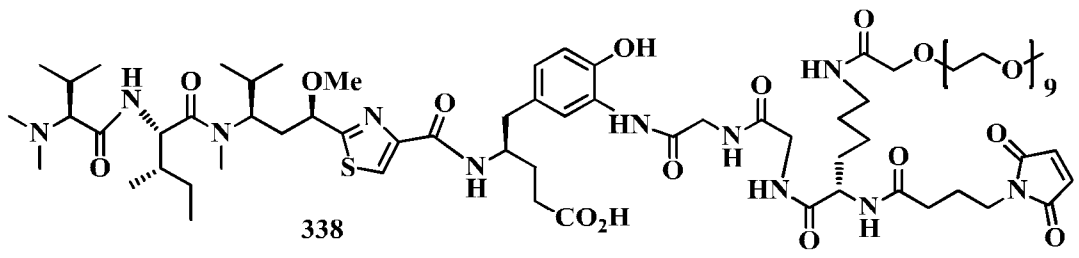
Example 397. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7-dioxo-12-oxa-2, 5, 8-triazatridecan-11-yl)thiazole-4-carboxamido)-5-(3-((37S, 40S, 43S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan -44-amido)-4-hydroxyphenyl)pentanoic acid (**337**).



337

To a solution of **Tub-2** (58.1 mg, 0.085 mmol) and compound **316** (128.0 mg, 0.094 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (20 mg, 0.17 mmol) at 0 °C. The reaction was warmed to r.t. and stirred for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **337** (26 mg, 19% yield). MS-ESI (m/z): calcd. for $C_{79}H_{132}N_{11}O_{24}S$ [M+H]$^+$ 1650.91; found, 1650.91.

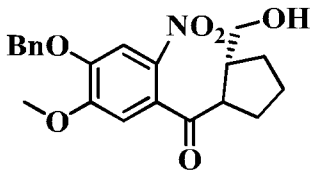
Example 398. Synthesis of (R)-4-(2-((3S, 6S, 9R, 11R)-6-((S)-sec-butyl)-3, 9-diisopropyl-2, 8-dimethyl-4, 7-dioxo-12-oxa-2, 5, 8-triazatridecan-11-yl)thiazole-4- carboxamido)-5-(3-((S)-37-(4-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)butanamido)-31, 38, 41-trioxo-2, 5, 8, 11, 14, 17, 20, 23, 26, 29-decaoxa-32, 39, 42-triazatetratetracontan-44-amido) -4-hydroxyphenyl)pentanoic acid (**338**).



338

To a solution of **Tub-2** (37.0 mg, 0.055 mmol) and compound **333** (60.0 mg, 0.055 mmol) in DMF (2 mL) was added N,N-diisopropylethylamine (0.009 mL, 0.055 mmol) at 0 °C. The reaction was warmed to r.t. and stirred for 2.5 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **338** (52 mg, 60% yield). MS-ESI (m/z): calcd. for $C_{75}H_{123}N_{11}O_{24}S$ [M+H]$^+$ 594.83; found, 1596.26.

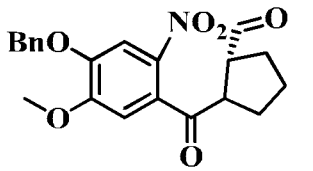
Example 399. Synthesis of (4-(benzyloxy)-5-methoxy-2-nitrophenyl) ((2R)-2-(hydroxymethyl)cyclopentyl)methanone (**342**).



342

To a solution of compound **340** (30.3 g, 0.1 mol), compound **341** (10.1 g, 0.1 mol) in DMF (500 mL) were added triethylamine (20.1 g, 0.2 mol) and HATU (49.4 g, 0.13mol) at r.t. The reaction was stirred at r.t. for 2 hours, diluted with DCM (2 L), washed twice with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product, which is purified by a silica gel column (EA/PE = 30%-100%) to give the title compound (36.8 g, 95% yield).

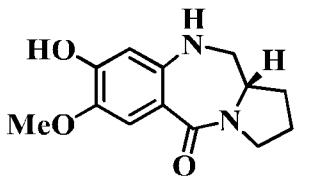
Example 400. Synthesis of (1R)-2-(4-(benzyloxy)-5-methoxy-2-nitrobenzoyl) cyclopentane-1 -carbaldehyde (**343**).



343

Under N_2, a solution of oxalyl chloride (4.34 g, 34.19 mmol) in DCM (150 mL) was cooled over dry ice/acetone batch, to which a solution of DMSO (5.57 g, 71.23 mmol) in 30 mL of DCM was added dropwise, and the reaction was kept below -65°C for 20 min. after the addition. A solution of compound **342** (11.01 g, 28.49 mmol) in 100 mL of DCM was added dropwise, and the reaction was kept below -65°C and stirred for 20 minutes. A solution of TEA (14.42 g, 142.47 mmol) in 60 mL of DCM was added dropwise at last. After the addition was completed, the dry ice/acetone bath was removed, and the reaction was gradually warmed to r.t. and stirred for 1 hour, then washed with 0.2 N HCl, brine, and dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatography purification (EA/PE = 20%-100%) gave the title compound (9.1 g, 83% yield).

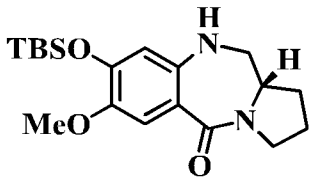
Example 401. Synthesis of (S)-8-hydroxy-7-methoxy-1,2,3,10,11,11a-hexahydro-5H-benzo[e] pyrrolo[1,2-a][1,4]diazepin-5-one (**344**).



344

Compound **343** (9.1 g, 23.67 mmol) in methanol (400 mL), palladium/carbon (2.0 g, 10 wt%) were charged into a hydrogenation reaction flask, and the reaction was stirred under hydrogen at r.t. overnight, filtered and concentrated to give the title compound (5.8 g, 98% yield).

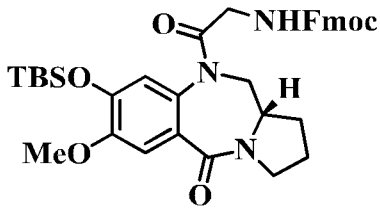
Example 402. Synthesis of (S)-8-((tert-butyldimethylsilyl)oxy)-7-methoxy-1,2,3,10,11,11a -hexahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (**345**).



345

To a solution of compound **344** (4.3 g, 17.32 mmol) in DCM (150 mL) were added imidazole (2.4 g, 34.64 mmol) and TBSCl (3.1 g, 20.78 mmol). After the addition, the mixture was reacted at r.t. for 2 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 20%-100%) to give the title compound (3.7 g, 59% yield).

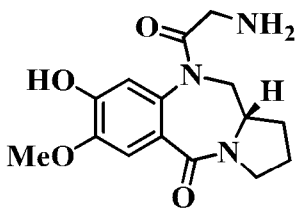
Example 403. Synthesis of (9H-fluoren-9-yl)methyl (S)-(2-(8-((tert-butyldimethylsilyl)oxy) -7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-2-oxoethyl)carbamate (**347**).



347

A solution of compound **345** (1.09 g, 3.00 mmol) and pyridine (0.36 g, 4.50 mmol) in DCM (50 mL) was cooled over ice/water. Compound **346** (1.14 g, 3.60 mmol) was added and stirred for 1 hour. The reaction solution was washed with 0.3 N HCl solution, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 20%-100%) to give the title compound (1.35 g, 70% yield).

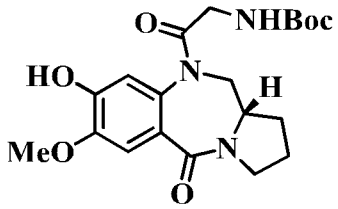
Example 404. Synthesis of (S)-10-glycyl-8-hydroxy-7-methoxy -1,2,3,10,11,11a-hexahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (**348**).



348

Compound **347** (1.01 g, 1.57 mmol) was dissolved in DCM (20 mL), to which pyrrolidine (1 mL) was added at r.t. After the addition, the reaction was stirred at r.t. for 3 hours and concentrated, purified by column chromatography (MeOH/DCM= 0%-30%) to give the title compound (0.41 g, 85% yield).

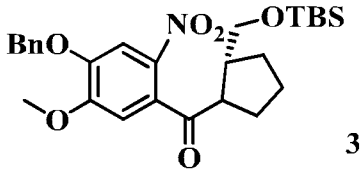
Example 405. Synthesis of tert-butyl (S)-(2-(8-hydroxy-7-methoxy-5-oxo- 2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-2-oxoethyl)carbamate (**349**).



349

Compound **348** (0.41 g, 1.34 mmol) was dissolved in DCM (20 mL) and di-t-butyl dicarbonate (0.35 g, 1.61 mmol) was added at room temperature. After the addition, the reaction was stirred at r.t. for 2 hours and concentrated, purified by column chromatography (EA/PE= 20%-100%) to give the title compound (0.20 g, 37% yield).

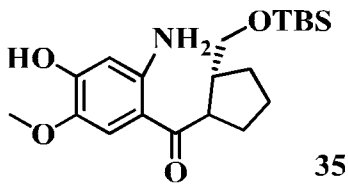
Example 406. Synthesis of (4-(benzyloxy)-5-methoxy-2-nitrophenyl)((2R)-2- (((tert-butyldimethylsilyl)oxy)methyl)cyclopentyl)methanone (**350**).



350

To a solution of compound **342** (38.0 g, 0.10 mol) in DCM (1000 mL) imidazole (27.2 g, 0.40 mol) and TBSCl (30.1 g, 0.20 mmol) were added at r.t. under stirring. After the addition, the reaction was stirred at r.t. for 2 hours, and then washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 10%-50%) to give the title compound (45.0 g, 90% yield).

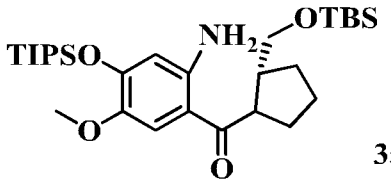
Example 407. Synthesis of (2-amino-4-hydroxy-5-methoxyphenyl) ((2R)-2-(((tert-butyldimethylsilyl)oxy)methyl)cyclopentyl)methanone (**351**).



351

Compound **350** (45 g, 90 mmol), methanol (800 mL), palladium/carbon (8.0 g, 10 wt%) were charged into a hydrogenation reaction flask. The flask was evacuated and back-filled with hydrogen for three times and stirred at r.t. overnight. Filtration and concentration under oil pump gave the title compound (34 g, 100% yield).

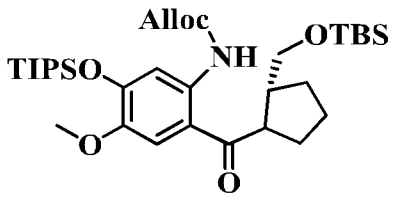
Example 408. Synthesis of (2-amino-5-methoxy-4-((triisopropylsilyl)oxy)phenyl) ((2R)-2-(((tert-butyldimethylsilyl)oxy)methyl)cyclopentyl)methanone (**352**).



352

A mixture of compound **351** (19.0 g, 0.05 mol), imidazole (6.8 g, 0.1 mol) and TIPSCl (14.4 g, 0.075 mmol) in ethyl acetate (30 mL) was heated to reflux and stirred for 1 hour. After cooling, DCM was added and the mixture was washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 10%-50%) to give the title compound (22 g, 82% yield).

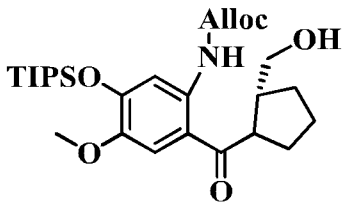
Example 409. Synthesis of allyl (2-((2R)-2-(((tert-butyldimethylsilyl)oxy)methyl) cyclopentane-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (**353**).



353

To a solution of compound **352** (92.7 g, 0.173 mol) in DCM (500 mL) was added pyridine (31.3 g, 0.397 mol) at r.t. under stirring. The mixture was cooled over dry ice/acetone bath, and allyl chloroformate (24.97 g, 0.207 mol) was added dropwise at about -65 °C. After the addition, dry ice/acetone bath was removed, and the reaction was naturally warmed to room temperature and stirred for 3 hours. The reaction solution was washed with 0.3 N HCl solution, brine, dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product (104 g, 99% yield).

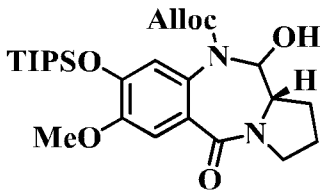
Example 410. Synthesis of allyl (2-((2R)-2-(hydroxymethyl)cyclopentane -1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (**354**).



354

A solution of compound **353** (104 g, 0.173 mol) in acetic acid (600 mL), methanol (85 mL), THF (85 mL) and water (170 mL) was stirred at room temperature for 8 hours, and then diluted with ethyl acetate and washed with water for 3 times, brine once, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 30%-70%) to give the title compound (59 g, 69% yield over 2 steps).

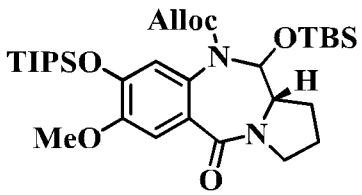
Example 411. Synthesis of allyl (11aS)-11-hydroxy-7-methoxy-5-oxo-8 -((triisopropylsilyl)oxy)-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**355**).



355

Under N_2, a solution of oxalyl chloride (19.9 g, 155 mmol) in DCM (400 mL) was cooled over dry ice/acetone batch, to which a solution of DMSO (23.4 g, 299 mmol) in 50 mL of DCM was added dropwise, and the reaction was kept below -65°C for 20 min. after the addition. A solution of compound **354** (59.0 g, 119 mmol) in 200 mL of DCM was added dropwise, and the reaction was kept below -65°C and stirred for 20 minutes. A solution of TEA (60.6 g, 598 mmol) in 100 mL of DCM was added dropwise at last. After the addition was completed, the dry ice/acetone bath was removed, and the reaction was gradually warmed to r.t. and stirred for 1 hour, then washed with 0.2 N HCl, brine, and dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatography purification (EA/PE = 10%-50%) gave the title compound (49.7 g, 84% yield).

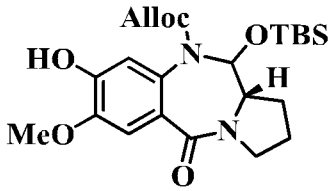
Example 412. Synthesis of allyl (11aS)-11-((tert-butyldimethylsilyl)oxy) -7-methoxy-5-oxo-8-((triisopropylsilyl)oxy)-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**356**).



356

To a solution of compound **355** (5.8 g, 11.82 mmol) in DCM (100 mL) were added 2,6-lutidine (5.07 g, 47.28 mmol) and TBSOTf (9.37 g, 35.46 mmol). After the addition, the reaction was stirred at r.t. for 2 hours, washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 10%-50%) to give the title compound (6.3 g, 88% yield).

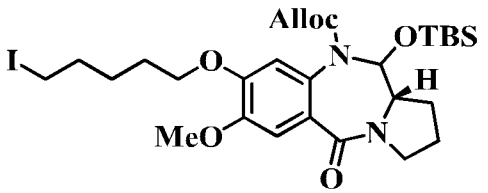
Example 413. Synthesis of allyl (11aS)-11-((tert-butyldimethylsilyl)oxy) -8-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**357**).



357

A mixture of compound **356** (6.3 g, 10.41 mmol) and lithium acetate (0.69 g, 10.41 mmol) in DMF (78 mL) and water (1.5 mL) was stirred at r.t. for 4 hours. The reaction solution was diluted with ethyl acetate, washed with water for 3 times, brine once, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 30%-100%) to give the title compound (3.3 g, 70% yield).

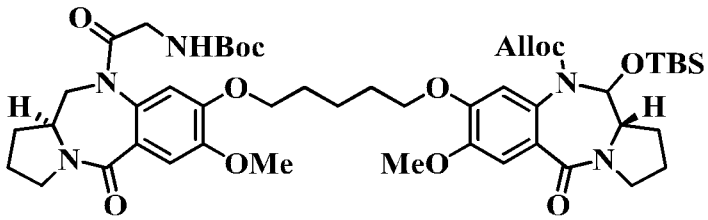
Example 414. Synthesis of allyl (11aS)-11-((tert-butyldimethylsilyl)oxy) -8-((5-iodopentyl)oxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**358**).



358

To a solution of compound **357** (1.39 g, 3 mmol) in acetone (100 mL) were added diiodopentane (4.86 g, 15 mmol) and potassium carbonate (0.62 g, 4.5 mmol) with stirring. After the addition, the reaction was heated under reflux for 8 hours, and after cooling, it was directly purified by column chromatography (EA/PE= 20%-80%) to give the title compound (1.85 g, 93% yield).

Example 415. Synthesis of allyl (11aS)-8-((5-(((S)-10-((tert-butoxycarbonyl) glycyl)-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**359**).

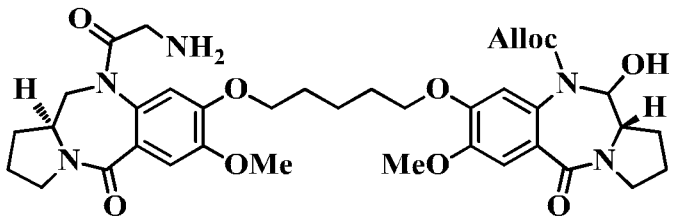


359

Compound **349** (121 mg, 0.3 mmol) was dissolved in acetone (20 mL), to which compound **359** (197 mg, 0.3 mmol) and potassium carbonate (83 mg, 0.6 mmol) were added with stirring. The reaction was heated under reflux for 8 hours, and after cooling, it was directly purified by column chromatography (MeOH/DCM= 0%-10%) to give the title compound (240 mg, 85% yield).

Example 416. Synthesis of allyl (11aS)-8-((5-(((S)-10-glycyl-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-

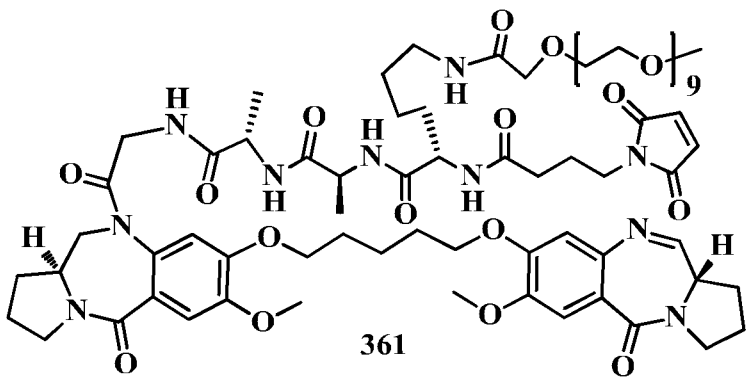
hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**360**).



360

Compound **359** (199.9 mg, 0.21 mmol) was dissolved in 2 mL of TFA and 6 mL of DCM, stirred at r.t. for ~3 h, diluted with 10 mL of DCM, washed with 5 mL of brine and 5 mL of saturated sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and concentrated to give a pale yellow foamy solid (177.5 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{37}H_{48}N_5O_{10}$ $[M+H]^+$ 721.33; found 721.33.

Example 417. Synthesis of N-((S)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-6-(((S)-1-(((S)-1-((2-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**361**).

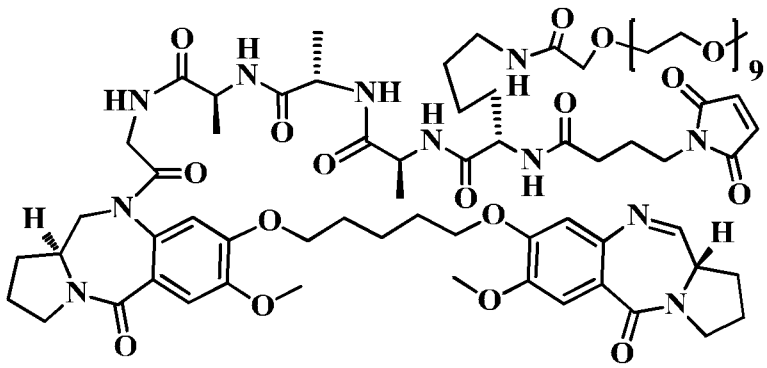


361

Compound **360** (77.0 mg, 0.11 mmol) was dissolved in 2 mL of DCM, to which pyrrolidine (7.6 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 20 min. The reaction was cooled to 0-5 °C, compound **291** (147.6 mg, 0.16 mmol) in 2 mL of DCM and EDC·HCl (40.9 mg, 0.21 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a pale yellow solid (65 mg, 41% yield). MS-ESI (m/z): calcd. for $C_{74}H_{111}N_{10}O_{24}$ $[M+H]^+$ 1523.77; found 1523.77.

Example 418. Synthesis of N-((5S,8S,11S,14S)-14-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-1-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-

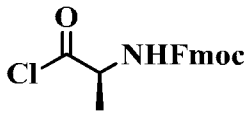
benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-5,8,11-trimethyl-1,4,7,10,13-pentaoxo-3,6,9,12-tetraazaoctadecan-18-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**362**).



362

Compound **360** (77.0 mg, 0.11 mmol) was dissolved in 4 mL of DCM, to which pyrrolidine (7.6 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 20 min. The reaction was cooled to 0-5 °C, compound **217** (175.3 mg, 0.17 mmol) in 2 mL of DCM and EDC·HCl (41.0 mg, 0.21 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a pale yellow solid (18.6 mg, 11% yield). MS-ESI (m/z): calcd. for $C_{77}H_{116}N_{11}O_{25}$ $[M+H]^+$ 1594.81; found 1594.81.

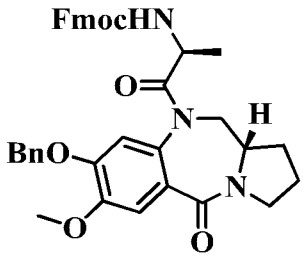
Example 419. Synthesis of (9H-fluoren-9-yl)methyl (S)-(1-chloro-1-oxopropan-2-yl)carbamate (**364**).



364

Fmoc-Ala-OH (10.4 g, 33.40 mmol) was dissolved in 100 mL of DCM, to which 6 drops of DMF, 12 mL of $SOCl_2$ were added. The reaction mixture was heated to 40-50 °C, refluxed for 1 h, cooled to r.t. and concentrated. The residue was triturated with 50 mL of n-hexane, filtered and dried to give a white solid (9.7 g, 88% yield). MS-ESI (m/z): calcd. for $C_{18}H_{18}NO_4$ $[M+H]^+$ 312.12; found 312.12.

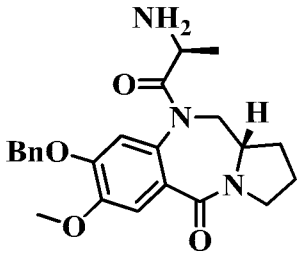
Example 420. Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-((S)-8-(benzyloxy)-7-methoxy -5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)carbamate (**366**).



366

Compound **364** (1.23 g, 3.03 mmol) and compound **365** (1.00 g, 3.03 mmol) were dissolved in 10 mL of DCM, and stirred at r.t. for ~5h. The reaction was diluted with 40 mL of DCM, washed with 40 mL of 0.3 N HCl, 50 mL of brine, dried over sodium sulfate, filtered, concentrated to dryness. The residue was purified by a silica gel column (ethyl acetate/petroleum ether) to give a pale yellow solid (1.4 g, 73% yield). MS-ESI (m/z): calcd. for $C_{38}H_{38}N_3O_6$ $[M+H]^+$ 632.27; found 632.27.

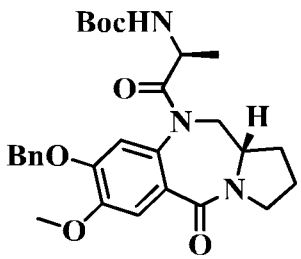
Example 421. Synthesis of (S)-10-(L-alanyl)-8-(benzyloxy)-7-methoxy-1,2,3,10,11,11a -hexahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (**367**).



367

A mixture of compound **366** (0.66 g, 1.04 mmol) in 10 mL of DCM, and 1 mL of pyrrolidine was stirred at r.t. for 1 h, and concentrated to give a pale yellow solid (1.06 g, 100% yield). MS-ESI (m/z): calcd. for $C_{23}H_{28}N_3O_4$ $[M+H]^+$ 410.20; found 410.20.

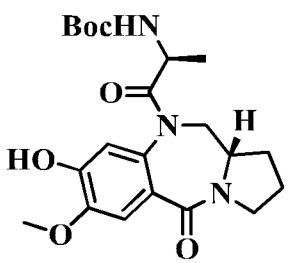
Example 422. Synthesis of tert-butyl ((S)-1-((S)-8-(benzyloxy)-7-methoxy-5-oxo- 2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)carbamate (**368**).



368

To a solution of compound **367** (430 mg, 1.05 mmol) in 10 mL of DCM, di-tert-butyl dicarbonate (901.1 mg, 4.13 mmol), DMAP (24 mg, 0.196 mmol) were added, and the mixture was stirred at r.t. for 3 hours and directly purified by a silica gel column (ethyl acetate/petroleum ether) to give a colorless oil (297.1 mg, 55% yield). MS-ESI (m/z): calcd. for $C_{28}H_{36}N_3O_6$ $[M+H]^+$ 510.25; found 510.25.

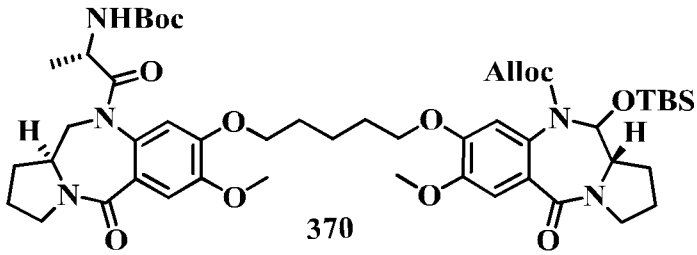
Example 423. Synthesis of tert-butyl ((S)-1-((S)-8-hydroxy-7-methoxy-5-oxo- 2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)carbamate (**369**).



369

Compound **368** (567 mg, 1.11 mmol), methanol (60 mL), palladium/carbon (126 mg, 10 wt%) were charged into a hydrogenation reaction flask. The flask was evacuated and back-filled with hydrogen for three times and stirred at r.t. overnight. Filtration and concentration under oil pump gave a colorless oil (469 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{21}H_{30}N_3O_6$ $[M+H]^+$ 420.21; found 420.21.

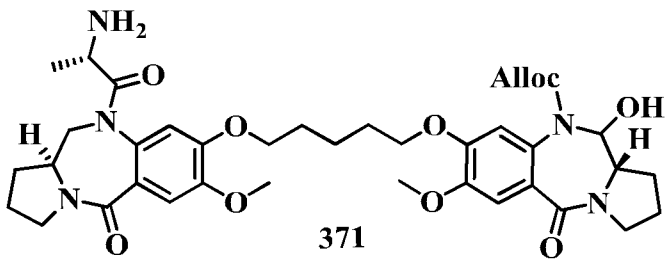
Example 424. Synthesis of allyl (11aS)-8-((5-(((S)-10-((tert-butoxycarbonyl)-L-alanyl) -7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**370**).



370

A mixture of compound **369** (254 mg, 0.61 mmol) and compound **358** (598 mg, 0.91 mmol) in 40 mL of acetone, and potassium carbonate (167.0 mg, 1.21 mmol) was heated to reflux and stirred for 7 hours. The reaction was concentrated and purified by a silica gel column (ethyl acetate/petroleum ether and then dichloromethane/methanol) to give a light yellow solid (413 mg, 72% yield). MS-ESI (m/z): calcd. for $C_{49}H_{72}IN_5O_{12}Si$ $[M+H]^+$ 950.49; found 950.49.

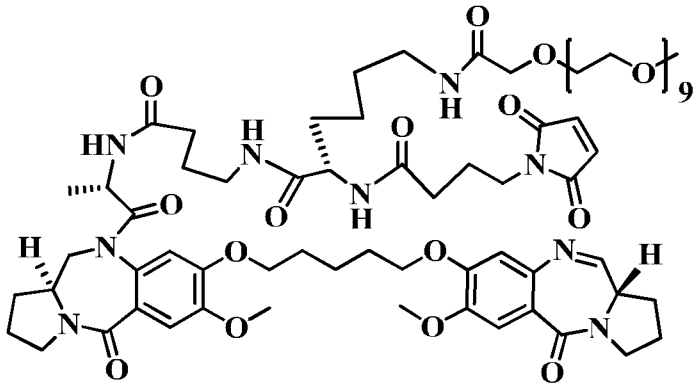
Example 425. Synthesis of allyl (11aS)-8-((5-(((S)-10-(L-alanyl)-7-methoxy-5 -oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**371**).



371

Compound **370** (413 mg, 0.43 mmol) was dissolved in 2 mL of TFA and 6 mL of DCM, stirred at r.t. for 2 h, and concentrated. The residue was diluted with 20 mL of DCM, washed with 6 mL of brine, 6 mL of saturated sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and concentrated to give a light yellow solid (334 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{38}H_{50}IN_5O_{10}$ $[M+H]^+$ 736.35; found 736.35.

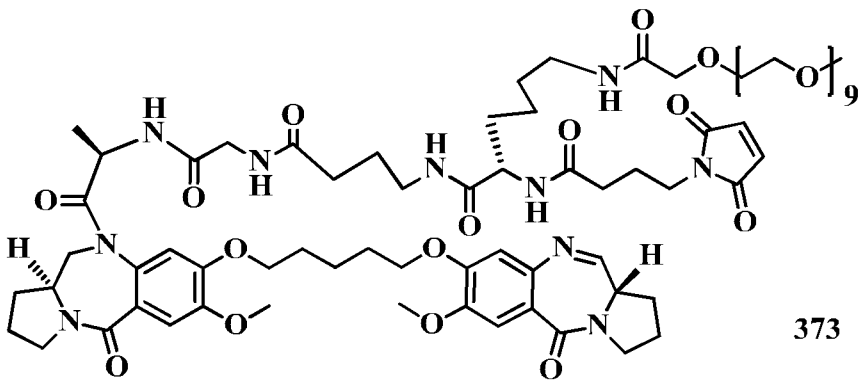
Example 426. Synthesis of N-((S)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-6-((4-(((S)-1-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)amino)-4-oxobutyl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**372**).



372

Compound **371** (79.9 mg, 0.11 mmol) was dissolved in 8 mL of DCM, to which pyrrolidine (7.7 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 30 min. The reaction was cooled to 0-5 °C, compound **242** (140 mg, 0.16 mmol) in 8 mL of DCM and EDC·HCl (42.0 mg, 0.22 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a brown oil (33 mg, 20% yield). MS-ESI (m/z): calcd. for $C_{73}H_{110}IN_9O_{23}$ $[M+H]^+$ 1480.76; found 1480.76.

Example 427. Synthesis of N-((S)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-6-((4-((2-(((R)-1-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-oxobutyl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**373**).

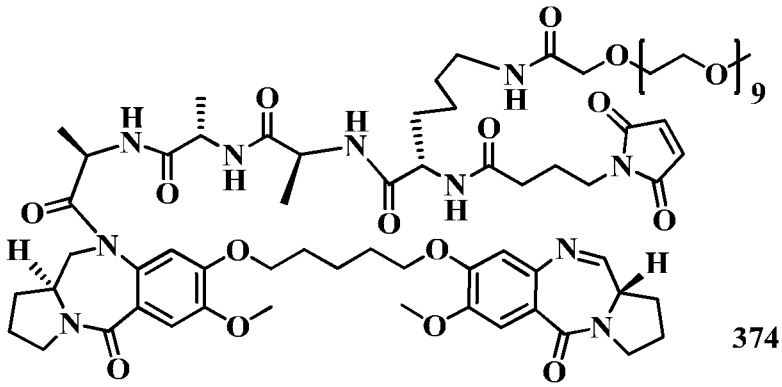


373

Compound **371** (79.9 mg, 0.11 mmol) was dissolved in 8 mL of DCM, to which pyrrolidine (7.7 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 30 min. The reaction was cooled to 0-5 °C, compound **234** (150 mg, 0.16 mmol) in 8 mL of DCM and

EDC·HCl (42.0 mg, 0.22 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a light yellow solid (32 mg, 19% yield). MS-ESI (m/z): calcd. for $C_{75}H_{113}IN_{10}O_{24}$ $[M+H]^+$ 1537.79; found 1537.79.

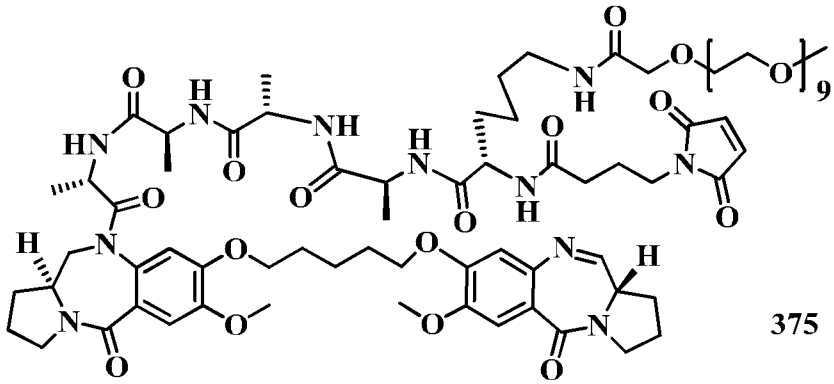
Example 428. Synthesis of N-((S)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-6-(((S)-1-(((S)-1-(((R)-1-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**374**).



374

Compound **371** (79.9 mg, 0.11 mmol) was dissolved in 8 mL of DCM, to which pyrrolidine (7.7 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 30 min. The reaction was cooled to 0-5 °C, compound **291** (164 mg, 0.17 mmol) in 10 mL of DCM and EDC·HCl (42.0 mg, 0.22 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a brown oil (56 mg, 32% yield). MS-ESI (m/z): calcd. for $C_{75}H_{113}IN_{10}O_{24}$ $[M+H]^+$ 1537.79; found 1537.79.

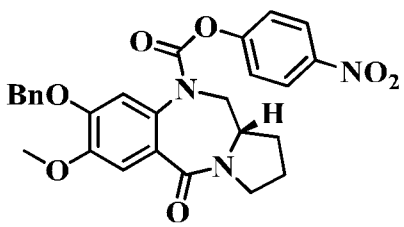
Example 429. Synthesis of N-((2S,5S,8S,11S,14S)-14-(4-(2,5-dioxo-2,5-dihydro-1H -pyrrol-1-yl)butanamido)-1-((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)-2,5,8,11-tetramethyl-1,4,7,10,13-pentaoxo-3,6,9,12-tetraazaoctadecan-18-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**375**).



375

Compound **371** (79.9 mg, 0.11 mmol) was dissolved in 10 mL of DCM, to which pyrrolidine (7.7 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 30 min. The reaction was cooled to 0-5 °C, compound **217** (162 mg, 0.16 mmol) in 10 mL of DCM and EDC·HCl (43.0 mg, 0.22 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a brown oil (45 mg, 25% yield). MS-ESI (m/z): calcd. for $C_{78}H_{118}IN_{11}O_{25}$ $[M+H]^+$ 1608.82; found 1608.82.

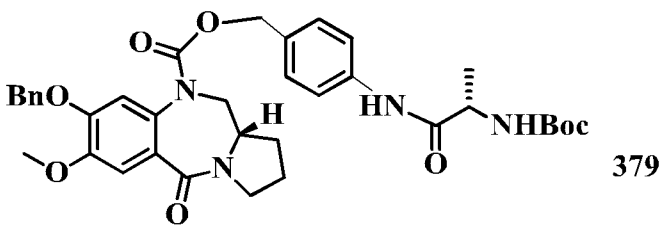
Example 430. Synthesis of 4-nitrophenyl (S)-8-(benzyloxy)-7-methoxy-5-oxo-2,3,11,11a -tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**377**).



377

To a solution of compound **365** (2.03 g, 6.0 mmol) in DCM (100 mL) were added DIPEA (0.93 g, 7.2 mmol) and 4-nitrophenyl chloroformate (1.33 g, 6.6 mmol) under stirring. After the addition, the mixture was stirred at r.t. overnight, washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 20%-100%) to give the title compound (2.6 g, 86% yield).

Example 431. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido) benzyl (S)-8-(benzyloxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**379**).

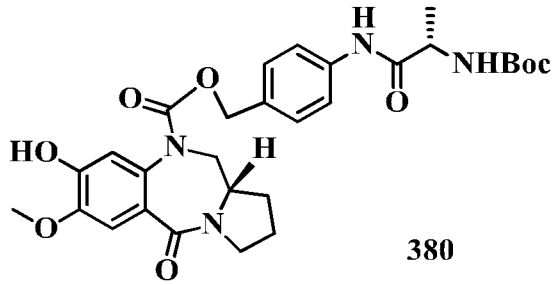


379

A solution of compound **378** (0.71 g, 2.4 mmol) in anhydrous THF (5 mL) and DMA (10 mL) was cooled to below 0°C in an ice-salt batch. LiHMDS (2.4 mL, 1 mol/L) was added dropwise under

N_2, and the reaction was kept below 0°C for 20 minutes. A solution of tert-butyl (S)-(1-((4-(hydroxymethyl)-phenyl)amino)-1-oxopropan-2-yl)carbamate (compound **377**) (1.01 g, 2 mmol) in 10 mL of THF was added dropwise, and the reaction was kept below 0°C for 20 minutes, and warmed to r.t. and stirred for 4 hours. The reaction solution was diluted with DCM, washed with ammonium chloride solution, brine, and dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/PE= 20%-100%) to give the title compound (0.84g, 63% yield).

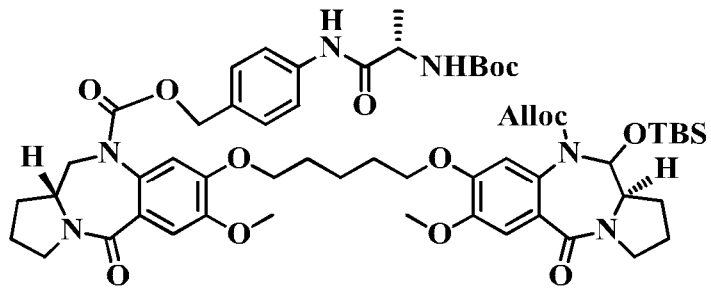
Example 432. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido) benzyl (S)-8-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**380**).



380

Compound **379** (1.17 g, 1.77 mmol), methanol (25 mL), palladium/carbon (0.20 g, 10 wt%) were charged into a hydrogenation reaction flask. The flask was evacuated and back-filled with hydrogen for three times and stirred at 0 °C for 1 h. Filtration and concentration under oil pump gave the title compound (0.91 g, 90% yield).

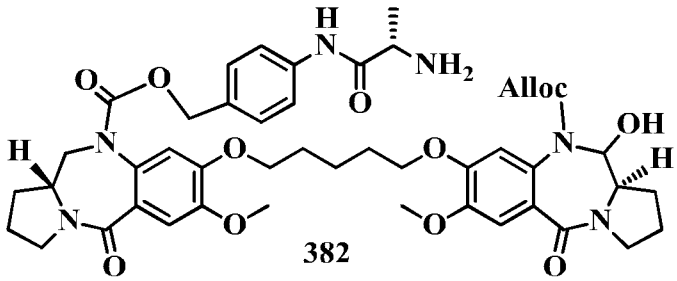
Example 433. Synthesis of allyl (11aR)-8-((5-(((R)-10-(((4-((S)-2-((tert-butoxycarbonyl) amino)propanamido)benzyl)oxy)carbonyl)-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**381**).



381

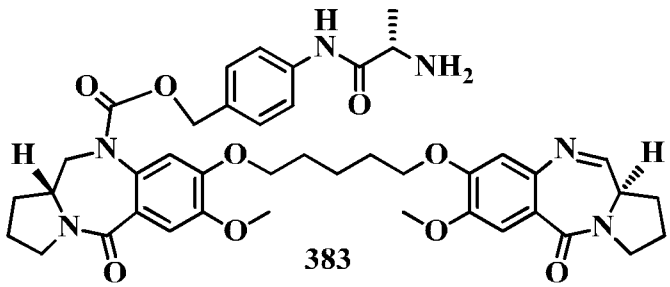
A mixture of compound **380** (0.91 g, 1.6 mmol) and compound **358** (1.37 g, 2.1 mmol) in 80 mL of acetone, and potassium carbonate (0.44 g, 3.2 mmol) was heated to reflux and stirred for 8 hours. The reaction was directly purified by a silica gel column (0-10% MeOH/DCM) to give the title compound (1.4 g, 79% yield).

Example 434. Synthesis of allyl (11aR)-8-((5-(((R)-10-(((4-((S)-2-aminopropanamido) benzyl)oxy)carbonyl)-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**382**).



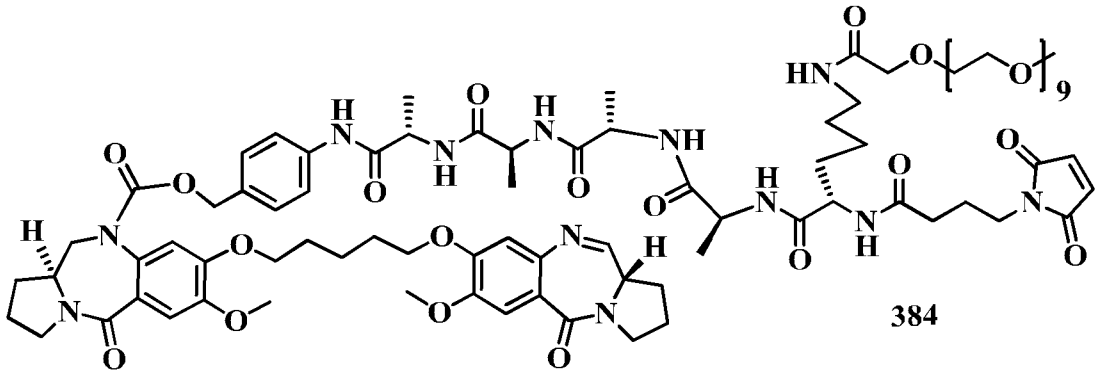
Compound **381** (0.70 g, 0.64 mmol) was dissolved in 15 mL of DCM and cooled to 5 °C, to which TFA (5 mL) was added and stirred at r.t. for 40 min. The reaction was diluted with DCM, washed with 10% saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by a silica gel column (0-15% MeOH/DCM) to give the title compound (0.38 g, 66% yield).

Example 435. Synthesis of 4-((S)-2-aminopropanamido)benzyl (R)-7-methoxy-8- ((5-(((R)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**383**).



Compound **382** (62 mg, 0.07 mmol) was dissolved in 2 mL of DCM, to which pyrrolidine (4.7 mg, 0.07 mmol) and catalytic amount of $Pd(PPh_3)_4$ (1 mg) were added, and then stirred at r.t. for 30 min. The reaction was diluted with DMF and evaporated to remove DCM. The resulting crude product in DMF was used directly in the next step. MS-ESI (m/z): calcd. for $C_{42}H_{50}N_6O_9[M+H]^+$:783.90; found 783.85.

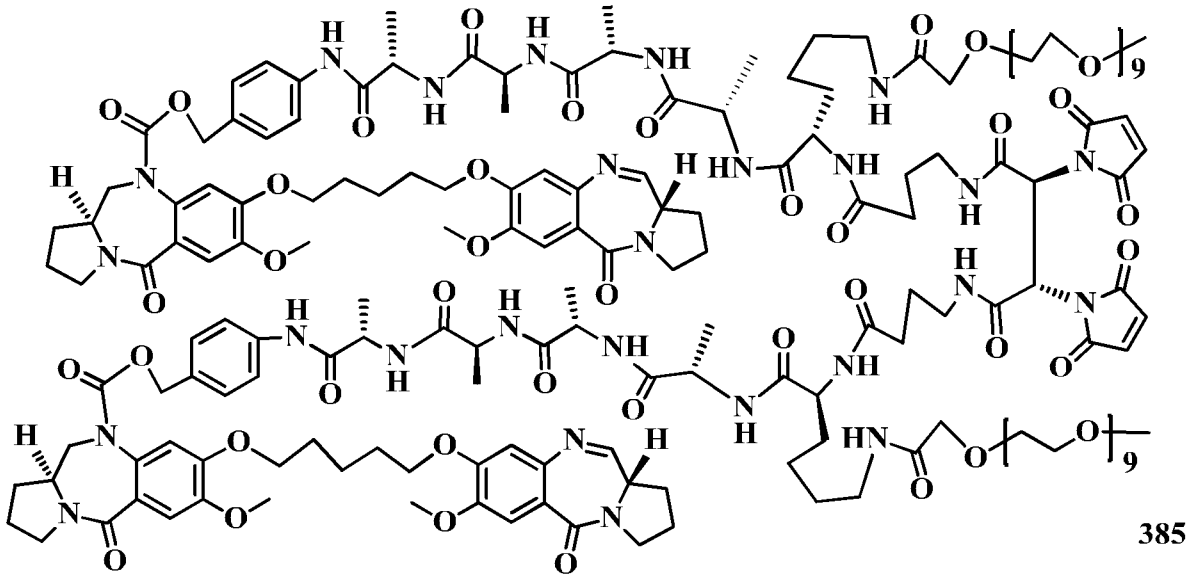
Example 436. Synthesis of 4-((37S,40S,43S,46S,49S)-37-(4-(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)butanamido)-40,43,46,49-tetramethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-amido)benzyl (S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**384**).



384

Compound **382** (100 mg, 0.11 mmol) was dissolved in 10 mL of DCM, to which pyrrolidine (8.0 mg, 0.11 mmol) and catalytic amount of $Pd(PPh_3)_4$ were added, and then stirred at r.t. for 30 min. The reaction was cooled to 0-5 °C, compound **217** (156 mg, 0.17 mmol) in 10 mL of DCM and EDC·HCl (86.6 mg, 0.45 mmol) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative HPLC to give a white solid (43 mg, 22% yield). MS-ESI (m/z): calcd. for $C_{86}H_{125}IN_{12}O_{27}$ $[M+H]^+$ 1756.87; found 1756.87.

Example 437. Synthesis of ((((2S,5S,8S,11S,14S,22S,23S,31S,34S,37S,40S,43S) -22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,11,34,37,40,43-octamethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4,1-phenylene))bis(methylene) (11aS,11a'S)-bis(7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a -tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate) (**385**).

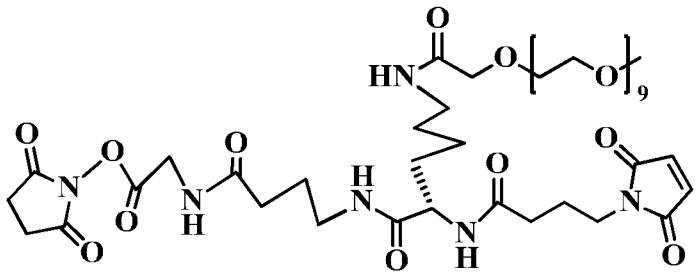


385

A solution of compound **136** (73 mg, 0.035 mmol), HATU (40 mg, 0.105 mmol) in DMF (1 mL) was stirred at r.t. for 15 min. and then the crude product of **383** (0.07 mmol) in DMF was added

to the reaction solution, followed by DIPEA (14 mg, 0.105 mmol). After stirring at r.t. for 30 min., the reaction mixture was directly purified by preparative HPLC to give a pale yellow solid (45 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{176}H_{252}N_{26}O_{56}[2M+H]^+$:1815.08; found 1815.11.

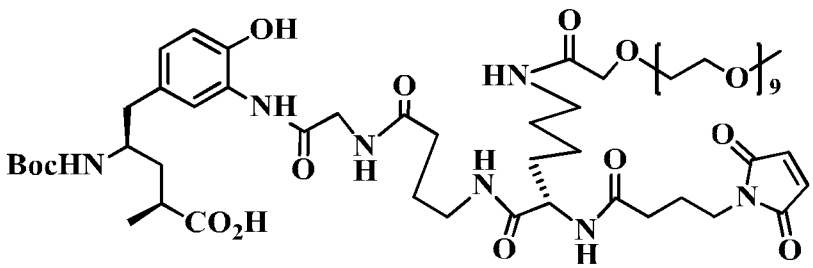
Example 438. Synthesis of (S)-2,5-dioxopyrrolidin-1-yl 37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-31,38,43-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,44-triazahexatetracontan-46-oate (**387**).



387

Compound **234** (12.6 g, 13.7 mmol) was dissolved in DCM (150 mL), N-hydroxysuccinimide (3.2 g, 27.8 mmol) and EDC·HCl (7.9 g, 41.2 mmol) were added over ice water bath. The reaction was stirred at r.t. for 3.5 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **387** (14.0 g), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{45}H_{75}N_6O_{20}$ $[M+H]^+$ 1019.50; found, 1019.58.

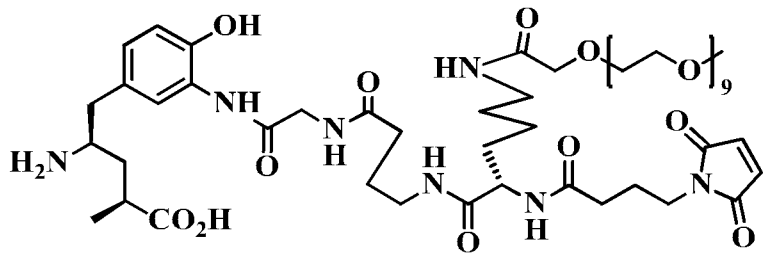
Example 439. Synthesis of (2S,4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37- (4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-31,38,43-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,44-triazahexatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**388**).



388

Compound **387** (14.0 g, 13.7 mmol) and compound 77 (4.2 g, 12.3 mmol) were dissolved in THF (150 mL), stirred at 25 °C for 8 hours, concentrated and diluted with ethyl acetate, washed with water. The aqueous phase was saturated with solidum chloride, extracted with dichloromathane twice. The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column ($MeOH/CH_2Cl_2$) to give compound **388** (7.6 g, 50% yield over 2 steps). MS-ESI (m/z): calcd. for $C_{58}H_{96}N_7O_{22}$ $[M+H]^+$ 1242.65; found, 1242.65.

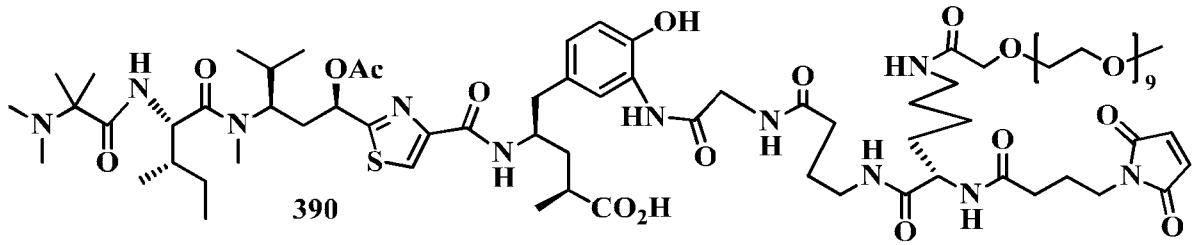
Example 440. Synthesis of (2S,4R)-4-amino-5-(3-((S)-37-(4-(2,5-dioxo-2,5-dihydro- 1H-pyrrol-1-yl)butanamido)-31,38,43-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,44-triazahexatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**389**).



389

Compound **388** (0.50 g, 0.40 mmol) was dissolved in DCM (10 mL) and TFA (5 mL). The reaction was stirred for 1 hour, and then concentrated to give compound **389** (0.46 g), which is used directly in the next step. MS-ESI (m/z): calcd. for $C_{53}H_{88}N_7O_{20}$ $[M+H]^+$ 1142.60; found, 1142.62.

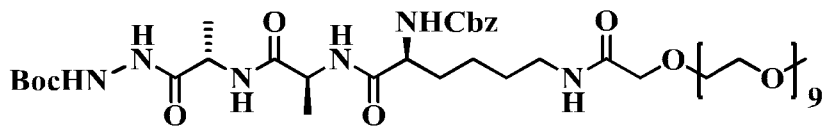
Example 441. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl -2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-31,38,43-trioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,44-triazahexatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**390**).



390

Compound **389** (0.46 g, 0.40 mmol) and **Tub-1** (0.30 g, 0.44 mol) were dissolved in DMF (5 mL) and N,N-diisopropylethylamine (0.52 g, 4.0 mol) was added over ice-salt bath. The reaction was stirred at r.t. for 2 hours and concentrated. The residue was dissolved in dichloromethane (10 mL) and formic acid (0.5 mL), concentrated again. The residue was purified by preparative HPLC to give compound **390** (0.26 g, 40% yield). MS-ESI (m/z): calcd. for $C_{78}H_{128}N_{11}O_{25}S$ $[M+H]^+$ 1650.87; found, 1650.87.

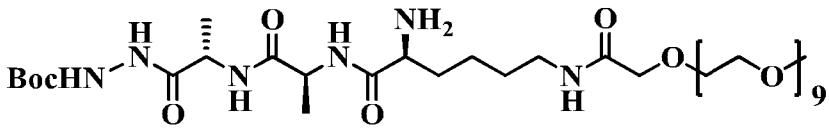
Example 442. Synthesis of (5S,8S,11S)-tert-butyl 11-(((benzyloxy)carbonyl)amino)-5,8 -dimethyl-4,7,10,17-tetraoxo-19,22,25,28,31,34,37,40,43,46-decaoxa-2,3,6,9,16-pentaazaheptatetracontan-1-oate (**392**).



392

To a solution of compound **391** (2.67 g, 3.00 mmol) and tert-butyl carbazate (0.48 g, 3.60 mmol) in DCM (20 mL), EDC·HCl (0.69 g, 3.60 mmol) was added, the reaction was stirred for 1 h, washed with brine, dried over anhydrous sodium sulfate, filtered, concentrate and purified by silica gel column ($MeOH/CH_2Cl_2$) to give the title compound (2.56 g, 85 % yield). MS-ESI (m/z): $[M+H]^+$ calcd. for $C_{46}H_{80}N_6O_{18}$, 1005.55; found, 1005.65.

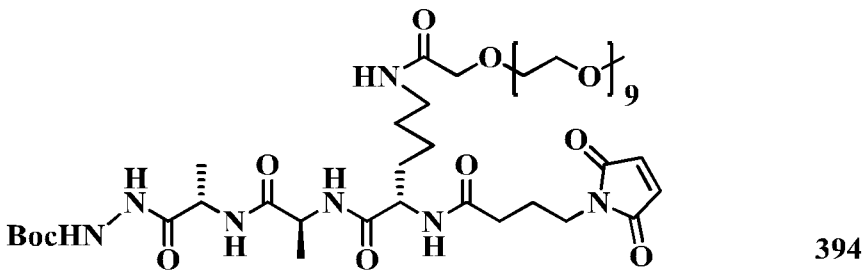
Example 443. Synthesis of (5S,8S,11S)-tert-butyl 11-amino-5,8-dimethyl-4,7,10,17-tetraoxo-19,22,25,28,31,34,37,40,43,46-decaoxa-2,3,6,9,16-pentaazaheptatetracontan-1-oate (**393**).



393

To a solution of compound **392** (2.56 g, 2.55 mmol) in methanol (20 mL), 10 wt% Pd/C (0.30 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 2 hours, filtered, and concentrated to dryness to afford compound **393** (2.10 g, 94% yield). MS-ESI (m/z): $[M+H]^+$ calcd. for $C_{38}H_{74}N_6O_{16}$, 871.52; found, 871.56.

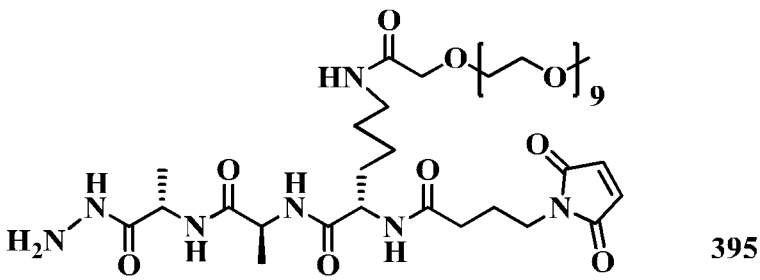
Example 444. Synthesis of (5S,8S,11S)-tert-butyl 11-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-5,8-dimethyl-4,7,10,17-tetraoxo-19,22,25,28,31,34,37,40,43,46-decaoxa-2,3,6,9,16-pentaazaheptatetracontan-1-oate (**394**).



394

To a solution of compound **393** (2.10 g, 2.41 mmol) and 4-maleimidobutyric acid N-hydroxysuccinimide ester (0.81 g, 2.89 mmol) in DCM (25 mL) was added N-methylmorpholine (0.29 g, 2.89 mmol). The reaction was stirred at r.t. overnight, concentrated, then purified by silica gel column ($MeOH/CH_2Cl_2$) to give compound **394** (2.30 g, 92% yield). MS-ESI (m/z): calcd. for $C_{46}H_{81}N_7O_{19}$ $[M+H]^+$ 1036.56; found, 1037.20.

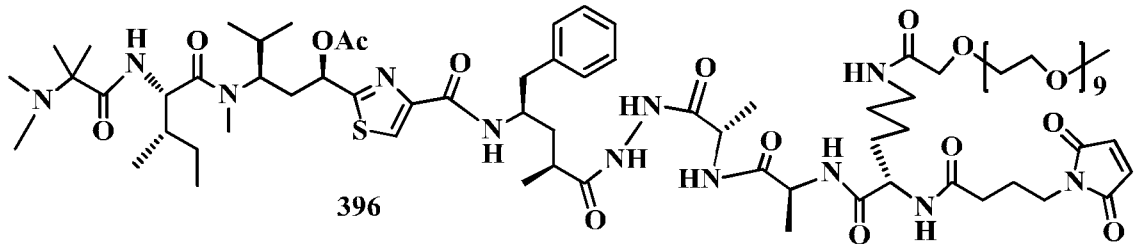
Example 445. Synthesis of N-((S)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-6-(((S)-1-(((S)-1-hydrazinyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**395**).



395

A solution of compound **394** (0.52 g, 0.50 mmol) in DCM (10 mL) was stirred with TFA (5 mL) for 30 min. and then concentrated to give a crude product (0.45 g), which is used directly in the next step. MS-ESI (m/z): calcd. for $C_{41}H_{73}N_7O_{17}$ $[M+H]^+$ 936.51; found, 936.55.

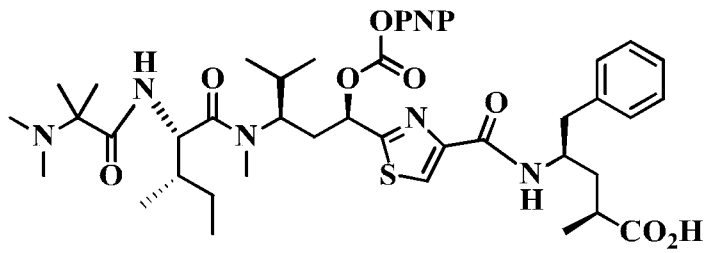
Example 446. Synthesis of (1R,3R)-3-((2S,3S)-2-(2-(dimethylamino)-2-methylpropanamido) -N,3-dimethylpentanamido)-1-(4-(((37S,40S,43S,48S,50R)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43,48-trimethyl-31,38,41,44,47-pentaoxo-51-phenyl-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,46-pentaazahenpentacontan-50-yl)carbamoyl)thiazol-2-yl)-4-methylpentyl acetate (**396**).



396

To a mixture of compound **395** (0.045 g, 0.050 mmol) and **Tub-5** (0.039 g, 0.055 mmol) in DMF (10 mL) were added HATU (0.021 g, 0.055 mmol) and trimethylamine (5.5 mg, 0.055 mmol) at 0 °C. The mixture was stirred at 0 °C until complete conversion, and then washed with brine (20 mL) twice, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by preparative HPLC(water/acetonitrile) to give compound **396** (0.042 g, 52% yield) as a white foam. MS-ESI (m/z): $[M+2H]^{2+}$ calcd. for $C_{78}H_{128}N_{12}O_{23}S$, 817.45; found, 817.56.

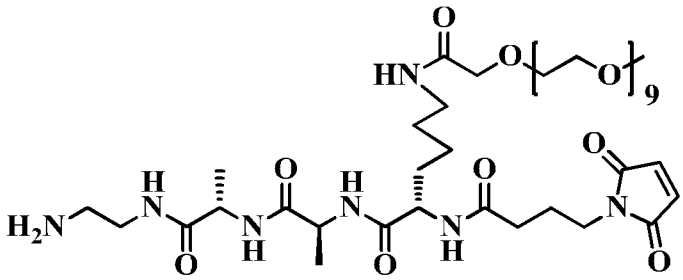
Example 447. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-9-isopropyl-2,3,3,8-tetramethyl- 13-(4-nitrophenoxy)-4,7,13-trioxo-6-propyl-12-oxa-2,5,8-triazatridecan-11-yl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (**397**).



397

To a mixture of compound **Tub-6** (0.034 g, 0.050 mmol) and 4-nitrobenzoyl chloride (0.011 g, 0.060 mmol) in 20 mL of dry dichloromethane was added N,N-DIISOPROPYLETHYLAMINE (8 mg, 0.060 mmol) at 0 °C. After stirring for 30 min, the reaction mixture was loaded on a short silica gel column and eluted with $MeOH/CH_2Cl_2$. Fractions were combined and concentrated to give the title compound (0.030 g, 72% yield). MS-ESI (m/z): $[M+H]^+$ calcd. for $C_{41}H_{56}N_6O_{10}S$ 825.38; found, 825.60.

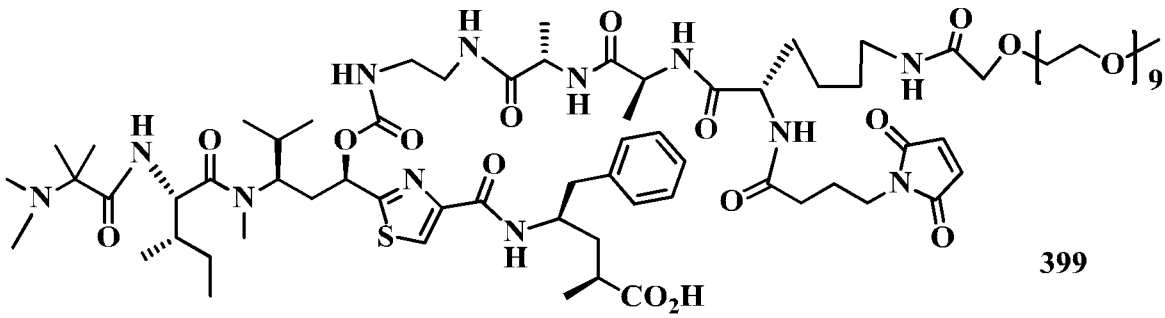
Example 448. Synthesis of N-((S)-6-(((S)-1-(((S)-1-((2-aminoethyl)amino)-1-oxopropan -2-yl)amino)-1-oxopropan-2-yl)amino)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**398**).



398

Tert-butyl ((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-yl)carbamate (0.043 g, 0.040 mmol) was dissolved in dichloromethane (5 mL) and treated with TFA (2.5 mL) for 30 min, and the reaction was then concentrated to give the title compound, which is used directly in the next step.

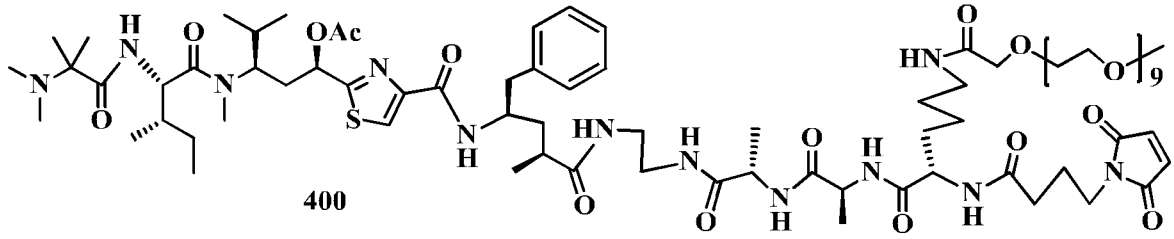
Example 449. Synthesis of (2S,4R)-4-(2-((37S,40S,43S,51R,53R,56S)-56-((S)-sec-butyl) -37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-53-isopropyl-40,43,54,59,59,60-hexamethyl-31,38,41,44,49,55,58-heptaoxo-2,5,8,11,14,17,20,23,26,29,50-undecaoxa-32,39,42,45,48,54,57,60-octaazahenhexacontan-51-yl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (**399**).



399

To a solution of **397** (0.030 g, 0.036 mmol) and compound **398** (0.040 mmol) in DMF (5 mL) was added N,N-diisopropylethylamine (13 mg, 0.10 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours and concentrated, then purified by preparative HPLC (water/acetonitrile) to give compound **399** (53 mg, 90% yield). MS-ESI (m/z): calcd. for $C_{79}H_{130}N_{12}O_{24}S$ $[M+2H]^{2+}$ 832.45; found, 832.56.

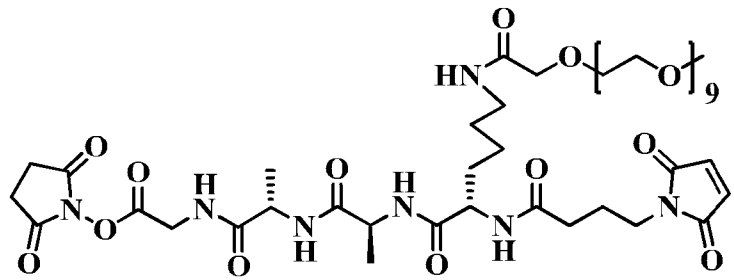
Example 450. Synthesis of (1R,3R)-3-((2S,3S)-2-(2-(dimethylamino)-2-methylpropanamido) -N,3-dimethylpentanamido)-1-(4-(((37S,40S,43S,50S,52R)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43,50-trimethyl-31,38,41,44,49-pentaoxo-53-phenyl-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazatripentacontan-52-yl)carbamoyl)thiazol-2-yl)-4-methylpentyl acetate (**400**).



400

To a solution of **Tub-5** (29 mg, 0.040 mmol) and compound **398** (0.040 mmol) in DMF (10 mL) cooled over an ice-water bath, were added HATU (0.19 g, 0.050 mmol) and triethylamine (10 mg, 0.10 mmol). The reaction was warmed to r.t. and stirred overnight, washed with brine, dried, concentrated, and purified by preparative HPLC (water/acetonitrile) to give a white foam (55 mg, 83% yield). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd. for $C_{80}H_{132}N_{12}O_{23}S$, 831.46; found, 832.58.

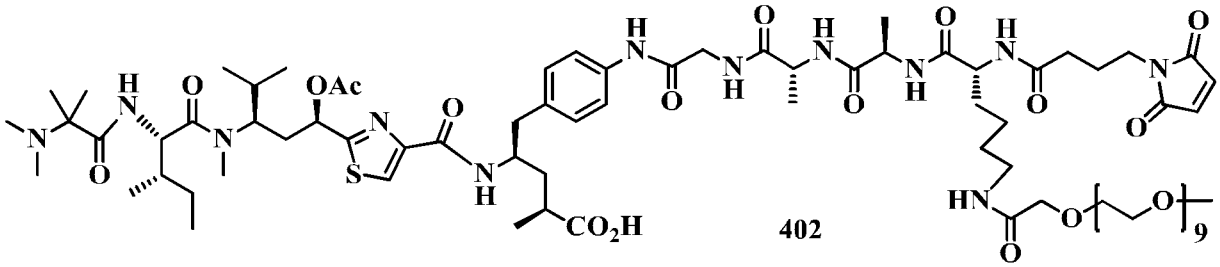
Example 451. Synthesis of (37S,40S,43S)-2,5-dioxopyrrolidin-1-yl 37-(4-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-oate (**401**).



401

To a solution of ((S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-31 -oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oyl)-L-alanyl-L-alanylglycine (0.98 g, 0.10 mmol) in DCM (10 mL), N-hydroxysuccinimide (14 mg, 0.12 mmol) and EDC·HCl (23 mg, 0.12 mmol) were added over ice water bath. The reaction was stirred at r.t. for 3.5 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give compound **401** (0.11 g), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{47}H_{77}N_7O_{21}$ $[M+H]^+$ 1076.52; found, 1077.50.

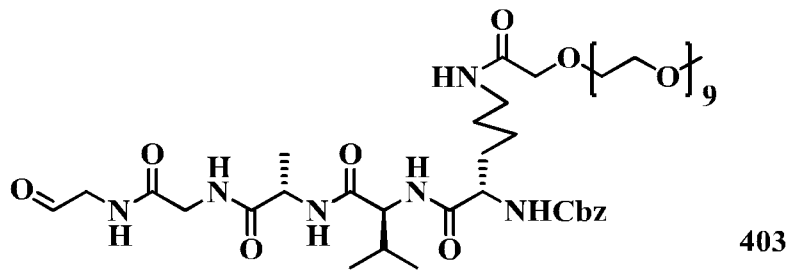
Example 452. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl- 2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(4-((37R,40R,43R)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontanamido)phenyl)-2-methylpentanoic acid (**402**).



402

Compound **401** (0.11 g, 0.10 mmol) and **Tub-7** (44 mg, 0.06 mmol) were dissolved in 2 mL of DMF, and N,N-diisopropylethylamine (13 mg, 0.10 mmol) was added. After stirring at r.t. for 2 hours, the reaction was concentrated, purified by preparative HPLC to give a light yellow liquid (62 mg, 61% yield). MS-ESI (m/z): calcd. for $C_{80}H_{130}N_{12}O_{25}S$ $[M+2H]^{2+}$ 846.45; found, 846.40.

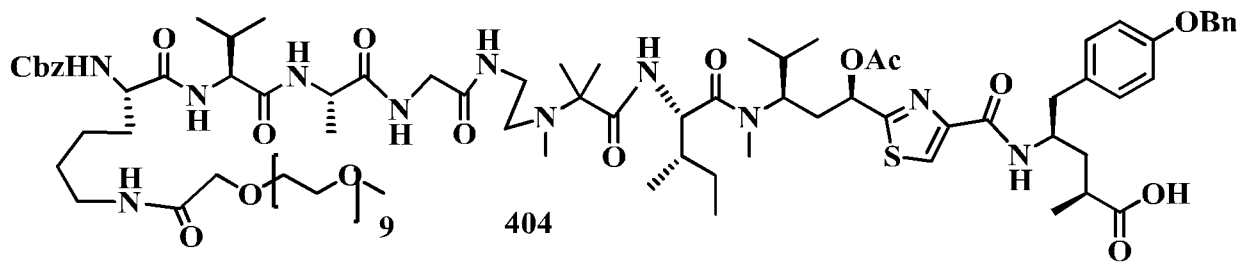
Example 453. Synthesis of benzyl ((37S,40S,43S)-40-isopropyl-43-methyl-31,38,41,44,47,50-hexaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-37-yl)carbamate (**403**).



403

To a solution of benzyl ((37S,40S,43S)-40-isopropyl-50-methoxy-43-methyl- 31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29,51-undecaoxa-32,39,42,45,48-pentaazadopentacontan-37-yl)carbamate (102 mg, 0.10 mmol) in dichloromethane (5 mL) was added p-toluenesulfonamide (1.7 mg, 0.010 mmol) and the reaction mixture was stirred overnight, concentrated and purified by fast silica gel column (ethyl acetate/dichloromethane) to give a colorless oil (69 mg, 68% yield). MS-ESI (m/z): calcd. for $C_{47}H_{80}N_6O_{18}$ $[M+2H]^{2+}$ 509.27; found, 509.26.

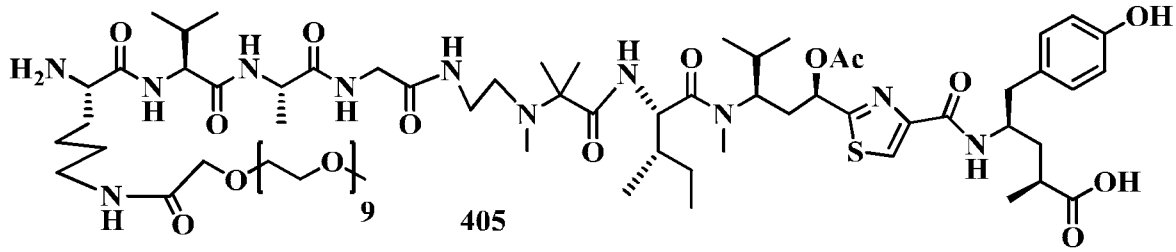
Example 454. Synthesis of (2S,4R)-4-(2-((37S,40S,43S,55S,58R,60R)-37-(((benzyloxy) carbonyl)amino)-55-((S)-sec-butyl)-40,58-diisopropyl-43,51,52,52,57-pentamethyl-31,38,41,44,47,53,56,62-octaoxo-2,5,8,11,14,17,20,23,26,29,61-undecaoxa-32,39,42,45,48,51,54,57-octaazatrihexacontan-60-yl)thiazole-4-carboxamido)-5-(4-(benzyloxy)phenyl)-2-methylpentanoic acid (**404**).



404

To a solution of compound **403** (69 mg, 0.068 mmol) and **Tub-8** (48 mg, 0.060 mmol) in isopropyl alcohol (2.0 mL) and acetic acid (0.2 mL), sodium triacetoxyborohydride (38 mg, 0.18 mmol) at 0 °C. The reaction was stirred at r.t. overnight and then concentrated and purified by preparative HPLC(water/acetonitrile) to give a white foam (92 mg, 85% yield). MS-ESI (m/z): calcd. for $C_{90}H_{141}N_{11}O_{25}S$ $[M+2H]^{2+}$ 904.99; found, 905.10.

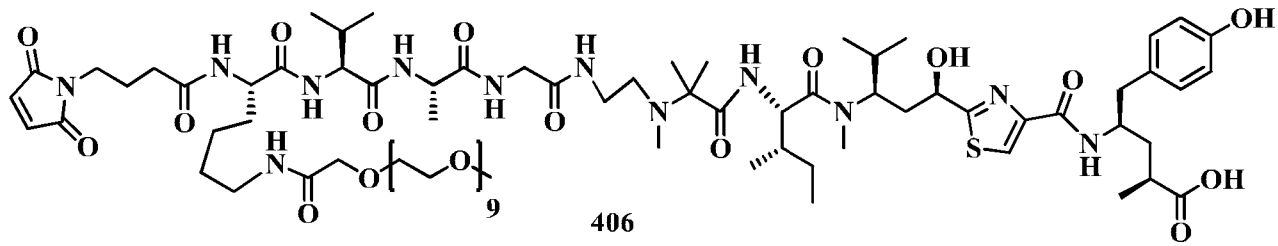
Example 455. Synthesis of (2S,4R)-4-(2-((37S,40S,43S,55S,58R,60R)-37-amino-55- ((S)-sec-butyl)-40,58-diisopropyl-43,51,52,52,57-pentamethyl-31,38,41,44,47,53,56,62-octaoxo-2,5,8,11,14,17,20,23,26,29,61-undecaoxa-32,39,42,45,48,51,54,57-octaazatrihexacontan-60-yl)thiazole-4-carboxamido)-5-(4-hydroxyphenyl)-2-methylpentanoic acid (**405**).



405

Compound **404** (92 mg, 0.051 mmol) was dissolved in methanol (5 mL), palladium on carbon (10 wt%, 10 mg) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times, stirred for 4 hours. The reaction mixture was filtered, and the filtrate was concentrated to the title compound (77 mg, 96% yield). MS-ESI (m/z): $[M+2H]^{2+}$ calcd. for $C_{75}H_{129}N_{11}O_{23}S$, 792.95; found, 973.91.

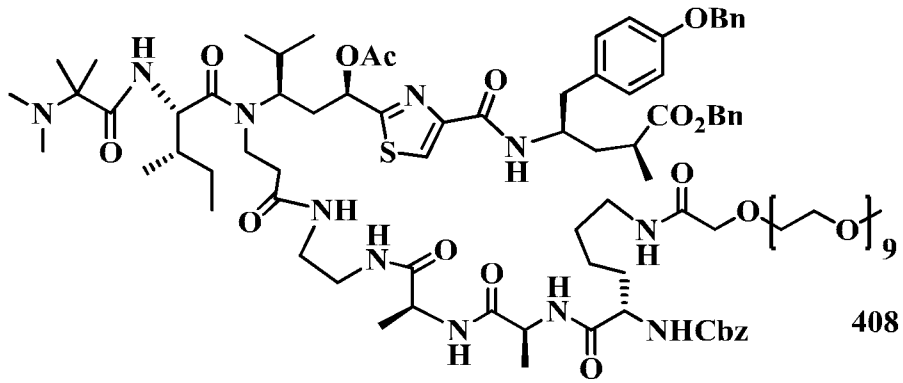
Example 456. Synthesis of (2S,4R)-4-(2-((37S,40S,43S,55S,58R,60R)-55-((S)-sec-butyl)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-60-hydroxy-40,58-diisopropyl-43,51,52,52,57-pentamethyl-31,38,41,44,47,53,56-heptaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48,51,54,57-octaazahexacontan-60-yl)thiazole-4-carboxamido)-5-(4-hydroxyphenyl)-2-methylpentanoic acid (**406**).



406

A solution of compound **405** (77 mg, 0.048 mmol) and 4-maleimidobutyric acid N-hydroxysuccinimide ester (13 mg, 0.048 mmol) in THF (1.5 mL) and PBS (pH 6.2, 1.0 mL) was stirred at r.t. overnight, concentrated, then purified by preparative HPLC(water/acetonitrile) to give compound **406** (47 mg, 58% yield). MS-ESI (m/z): calcd. for $C_{81}H_{134}N_{12}O_{25}S$ $[M+2H]^{2+}$ 854.46; found, 854.20.

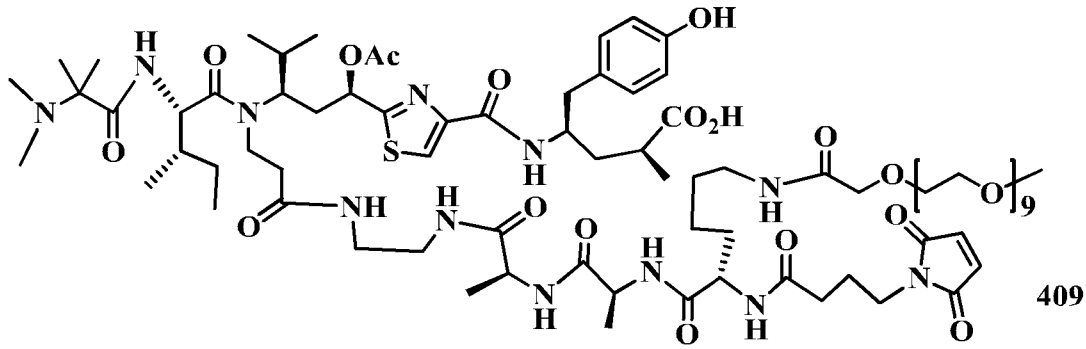
Example 457. Synthesis of (2S,4R)-benzyl 4-(2-((37S,40S,43S,53R,55R)-37-(((benzyloxy) carbonyl)amino)-52-((2S,3S)-2-(2-(dimethylamino)-2-methylpropanamido)-3-methylpentanoyl)-53-isopropyl-40,43-dimethyl-31,38,41,44,49,57-hexaoxo-2,5,8,11,14,17,20,23,26,29,56-undecaoxa-32,39,42,45,48,52-hexaazaoctapentacontan-55-yl)thiazole-4-carboxamido)-5-(4-(benzyloxy)phenyl)-2-methylpentanoate (**408**).



408

Compound **407** (50 mg, 0.05 mmol) and **Tub-9** (58 mg, 0.06 mmol) were dissolved in DMF (2.5 mL), to which EDC·HCl (17 mg, 0.09 mmol) and N,N-diisopropylethylamine (19 mg, 0.15 mmol) were added, and the reaction was stirred for 0.5 hours, and then concentrated to dryness. The crude product was purified by preparative HPLC (water/acetonitrile) to give compound **408** as an oil (83 mg, 88% yield). MS-ESI (m/z): $[M + 2H]^{2+}$ calcd. for $C_{96}H_{145}N_{11}O_{25}S$, 942.00; found, 942.12.

Example 458. Synthesis of (2S,4R)-4-(2-((37S,40S,43S,53R,55R)-52-((2S,3S)-2-(2-(dimethylamino)-2-methylpropanamido)-3-methylpentanoyl)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-53-isopropyl-40,43-dimethyl-31,38,41,44,49,57-hexaoxo-2,5,8,11,14,17,20,23,26,29,56-undecaoxa-32,39,42,45,48,52-hexaazaoctapentacontan-55-yl)thiazole-4-carboxamido)-5-(4-hydroxyphenyl)-2-methylpentanoic acid (**409**).

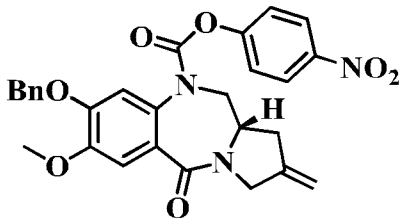


409

Compound **408** (83 mg, 0.044 mmol) was dissolved in methanol (5 mL), palladium on carbon (10 wt%, 10 mg) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times, stirred overnight. The reaction mixture was filtered, and the filtrate was concentrated, re-dissolved in THF (1.5 mL) and PBS (pH 6.2, 1.0 mL), 4-maleimidobutyric acid N-hydroxysuccinimide ester (12 mg, 0.043 mmol) was added and stirred at r.t. overnight. The reaction

was concentrated, and purified by preparative HPLC(water/acetonitrile) to give the title compound (34 mg, 45% yield). MS-ESI (m/z): calcd. for $C_{82}H_{134}N_{12}O_{26}S$ $[M+2H]^{2+}$ 867.46; found, 868.02.

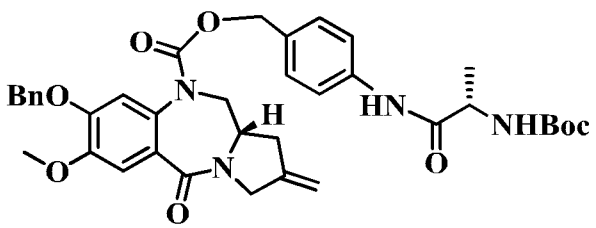
Example 459. Synthesis of 4-nitrophenyl (S)-8-(benzyloxy)-7-methoxy-2-methylene-5 -oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**410**).



410

To a solution of (S)-8-(benzyloxy)-7-methoxy-2-methylene-1,2,3,10,11,11a- hexahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (2.118 g, 6.051 mmol) in DCM (100 mL) were added DIPEA (0.931 g, 7.201 mmol) and 4-nitrophenyl chloroformate (1.331 g, 6.601 mmol) under stirring. After the addition, the mixture was stirred at r.t. overnight, washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/DCM= 15%-45%) to give the title compound (2.618 g, 84% yield). $C_{28}H_{26}N_3O_7$ $[M+H]^+$ 516.177; found, 516.195.

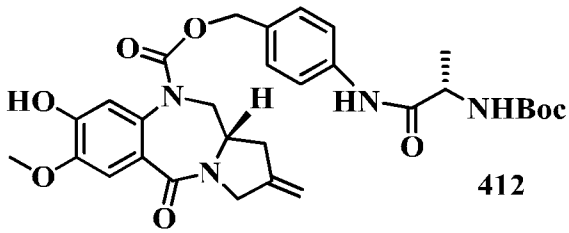
Example 460. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido)benzyl (S)-8-(benzyloxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**411**).



411

A solution of compound **410** (0.70 g, 1.36 mmol) in anhydrous THF (5 mL) and DMA (10 mL) was cooled to below 0°C in an ice-salt batch. LiHMDS (2.4 mL, 1 mol/L) was added dropwise under N_2, and the reaction was kept below 0°C for 20 minutes. A solution of tert-butyl (S)-(1-((4-(hydroxymethyl)-phenyl)amino)-1-oxopropan-2-yl)carbamate (compound **377)** (1.01 g, 2.0 mmol) in 10 mL of THF was added dropwise, and the reaction was kept below 0°C for 20 minutes, and warmed to r.t. and stirred for 4 hours. The reaction solution was diluted with DCM, washed with ammonium chloride solution, brine, and dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (EA/DCM= 15%-40%) to give the title compound (0.59 g, 65% yield). MS, $C_{37}H_{43}N_4O_8$ $[M+H]^+$ 671.31; found, 671.60.

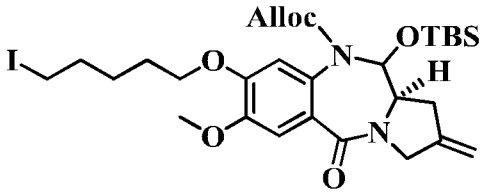
Example 461. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido)benzyl (S)-8-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**412**).



412

Compound **411** (1.10 g, 1.64 mmol) in DCM (15 mL) was treated with $AlCl_3$ (0.65 g, 4.93 mmol) and N,N-dimethylaniline (0.30 g, 2.48 mmol) at r. t. for 45 min. The mixture was diluted with DCM (15 ml), washed with 0.1 M HCl (10 ml), brine (10 ml), 5% $NaHCO_3$ solution and brine (10 ml), dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography (EA/DCM= 20%-40%) to give the title compound (0.685 g, 72% yield). MS, $C_{30}H_{37}N_4O_8$ $[M+H]^+$ 581.26; found, 581.40.

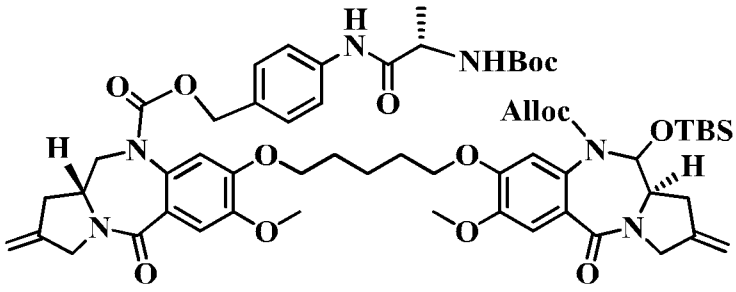
Example 462. Synthesis of (11aR)-allyl 11-((tert-butyldimethylsilyl)oxy)-8- ((5-iodopentyl)oxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**413**).



413

To a solution of (11aR)-allyl 11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7- methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (1.45 g, 3.06 mmol) in acetone (100 mL) were added diiodopentane (4.86 g, 15 mmol) and potassium carbonate (0.62 g, 4.5 mmol) with stirring. After the addition, the reaction was heated under reflux for 8 hours, and after cooling, it was directly purified by column chromatography (EA/DCM= 15%-30%) to give the title compound (1.87 g, 91% yield). MS, $C_{29}H_{44}IN_2O_6Si$ $[M+H]^+$ 671.20; found, 671.45.

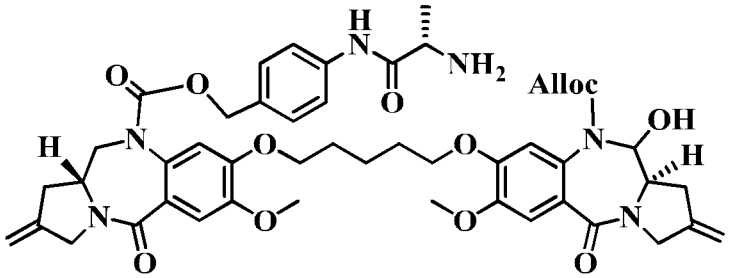
Example 463. Synthesis of (11aR)-allyl 8-((5-(((R)-10-(((4-((S)-2-((tert-butoxycarbonyl)amino) -propanamido)benzyl)oxy)carbonyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**414**).



414

A mixture of compound **413** (0.90 g, 1.34 mmol) and compound **412** (0.80g, 1.38 mmol) in 80 mL of acetone, and potassium carbonate (0.44 g, 3.2 mmol) was heated to reflux and stirred for 8 hours. The reaction was directly purified by a silica gel column (0-10% MeOH/DCM) to give the title compound (1.13 g, 75% yield). MS, $C_{59}H_{79}N_6O_{14}Si$ $[M+H]^+$ 1123.542; found, 1123.565.

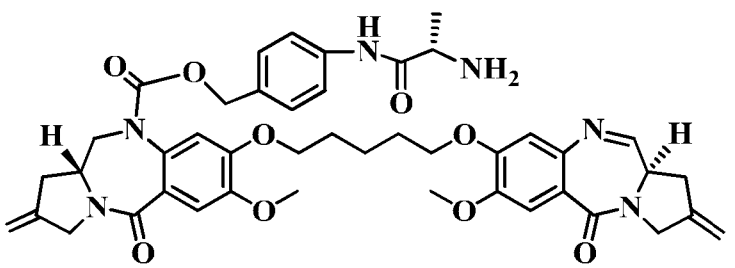
Example 464. Synthesis of (11aR)-allyl 8-((5-(((R)-10-(((4-((S)-2-aminopropanamido)benzyl) -oxy)carbonyl)-7-methoxy-2-methylene-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**415**).



414

Compound **414** (0.70 g, 0.62 mmol) was dissolved in 15 mL of dioxane and cooled to 5 °C, to which HCl (conc., 5 mL) was added and stirred at r.t. for 40 min. The reaction was diluted with dioxane/toluene and concentrated. The crude product was purified by a silica gel column (1:5:25, Et3N/MeOH/acetone) to give the title compound (0.41 g, 72% yield). MS, $C_{48}H_{57}N_6O_{12}$ $[M+H]^+$ 909.40; found, 909.60.

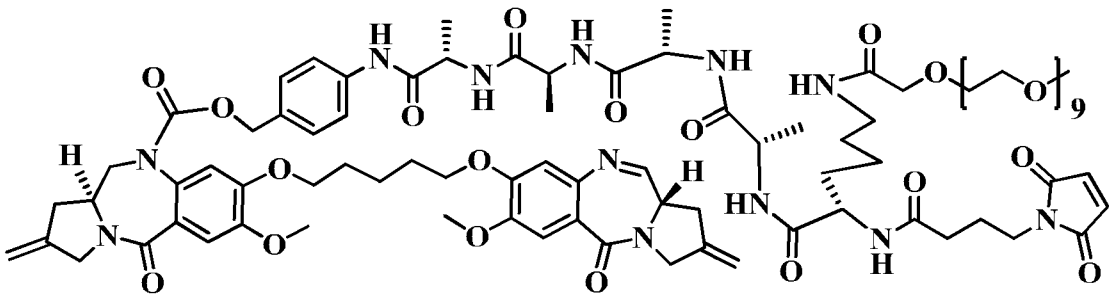
Example 465. Synthesis of (R)-4-((S)-2-aminopropanamido)benzyl 7-methoxy-8-((5- (((R)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**415**).



415

Compound **414** (115 mg, 0.126 mmol) was dissolved in 2 mL of DCM, to which pyrrolidine (19.0 mg, 0.28 mmol) and catalytic amount of $Pd(PPh_3)_4$ (3 mg) were added, and then stirred at r.t. for 30 min. The reaction was diluted with DMF and evaporated to remove DCM. The resulting crude product in DMF was used directly in the next step. MS-ESI (m/z): calcd. for $C_{44}H_{51}N_6O_9[M+H]^+$:807.37; found 807.50.

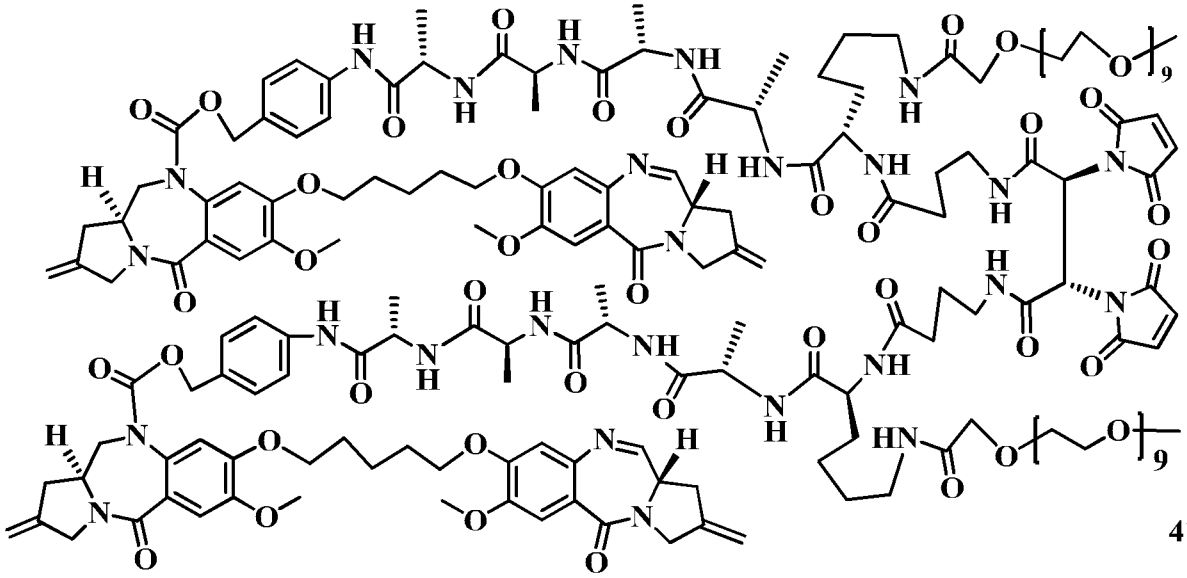
Example 466. Synthesis of 4-((37S,40S,43S,46S,49S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43,46,49-tetramethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-amido)benzyl (S)-7-methoxy-8-((5-(((S)-7- methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**416**).



416

Compound **415** (~105 mg, ~0.131 mmol) was dissolved in 10 mL of DMF, to which compound **218** (158.0 mg, 0.145 mmol) and DIPEA (0.1 ml) were added. After stirring for 2 h, the reaction was concentrated, purified by preparative C-18 HPLC to give a white solid (118 mg, 51% yield). MS-ESI (m/z): calcd. for $C_{88}H_{125}IN_{12}O_{27}$ $[M+H]^+$ 1780.87; found 1781.25.

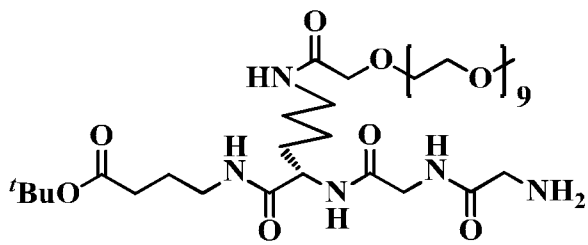
Example 467. Synthesis of ((((2S,5S,8S,11S,14S,22S,23S,31S,34S,37S,40S,43S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,11,34,37,40,43-octamethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4,1-phenylene))bis(methylene) (11aS,11a'S)-bis(7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo- 2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methylene-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate) (**417**).



417

A solution of compound **136** (73 mg, 0.035 mmol), HATU (40 mg, 0.105 mmol) in DMF (1 mL) was stirred at r.t. for 15 min. and then the crude product of **415** (60 mg, 0.074 mmol) in DMF was added to the reaction solution, followed by DIPEA (5 mg, 0.039 mmol). After stirring at r.t. for 90 min., the reaction mixture was directly purified by preparative HPLC to give a pale yellow solid (61 mg, 47% yield). MS-ESI (m/z): calcd. for $C_{180}H_{253}N_{26}O_{56}[M+H]^{2+}$:1837.3875; found 1837.3960.

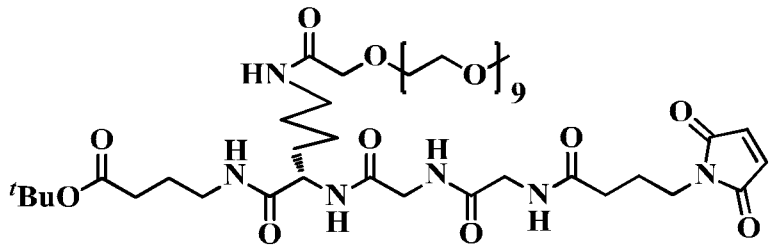
Example 468. Synthesis of (S)-tert-butyl 37-(2-(2-aminoacetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontan-43-oate (**421**).



421

Compound **420** (10 g, 9.2 mmol) was dissolved in THF (100 mL), 10 wt% palladium on carbon (2 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 16 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (8 g, 100% yield). MS-ESI (m/z): calcd for $C_{39}H_{75}N_5O_{16}$, $[M+H]^+$ 871.05; found, 870.48.

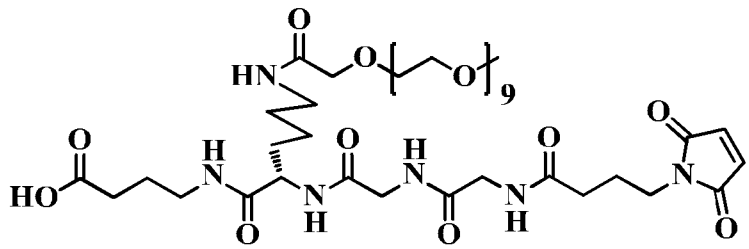
Example 469. Synthesis of (S)-tert-butyl 37-(2-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontan-43-oate (**422**).



422

To a solution of compound **421** (8.0 g, 9.2 mmol) and 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanoic acid (2.2 g, 12 mmol) in dichloromethane (150 mL), EDC·HCl (4.4 g, 23 mmol) was added. The reaction was stirred at r.t. for 2 hours, washed with water (50 mL), 5% sodium carbonate (50 mL), 0.5 N HCl (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified by a silica gel column (MeOH/DCM) to give the title compound (6.6 g, 70% yield). MS-ESI (m/z): calcd for $C_{47}H_{82}N_6O_{19}$, $[M+H]^+$ 1036.20; found, 1035.61.

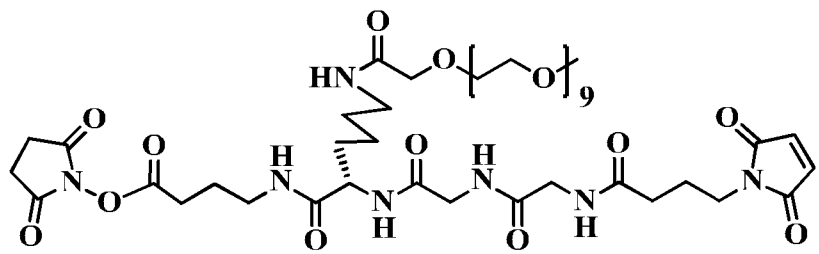
Example 470. Synthesis of (S)-37-(2-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido) acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontan-43-oic acid (**423**).



423

Compound **422** (6.6 g, 6.4 mmol) was dissolved in formic acid (50 mL) and dichloromethane (50 mL), and stirred at r.t. overnight. The reaction mixture was concentrated and purified by a silica gel column (MeOH/DCM) to give the title compound (6.3 g, 100% yield). MS-ESI (m/z): calcd for $C_{43}H_{74}N_6O_{19}$, $[M + H]^+$ 980.09; found, 979.68.

Example 471. Synthesis of (S)-2,5-dioxopyrrolidin-1-yl 37-(2-(2-(4-(2,5-dioxo-2,5-dihydro - 1H-pyrrol-1-yl)butanamido)acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontan-43-oate (**424**).

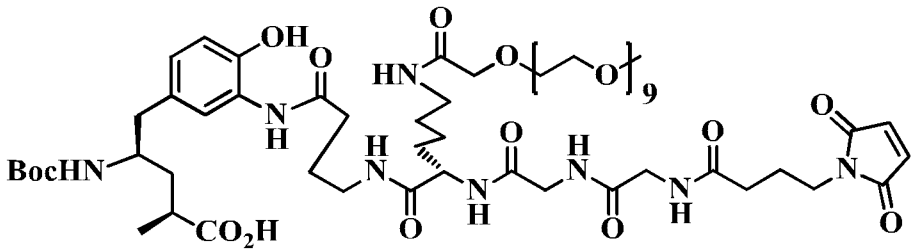


424

Compound **423** (3.2 g, 3.3 mmol) was dissolved in dichloromethane (60 mL), NHS (0.75 g, 6.6 mmol) and EDC·HCl (1.8 g, 9.8 mmol) were added, and the reaction was stirred at r.t. for 2 hours, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give the title

compound (3.65 g, 100% yield). MS-ESI (m/z): calcd for $C_{47}H_{77}N_7O_{21}$, $[M + H]^+$ 1077.16; found, 1077.05.

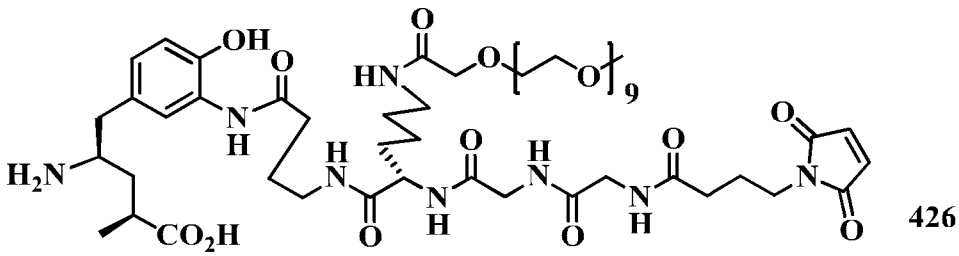
Example 472. Synthesis of (2S,4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((S)-37-(2-(2-(4- (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**425**).



425

Compound **424** (3.65 g, 3.3 mmol) and compound **77** (1.26 g, 3.7 mmol) were dissolved in tetrahydrofuran (100 mL) and stirred under reflux for 16 hours. The reaction mixture was concentrated under reduced pressure, and then the residue was purified by a silica gel column to give the title compound (1.5 g, 34% yield). MS-ESI (m/z): calcd for $C_{60}H_{98}N_8O_{23}$, $[M+H]^+$: 1300.48; found 1300.11.

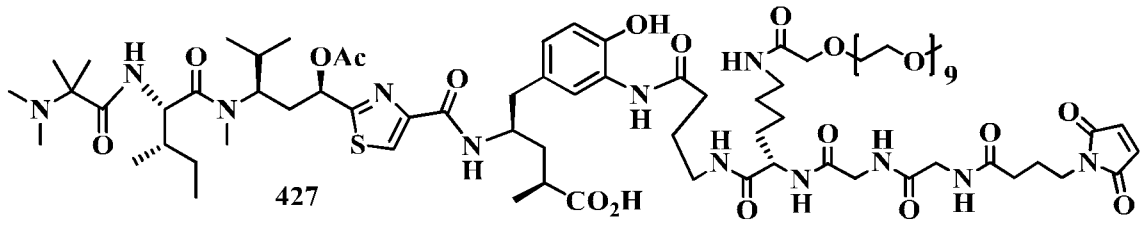
Example 473. Synthesis of (2S,4R)-4-amino-5-(3-((S)-37-(2-(2-(4-(2,5-dioxo -2,5-dihydro-1H-pyrrol-1-yl)butanamido)acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**426**).



426

Compound **425** (0.75 g, 0.58 mmol) was dissolved in dichloromethane (10 mL) and treated with TFA (10 mL) for 1 h, concentrated to give the title compound (1.1 g). MS-ESI (m/z): calcd for $C_{55}H_{90}N_8O_{21}$, $[M + H]^+$ 1200.36; found, 1200.12.

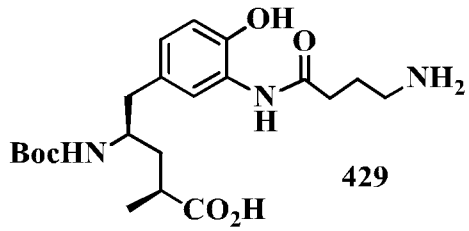
Example 474. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl -2,3,3,8 -tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((S)-37-(2-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)acetamido)acetamido)-31,38-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39-diazatritetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**427**).



427

To a solution of compound **426** (1.1 g, 0.58 mmol) in DMF (12 mL), **Tub-1** (0.40 g, 0.58 mmol) and N,N-diisopropylethylamine (0.75 g, 5.8 mmol) were added at 0 °C. The reaction was stirred at 0 °C for 1 hour, and r.t. for 1 hour and concentrated. The residue was purified by a silica gel column (MeOH/DCM) and then preparative HPLC to give the title compound (0.50 g, 51% yield). MS-ESI (m/z): calcd. for $C_{80}H_{130}N_{12}O_{26}S$, $[M+H]^+$ 1709.04; found, 1708.85.

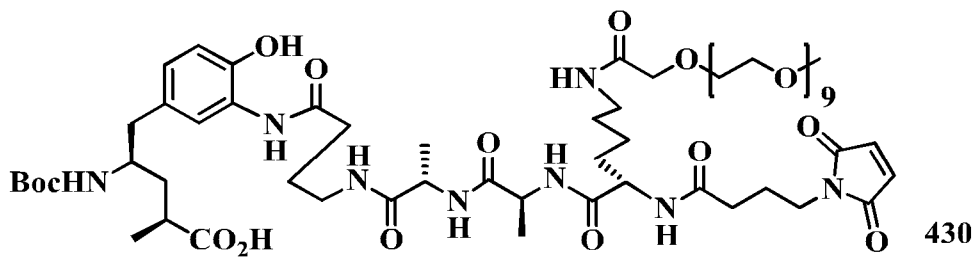
Example 475. Synthesis of (2S,4R)-5-(3-(4-aminobutanamido)-4-hydroxyphenyl) -4-((tert-butoxycarbonyl)amino)-2-methylpentanoic acid (**429**).



429

To a solution of compound **428** (2.80 g, 5.0 mmol) in methanol (30 mL), palladium carbon (0.30 g, 10% wt) was added and the reaction flask was evacuated and back-filled with H_2 for three times. The reaction mixture was stirred under H_2 overnight, filtered and the filtrate was concentrated to give a crude material (1.96 g, 92% yield), which was directly used for the next reaction. MS-ESI (m/z): calcd. for $C_{21}H_{33}N_3O_6$ $[M+H]^+$ 424.24; found, 424.24.

Example 476. Synthesis of (2S,4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S,40S,43S) -37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazanonatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**430**).

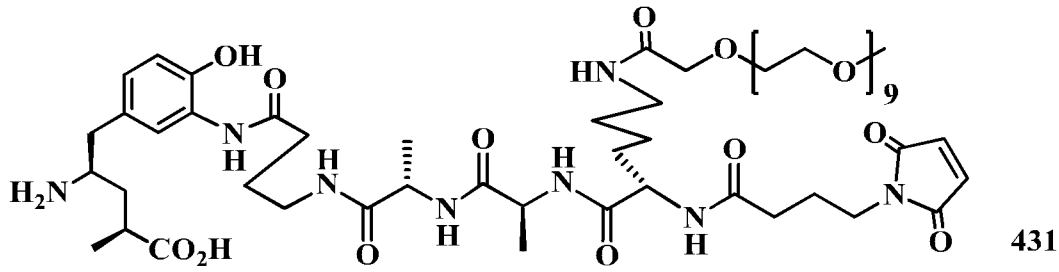


430

Compound **429** (0.23 g, 0.50 mmol) and compound **302** (0.56 g, 0.50 mmol) were dissolved in tetrahydrofuran (10 mL) and stirred at 50 °C for 3 hours. The reaction mixture was concentrated under reduced pressure, and then the residue was purified by preparative HPLC (acetonitrile/water

containing 0.1% formic acid) to give the title compound (0.27 g, 37% yield). MS-ESI (m/z): calcd for $C_{62}H_{102}N_8O_{23}$, $[M+H]^+$: 1327.71; found 1327.71.

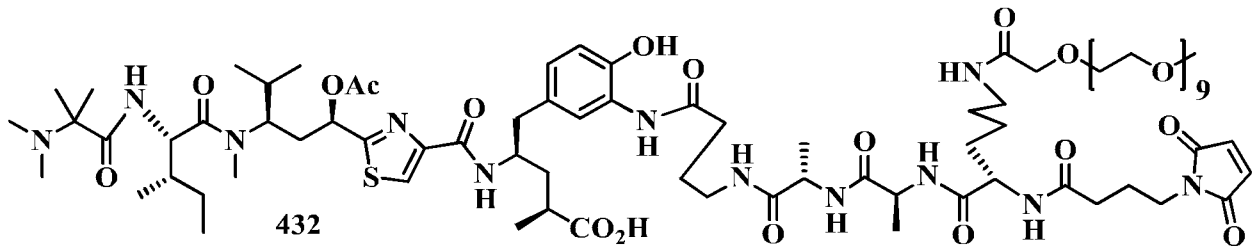
Example 477. Synthesis of (2S,4R)-4-amino-5-(3-((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazanonatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**431**).



431

Compound **430** (0.27 g, 0.20 mmol) was dissolved in dichloromethane (2 mL) and treated with TFA (4 mL) for 0.5 hours, then concentrated to give the title compound. MS-ESI (m/z): calcd for $C_{57}H_{94}N_8O_{21}$, $[M + H]^+$ 1227.65; found, 1227.65.

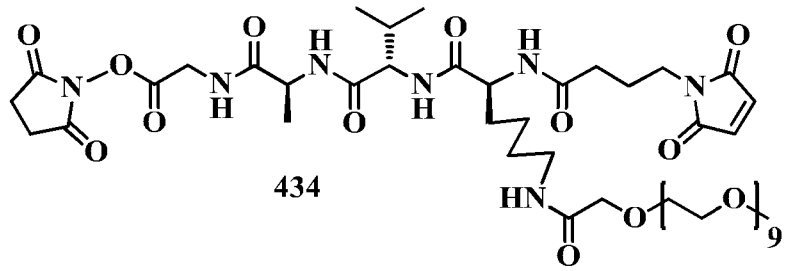
Example 478. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl -2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43-dimethyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazanonatetracontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**432**).



432

To a solution of compound **431** (0.25 g, 0.20 mmol) and **Tub-1** (0.14 g, 0.20 mmol) in DMF (1 mL), N,N-diisopropylethylamine (101 μL, 0.60 mmol) were added at 0 °C. The reaction was stirred at r.t. for 3 hours and directly purified by preparative HPLC to give the title compound (210 mg, 60% yield). MS-ESI (m/z): calcd. for $C_{82}H_{134}N_{12}O_{26}S$, $[M+2H]^{2+}$ 868.96; found, 868.86.

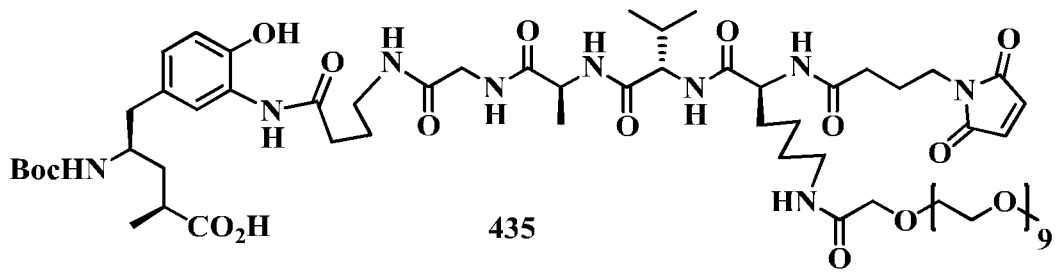
Example 479. Synthesis of (37S,40S,43S)-2,5-dioxopyrrolidin-1-yl 37-(4-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-oate (**434**).



434

Compound **433** (0.090 g, 0.10 mmol) was dissolved in dichloromethane (10 mL), NHS (0.012 g, 0.10 mmol) and EDC·HCl (0.034 g, 0.20 mmol) were added, and the reaction was stirred at r.t. for 3 hours, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated to give the title compound (72 mg, 72% yield). MS-ESI (m/z): calcd for $C_{49}H_{81}N_7O_{21}$, $[M + H]^+$ 1104.55; found, 1104.55.

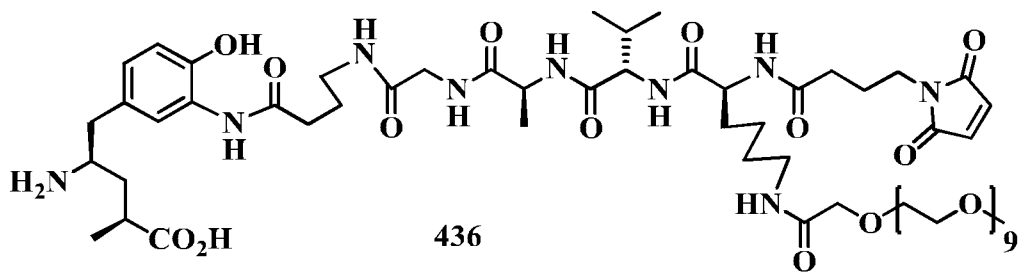
Example 480. Synthesis of (2S,4R)-4-((tert-butoxycarbonyl)amino)-5-(3-((37S,40S,43S) -37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazadopentacontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**435**).



435

Compound **434** (0.072 g, 0.07 mmol) and compound **429** (0.028 g, 0.07 mmol) were dissolved in tetrahydrofuran (5 mL) and stirred at 50 °C for 4 hours. The reaction mixture was concentrated under reduced pressure, and then the residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (49 mg, 53% yield). MS-ESI (m/z): calcd for $C_{66}H_{109}N_9O_{24}$, $[M+2H]^{2+}$: 706.38; found 706.28.

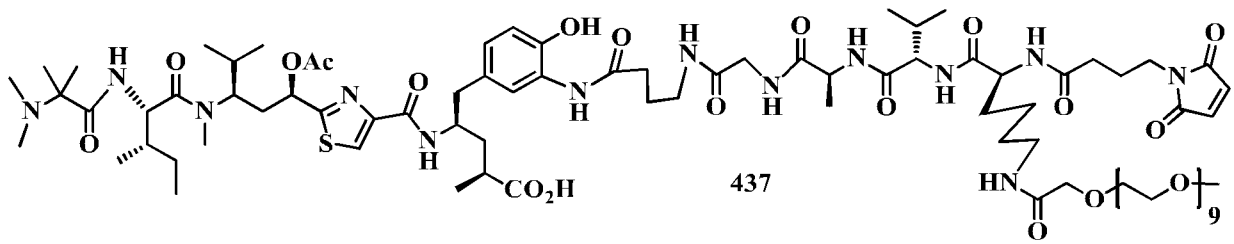
Example 481. Synthesis of (2S,4R)-4-amino-5-(3-((37S,40S,43S)-37-(4-(2,5-dioxo -2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazadopentacontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**436**).



436

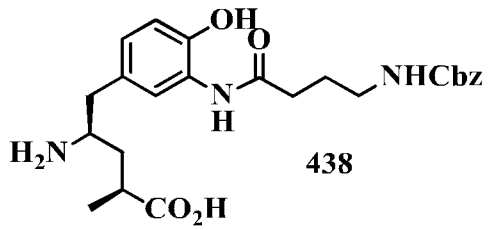
Compound **435** (0.049 g, 0.04 mmol) was dissolved in dichloromethane (1 mL) and treated with TFA (2 mL) for 1 h, concentrated to give the title compound, which was used directly in the next step. MS-ESI (m/z): calcd for $C_{61}H_{101}N_9O_{22}$, $[M + 2H]^{2+}$ 657.36; found, 657.48.

Example 482. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8 -tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazadopentacontanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**437**).



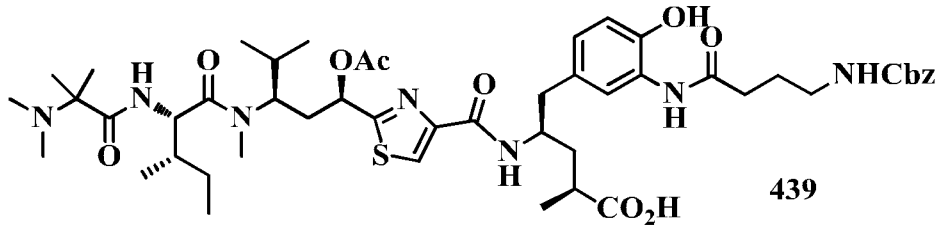
To a solution of compound **436** (0.046 g, 0.04 mmol) and **Tub-1** (0.024 g, 0.04 mmol) in DMF (1 mL), N,N-diisopropylethylamine (17 μL, 0.10 mmol) was added at 0 °C. The reaction was stirred at r.t. for 4 hours and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (35 mg, 58% yield). MS-ESI (m/z): calcd. for $C_{86}H_{141}N_{13}O_{27}S$, $[M+2H]^{2+}$ 911.49; found, 911.56.

Example 483. Synthesis of (2S,4R)-4-amino-5-(3-(4-(((benzyloxy)carbonyl)amino) butanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**438**).



Compound **428** (0.83 g, 1.50 mmol) was dissolved in dichloromethane (5 mL) and treated with TFA (5 mL) for 3 hours, then concentrated to give the title compound (1.01 g, crude). MS-ESI (m/z): calcd for $C_{24}H_{31}N_3O_6$, $[M + H]^+$ 458.22; found, 458.22.

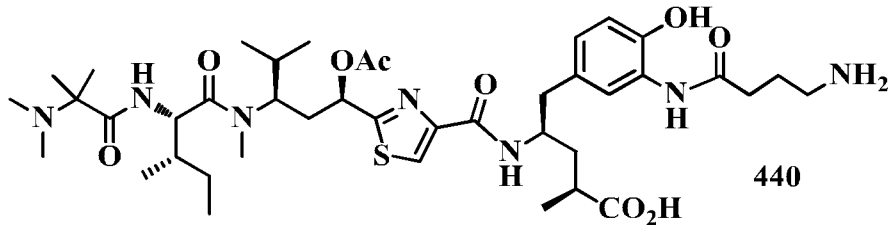
Example 484. Synthesis of (2S,4R)-5-(3-(4-(((benzyloxy)carbonyl)amino)butanamido) -4-hydroxyphenyl)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoic acid (**439**).



439

To a solution of compound **438** (0.68 g, 1.50 mmol) and **Tub-1** (1.03 g, 1.50 mmol) in DMF (5mL), N,N-diisopropylethylamine (0.74 mL, 4.50 mmol) were added at 0 °C. The reaction was stirred at r.t. for 1 hour and concentrated. The residue was purified by a silica gel column (7% MeOH/DCM) to give the title compound (1.22 mg, 85% yield). MS-ESI (m/z): calcd. for $C_{49}H_{71}N_7O_{11}S$, $[M+H]^+$ 966.49; found, 966.49.

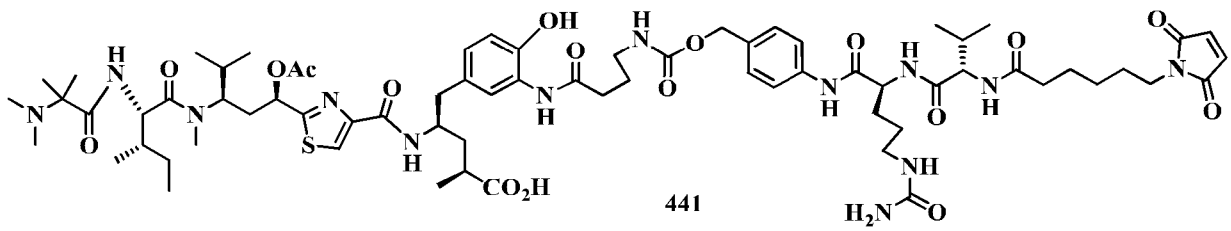
Example 485. Synthesis of (2S,4R)-5-(3-(4-aminobutanamido)-4-hydroxyphenyl) -4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-2-methylpentanoic acid (**440**).



440

Compound **439** (0.44 g, 0.46 mmol) was dissolved in methanol (10 mL), 10 wt% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 days, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (0.19 g, 50% yield). MS-ESI (m/z): calcd for $C_{41}H_{65}N_7O_9S$, $[M+H]^+$ 832.46; found, 832.48.

Example 486. Synthesis of (2S,4R)-4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8 -tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)-5-(3-(4-((((4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)amino)butanamido)-4-hydroxyphenyl)-2-methylpentanoic acid (**441**).

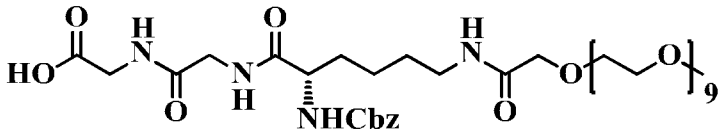


441

To a solution of compound **440** (0.076 g, 0.09 mmol) in DMF (1 mL), MC-Val-Cit-PAB-PNP (0.068 g, 0.09 mmol) and N,N-diisopropylethylamine (15 μL, 0.09 mmol) were added at 0 °C. The reaction was stirred at r.t. for 1 hour and concentrated. The residue was purified by preparative HPLC

to give the title compound (49 mg, 38% yield). MS-ESI (m/z): calcd. for $C_{70}H_{103}N_{13}O_{17}S$, $[M+2H]^{2+}$ 716.36; found, 716.58.

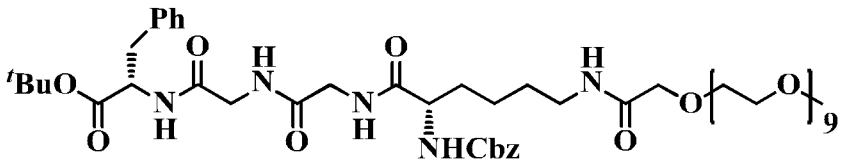
Example 487. Synthesis of (S)-37-(((benzyloxy)carbonyl)amino)-31,38,41-trioxo -2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42-triazatetratetracontan-44-oic acid (**442**).



442

Compound **26** (12 g, 13.1 mmol) was dissolved in dichloromethane (20 mL) and treated with formic acid (40 mL) for 3 hours. The mixture was concentrated, dissolved in dichloromethane, washed with water twice, brine once, dried over anhydrous sodium sulfate, filtered and concentrated again to give the title compound (14.0 g, 92% yield). MS-ESI (m/z): calcd for $C_{39}H_{67}N_4O_{17}$, $[M + H]^+$ 863.44; found, 863.44.

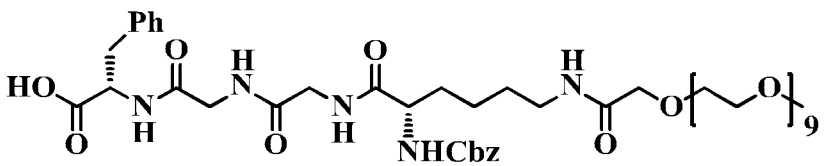
Example 488. Synthesis of (37S,46S)-tert-butyl 46-benzyl-37-(((benzyloxy)carbonyl)amino)-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-oate (**443**).



443

To a solution of compound **442** (3.21 g, 3.7 mmol) and H-Phe-O^{t}Bu·HCl (0.86 g, 3.3 mmol) in dichloromethane (50 mL), HATU (1.80 g, 4.8 mmol) and N,N-diisopropylethylamine (0.96 g, 7.4 mmol) were added. The reaction was stirred at r.t. for 30 min, diluted with water. The organic layer was separated and washed with water, 0.5 N HCl, 0.5 N $NaHCO_3$, and brine, dried over anhydrous sodium sulfate, filtered, concentrated to give the title compound (4.12 g). MS-ESI (m/z): calcd. for $C_{52}H_{84}N_5O_{18}$, $[M+2H]^{2+}$ 1066.57; found, 1066.57.

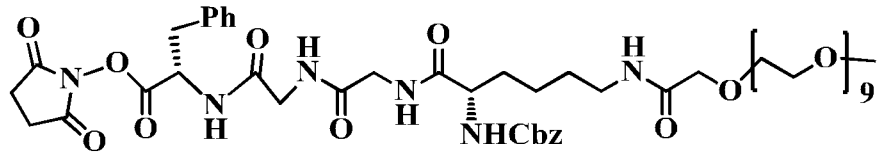
Example 489. Synthesis of (37S,46S)-46-benzyl-37-(((benzyloxy)carbonyl)amino)-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-oic acid (**444**).



444

Compound **443** (4.12 g, crude) was dissolved in dichloromethane (10 mL) and treated with formic acid (30 mL) at 50 °C overnight. The mixture was concentrated, co-evaporated with acetonitrile twice to give the title compound (3.26 g, 87% yield). MS-ESI (m/z): calcd for $C_{48}H_{76}N_5O_{18}$, $[M + H]^+$ 1010.51; found, 1010.51.

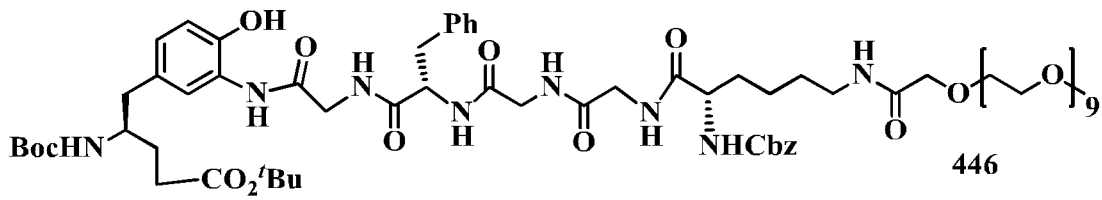
Example 490. Synthesis of (37S,46S)-2,5-dioxopyrrolidin-1-yl 46-benzyl-37-(((benzyloxy)carbonyl)amino)-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-oate (**445**).



445

To a solution of compound **444** (3.26 g, 3.0 mmol) in dichloromethane (50 mL), NHS (0.41 g, 4.0 mmol) and EDC·HCl (1.25 g, 6.0 mmol) were added at 0 °C, and the reaction was stirred at r.t. for 3 hours, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated to give the title compound (3.29 g). MS-ESI (m/z): calcd for $C_{52}H_{79}N_6O_{20}$, $[M + H]^+$ 1107.53; found, 1107.53.

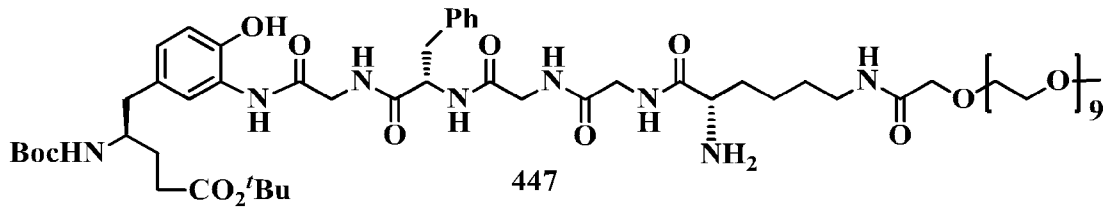
Example 491. Synthesis of (R)-tert-butyl 5-(3-((37S,46S)-46-benzyl-37- (((benzyloxy)carbonyl) amino)-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**446**).



446

Compound **445** (3.29 g, crude) and compound **85** (1.26 g, 2.9 mmol) were dissolved in tetrahydrofuran (50 mL) and stirred at 30 °C for 4 hours. The reaction mixture was concentrated under reduced pressure, and then the residue was purified by a silica gel column (0-8% MeOH/DCM) to give the title compound (1.80 g, 42% yield). MS-ESI (m/z): calcd for $C_{70}H_{109}N_8O_{23}$, $[M+2H]^{2+}$: 714.88; found 714.28.

Example 492. Synthesis of (R)-tert-butyl 5-(3-((37S,46S)-37-amino-46-benzyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**447**).

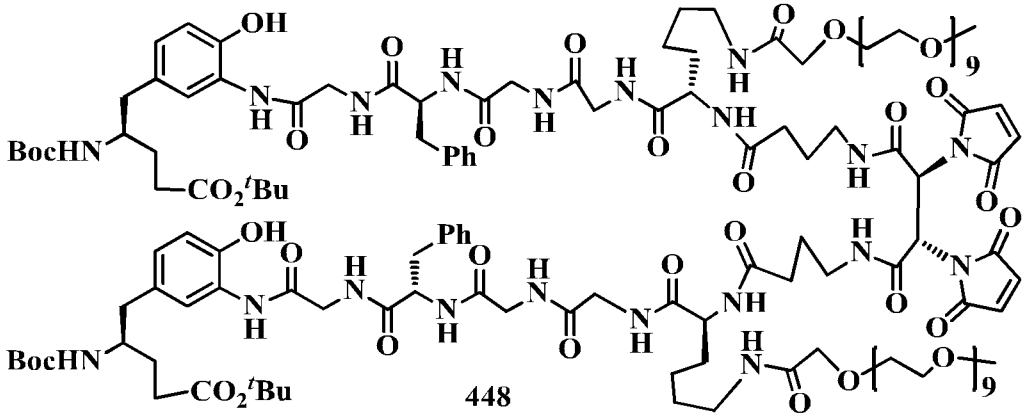


447

Compound **446** (1.80 g, 1.26 mmol) was dissolved in ethyl acetate (20 mL), 10 wt% palladium on carbon (0.1 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was

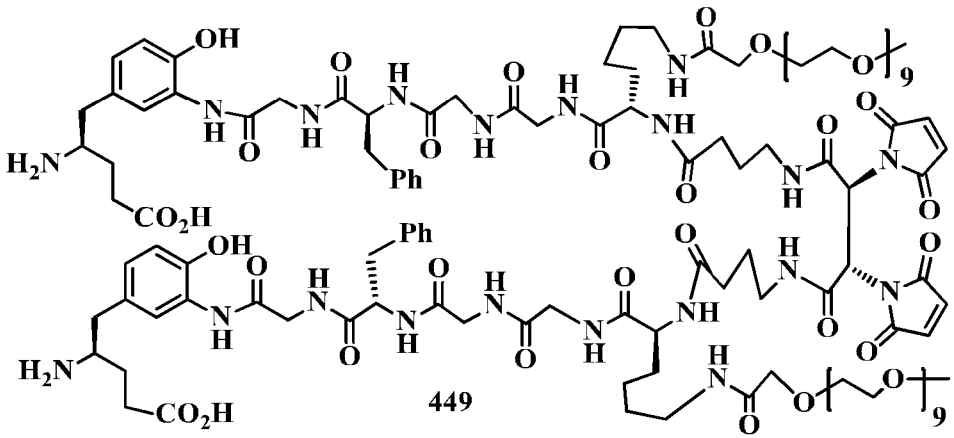
concentrated to give the title compound (2.01 g). MS-ESI (m/z): calcd for $C_{62}H_{103}N_8O_{21}$, $[M+2H]^{2+}$ 648.86; found, 648.68.

Example 493. Synthesis of di-tert-butyl 5,5'-((((5S,14S,22S,23S,31S,40S)-5,40-dibenzyl-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))(4R,4'R)-bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**448**).



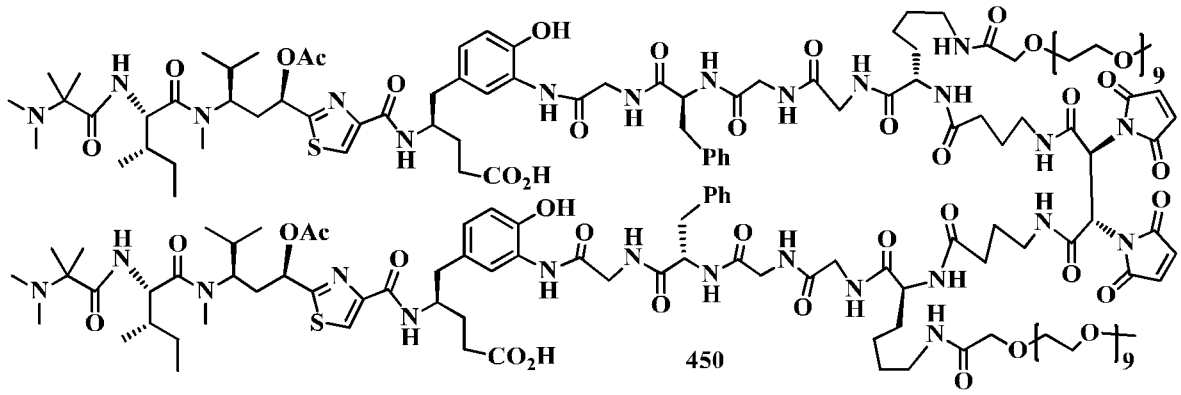
To a solution of compound **447** (2.01 g, crude) in dichloromethane (20 mL), compound **80** (0.36 g, 0.42 mmol) was added, followed by N,N-diisopropylethylamine (110 mg, 0.84 mmol). The reaction mixture was stirred at 35 °C for 2 hours, and then washed with water, brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (102 mg, 8% yield). MS-ESI (m/z): calcd for $C_{144}H_{223}N_{20}O_{50}$, $[M+3H]^{3+}$: 1011.85; found 1012.56.

Example 494. Synthesis of (4R,4'R)-5,5'-((((5S,14S,22S,23S,31S,40S)-5,40-dibenzyl-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontane-1,44-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**449**).



Compound **448** (0.093 g, 0.031 mmol) was dissolved in dichloromethane (1 mL) and treated with TFA (2 mL) at 30 °C for 10 min. The reaction was concentrated to give the title compound (86 mg), which was used directly in the next step. MS-ESI (m/z): calcd for $C_{134}H_{207}N_{20}O_{46}$, $[M + 3H]^{3+}$ 945.14; found, 945.48.

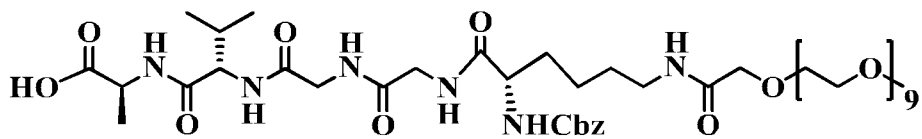
Example 495. Synthesis of compound **450.**



450

To a solution of compound **449** (86 mg, 0.031 mmol) and **Tub-1** (48 mg, 0.068 mmol) in DMF (2 mL), N,N-diisopropylethylamine (13 mg, 0.093 mmol) was added at 0 °C. The reaction was stirred for 2 hours and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (35 mg, 29% yield). MS-ESI (m/z): calcd. for $C_{176}H_{271}N_{28}O_{56}S_2$, $[M+3H]^{3+}$ 1246.62; found, 1246.59.

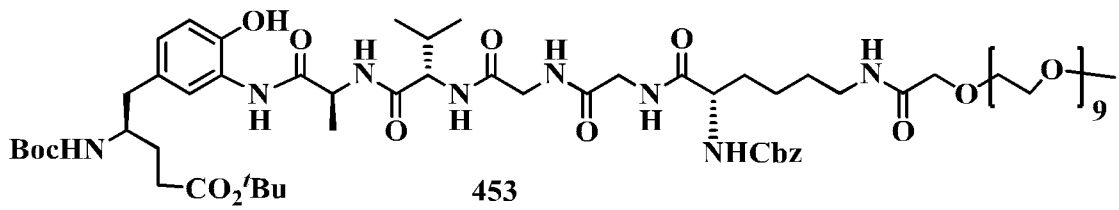
Example 496. Synthesis of (37S,46S,49S)-37-(((benzyloxy)carbonyl)amino)-46-isopropyl -49-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-oic acid (**452**).



452

Compound **451** (1.50 g, 1.38 mmol) was dissolved in dichloromethane (10 mL) and treated with TFA (5 mL) for 2 h, and then concentrated to give the title compound, which was used directly in the next step. MS-ESI (m/z): calcd for $C_{47}H_{80}N_6O_{19}$, $[M + H]^+$ 1033.55; found, 1033.91.

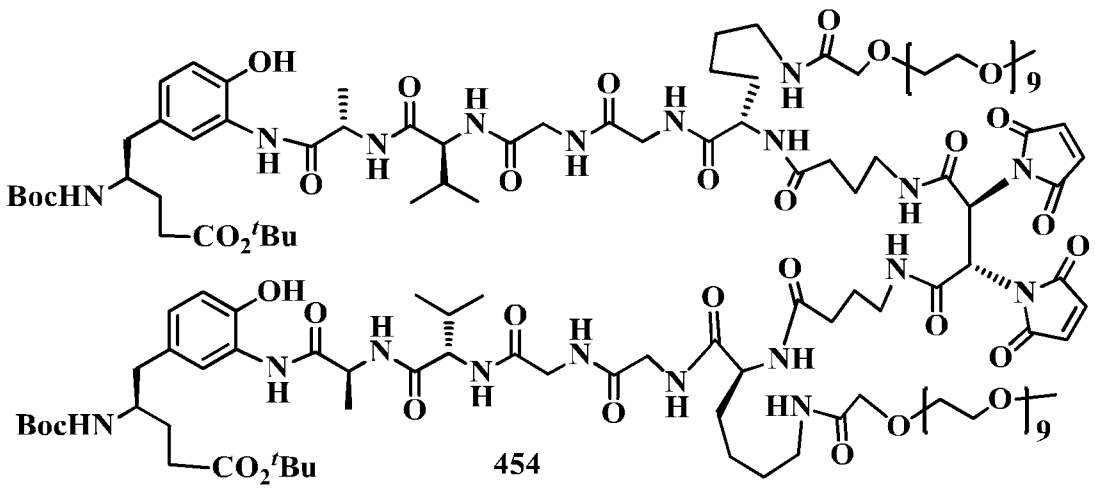
Example 497. Synthesis of (R)-tert-butyl 5-(3-((37S,46S,49S)-37-(((benzyloxy)carbonyl) amino)-46-isopropyl-49-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontanamido)-4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**453**).



453

To a solution of compound **452** (1.40 g, 1.36 mmol) in dichloromethane (30 mL), NHS (1.60 g, 14.6 mmol) and EDC·HCl (2.60 g, 14.6 mmol) were added at 0 °C, and the reaction was stirred at °C for 1 hour, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The obtained crude product was dissolved in tetrahydrofuran (20 mL) and compound **69** (1.00 g, 2.72 mmol) was added. The reaction solution was heated to 60 °C and stirred overnight, and then concentrated under reduced pressure. The residue was purified by a silica gel column (5-10% MeOH/DCM) to give a light brown oil (1.10 g, 58% yield). MS-ESI (m/z): calcd for $C_{67}H_{110}N_8O_{23}$, $[M+H]^+$: 1395.77; found 1396.10.

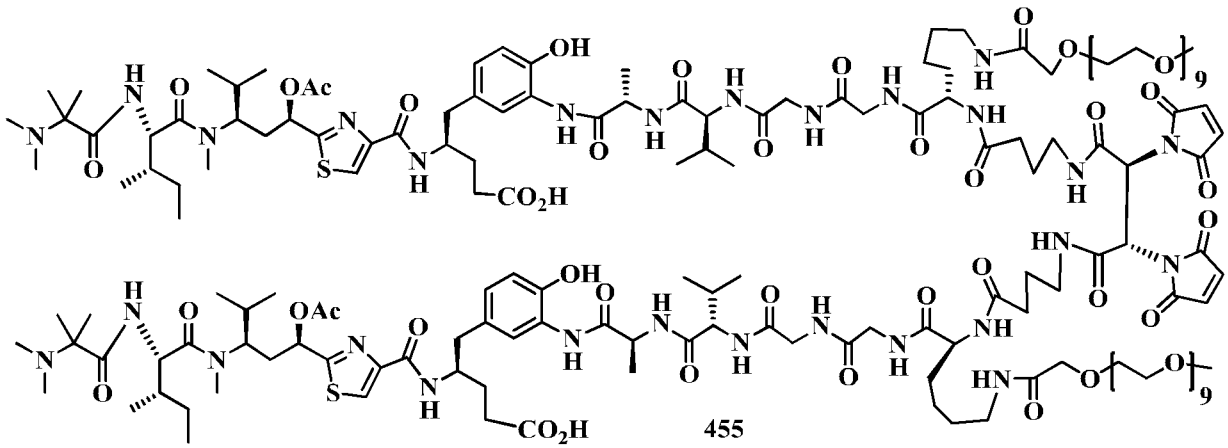
Example 498. Synthesis of (4R,4'R)-di-tert-butyl 5,5'-((((2S,5S,14S,22S,23S,31S,40S,43S)-22,23-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,40-diisopropyl-2,43-dimethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontane-1,44-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**454**).



454

Compound **453** (1.10 g, 0.79 mmol) was dissolved in isopropanol (10 mL), 10 wt% palladium on carbon (0.15 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring at 30 °C overnight, the reaction mixture was filtered and the filtrate was concentrated to a crude compound (0.91 g). The crude product was dissolved in DMF (10 mL) and stirred with compound **80** (0.18 g, 0.27 mmol) at 35 °C overnight. The reaction mixture was diluted with dichloromethane (50 mL), and washed with brine (4 × 50 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by a silica gel column (5-10% MeOH/DCM) to give the title compound (620 mg, 78% yield). MS-ESI (m/z): calcd for $C_{138}H_{226}N_{20}O_{50}$, $[M+3H]^{3+}$: 989.19; found 989.19.

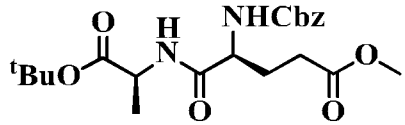
Example 499. Synthesis of compound **455.**



455

Compound **454** (0.62 g, 0.21 mmol) was dissolved in dichloromethane (10 mL) and treated with TFA (5 mL) at 30 °C for 2 hours. The reaction was concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give a white solid (240 mg), which was dissolved in DMF (3 mL). **Tub-1** (138 mg, 0.20 mmol) was then added to the solution and the mixture was cooled to 0 °C, N,N-diisopropylethylamine (30 mg, 0.23 mmol) was added. The reaction was stirred for 3 hours and then directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound as a white solid (64 mg, 19% yield). MS-ESI (m/z): calcd. for $C_{170}H_{274}N_{28}O_{56}S_2$, $[M+3H]^{3+}$ 1223.96; found, 1223.96.

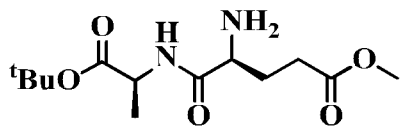
Example 500. Synthesis of (S)-methyl 4-(((benzyloxy)carbonyl)amino)-5-(((S)-1-(tert-butoxy) -1-oxopropan-2-yl)amino)-5-oxopentanoate (**456**).



456

To a solution of Cbz-Glu(OMe)-OH (5.00 g, 28 mmol) and H-Ala-O^{t}Bu (8.13 g, 28 mmol) in dichloromethane (100 mL), EDC·HCl (6.40 g, 33 mmol) and N,N-diisopropylethylamine (13.6 mL, 83 mmol) were added. The reaction was stirred for 10 min, washed with water (100 mL), 1 N HCl (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by a silica gel column (40% ethyl acetate/petroleum ether) to give the title compound (5.04 g, 43% yield). MS-ESI (m/z): calcd for $C_{21}H_{30}N_2O_7$, $[M+H]^+$ 423.21; found 423.21.

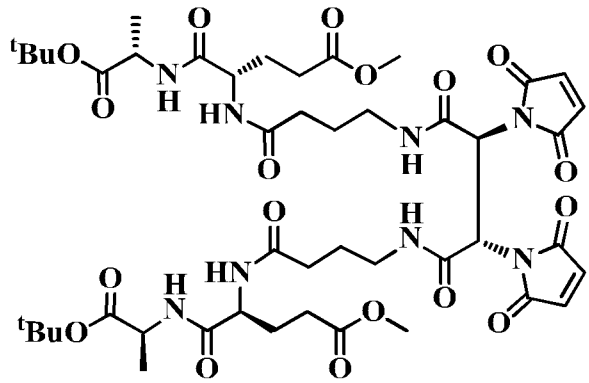
Example 501. Synthesis of (S)-methyl 4-amino-5-(((S)-1-(tert-butoxy)-1-oxopropan-2-yl) amino)-5-oxopentanoate (**457**).



457

Compound **456** (5.04 g, 12.0 mmol) was dissolved in methanol (100 mL), 10 wt% palladium on carbon (0.50 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (3.22 g, 94% yield). MS-ESI (m/z): calcd for $C_{13}H_{24}N_2O_5$, $[M+H]^+$ 289.17; found, 289.17.

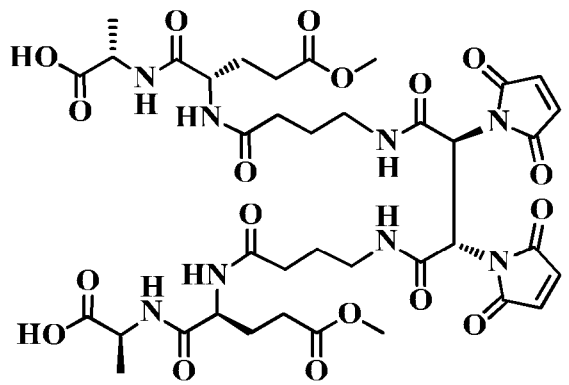
Example 502. Synthesis of (2S,5S,13S,14S,22S,25S)-di-tert-butyl 13,14-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,22-bis(3-methoxy-3-oxopropyl)-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-3,6,11,16,21,24-hexaazahexacosane-1,26-dioate (**458**).



458

To a solution of compound **457** (2.00 g, 7.0 mol) and compound **19** (1.58 g, 3.0 mmol) in dichloromethane (50 mL) and DMF (5 mL), HATU (2.76 g, 7.0 mmol) was added, followed by N,N-diisopropylethylamine (2.2 mL, 13.0 mmol). The reaction was stirred at r.t. for 30 min, washed with water (100 mL) and brine (100 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (5.90 g). MS-ESI (m/z): calcd. for $C_{46}H_{66}N_8O_{18}$, $[M+H]^+$ 1019.45; found, 1019.45.

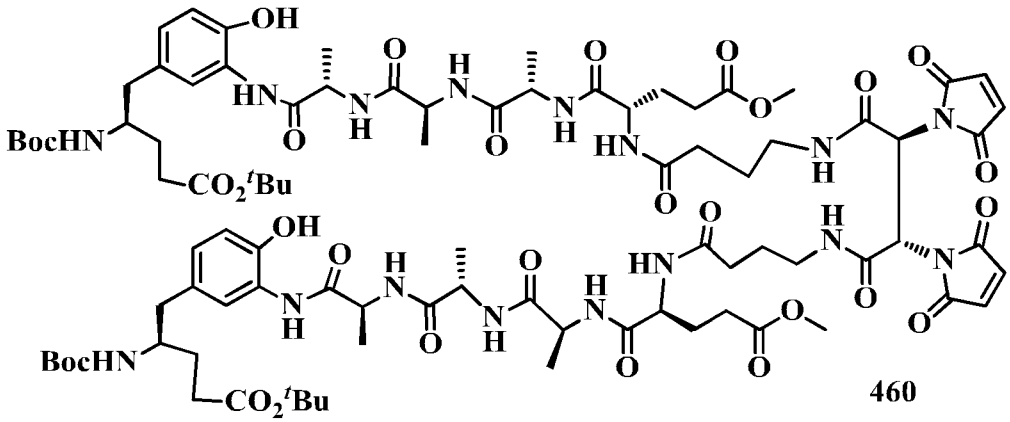
Example 503. Synthesis of (2S,5S,13S,14S,22S,25S)-13,14-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,22-bis(3-methoxy-3-oxopropyl)-2,25-dimethyl-4,7,12,15,20,23-hexaoxo-3,6,11,16,21,24-hexaazahexacosane-1,26-dioic acid (**459**).



459

A solution of compound **458** (3.37 g, 3.0 mmol) in DCM (10 mL) and TFA (10 mL) was stirred at r.t. for about 1 hour and concentrated to give the title compound (1.59 g, 53% yield). MS-ESI (m/z): calcd. for $C_{38}H_{50}N_8O_{18}$, $[M+H]^+$ 907.32; found, 907.32.

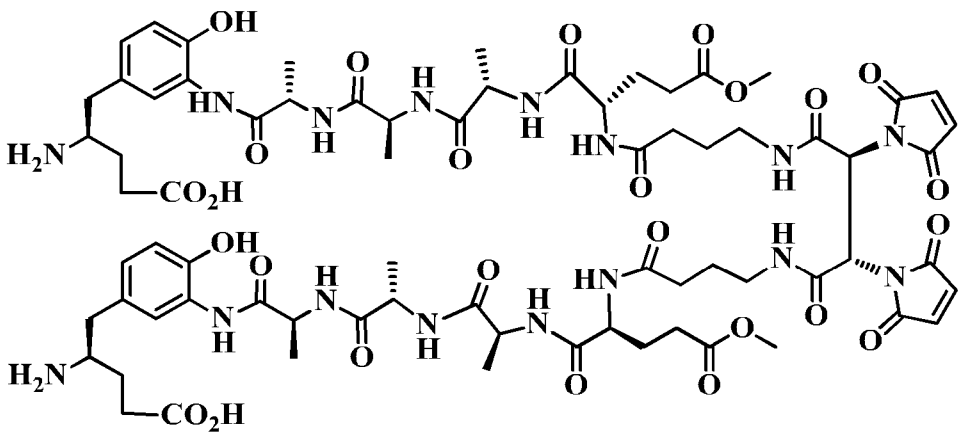
Example 504. Synthesis of (4S,12S,13S,21S)-dimethyl 4,21-bis(((S)-1-(((S)-1-(((S)-1-((5-((R)-5-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-5-oxopentyl)-2-hydroxyphenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-12,13-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,11,14,19-tetraoxo-5,10,15,20-tetraazatetracosane-1,24-dioate (**460**).



460

Compound **459** (0.33 g, 0.40 mmol) and compound **117** (0.58 g, 1.10 mmol) were dissolved in dichloromethane (80 mL) and DMF (4 mL). To which, EDC·HCl (0.35 g, 1.80 mmol) was added, and the reaction was stirred for 2 hours. The solvent was removed and the residue was purified by preparative HPLC (acetonitrile/water containing 0.1% HCO_2H) to give the title compound (64 mg, 9% yield). MS-ESI (m/z): calcd. for $C_{90}H_{130}N_{16}O_{30}$, $[M+H]^+$1915.91; found, 1915.91.

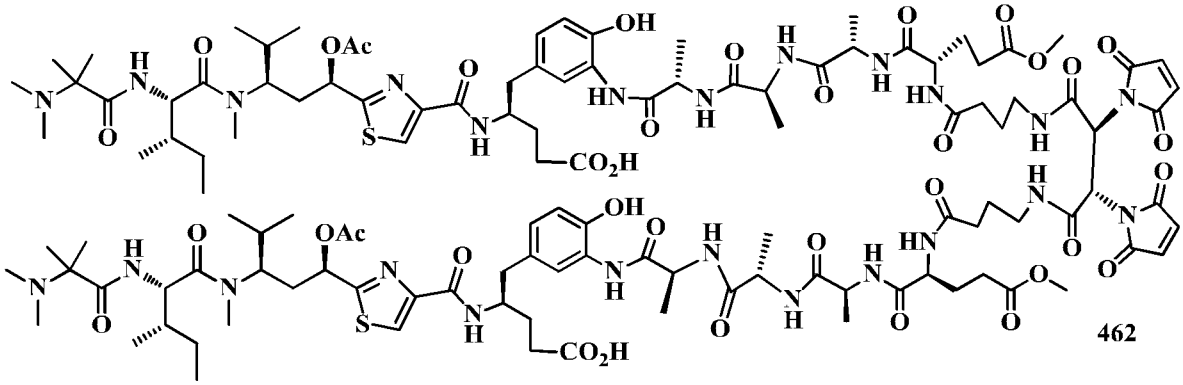
Example 505. Synthesis of (4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-11,28-bis(3-methoxy-3-oxopropyl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontane-1,38-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-aminopentanoic acid) (**461**).



461

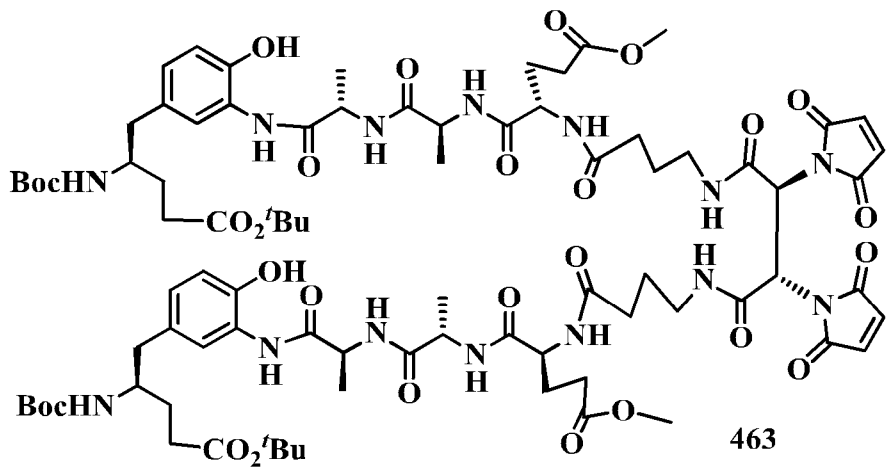
A solution of compound **460** (64 mg, 0.03 mmol) in DCM (1 mL) and TFA (3 mL) was stirred at r.t. for about 30 min and concentrated to give the title compound (50 mg). MS-ESI (m/z): calcd. for $C_{72}H_{98}N_{16}O_{26}$, $[M+H]^+$ 1603.68; found, 1603.68.

Example 506. Synthesis of (R,R,S,S,4R,4'R)-5,5'-((((2S,5S,8S,11S,19S,20S,28S,31S,34S,37S)-19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-11,28-bis(3-methoxy-3-oxopropyl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontane-1,38-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**462**).



To a solution of compound **461** (0.050 g, 0.03 mmol) and **Tub-1** (0.043 g, 0.06 mmol) in DMF (2mL), N,N-diisopropylethylamine (21 μL, 0.12 mmol) was added at 0 °C. The reaction was stirred at r.t. for 1 hour and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (24 mg, 29% yield). MS-ESI (m/z): calcd. for $C_{122}H_{178}N_{24}O_{36}S_2$, $[M+2H]^{2+}$ 1310.81; found, 1310.81.

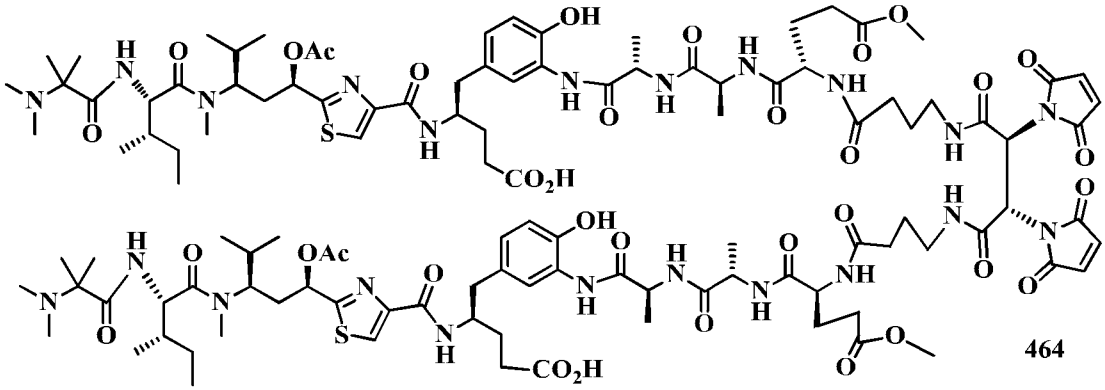
Example 507. Synthesis of (4S,12S,13S,21S)-dimethyl 4,21-bis(((S)-1-(((S)-1-((5-((R)-5 -(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-5-oxopentyl)-2-hydroxyphenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-12,13-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,11,14,19-tetraoxo-5,10,15,20-tetraazatetracosane-1,24-dioate (**463**).



Compound **459** (0.37 g, 0.80 mmol) and compound **99** (0.34 g, 0.40 mmol) were dissolved in dichloromethane (100 mL) and DMF (5 mL). To which, EDC·HCl (0.28 g, 1.50 mmol) was added, and the reaction was stirred for 6 hours. The reaction mixture was washed with water (100 mL) and

brine (100 mL), dried and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% HCO_2H) to give the title compound (100 mg, 15% yield). MS-ESI (m/z): calcd. for $C_{84}H_{120}N_{14}O_{28}$, $[M+H]^+$1773.84; found, 1773.84.

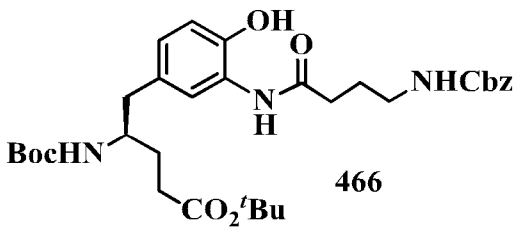
Example 508. Synthesis of (R,R,S,S,4R,4'R)-5,5'-((((2S,5S,8S,16S,17S,25S,28S,31S)-16,17 -bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-8,25-bis(3-methoxy-3-oxopropyl)-2,5,28,31-tetramethyl-4,7,10,15,18,23,26,29-octaoxo-3,6,9,14,19,24,27,30-octaazadotriacontane-1,32-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-(2-((6S,9R,11R)-6-((S)-sec-butyl)-9-isopropyl-2,3,3,8-tetramethyl-4,7,13-trioxo-12-oxa-2,5,8-triazatetradecan-11-yl)thiazole-4-carboxamido)pentanoic acid) (**464**).



464

A solution of compound **463** (100 mg, 0.056 mmol) in DCM (1 mL) and TFA (3 mL) was stirred at r.t. for about 30 min and concentrated to give an amino compound (80 mg). To a solution of the amino compound **461** (0.080 g, crude) and **Tub-1** (0.076 g, 0.109 mmol) in DMF (2mL), N,N-diisopropylethylamine (36 μL, 0.219 mmol) was added at 0 °C. The reaction was stirred at r.t. for 3 hours and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (92 mg, 68% yield). MS-ESI (m/z): calcd. for $C_{116}H_{168}N_{22}O_{34}S_2$, $[M+2H]^{2+}$ 1240.08; found, 1240.20.

Example 509. Synthesis of (R)-tert-butyl 5-(3-(4-(((benzyloxy)carbonyl)amino)butanamido) -4-hydroxyphenyl)-4-((tert-butoxycarbonyl)amino)pentanoate (**464**).

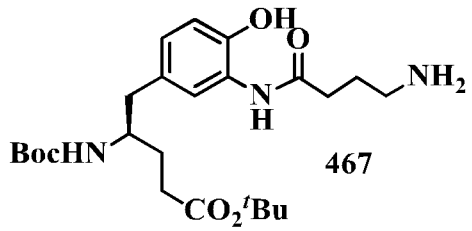


466

To a solution of compound **69** (1.00 g, 2.6 mmol) and 4-(((benzyloxy)carbonyl)amino)butanoic acid (0.62 g, 2.6 mmol) in dichloromethane (10 mL), HATU (1.20 g, 3.2 mmol) and N,N-diisopropylethylamine (0.87 mL, 5.3 mmol) were added. The reaction was stirred for 1 hour, washed with water (10 mL), 1 N HCl (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate,

filtered and concentrated. The residue was purified by a silica gel column (5% MeOH/DCM) to give the title compound (1.70 g). MS-ESI (m/z): calcd for $C_{32}H_{45}N_3O_8$, $[M+H]^+$ 600.32; found 600.32.

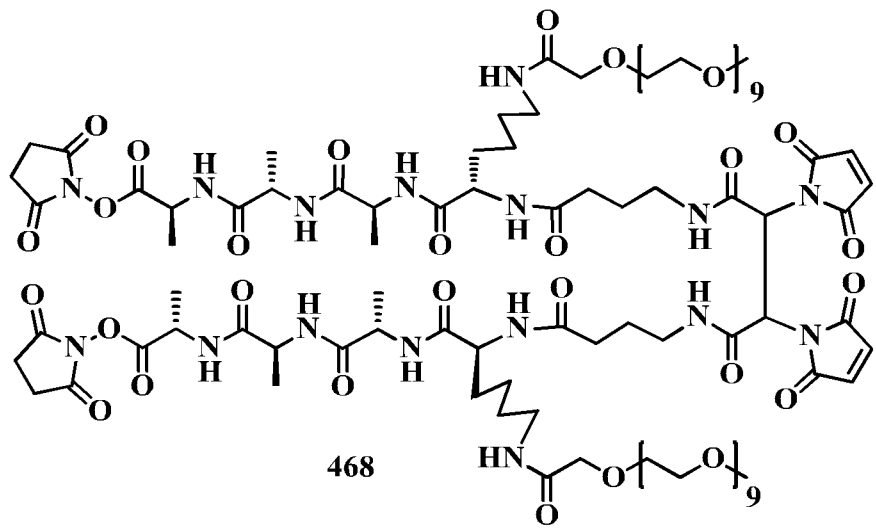
Example 510. Synthesis of (R)-tert-butyl 5-(3-(4-aminobutanamido)-4-hydroxyphenyl)- 4-((tert-butoxycarbonyl)amino)pentanoate (**467**).



467

Compound **466** (0.80 g, 1.30 mmol) was dissolved in methanol (20 mL), 10 wt% palladium on carbon (0.20 g) was added, and the reaction flask was evacuated and back-filled with hydrogen for three times. After stirring for 2 hours, the reaction mixture was filtered and the filtrate was concentrated to give the title compound (0.60 g, 97% yield). MS-ESI (m/z): calcd for $C_{24}H_{39}N_3O_6$, $[M+H]^+$ 466.28; found, 466.28.

Example 511. Synthesis of (2S,5S,8S,11S,28S,31S,34S,37S)-bis(2,5-dioxopyrrolidin-1-yl) 19,20-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,31,34,37-hexamethyl-4,7,10,13,18,21,26,29,32,35-decaoxo-11,28-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-3,6,9,12,17,22,27,30,33,36-decaazaoctatriacontane-1,38-dioate (**468**).

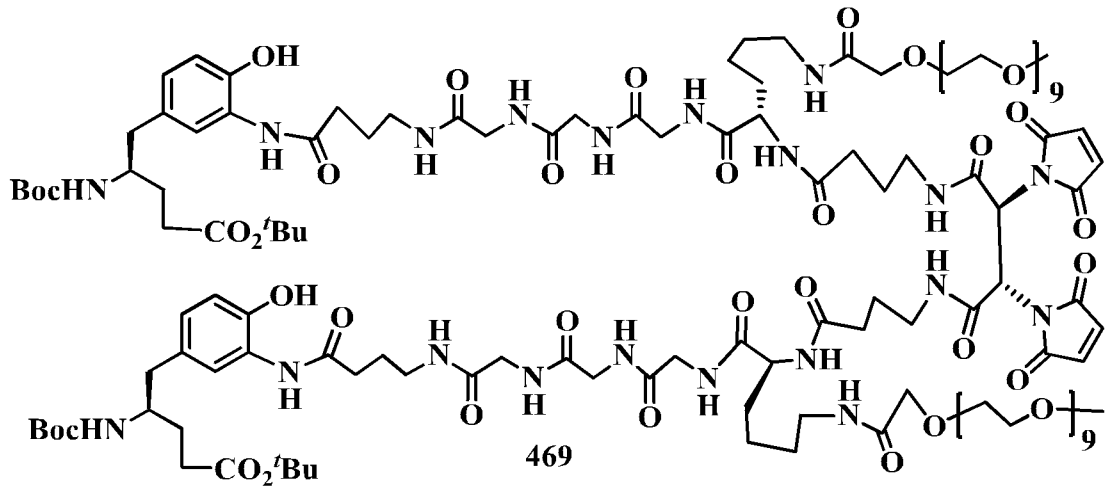


468

To a solution of compound **467** (1.01 g, 0.50 mmol) in dichloromethane (30 mL), NHS (0.12 g, 1.0 mmol) and EDC·HCl (0.37 g, 2.0 mmol) were added, and the reaction was stirred for 4 hours. The reaction mixture was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give the title compound (1.09 g). MS-ESI (m/z): calcd. for $C_{100}H_{162}N_{16}O_{44}$, $[M+2H]^{2+}$1147.04; found, 1147.14.

Example 512. Synthesis of (4R,4'R)-di-tert-butyl 5,5'-((((16S,24S,25S,33S)-24,25-bis (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,9,12,15,18,23,26,31,34,37,40,43-dodecaoxo-16,33-bis(31-oxo-

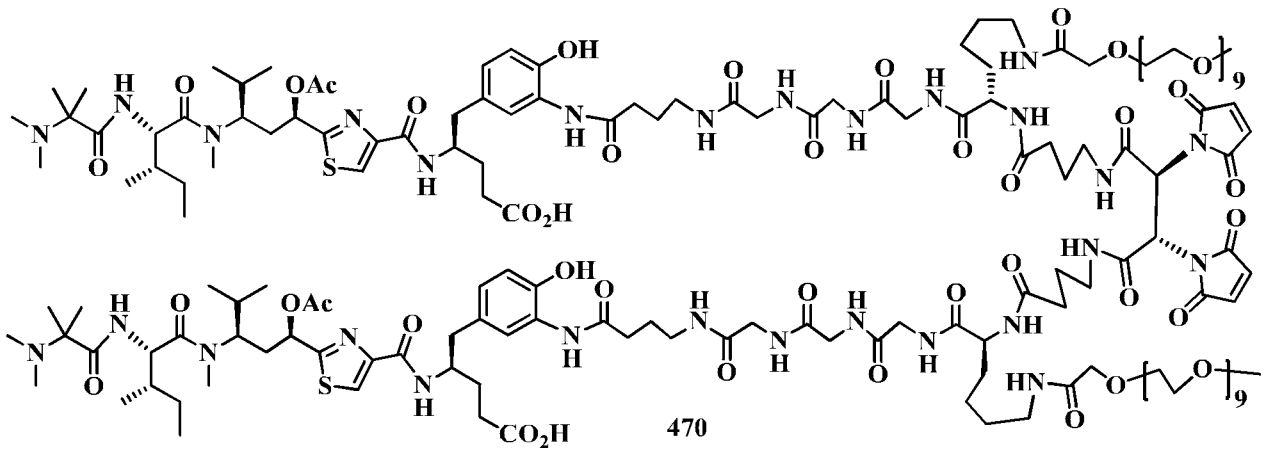
2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-5,8,11,14,17,22,27,32,35,38,41,44-dodecaazaoctatetracontane-1,48-dioyl)bis(azanediyl))bis(4-hydroxy-3,1-phenylene))bis(4-((tert-butoxycarbonyl)amino)pentanoate) (**469**).



469

Compound **467** (0.49 g, 1.0 mmol) and compound **468** (1.09 g, crude) were dissolved in tetrahydrofuran (20 mL) and stirred at 45 °C for 16 hours. The reaction mixture was concentrated under reduced pressure, and then the residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (99 mg, 7% yield). MS-ESI (m/z): calcd for $C_{140}H_{230}N_{20}O_{50}$, $[M+2H]^{2+}$: 1496.80; found 1496.80.

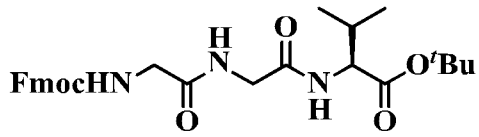
Example 513. Synthesis of compound **470.**



470

Compound **469** (0.099 g, 0.03 mmol) was dissolved in dichloromethane (3 mL) and treated with TFA (3 mL) at r.t. for 1 hour. The reaction mixture was concentrated to a crude product, which was used directly in the next step. To a solution of above crude product in DMF (1 mL), **Tub-1** (50 mg, 0.07 mmol) and N,N-diisopropylethylamine (22 μL, 0.13 mmol) were added. The reaction was stirred for 2 hours and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (42 mg, 35% yield). MS-ESI (m/z): calcd. for $C_{172}H_{278}N_{28}O_{56}S_2$, $[M+3H]^{3+}$ 1232.97; found, 1232.97.

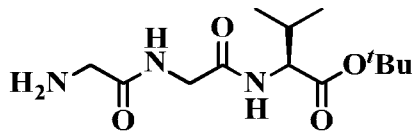
Example 514. Synthesis of (S)-tert-butyl 1-(9H-fluoren-9-yl)-11-isopropyl-3,6,9-trioxo- 2-oxa-4,7,10-triazadodecan-12-oate (**472**).



472

To a solution of Fmoc-Gly-Gly-OH (2.0 g, 11.5 mmol) in 5 mL of DMF, were added HATU (6.6 g, 17.3 mmol) and N,N-diisopropylethylamine (5.7 mL, 34.6 mmol). After stirring at r.t. for 10 min, H-Val-O^{t}Bu (2.4 g, 11.5 mmol) was added and the reaction was stirred for 15 min, then quenched with water (100 mL), extracted with ethyl acetate (3 × 50 mL). The combined organic phase was washed with water (50 mL), 2 N HCl (50 mL) and brine (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was triturated with petroleum ether/ethyl acetate (1:1) to give a white solid (3.0 g, 51% yield). MS-ESI (m/z): calcd. for $C_{28}H_{35}N_3O_6$ $[M+H]^+$ 510.60; found, 510.60.

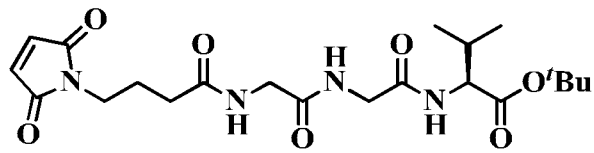
Example 515. Synthesis of (S)-tert-butyl 2-(2-(2-aminoacetamido)acetamido)-3-methyl butanoate (**473**).



473

A solution of compound **472** (3.0 g) in DMF (25 mL) and was stirred with piperidine (5 mL) at r.t. for 15 min and concentrated under vacuum to give a white solid, which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{13}H_{25}N_3O_4$ $[M+H]^+$ 288.36; found, 288.36.

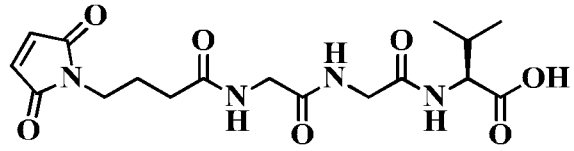
Example 516. Synthesis of (S)-tert-butyl 2-(2-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)acetamido)acetamido)-3-methylbutanoate (**474**).



474

Compound **473** (1.7 g, 5.8 mmol) was dissolved in DMF (30 mL) and cooled to 0 °C, N,N-diisopropylethylamine (1.1 mL) and 4-maleimidobutyric acid N-hydroxysuccinimide ester (2.0 g, 7.0 mmol) were added. The reaction was stirred at r.t. for 15 min and quenched with water, extracted with dichloromethane (3 × 100 mL), and the combined organic phase was washed with 2 N HCl (50 mL) and water (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was triturated with petroleum ether/ethyl acetate to give a white solid (2.66 g, 100% yield). MS-ESI (m/z): calcd. for $C_{21}H_{32}N_4O_7$ $[M+H]^+$ 453.51; found, 453.51.

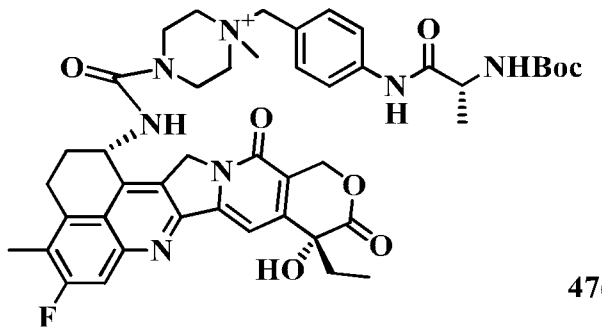
Example 517. Synthesis of (S)-2-(2-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido) acetamido)acetamido)-3-methylbutanoic acid (**475**).



475

Compound **474** (650 mg) was dissolved in formic acid (8 mL) and dichloromethane (4 mL), and then heated to 60 °C, stirred for 4 hours. The reaction was then concentrated, during which a white precipitate formed. Trituration with petroleum ether/ethyl acetate (1:1, 10 mL) gave the title compound (0.45 g, 78% yield). MS-ESI (m/z): calcd. for $C_{17}H_{24}N_4O_7$ $[M+H]^+$ 397.40; found, 397.40.

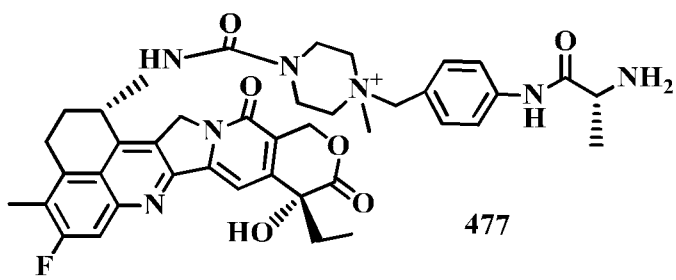
Example 518. Synthesis of 1-(4-((R)-2-((tert-butoxycarbonyl)amino)propanamido)benzyl) -4-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)-1-methylpiperazin-1-ium (**476**).



476

To a solution of exatecan mesylate (264 mg, 0.49 mmol) and N,N-diisopropylethylamine (246μL, 1.49 mmol) in DMF (5 mL), methyl (4-nitrophenyl) carbonate (100 mg, 0.49 mmol) was added at 0 °C. After the reaction was stirred for about 10 min, compound **165** (187 mg, 0.49 mmol) was added and stirred for 15 min. Ethyl acetate was added to the reaction mixture and a white solid crushed out, which was collected by filtrateion to give the title compound **476** (420 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{45}H_{53}FN_7O_8$, $[M]^+$ 838.96; found, 838.96.

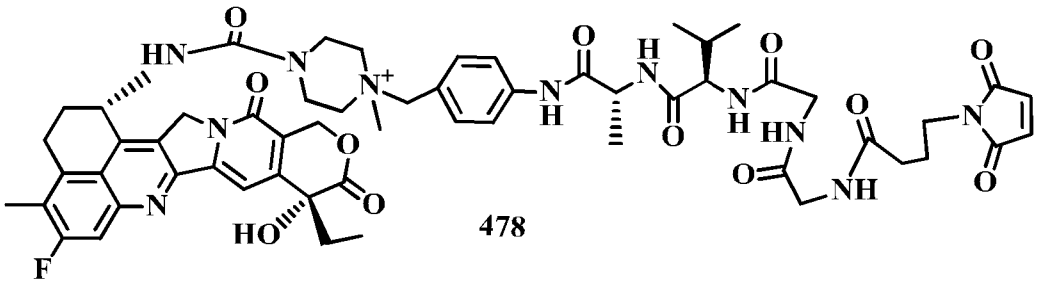
Example 519. Synthesis of 1-(4-((R)-2-aminopropanamido)benzyl)-4-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)-1-methylpiperazin-1-ium (**477**).



477

A solution of compound **476** (0.33 g) in dichloromethane (9 mL) and TFA (3 mL) was stirred at r.t. for 30 min and concentrated under vacuum to give the title compound, which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{40}H_{45}FN_7O_6$, $[M]^+$ 738.84; found, 738.84.

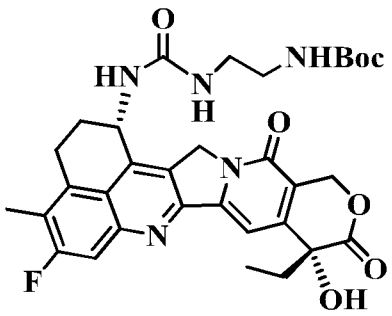
Example 520. Synthesis of 1-(4-((2R,5R)-16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazahexadecanamido)benzyl)-4-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)-1-methylpiperazin-1-ium (**478**).



478

To a solution of compound **475** (155 mg, 0.39 mmol) in DMF (5 mL) were added HATU (224 mg, 0.59 mmol) and N,N-diisopropylethylamine (195 μL, 1.18 mmol), and the reaction was stirred for 15 min. A solution of compound 477 (290 mg, 0.39 mmol) in DMF (5 mL) was added and stirred for another 15 min. The reaction mixture was dissolved in 80% water/acetonitrile and filtered. The filtrate was purified by preparative HPLC to give the title compound (43 mg, 9% yield). MS-ESI (m/z): calcd. for $C_{57}H_{67}FN_{11}O_{12}$, $[M]^{3+}$ 1117.23; found, 1117.23.

Example 521. Synthesis of tert-butyl (2-(3-((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)ureido)ethyl)carbamate (**479**).

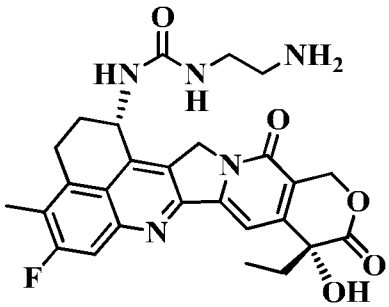


479

To a solution of tert-butyl (2-aminoethyl)carbamate (0.16 g, 1.0 mmol) in THF (20 mL), N,N-diisopropylethylamine (0.51 g, 4.0 mmol) and 4-nitrophenyl carbonochloridate (0.20 g, 1.0 mmol) were added over ice-water bath and stirred for 3 hours. THF was then evaporated under vacuum. To the residue, exatecan mesylate (0.53 g, 1.0 mmol) and N,N-diisopropylethylamine (0.31 g, 2.4 mmol) in DMF (5 mL) was added. The reaction was stirred at r.t. for 3 hours and diluted with dichloromethane, washed with water. The aqueous layer was extracted with dichloromethane twice.

The combined organic phase was washed with brine, water, dried over sodium sulfate, filtered and concentrated. The residue was purified by a silica gel column (ethyl acetate then 0-10% MeOH/dichloromethane) to give the title compound (0.49 g, 79% yield).

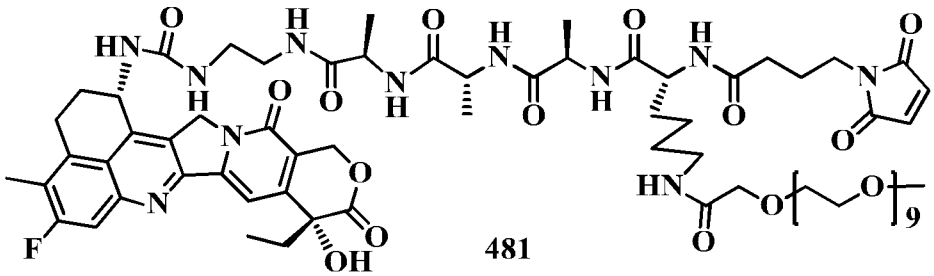
Example 522. Synthesis of 1-(2-aminoethyl)-3-((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl -10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)urea (**480**).



480

Compound **479** (100 mg, 0.161 mmol) in DCM (6 mL) and TFA (1 mL) was stirred at r.t. for about 1 hour and concentrated to give a light yellow solid (113 mg, 100% yield). MS-ESI (m/z): calcd. for $C_{27}H_{29}FN_5O_5$, $[M+H]^+$ 522.21; found, 522.21.

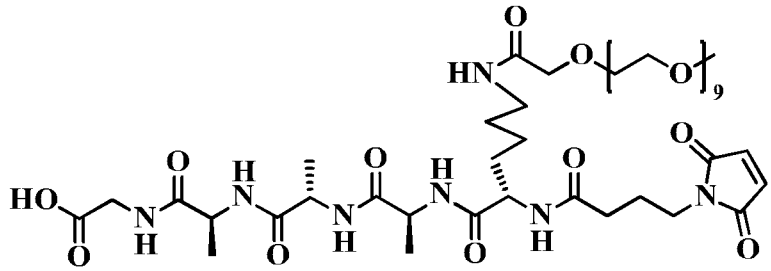
Example 523. Synthesis of N-((7R,10R,13R,16R)-16-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-1-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-7,10,13-trimethyl-1,6,9,12,15-pentaoxo-2,5,8,11,14-pentaazaicosan-20-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**481**).



481

Compound **480** (69 mg, 0.132 mmol) and compound **217** (137 mg, 0.132 mmol) were dissolved in DMF (6 mL) and cooled to about 0 °C. HATU (60 mg, 0.158 mmol) and N,N-diisopropylethylamine (126 mg, 0.974 mmol) were added, and the reaction was warmed to r.t. and stirred for 2 hours. The solvent was removed and the residue was purified by preparative HPLC to give the title compound (43 mg, 21% yield). MS-ESI (m/z): calcd. for $C_{71}H_{103}FN_{11}O_{23}$, $[M+H]^+$1496.71; found, 1496.71.

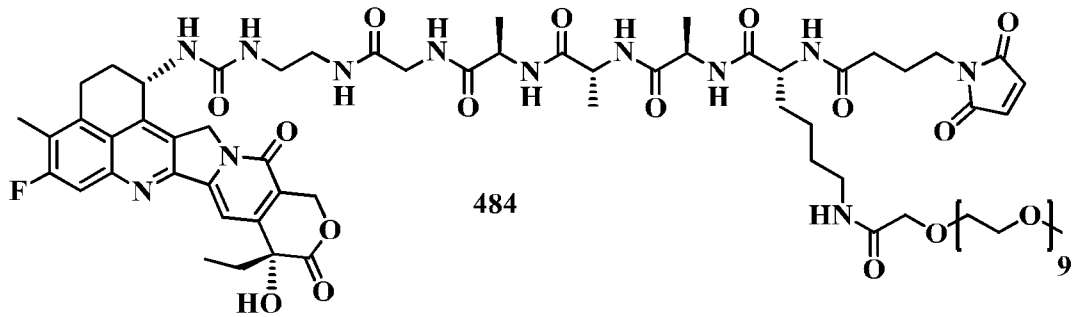
Example 524. Synthesis of (37S,40S,43S,46S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-oic acid (**483**).



483

Compound **482** (0.66 g, 0.60 mmol) was dissolved in dichloromethane (5 mL) and formic acid (20 mL), stirred at 50 °C for 1 h, concentrated to dryness, and purified by preparative HPLC to give the title compound (228 mg, 36% yield). MS-ESI (m/z): calcd. for $C_{46}H_{79}N_7O_{20}$, $[M+H]^+$ 1050.54; found, 1050.54.

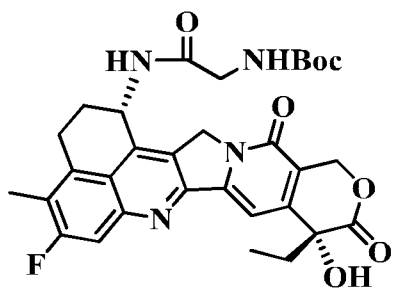
Example 525. Synthesis of N-((10R,13R,16R,19R)-19-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol- 1-yl)butanamido)-1-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-10,13,16-trimethyl-1,6,9,12,15,18-hexaoxo-2,5,8,11,14,17-hexaazatricosan-23-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**484**).



484

To a solution of compound **480** (50.0 mg, 0.096 mmol) and compound **483** (101 mg, 0.096 mmol) in DMF (1 mL), HATU (43.7 mg, 0.115 mmol) and N,N-diisopropylethylamine (24 μL, 0.144 mmol) were added. The reaction was stirred at r.t. for 15 min and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (12 mg, 8% yield). MS-ESI (m/z): calcd. for $C_{73}H_{105}FN_{12}O_{24}$, $[M+H]^+$ 1553.73; found, 1553.73.

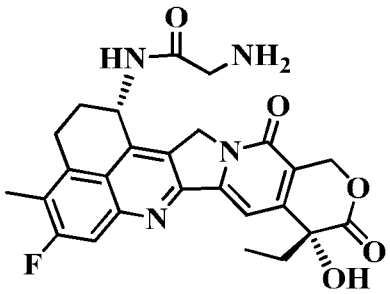
Example 526. Synthesis of tert-butyl (2-(((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)carbamate (**485**).



485

To a solution of exatecan mesylate (1.00 g, 1.88 mmol) in DMF (5 mL) were added Boc-Gly-OH (0.40 g, 2.26 mmol), HATU (1.10 g, 2.82 mmol) and N,N-diisopropylethylamine (0.90 mL, 5.64 mmol), and the reaction was stirred at r.t. for 1 hour. The reaction mixture was quenched with water (30 mL) and filtered. The solid was collected and vacuum dried to give the title compound (0.63 g, 65% yield). MS-ESI (m/z): calcd. for $C_{31}H_{33}FN_4O_7$, $[M+H]^+$ 593.62; found, 595.62.

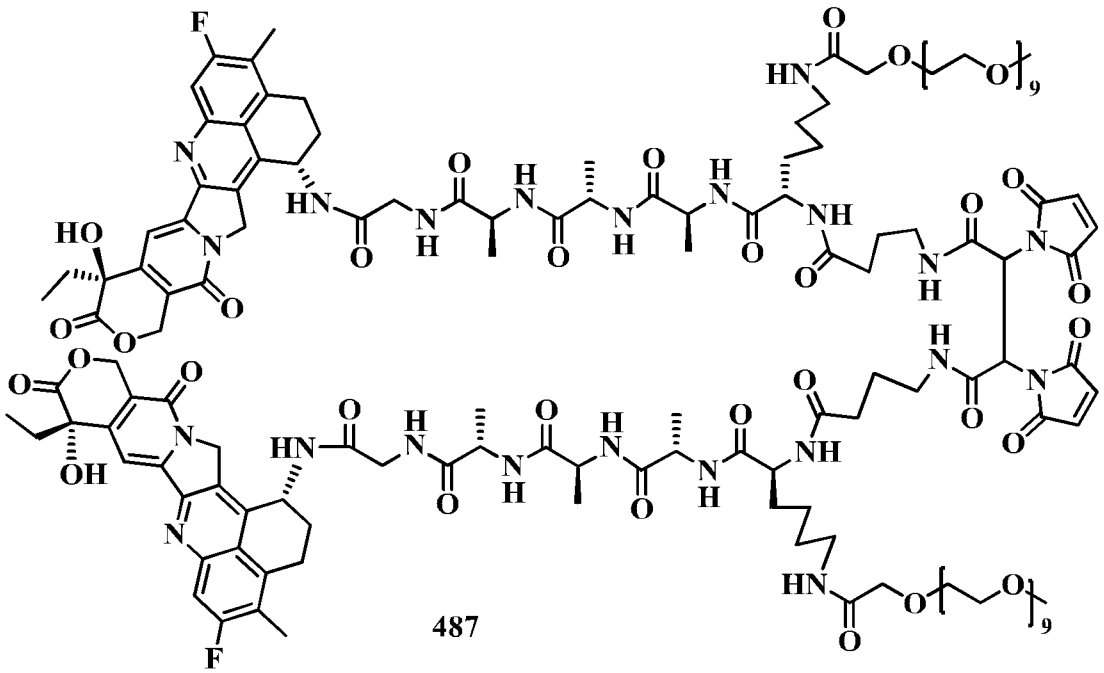
Example 527. Synthesis of 2-amino-N-((1S,9R)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (**486**).



486

Compound **485** (630 mg) was treated with TFA (3 mL) and dichloromethane (9 mL) at r.t. for 1 hour, and then concentrated. The residue was triturated with petroleum ether/ethyl acetate (1:1, 20 mL) to give the title compound (0.40 g, 77% yield). MS-ESI (m/z): calcd. for $C_{26}H_{25}FN_4O_5$ $[M+H]^+$ 493.51; found, 493.51.

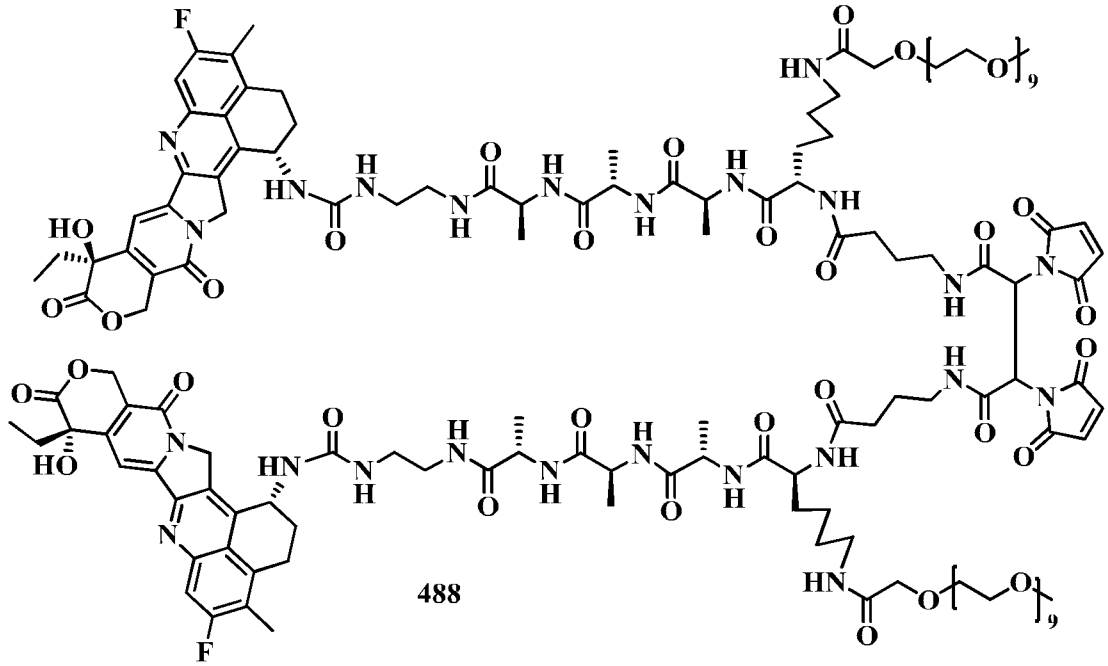
Example 528. Synthesis of 2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1-((S)-37-(((S)-1-(((S)-1-(((S)-1-((2-(((1R,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)-N4-((S)-37-(((S)-1-(((S)-1-(((S)-1-((2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**487**).



487

To a solution of compound **486** (94 mg, 0.191 mmol) and compound **139** (101 mg, 0.096 mmol) in DMF (5 mL), HATU (90 mg, 0.238 mmol) and N,N-diisopropylethylamine (61 mg, 0.477 mmol) were added. The reaction was stirred at r.t. for 2 hours, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (136 mg, 47% yield). MS-ESI (m/z): calcd. for $C_{144}H_{202}F_2N_{22}O_{48}$, $[M+3H]^{3+}$ 1017.10; found, 1017.55.

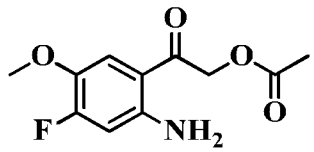
Example 529. Synthesis of 2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1-((S)-37-(((7S,10S,13S)-1-(((1R,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-7,10-dimethyl-1,6,9,12-tetraoxo-2,5,8,11-tetraazatetradecan-13-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)-N4-((S)-37-(((7S,10S,13S)-1-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-7,10-dimethyl-1,6,9,12-tetraoxo-2,5,8,11-tetraazatetradecan-13-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**488**).



488

To a solution of compound **139** (0.31 g, 0.15 mol) in DMF (2 mL), HATU (141 mg, 0.37 mmol) was added, followed by compound **480** (0.17 g, 0.33 mmol) in DMF (1 mL) and N,N-diisopropylethylamine (122 μL, 0.74 mmol). The reaction was stirred at r.t. for 1 hour, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (38 mg, 8% yield). MS-ESI (m/z): calcd. for $C_{146}H_{208}F_2N_{24}O_{48}$, $[M+2H]^{2+}$ 1552.72; found, 1552.72.

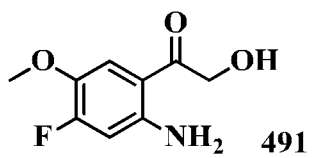
Example 530. Synthesis of 2-(2-amino-4-fluoro-5-methoxyphenyl)-2-oxoethyl acetate (**490**).



490

A solution of 1-(2-amino-4-fluoro-5-methoxyphenyl)-2-chloroethanone (20.0 g, 92 mmol) and sodium acetate (7.54 g, 92 mmol) in DMF (200 mL) was stirred at r.t. overnight. The reaction was quenched with water (400 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic phase was washed with water (200 mL), and brine (200 mL), dried over sodium sulfate, filtered and concentrated. The residue was triturated with petroleum ether (100 mL) and then filtered to give a yellow solid (20.8 g, 94% yield). MS-ESI (m/z): calcd. for $C_{11}H_{12}FNO_4$, $[M+H]^+$ 242.08; found, 242.08.

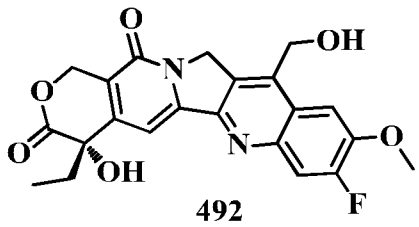
Example 531. Synthesis of 1-(2-amino-4-fluoro-5-methoxyphenyl)-2-hydroxyethanone (**491**).



491

A solution of compound **490** (0.57 g, 2 mmol) dissolved in 6 N HCl (10 mL) was stirred at 65 °C for 2 hours and cooled to r.t. The reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phase was dried over sodium sulfate, filtered and concentrated to give the title compound (0.40 g, 85% yield). MS-ESI (m/z): calcd. for $C_9H_{10}FNO_3$, $[M+H]^+$ 200.06; found, 200.06.

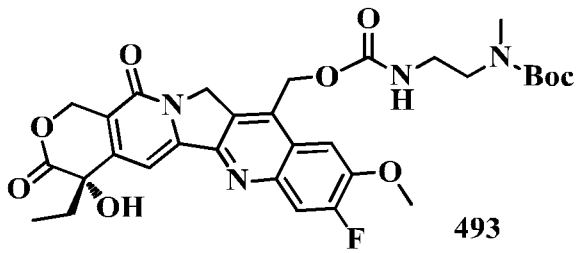
Example 532. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-(hydroxymethyl)-9-methoxy -1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione (**492**).



492

Compound **491** (0.40 g, 2.0 mmol) and compound **159** (0.53 g, 2.0 mmol) were dissolved in anhydrous toluene (15 mL), and *p*-toluenesulfonic acid (38 mg, 0.20 mmol) was added. The suspension was heated at reflux overnight and allowed to cool to r.t. and filtered. The filter cake was washed with ethyl acetate, air-dried to give compound **492** (0.40 g, 47% yield). ESI-MS m/z: calcd. for $C_{22}H_{19}FN_2O_6$, $[M+H]^+$ 427.12; found, 427.12.

Example 533. Synthesis of (S)-tert-butyl (2-((((4-ethyl-8-fluoro-4-hydroxy-9-methoxy- 3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methoxy)carbonyl)amino)ethyl)(methyl)carbamate (**493**).



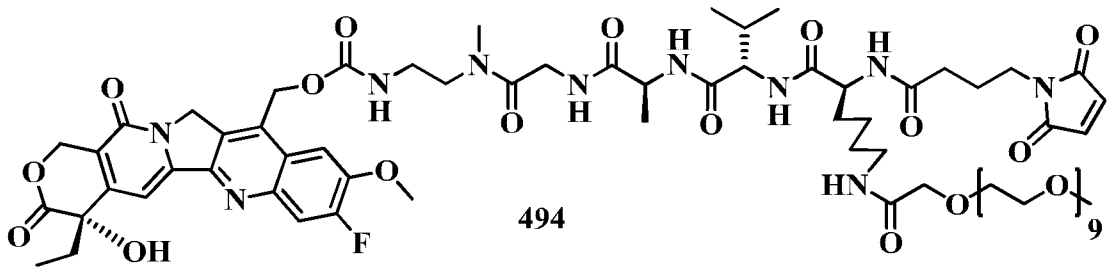
493

To a solution of tert-butyl (2-aminoethyl)(methyl)carbamate (0.23 g, 1.3 mmol) in dichloromethane (8 mL) was added pyridine (156 μL, 1.9 mmol) at 0 °C, followed by triphosgene (0.46 g, 1.6 mmol). The reaction mixture was warmed to r.t. and stirred for 2 hours, and then washed with 2 N HCl (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give tert-butyl (2-((chlorocarbonyl)amino)ethyl)(methyl)carbamate (253 mg, 82% yield).

To a solution of compound **492** (0.20 g, 0.5 mmol) in DMF (4 mL) was added the above compound (0.22 g, 0.9 mmol), followed by DMAP (0.012 g, 0.09 mmol) and N,N-diisopropylethylamine (153 μL, 0.9 mmol). The reaction was stirred overnight and quenched with water (20 mL), extracted with ethyl acetate (3 × 20 mL) and dichloromethane (3 × 20 mL). The

combined organic phase was dried over sodium sulfate, filtered and concentrated, purified by a silica gel column to give the title compound (81 mg, 28% yield). MS-ESI (m/z): calcd. for $C_{31}H_{35}FN_4O_9$, $[M+H]^+$ 627.24; found, 627.24.

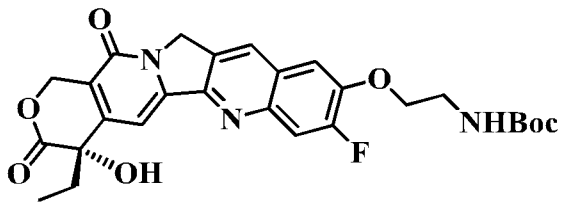
Example 534. Synthesis of ((S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-3,14-dioxo- 3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl ((37S,40S,43S)-37 -(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43,48-dimethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)carbamate (**494**).



494

Compound **493** (81 mg, 0.13 mmol) was dissolved in a mixture of dichloromethane (2 mL) and TFA (4 mL), and stirred at r.t. for 20 minutes. The mixture was then concentrated and dried on a vacuum pump and redissolved in DMF (1 mL). To this solution, compound **433** (130 mg, 0.13 mmol) was added, followed by HATU (60.4 mg, 0.16 mmol) and N,N-diisopropylethylamine (43 μL, 0.26 mmol). The mixture was stirred for 20 min and directly purified by preparative HPLC (acetonitrle/water) to give the title compound (77 mg, 39% yield). ESI-MS m/z: calcd. for $C_{71}H_{103}FN_{10}O_{25}$, $[M+H]^+$ 1515.71; found, 1515.71.

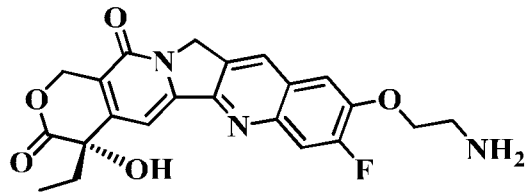
Example 535. Synthesis of (S)-tert-butyl (2-((4-ethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)carbamate (**495**).



495

To a solution of (S)-4-ethyl-8-fluoro-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino [1,2-b]quinoline-3,14(4H,12H)-dione (0.56 g, 1.5 mmol) in THF (40 mL) were added triphenylphosphine (1.15 g, 4.4 mmol), tert-butyl (2-hydroxyethyl)carbamate (0.94 g, 5.8 mmol) and DEAD (0.76 g, 4.4 mmol) at 0 °C. The reaction was warmed to r.t. gradually and stirred for 2 days, concentrated and purified by a silica gel column (3% MeOH/dichloromethane) to give a brown solid (0.56 g, 73% yield). MS-ESI (m/z): calcd. for $C_{27}H_{28}FN_3O_7$, $[M+H]^+$ 526.19; found, 526.19.

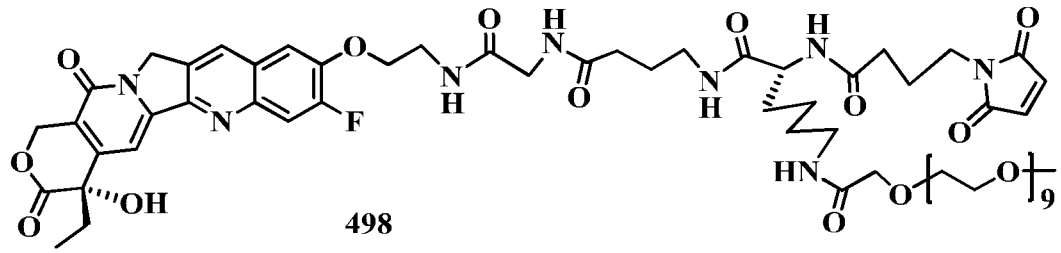
Example 536. Synthesis of (S)-9-(2-aminoethoxy)-4-ethyl-8-fluoro-4-hydroxy-1H-pyrano [3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione (**496**).



496

Compound **495** (0.24 g, 0.5 mmol) in dichloromethane (10 mL) was treated with TFA (10 mL) at r.t. for 0.5 hours, and then concentrated. The residue was used directly in the next step. MS-ESI (m/z): calcd. for $C_{41}H_{71}N_5O_{18}$ $[M+H]^+$ 922.48; found, 922.48.

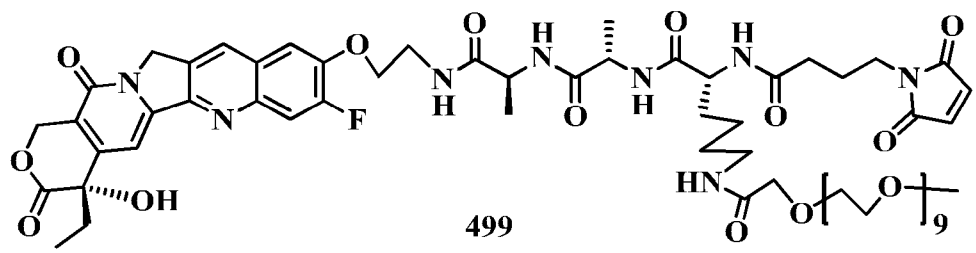
Example 537. Synthesis of N-((R)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido) -6-((4-((2-((2-(((S)-4-ethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)amino)-2-oxoethyl)amino)-4-oxobutyl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**498**).



498

To a solution of compound **496** (0.06 g, 0.14 mmol) and **497** (0.13 g, 0.14 mmol) in DMF (2 mL), HATU (82 mg, 0.21 mmol) and N,N-diisopropylethylamine (47 μL, 0.28 mmol) were added. The reaction was stirred at r.t. for 20 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (65 mg, 34% yield). MS-ESI (m/z): calcd. for $C_{63}H_{89}FN_8O_{22}$, $[M+2H]^{2+}$ 665.81; found, 665.89.

Example 538. Synthesis of N-((R)-5-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido) -6-(((S)-1-(((S)-1-((2-(((S)-4-ethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-6-oxohexyl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**499**).

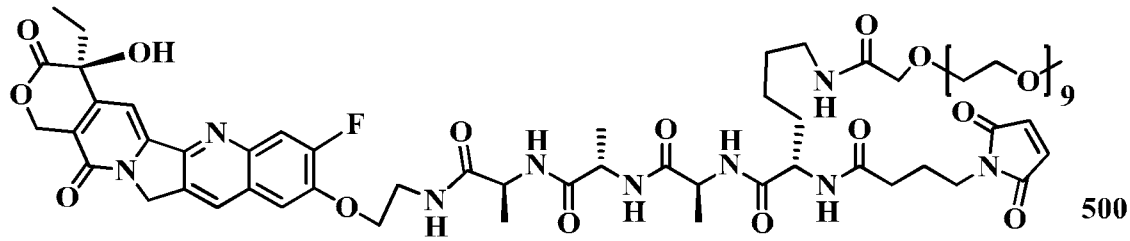


499

To a solution of compound **496** (0.06 g, 0.14 mmol) and **291** (0.13 g, 0.14 mmol) in DMF (2 mL), HATU (82 mg, 0.21 mmol) and N,N-diisopropylethylamine (47 μL, 0.28 mmol) were added. The reaction was stirred at r.t. for 20 min, concentrated and purified by preparative HPLC

(acetonitrile/water containing 0.1% formic acid) to give a light yellow solid (104 mg, 55% yield). MS-ESI (m/z): calcd. for $C_{63}H_{89}FN_8O_{22}$, $[M+2H]^{2+}$ 665.81; found, 665.92.

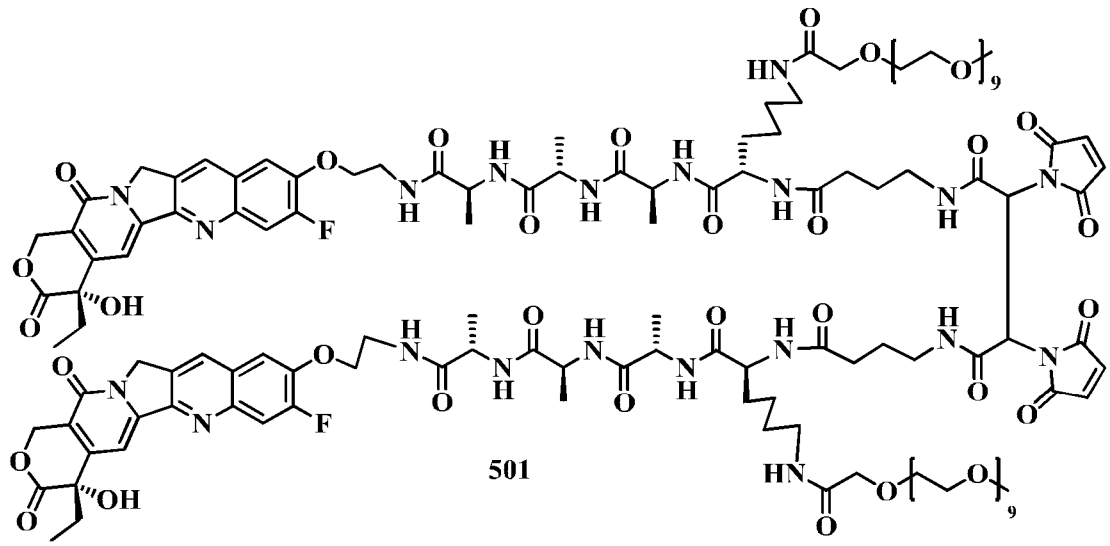
Example 539. Synthesis of N-((5S,8S,11S,14S)-14-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-1-(((S)-4-ethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-5,8,11-trimethyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazaoctadecan-18-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**500**).



500

To a solution of compound **496** (0.05 g, 0.10 mmol) and **217** (0.12 g, 0.10 mmol) in DMF (2 mL), HATU (67 mg, 0.20 mmol) and N,N-diisopropylethylamine (39 μL, 0.20 mmol) were added. The reaction was stirred at r.t. for 20 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (36 mg, 23% yield). MS-ESI (m/z): calcd. for $C_{66}H_{94}FN_9O_{23}$, $[M+2H]^{2+}$701.32; found, 701.36.

Example 540. Synthesis of 2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N1,N4-bis((S)-37 -(((S)-1-(((S)-1-(((S)-1-((2-(((S)-4-ethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-31,39-dioxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,38-diazadotetracontan-42-yl)succinamide (**501**).

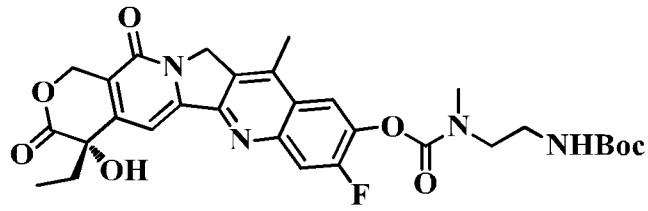


501

To a solution of compound **139** (224 mg, 0.10 mmol) in DMF (1 mL), HATU (102 mg, 0.30 mmol) was added and stirred for 10 min. A solution of compound **496** (100 mg, 0.20 mmol) and N,N-diisopropylethylamine (79 μL, 0.50 mmol) in DMF (1 mL) was then added. The reaction was

stirred at r.t. for 10 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give a yellow solid (176 mg, 57% yield). MS-ESI (m/z): calcd. for $C_{136}H_{192}F_2N_{20}O_{48}$, $[M+2H]^{2+}$ 729.33; found, 729.30.

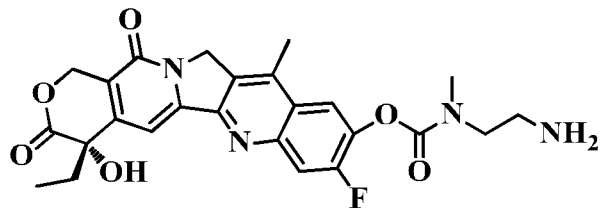
Example 541. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino) ethyl)(methyl)carbamate (**502**).



502

To a solution of compound **171** (673 mg, 1.7 mmol) in THF (60 mL), N,N-diisopropylethylamine (877 mg, 6.8 mmol) and 4-nitrophenyl carbonochloridate (691 mg, 3.4 mmol) were added and stirred for 3 hours. tert-Butyl (2-(methylamino)ethyl)carbamate (284 mg, 1.8 mmol) was added. The reaction was stirred at r.t. overnight and concentrated. The residue was stirred with 0.2 N HCl and a solid formed, which was collected by filtration and dried under vacuum to give the title compound (0.69 g, 68% yield). MS-ESI (m/z): calcd. for $C_{30}H_{33}FN_4O_8$, $[M+H]^+$ 597.61; found, 597.58.

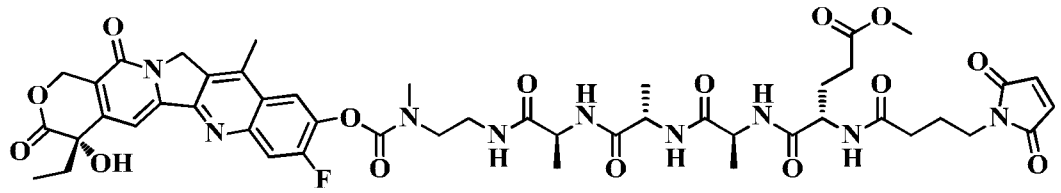
Example 542. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**503**).



503

Compound **502** (0.12 g, 0.20 mmol) in dichloromethane (2 mL) was treated with TFA (2 mL) at r.t. for 1 hour, and then concentrated. The residue was co-evaporated with dichloromethane and triturated with ethyl acetate to give a solid (0.10 g, 100% yield). MS-ESI (m/z): calcd. for $C_{25}H_{25}FN_4O_6$ $[M+H]^+$ 497.18; found, 497.20.

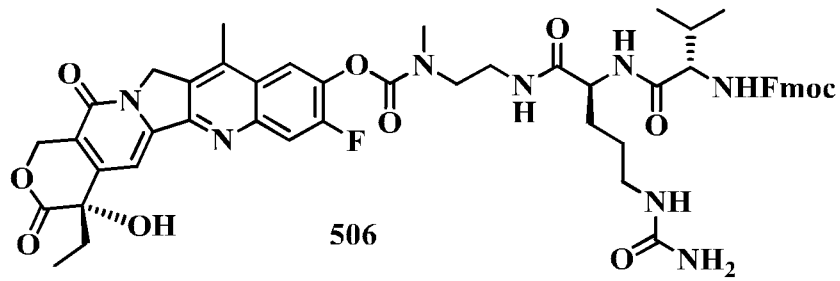
Example 543. Synthesis of (7S,10S,13S,16S)-methyl 16-(4-(2,5-dioxo-2,5-dihydro-1H- pyrrol-1-yl)butanamido)-1-(((S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-2,7,10,13-tetramethyl-1,6,9,12,15-pentaoxo-2,5,8,11,14-pentaazanonadecan-19-oate (**504**).



504

To a solution of compound **503** (90 mg, 0.18 mmol) and **251** (98 mg, 0.18 mmol) in DMF (5 mL), HATU (104 mg, 0.27 mmol) and N,N-diisopropylethylamine (81 mg, 0.63 mmol) were added. The reaction was stirred at r.t. for 1 hour and then concentrated. The residue was diluted with dichloromethane, washed with 0.2 N HCl and brine, dried over anhydrous sodium sulfate, filtered and concentrated again. The crude product was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (108 mg, 60% yield). MS-ESI (m/z): calcd. for $C_{48}H_{56}FN_9O_{15}$, $[M+H]^+$ 1018.39; found, 1018.39.

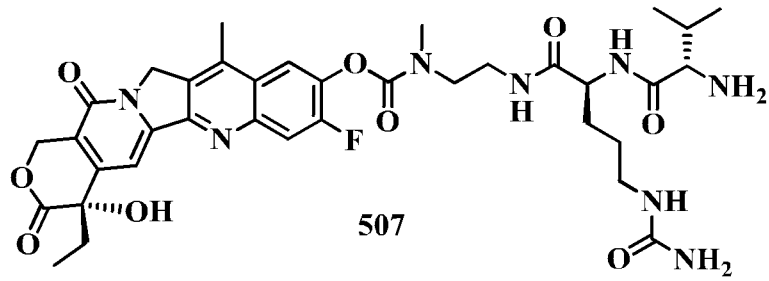
Example 544. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ((5S,8S)-1-(9H-fluoren-9-yl)-5-isopropyl-3,6,9-trioxo-8-(3-ureidopropyl)-2-oxa-4,7,10-triazadodecan-12-yl)(methyl)carbamate (**506**).



506

To a solution of compound **503** (124 mg, 0.25 mmol) and **505** (124 mg, 0.25 mmol) in DMF (4 mL), HATU (114 mg, 0.30 mmol) and N,N-diisopropylethylamine (83 μL, 0.50 mmol) were added. The reaction was stirred at r.t. for 10 min and then triturated with ethyl acetate (80 mL). A yellow solid was obtain after filtration (228 mg, 94% yield). MS-ESI (m/z): calcd. for $C_{51}H_{55}FN_8O_{11}$, $[M+H]^+$ 975.40; found, 975.40.

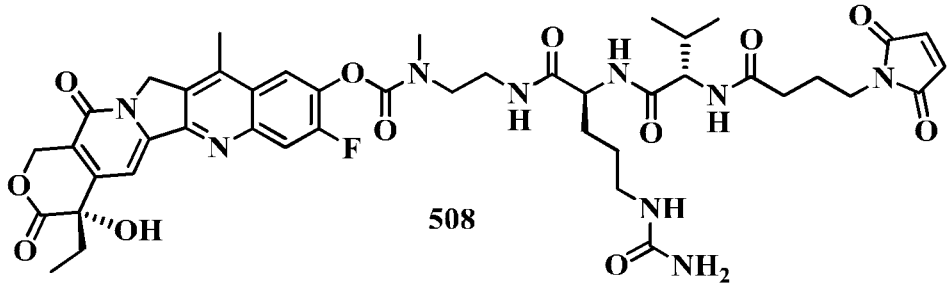
Example 545. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((S)-2-((S)-2-amino-3-methyl butanamido)-5-ureidopentanamido)ethyl)(methyl)carbamate (**507**).



507

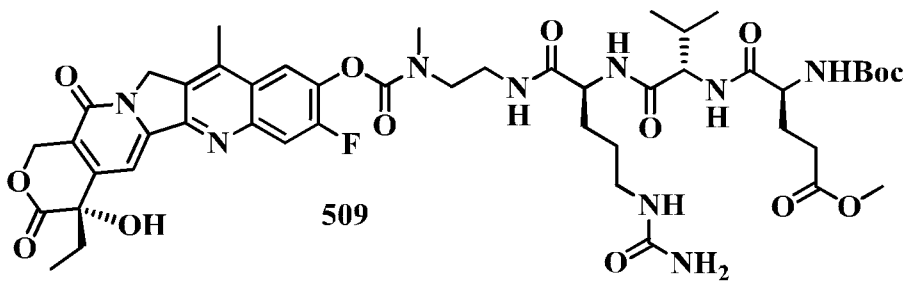
A solution of compound **506** (3.0 g) in DMF (4 mL) and piperidine (0.5 mL) were stirred at r.t. for 10 min and concentrated. The crude product was used directly in the next step. MS-ESI (m/z): calcd. for $C_{36}H_{45}FN_8O_9$ $[M+H]^+$ 753.33; found, 753.33.

Example 546. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((S)-2-((S)-2-(4-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)butanamido)-3-methylbutanamido)-5-ureidopentanamido)ethyl)(methyl)carbamate (**508**).



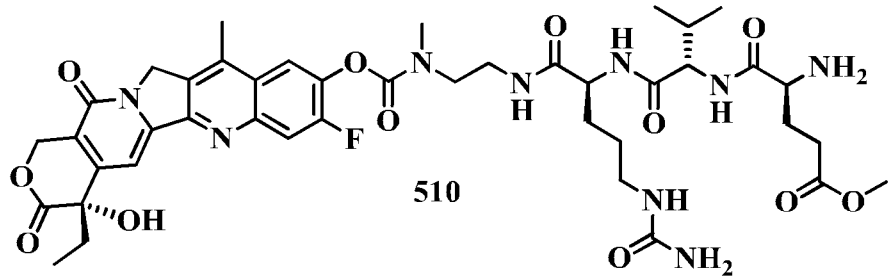
To a solution of compound **507** (52 mg, 0.07 mmol) and 4-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)butanoic acid (14 mg, 0.08 mmol) in DMF (1 mL), HATU (32 mg, 0.08 mmol) and N,N-diisopropylethylamine (23 μL, 0.14 mmol) were added. The reaction was stirred at r.t. for 10 min and then purfied by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (20 mg, 32% yield). MS-ESI (m/z): calcd. for $C_{44}H_{52}FN_9O_{12}$, $[M+H]^+$ 918.37; found, 918.36.

Example 547. Synthesis of (7S,10S,13S)-methyl 13-((tert-butoxycarbonyl)amino)-1- (((S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-10-isopropyl-2-methyl-1,6,9,12-tetraoxo-7-(3-ureidopropyl)-2,5,8,11-tetraazahexadecan-16-oate (**509**).



To a solution of compound **507** (50 mg, 0.07 mmol) and Boc-Glu(OMe)-OH (18 mg, 0.07 mmol) in DMF (1 mL), HATU (31 mg, 0.08 mmol) and N,N-diisopropylethylamine (22 μL, 0.13 mmol) were added. The reaction was stirred at r.t. for 10 min and then triturated with ethyl acetate (20 mL) to give the title compound as a yellow solid (44 mg, 67% yield). MS-ESI (m/z): calcd. for $C_{47}H_{62}FN_9O_{14}$, $[M+H]^+$ 996.44; found, 996.48.

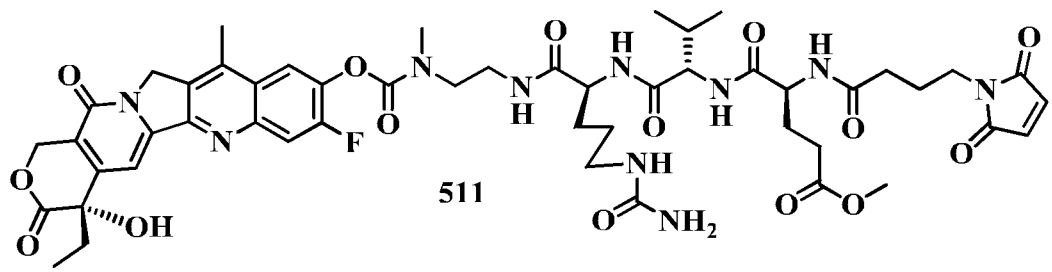
Example 548. Synthesis of (7S,10S,13S)-methyl 13-amino-1-(((S)-4-ethyl-8-fluoro-4-hydroxy -11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-10-isopropyl-2-methyl-1,6,9,12-tetraoxo-7-(3-ureidopropyl)-2,5,8,11-tetraazahexadecan-16-oate (**510**).



510

A solution of compound **509** (44 mg, 0.04 mmol) in dichloromethane (3 mL) and TFA (1 mL) were stirred at r.t. for 1 hour and concentrated. The crude product was used directly in the next step. MS-ESI (m/z): calcd. for $C_{42}H_{54}FN_9O_{12}$ $[M+H]^+$ 896.39; found, 896.39.

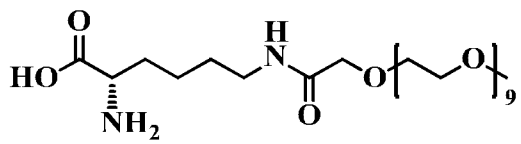
Example 549. Synthesis of (7S,10S,13S)-methyl 13-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) butanamido)-1-(((S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-10-isopropyl-2-methyl-1,6,9,12-tetraoxo-7-(3-ureidopropyl)-2,5,8,11-tetraazahexadecan-16-oate (**511**).



511

To a solution of compound **510** (39 mg, 0.04 mmol) and 4-(2,5-dioxo-2,5-dihydro-1H -pyrrol-1-yl)butanoic acid (8 mg, 0.04 mmol) in DMF (1 mL), HATU (20 mg, 0.05 mmol) and N,N-diisopropylethylamine (22 μL, 0.13 mmol) were added. The reaction was stirred at r.t. for 10 min and then triturated with ethyl acetate (20 mL) to give the title compound (40 mg, 87% yield). MS-ESI (m/z): calcd. for $C_{50}H_{61}FN_{10}O_{15}$, $[M+H]^+$ 1061.43; found, 1061.43.

Example 550. Synthesis of (S)-37-amino-31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa -32-azaoctatriacontan-38-oic acid (**512**).

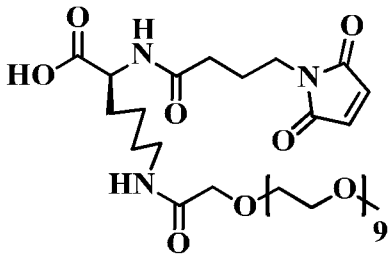


512

Compound **20** (2.0 g, 2.7 mmol) was dissolved in methanol (30 mL), and 10 wt% Pd/C (0.20 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. overnight, filtered, and concentrated to dryness to give the

title compound (1.64 g, 100% yield). MS-ESI (m/z): calcd. for $C_{27}H_{54}N_2O_{13}$, $[M+H]^+$ 615.36; found, 615.36.

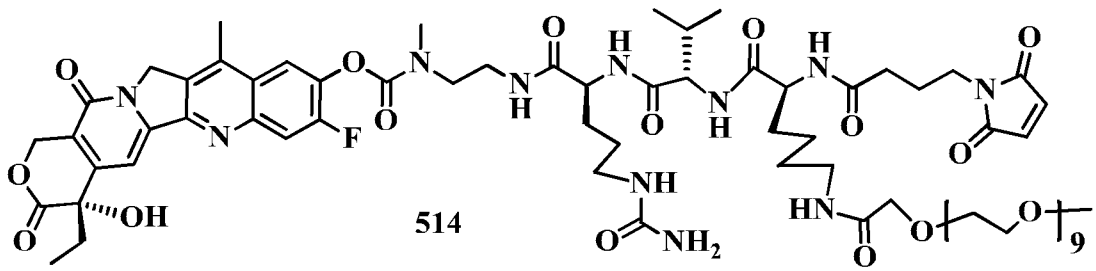
Example 551. Synthesis of (S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido) -31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azaoctatriacontan-38-oic acid (**513**).



513

A solution of compound **512** (1.64 g, 3.0 mmol) and N-(4-maleimidobutyryloxy) succinimide (0.75 g, 3.0 mmol) in THF (30 mL) was stirred at r.t. overnight, and then concentrated and purfied by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (0.76 g, 37% yield). MS-ESI (m/z): calcd. for $C_{35}H_{61}N_3O_{16}$, $[M+H]^+$ 780.41; found, 780.41.

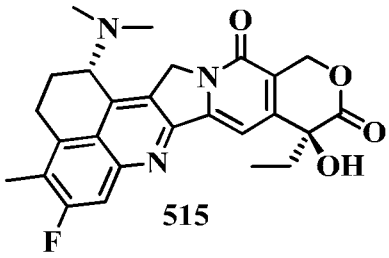
Example 552. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ((37S,40S,43S)-37-(4-(2,5-dioxo -2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-31,38,41,44-tetraoxo-43-(3-ureidopropyl)-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-yl)(methyl)carbamate (**514**).



514

To a solution of compound **507** (50 mg, 0.07 mmol) and compound **513** (52 mg, 0.07 mmol) in DMF (1 mL), HATU (31 mg, 0.08 mmol) and N,N-diisopropylethylamine (22 μL, 0.13 mmol) were added. The reaction was stirred at r.t. for 10 min and then purfied by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (23 mg, 23% yield). MS-ESI (m/z): calcd. for $C_{71}H_{104}FN_{11}O_{24}$, $[M+H]^+$ 1514.72; found, 1514.72.

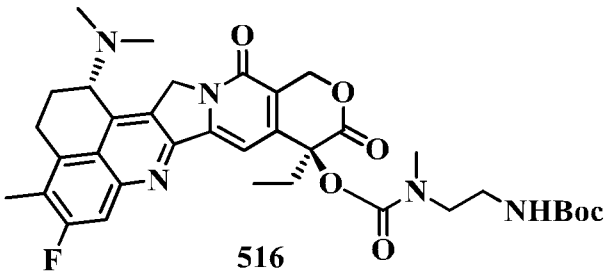
Example 553. Synthesis of (1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-9-hydroxy- 4-methyl-2,3,12,15-tetrahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(1H,9H)-dione (**515**).



515

To a mixture of exatecan (2.0 g, 4.6 mmol) in methanol (50 mL) and water (50 mL), formaldehyde (1.86 g, 37 wt%, 23 mmol) and Pd/C (200 mg) were added. The reaction flask was evacuated and back-filled with hydrogen, heated to 30 °C, and stirred under H_2 overnight. The solution was cooled to r.t. and filtered. The filtrated was concentrated to the title compound (2.0 g, 94% yield). MS-ESI (m/z): calcd. for $C_{26}H_{26}FN_3O_4$, $[M+H]^+$ 464.19; found, 464.20.

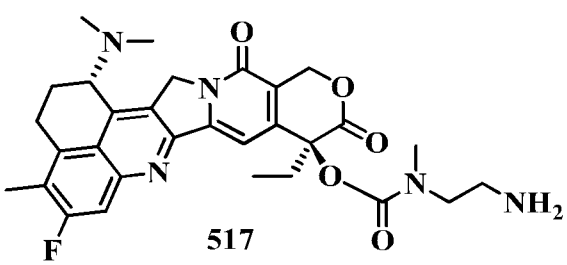
Example 554. Synthesis of (1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-4-methyl-10,13- dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**516**).



516

To a suspension of compound **515** (1.0 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), DMAP (2.6 g, 21.6 mmol) and 4-nitrophenyl carbonochloridate (4.3 g, 21.6 mmol) were added at 0 °C and then stirred at 30 °C for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate, and filtered. Tert-butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was added to the filtrate and stirred at 30 °C for 2 hours. The reaction mixture was washed with brine, dried, filtered and concentrated. The residue was purified by a silica gel column (MeOH/DCM 1:20) to give the title compound (1.0 g, 70% yield). MS-ESI (m/z): calcd. for $C_{35}H_{42}FN_5O_7$, $[M+H]^+$ 664.31; found, 664.40.

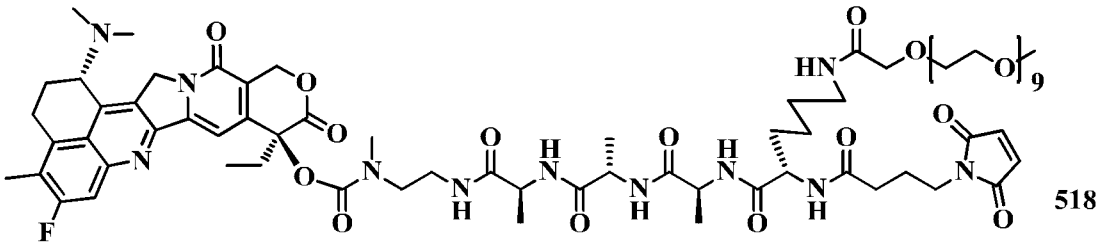
Example 555. Synthesis of (1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-4-methyl-10,13- dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**517**).



517

A solution of compound **516** (1.0 g, 1.5 mmol) in methanolic HCl (15 mL) was stirred at 30 °C for 6 hours and concentrated to give a yellow solid (900 mg), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{30}H_{34}FN_5O_5$ $[M+H]^+$ 564.25; found, 564.30.

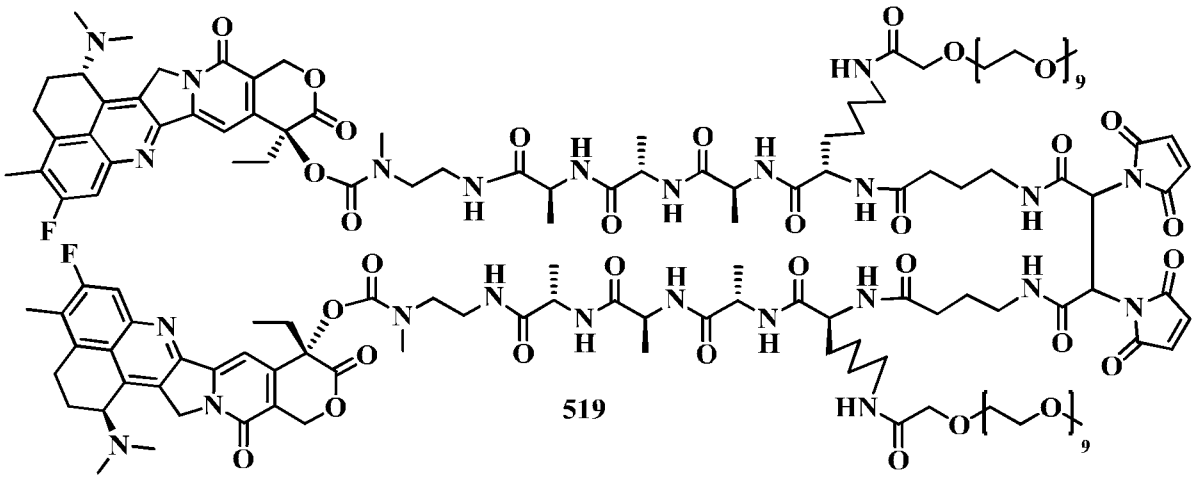
Example 556. Synthesis of (1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-4-methyl-10,13-dioxo -1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ((37S,40S,43S,46S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)(methyl)carbamate (**518**).



518

To a solution of compound **217** (150 mg, 0.15 mmol) and compound **517** (86 mg, 0.15 mmol) in DMF (3 mL), HATU (120 mg, 0.30 mmol) and N,N-diisopropylethylamine (40 mg, 0.30 mmol) were added. The reaction was stirred at 30 °C for 2 hours and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound as a brown solid (56 mg, 24% yield). MS-ESI (m/z): calcd. for $C_{74}H_{108}FN_{11}O_{23}$, $[M+H]^+$ 1538.76; found, 1539.10.

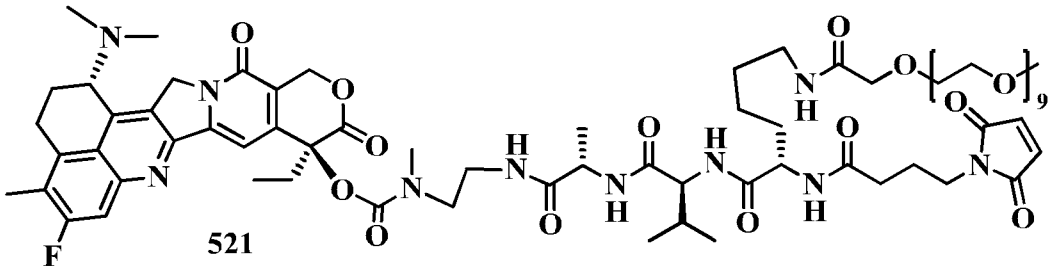
Example 557. Synthesis of bis((1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**519**).



519

To a solution of compound **139** (200 mg, 95μmol) and compound **517** (124 mg, 220 μmol) in DMF (5 mL), HATU (108 mg, 286 μmol) and N,N-diisopropylethylamine (75 mg, 572 μmol) were added. The reaction was stirred at 30 °C for 2 hours and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound as a yellow solid (40 mg, 13% yield). MS-ESI (m/z): calcd. for $C_{151}H_{220}F_2N_{24}O_{48}$, $[M+3H]^{3+}$ 1059.85; found, 1059.56.

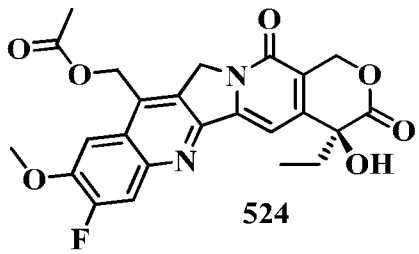
Example 558. Synthesis of (1S,9S)-1-(dimethylamino)-9-ethyl-5-fluoro-4-methyl-10,13-dioxo -1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44-tetraoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45-tetraazaheptatetracontan-47-yl)(methyl)carbamate (**521**).



521

To a solution of compound **520** (220 mg, 0.23 mmol) and compound **517** (145 mg, 0.26 mmol) in DCM (5 mL), EDC·HCl (445 mg, 2.3 mmol) and N,N-diisopropylethylamine (300 mg, 2.3 mmol) were added. The reaction was stirred at 30 °C for 2 hours, diluted with dichloromethane and then washed with brine, dried and concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (223 mg, 65% yield). MS-ESI (m/z): calcd. for $C_{73}H_{107}FN_{10}O_{22}$, $[M+H]^+$ 1495.75; found, 1495.80.

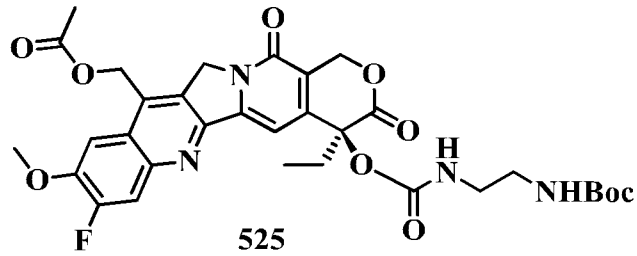
Example 559. Synthesis of (S)-(4-ethyl-8-fluoro-4-hydroxy-9-methoxy-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**524**).



524

Compound **490** (5.00 g, 20.7 mmol) and compound **159** (5.18 g, 19.7 mmol) were dissolved in anhydrous toluene (150 mL), and *p*-toluenesulfonic acid (390 mg, 2.07 mmol) was added. The suspension was heated at reflux overnight and allowed to cool to r.t. and filtered. The filter cake was washed with ethyl acetate, air-dried to give compound **524** (9.0 g, 92% yield). ESI-MS m/z: calcd. for $C_{23}H_{19}FN_2O_7$, $[M+H]^+$ 455.41; found, 455.38.

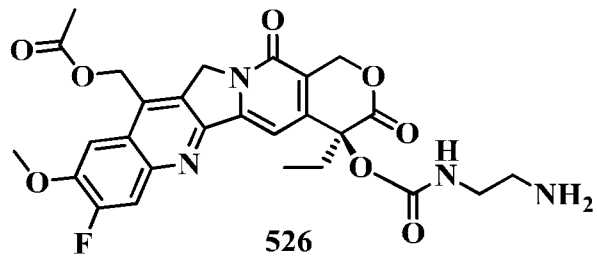
Example 560. Synthesis of (S)-(4-(((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)oxy)- 4-ethyl-8-fluoro-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**525**).



525

To a suspension of compound **524** (0.48 g, 1.0 mmol) in DMF (2.5 mL) and dichloromethane (15 mL), DMAP (0.25 g, 2.0 mmol) and 4-nitrophenyl carbonochloridate (0.31 g, 1.5 mmol) were added at 0 °C and then stirred at r.t. for 3 days. The reaction solution was washed with water and dried over anhydrous sodium sulfate, and filtered. Tert-butyl (2-(methylamino)ethyl)carbamate (0.33 g, 2.0 mmol) was added to the filtrate and stirred at r.t. overnight. The reaction mixture was concentrated and the residue was purified by a silica gel column (MeOH/DCM 1:10) to give the title compound (0.70 g). MS-ESI (m/z): calcd. for $C_{32}H_{35}FN_4O_{10}$, $[M+H]^+$ 655.23; found, 655.23.

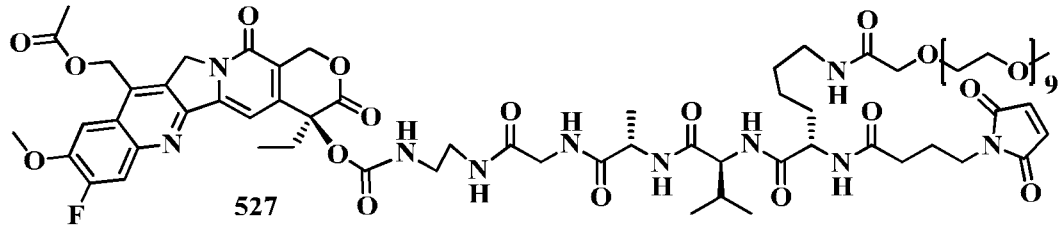
Example 561. Synthesis of (S)-(4-(((2-aminoethyl)carbamoyl)oxy)-4-ethyl-8-fluoro-9-methoxy -3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**526**).



526

A solution of compound **525** (0.40 g) in dichloromethane (3 mL) was stirred with TFA (10 mL) for 30 min and then concentrated. The residue was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (96 mg, 28% yield for two steps). MS-ESI (m/z): calcd. for $C_{27}H_{27}FN_4O_8$ $[M+H]^+$ 555.18; found, 555.18.

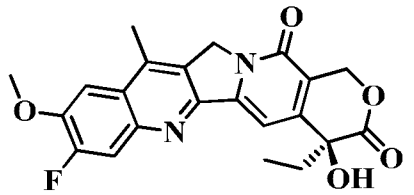
Example 562. Synthesis of ((S)-4-((((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H- pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)carbamoyl)oxy)-4-ethyl-8-fluoro-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**527**).



527

To a solution of compound **526** (96 mg, 0.17 mmol) and compound **433** (174 mg, 0.17 mmol) in DMF (1.5 mL), HATU (79 mg, 0.21 mmol) and N,N-diisopropylethylamine (57 μL, 0.35 mmol) were added. The reaction was stirred at r.t. for 10 min and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (32 mg, 12% yield). MS-ESI (m/z): calcd. for $C_{72}H_{103}FN_{10}O_{26}$, $[M+2H]^{2+}$ 772.85; found, 772.56.

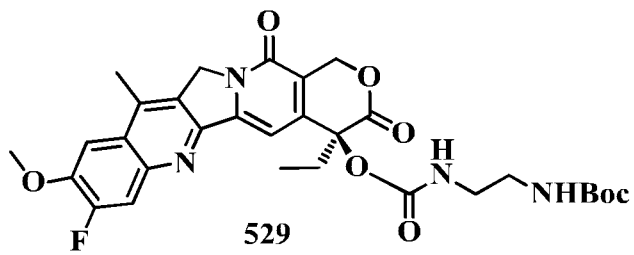
Example 563. Synthesis of (S)-4-ethyl-8-fluoro-4-hydroxy-9-methoxy-11-methyl-1H-pyrano [3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione (**528**).



528

Compound **160** (1.08 g, 2.4 mmol) was dissolved in methanol (200 mL), and 10 wt% Pd/C (1.10 g) was added. The reaction flask was evacuated and back-filled with hydrogen for three times, and then stirred under a hydrogen balloon at r.t. for 3 hours. The reaction mixture was filtered, and the filter cake was collected as the title compound (0.99 g, 100% yield). MS-ESI (m/z): calcd. for $C_{22}H_{19}FN_2O_5$, $[M+H]^+$ 411.13; found, 411.13.

Example 564. Synthesis of (S)-tert-butyl (4-ethyl-8-fluoro-9-methoxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl) ethane-1,2-diyldicarbamate (**529**).

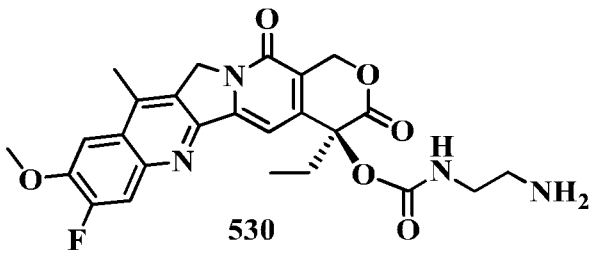


529

To a solution of compound **528** (1.11 g, 2.7 mmol) in dichloromethane (100 mL), DMAP (1.32 g, 11 mmol) and 4-nitrophenyl carbonochloridate (1.09 g, 5.4 mmol) were added and stirred at r.t. for 0.5 hours. The reaction solution was cooled to 0 °C and tert-butyl (2-aminoethyl) carbamate (0.87 g, 5.4 mmol) was added. After stirring for 1 hour, the reaction mixture was washed with 2 N HCl, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by a silica gel

column to give the title compound (0.62 g, 38% yield over two steps). MS-ESI (m/z): calcd. for $C_{30}H_{33}FN_4O_8$, $[M+H]^+$ 597.23; found, 597.23.

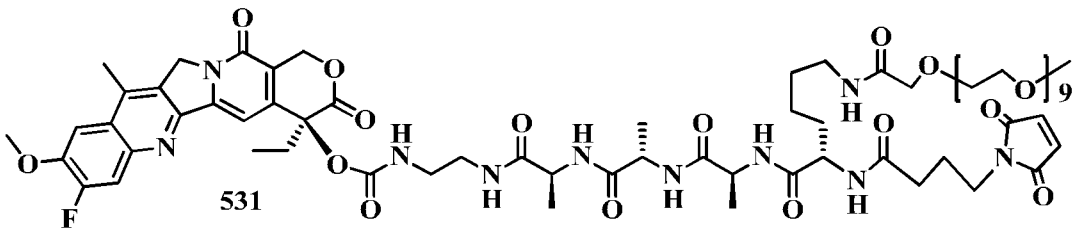
Example 565. Synthesis of (S)-4-ethyl-8-fluoro-9-methoxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl (2-aminoethyl)carbamate (**530**).



530

A solution of compound **529** (0.23 g, 0.39 mmol) in dichloromethane (4 mL) was stirred with TFA (8 mL) for 40 min and then concentrated to give the title compound (193 mg). MS-ESI (m/z): calcd. for $C_{25}H_{25}FN_4O_6$ $[M+H]^+$ 497.18; found, 497.18.

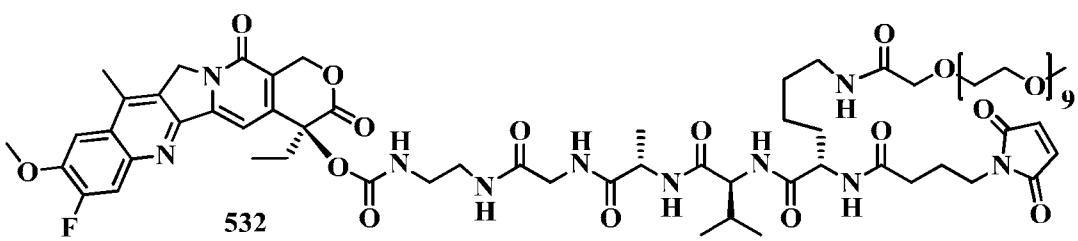
Example 566. Synthesis of (S)-4-ethyl-8-fluoro-9-methoxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl ((37S,40S,43S,46S)-37 -(4-(2,5 -dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)carbamate (**531**).



531

To a solution of compound **530** (94 mg, 0.19 mmol) and compound **217** (188 mg, 0.19 mmol) in DMF (2.0 mL), HATU (86 mg, 0.23 mmol) and N,N-diisopropylethylamine (63 μL, 0.38 mmol) were added. The reaction was stirred at r.t. for 1 hour and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (59 mg, 21% yield). MS-ESI (m/z): calcd. for $C_{69}H_{99}FN_{10}O_{24}$, $[M+2H]^{2+}$ 736.84; found, 736.56.

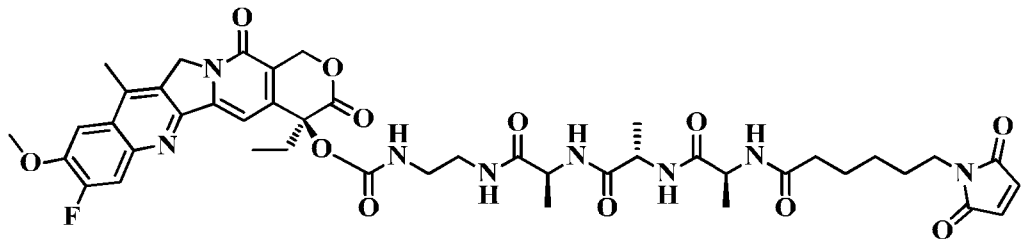
Example 567. Synthesis of (S)-4-ethyl-8-fluoro-9-methoxy-11-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl ((37S,40S,43S)-37-(4-(2,5-dioxo-2,5 -dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)carbamate (**532**).



532

To a solution of compound **530** (96 mg, 0.19 mmol) and compound **433** (195 mg, 0.19 mmol) in DMF (2.0 mL), HATU (88 mg, 0.23 mmol) and N,N-diisopropylethylamine (64 μL, 0.39 mmol) were added. The reaction was stirred at r.t. for 0.5 hours and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (110 mg, 34% yield). MS-ESI (m/z): calcd. for $C_{70}H_{101}FN_{10}O_{24}$, $[M+2H]^{2+}$ 743.85; found, 743.85.

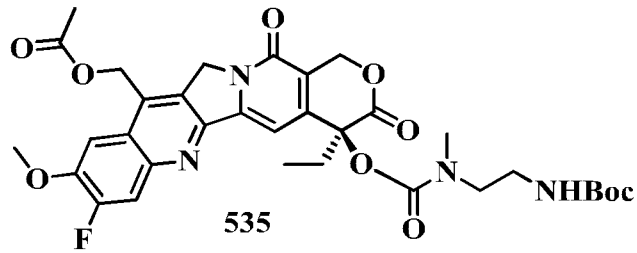
Example 568. Synthesis of (S)-4-ethyl-8-fluoro-9-methoxy-11-methyl-3,14-dioxo-3,4,12,14 -tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl ((5S,8S,11S)-18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11-trimethyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazaoctadecyl)carbamate (**534**).



534

To a solution of compound **530** (96 mg, 0.19 mmol) and compound **533** (82 mg, 0.19 mmol) in DMF (2.0 mL), HATU (88 mg, 0.23 mmol) and N,N-diisopropylethylamine (64 μL, 0.39 mmol) were added. The reaction was stirred at r.t. for 0.5 hours and then purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (32 mg, 19% yield). MS-ESI (m/z): calcd. for $C_{45}H_{52}FN_7O_{12}$, $[M+2H]^{2+}$ 902.37; found, 902.98.

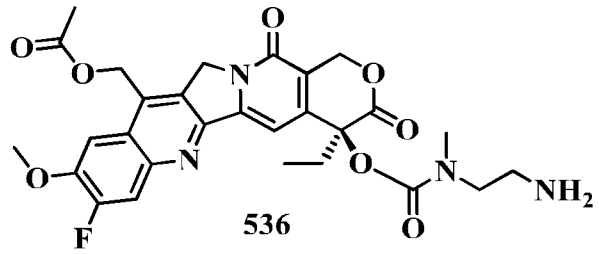
Example 569. Synthesis of (S)-(4-(((2-((tert-butoxycarbonyl)amino)ethyl)(methyl) carbamoyl)oxy)-4-ethyl-8-fluoro-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**535**).



535

To a solution of compound **524** (0.50 g, 1.1 mmol) in dichloromethane (100 mL), DMAP (0.52 g, 4.3 mmol) and 4-nitrophenyl carbonochloridate (0.43 g, 2.1 mmol) were added at 0 °C and stirred for 0.5 hours. Tert-butyl (2-aminoethyl) carbamate (0.37 g, 2.1 mmol) was added. After stirring for 1 hour at 0 °C, the reaction mixture was washed with 1 N HCl, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by a silica gel column (75% ethyl acetate/ petroleum ether) to give the title compound (0.39 g, 55% yield). MS-ESI (m/z): calcd. for $C_{33}H_{38}FN_4O_{10}$, $[M+H]^+$ 669.25; found, 669.25.

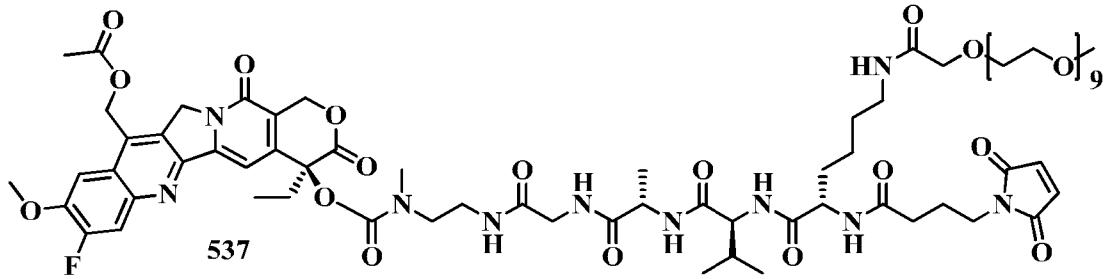
Example 570. Synthesis of (S)-(4-(((2-aminoethyl)(methyl)carbamoyl)oxy)-4-ethyl-8-fluoro -9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**536**).



536

A solution of compound **535** (0.35 g, 0.52 mmol) in 2 M HCl/ethyl acetate (5 mL) was stirred at r.t. for 30 min and then filtered. The filter cake was washed with ethyl acetate, dried on a vacuum pump to give the title compound (285 mg, 90% yield). MS-ESI (m/z): calcd. for $C_{28}H_{29}FN_4O_8$ $[M+H]^+$ 569.20; found, 569.50.

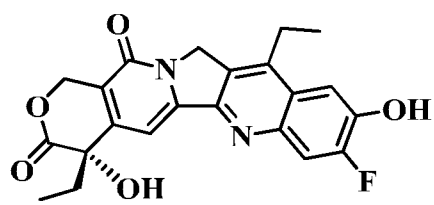
Example 571. Synthesis of ((S)-4-((((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol -1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)(methyl)carbamoyl)oxy)-4-ethyl-8-fluoro-9-methoxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)methyl acetate (**537**).



537

To a solution of compound **433** (83 mg, 0.82 mmol) and compound **536** (50 mg, 0.82 mmol) in DMF (1 mL), EDC·HCl (64 mg, 0.33 mmol) and HOBt (45 mg, 0.33 mmol) were added at 0 °C, followed by N,N-diisopropylethylamine (300 mg, 2.3 mmol. The reaction was stirred at 0 °C for 15 min, and then directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (71 mg, 55% yield). MS-ESI (m/z): calcd. for $C_{73}H_{106}FN_{10}O_{26}$, $[M+H]^+$ 1557.72; found, 1557.72.

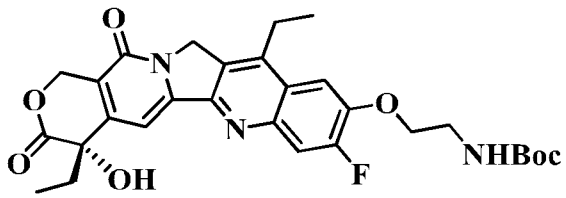
Example 572. Synthesis of (S)-4,11-diethyl-8-fluoro-4,9-dihydroxy-1,12-dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione (**539**).



539

Compound **538** (0.45 g, 2.5 mmol) and compound **159** (0.62 g, 2.4 mmol) were dissolved in anhydrous toluene (20 mL), and *p*-toluenesulfonic acid (50.2 mg, 0.20 mmol) was added. The suspension was heated at reflux overnight and allowed to cool to r.t. and filtered. The filter cake was collected and triturated with dichloromethane to give compound **539** (1.0 g, 98% yield). ESI-MS m/z: calcd. for $C_{22}H_{19}FN_2O_5$, $[M+H]^+$ 411.13; found, 411.13.

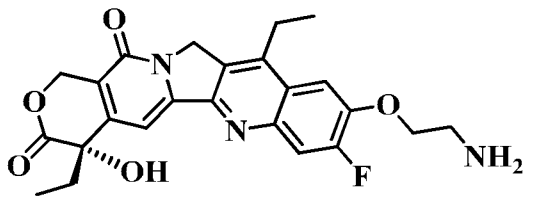
Example 573. Synthesis of tert-butyl (S)-(2-((4,11-diethyl-8-fluoro-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)carbamate (**540**).



540

To a solution of compound **539** (0.45 g, 1.1 mmol) in THF (40 mL) were added triphenylphosphine (0.86 g, 3.3 mmol), tert-butyl (2-hydroxyethyl)carbamate (0.70 g, 4.4 mmol) and DEAD (0.57 g, 3.3 mmol) at 0 °C. The reaction was warmed to r.t. gradually and stirred for 3 hours, concentrated and purified by a silica gel column to give the title compound (0.37 g, 62% yield). MS-ESI (m/z): calcd. for $C_{29}H_{32}FN_3O_7$, $[M+H]^+$ 554.22; found, 554.22.

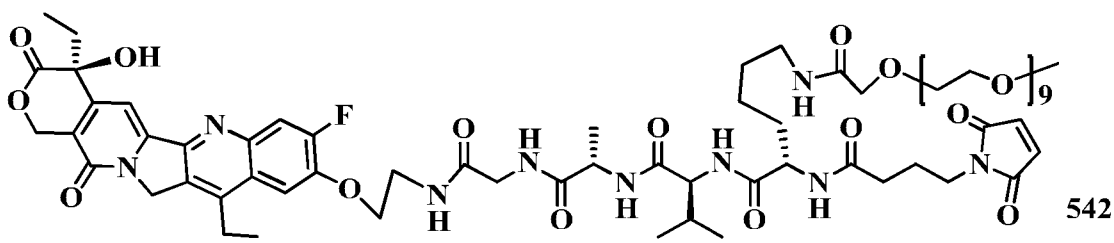
Example 574. Synthesis of (S)-9-(2-aminoethoxy)-4,11-diethyl-8-fluoro-4-hydroxy-1,12 -dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione (**541**).



541

Compound **540** (0.37 g, 0.67 mmol) in dichloromethane (3 mL) was treated with TFA (6 mL) at r.t. for 1 hour, and then concentrated to give a yellow solid (0.64 g). MS-ESI (m/z): calcd. for $C_{24}H_{24}FN_3O_5$ $[M+H]^+$ 454.17; found, 454.17.

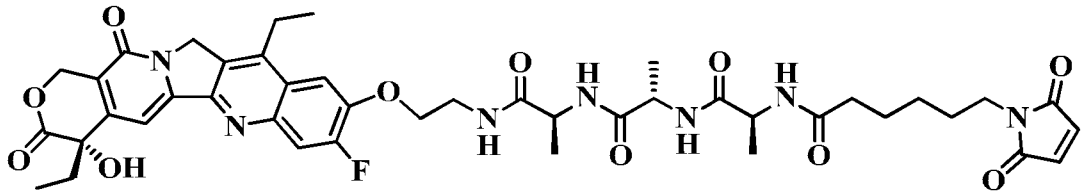
Example 575. Synthesis of N-((8S,11S,14S)-1-(((S)-4,11-diethyl-8-fluoro-4-hydroxy-3,14 -dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-14-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-11-isopropyl-8-methyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazaoctadecan-18-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**542**).



542

To a solution of compound **541** (43 mg, 0.10 mmol) and **433** (96 mg, 0.10 mmol) in DMF (2 mL), HATU (44 mg, 0.11 mmol) and N,N-diisopropylethylamine (63 μL, 0.40 mmol) were added. The reaction was stirred at r.t. for 30 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (103 mg, 73% yield). MS-ESI (m/z): calcd. for $C_{69}H_{100}FN_9O_{23}$, $[M+2H]^{2+}$ 722.34; found, 722.80.

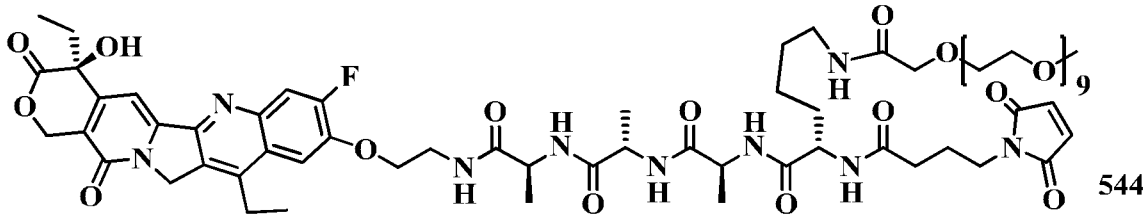
Example 576. Synthesis of N-((S)-1-(((S)-1-(((S)-1-((2-(((S)-4,11-diethyl-8-fluoro-4- hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (**543**).



543

To a solution of compound **541** (0.062 g, 0.14 mmol) and **533** (0.058 g, 0.14 mmol) in DMF (2 mL), HATU (62 mg, 0.16 mmol) and N,N-diisopropylethylamine (90 μL, 0.55 mmol) were added. The reaction was stirred at r.t. for 30 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (24 mg, 20% yield). MS-ESI (m/z): calcd. for $C_{43}H_{50}FN_7O_{11}$, $[M+H]^+$ 860.36; found, 860.36.

Example 577. Synthesis of N-((5S,8S,11S,14S)-1-(((S)-4,11-diethyl-8-fluoro-4-hydroxy- 3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-14-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-5,8,11-trimethyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazaoctadecan-18-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**544**).

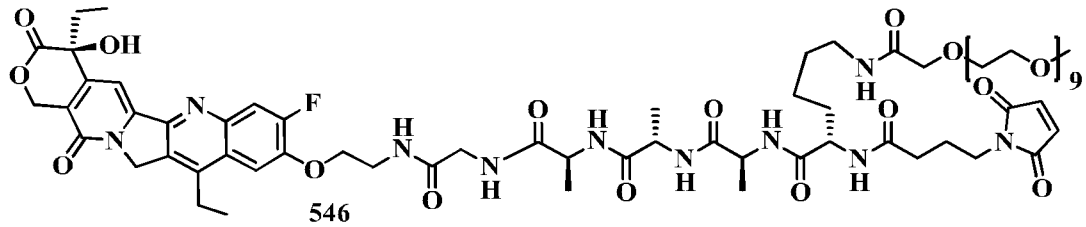


544

To a solution of compound **541** (0.076 g, 0.17 mmol) and **217** (0.167 g, 0.17 mmol) in DMF (2 mL), HATU (76 mg, 0.20 mmol) and N,N-diisopropylethylamine (111 μL, 0.67 mmol) were added. The reaction was stirred at r.t. for 20 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (101 mg, 42% yield). MS-ESI (m/z): calcd. for $C_{68}H_{98}FN_9O_{23}$, $[M+2H]^{2+}$ 714.34; found, 714.23.

Example 578. Synthesis of N-((8S,11S,14S,17S)-1-(((S)-4,11-diethyl-8-fluoro-4-hydroxy - 3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-17-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-8,11,14-trimethyl-4,7,10,13,16-pentaoxo-

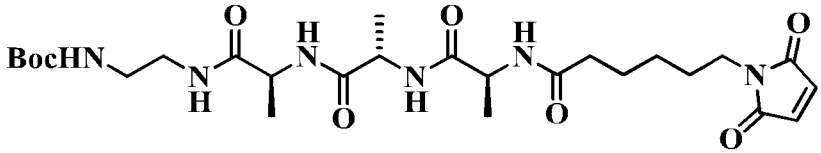
3,6,9,12,15-pentaazahenicosan-21-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**546**).



546

To a solution of compound **541** (0.050 g, 0.11 mmol) and **545** (0.115 g, 0.11 mmol) in DMF (2 mL), HATU (50 mg, 0.13 mmol) and N,N-diisopropylethylamine (54 μL, 0.33 mmol) were added. The reaction was stirred at r.t. for 30 min, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (110 mg, 69% yield). MS-ESI (m/z): calcd. for $C_{70}H_{101}FN_{10}O_{24}$, $[M+2H]^{2+}$ 743.85; found, 743.89.

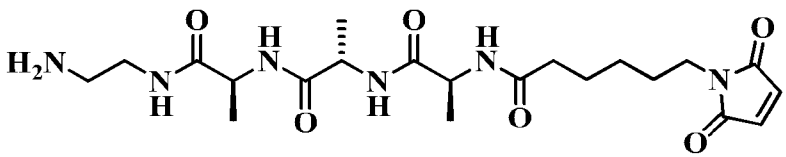
Example 579. Synthesis of tert-butyl ((5S,8S,11S)-18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -5,8,11-trimethyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazaoctadecyl)carbamate (**548**).



548

To a solution of compound **534** (0.85 g, 2.00 mmol) and tert-butyl (2-aminoethyl)carbamate (0.32 g, 2.00 mmol) in DMF (20 mL), HATU (0.91 g, 2.40 mmol) and N,N-diisopropylethylamine (0.52 g, 4.00 mmol) were added. The reaction was stirred at r.t. for 1 hour and then concentrated. The residue was diluted with dichloromethane, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated again. The crude product was purified by a silica gel column (0-10% MeOH/dichloromethane) to give the title compound (0.30 g, 26% yield).

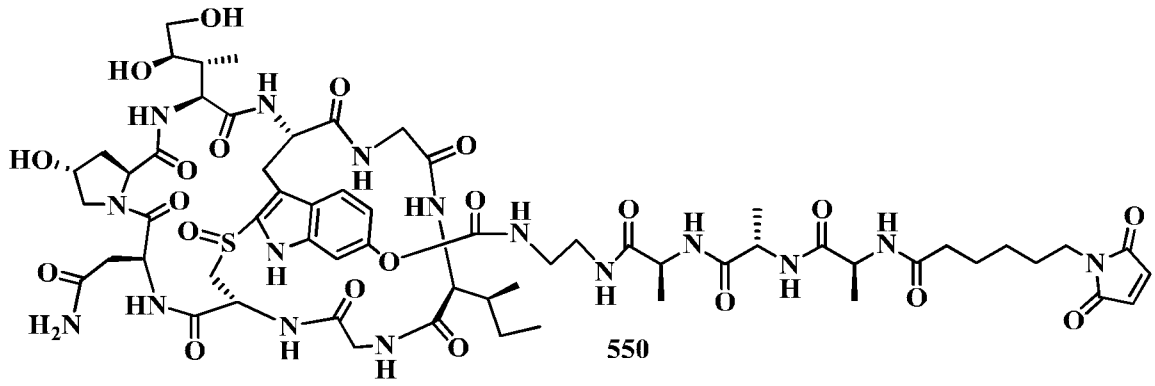
Example 580. Synthesis of N-((S)-1-(((S)-1-(((S)-1-((2-aminoethyl)amino)-1-oxopropan -2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (**549**).



549

A solution of compound **548** (200 mg, 0.353 mmol) in dichloromethane (4 mL) and TFA (2 mL) were stirred at r.t. for 1 hour and concentrated. The crude product was co-evaporated with dichloromethane to give the title compound (162 mg).

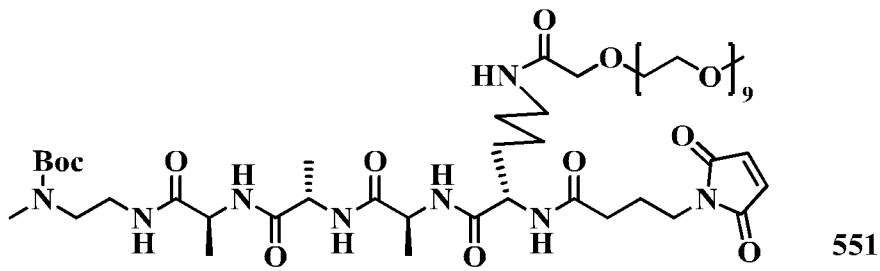
Example 581. Synthesis of compound **550.**



550

To a solution of α-amanitin **182** (5.1 mg, 0.006 mmol) in DMF (0.50 mL), N,N-diisopropylethylamine (4.3 mg, 0.033 mmol), 4-nitrophenyl carbonochloridate (1.7 mg, 0.008 mmol) was added and stirred for 3 hours. Compound **549** (2.6 mg, 0.006 mmol) was added and stirred at r.t. for 1 hour. The reaction mixture was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (3.1 mg, 39% yield). MS-ESI (m/z): calcd. for $C_{61}H_{86}N_{16}O_{21}S$, $[M+H]^+$ 1412.51; found, 1412.64.

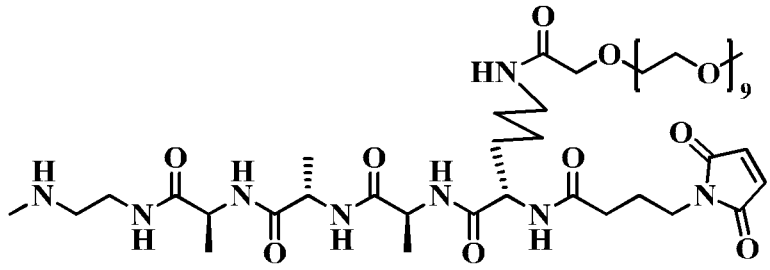
Example 582. Synthesis of tert-butyl ((37S,40S,43S,46S)-37-(4-(2,5-dioxo-2,5-dihydro -1H-pyrrol-1-yl)butanamido)-40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)(methyl)carbamate (**551**).



551

To a solution of compound **217** (2.00 g, 2.00 mmol) and tert-butyl (2-aminoethyl)(methyl)carbamate (0.35 g, 2.00 mmol) in DMF (20 mL), HATU (0.91 g, 2.40 mmol) and N,N-diisopropylethylamine (0.52 g, 4.00 mmol) were added. The reaction was stirred at r.t. for 1 hour and then concentrated. The residue was diluted with dichloromethane, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated again. The crude product was purified by a silica gel column (0-10% MeOH/dichloromethane) to give the title compound (0.65 g, 28% yield).

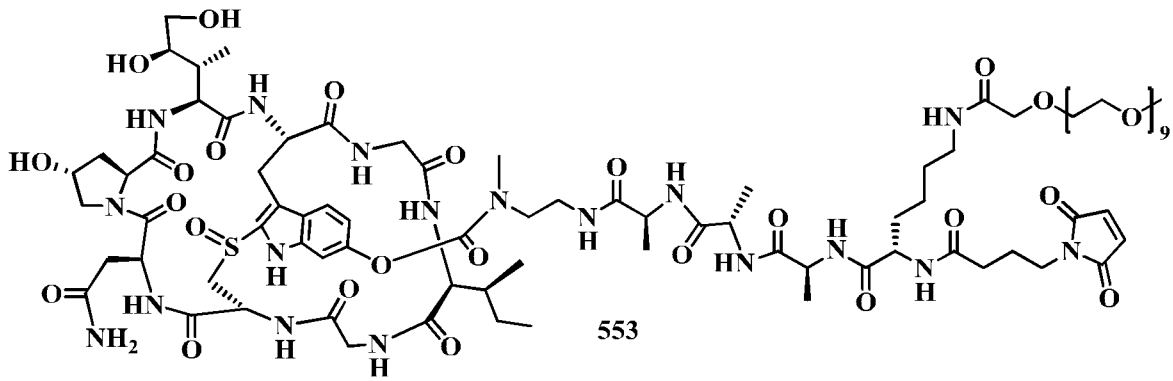
Example 583. Synthesis of N-((7S,10S,13S,16S)-16-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-7,10,13-trimethyl-6,9,12,15-tetraoxo-2,5,8,11,14-pentaazaicosan-20-yl)-2,5,8,11,14,17,20,23,26,29-decaoxahentriacontan-31-amide (**552**).



552

A solution of compound **551** (100 mg, 0.087 mmol) in dichloromethane (2 mL) and TFA (1 mL) were stirred at r.t. for 1 hour and concentrated. The crude product was co-evaporated with dichloromethane to give the title compound (90 mg, 99% yield).

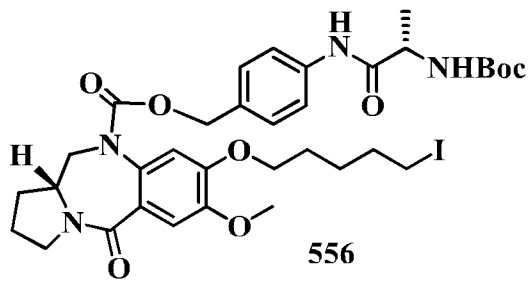
Example 584. Synthesis of compound **553.**



553

To a solution of α-amanitin **182** (5.1 mg, 0.006 mmol) in DMF (0.50 mL), N,N-diisopropylethylamine (4.3 mg, 0.033 mmol), 4-nitrophenyl carbonochloridate (1.7 mg, 0.008 mmol) was added and stirred for 3 hours. Compound **552** (6.3 mg, 0.006 mmol) was added and stirred at r.t. for 1 hour. The reaction mixture was purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (2.2 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{87}H_{136}N_{18}O_{33}S$, $[M+H]^+$ 1995.20; found, 1995.14.

Example 585. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido)benzyl (R)-8-((5-iodopentyl)oxy)-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**556**).

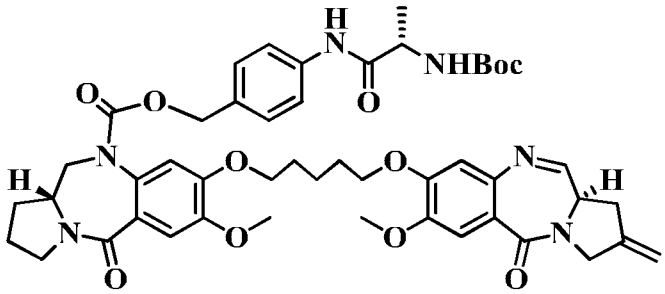


556

To a solution of compound **380** (0.57 g, 1.0 mmol) in acetone (40 mL) were added diiodopentane (1.62 g, 5.0 mmol) and potassium carbonate (0.21 g, 1.5 mmol) with stirring. After the addition, the reaction was heated under reflux for 8 hours, and after cooling, it was directly purified

by column chromatography (20%-100% ethyl acetate/petroleum ether) to give the title compound (0.62 g, 81% yield). MS-ESI (m/z): calcd. for $C_{34}H_{45}IN_4O_8$, $[M+H]^+$ 765.66; found, 765.59.

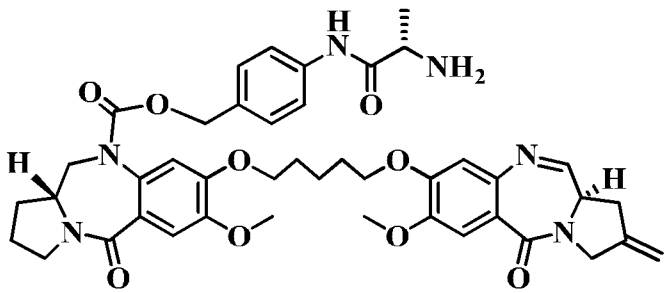
Example 586. Synthesis of 4-((S)-2-((tert-butoxycarbonyl)amino)propanamido)benzyl (R)-7-methoxy-8-((5-(((R)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**558**).



558

A mixture of compound **556** (0.62 g, 0.80 mmol) and compound **557** (0.21 g, 0.80 mmol) in 40 mL of acetone, and potassium carbonate (0.38 g, 1.6 mmol) was heated to reflux and stirred for 8 hours. The reaction was directly purified by a silica gel column (0-10% MeOH/DCM) to give the title compound (0.68 g, 76% yield). MS-ESI (m/z): calcd. for $C_{48}H_{58}N_6O_{11}$, $[M+H]^+$ 896.02; found, 896.12.

Example 587. Synthesis of 4-((S)-2-aminopropanamido)benzyl (R)-7-methoxy-8-((5-(((R)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**559**).

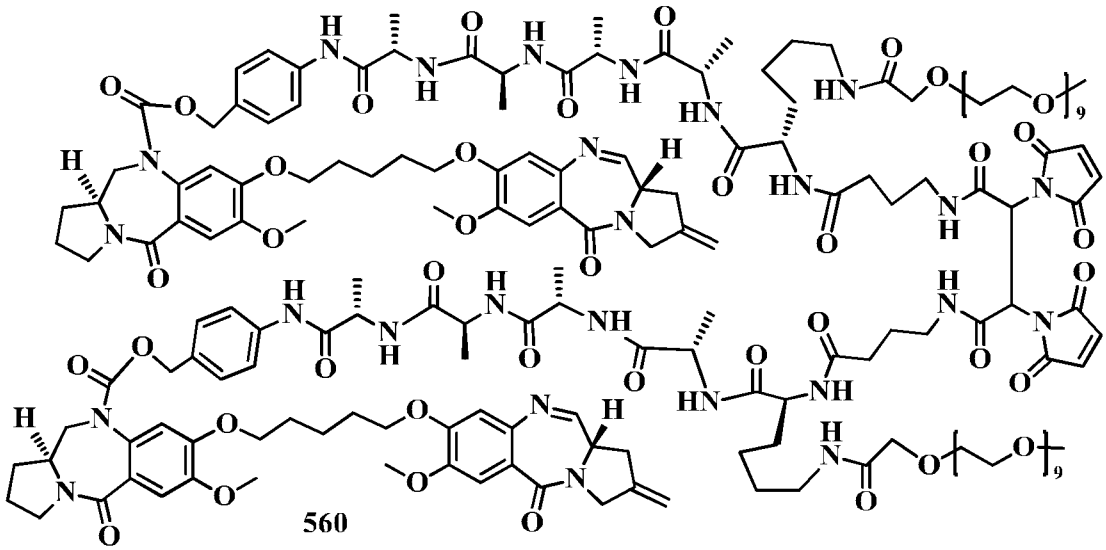


559

To a solution of compound **558** (90 mg, 0.10 mmol) in dichloromethane (15 mL) cooled to 5 °C, TFA (1 mL) was added and stirred for 30 min. The reaction was diluted with dichloromethane and washed with 10% $NaHCO_3$, and brine, dried over sodium sulfate, filtered and concentrated. The residue was dissolved in DMF (2 mL) and used as a solution. MS-ESI (m/z): calcd. for $C_{43}H_{50}N_6O_9$, $[M+H]^+$ 795.91; found, 795.88.

Example 588. Synthesis of ((((2S,5S,8S,11S,14S,31S,34S,37S,40S,43S)-22,23-bis(2,5- dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5,8,11,34,37,40,43-octamethyl-4,7,10,13,16,21,24,29,32,35,38,41-dodecaoxo-14,31-bis(31-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32-azahexatriacontan-36-yl)-

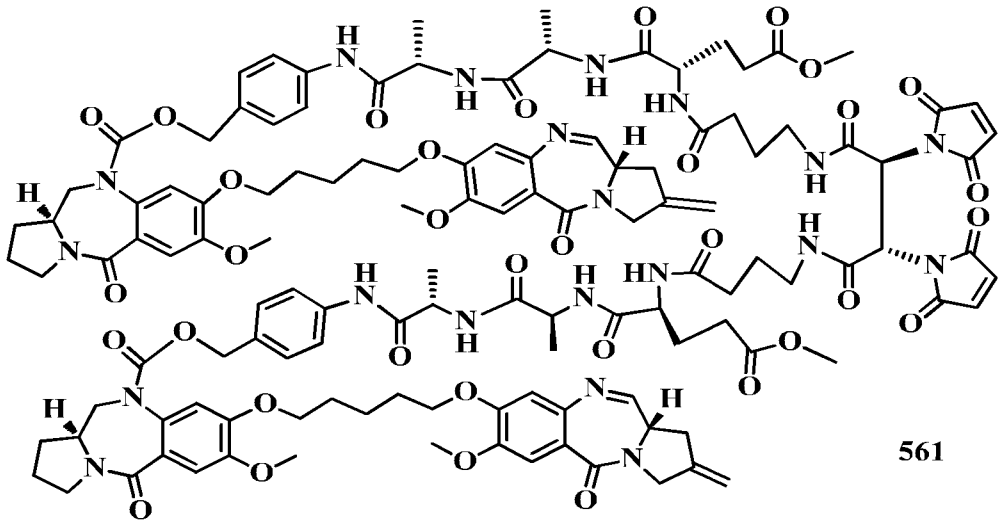
3,6,9,12,15,20,25,30,33,36,39,42-dodecaazatetratetracontanedioyl)bis(azanediyl))bis(4,1-phenylene))bis(methylene) (11aS,11a'S)-bis(7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a- tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate) (**560**).



560

A solution of compound **139** (105 mg, 0.05 mmol), HATU (60 mg, 0.15 mmol) in DMF (1 mL) was stirred at r.t. for 15 min. The above crude product of **559** in DMF was added to the reaction solution, followed by DIPEA (21 mg, 0.15 mmol). After stirring at r.t. for 30 min., the reaction mixture was directly purified by preparative HPLC to give a light yellow solid (64 mg, 35% yield). MS-ESI (m/z): calcd. for $C_{178}H_{252}N_{26}O_{56}[M+2H]^{2+}$:1827.05; found 1827.24.

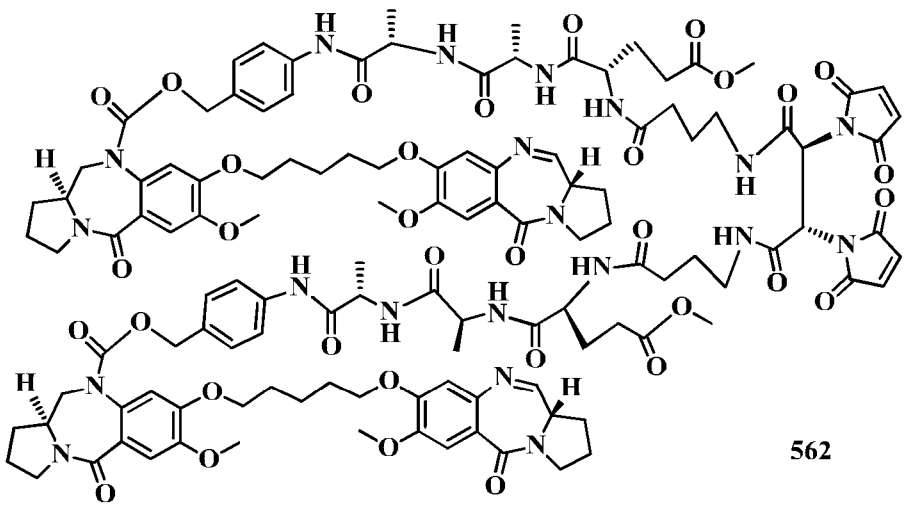
Example 589. Synthesis of dimethyl (4S,12S,13S,21S)-12,13-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,21-bis(((S)-1-(((S)-1-((4-((((S)-7-methoxy-8-((5-(((S)-7-methoxy-2-methylene-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-6,11,14,19-tetraoxo-5,10,15,20-tetraazatetracosanedioate (**561**).



561

A solution of compound **459** (45 mg, 0.05 mmol), HATU (60 mg, 0.15 mmol) in DMF (1 mL) was stirred at r.t. for 15 min. Another batch of **559** in DMF was added to the reaction solution, followed by DIPEA (21 mg, 0.15 mmol). After stirring at r.t. for 30 min., the reaction mixture was directly purified by preparative HPLC to give a light yellow solid (46 mg, 37% yield). MS-ESI (m/z): calcd. for $C_{124}H_{146}N_{20}O34[M+2H]^{2+}$:1231.32; found 1231.24.

Example 590. Synthesis of dimethyl (4S,12S,13S,21S)-12,13-bis(2,5-dioxo-2,5-dihydro- 1H-pyrrol-1-yl)-4,21-bis(((S)-1-(((S)-1-((4-((((S)-7-methoxy-8-((5-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-5-oxo-2,3,5,10,11,11a-hexahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10-carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-6,11,14,19-tetraoxo-5,10,15,20-tetraazatetracosanedioate (**562**).

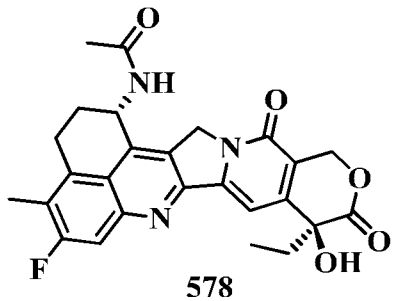


562

A solution of compound **459** (26 mg, 0.028 mmol), HATU (41 mg, 0.108 mmol) in DMF (1 mL) was stirred at r.t. for 15 min. and then the crude product of **383** (0.07 mmol) in DMF was added to the reaction solution, followed by DIPEA (13.6 mg, 0.105 mmol). After stirring at r.t. for 30 min.,

the reaction mixture was directly purified by preparative HPLC to give a light yellow solid (30 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{122}H_{146}N_{20}O_{36}$, $[M+2H]^{2+}$:1219.81; found 1219.89.

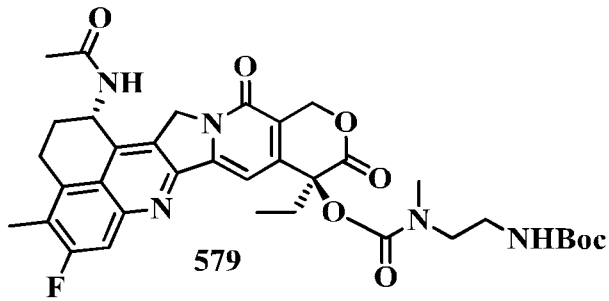
Example 591. Synthesis of N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo -2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (**578**).



578

To a solution of exatecan mesylate (1.0 g) and acetic acid (0.22 g) in DMF (20 mL), N,N-diisopropylethylamine (0.90 g) and DMTMM (2.0 g) were added. The reaction was stirred at r.t. overnight, diluted with dichloromethane (20 mL) and washed with brine. The precipitate formed in the solution was collected by filtration, dried to give a light brown solid (0.76 g, 69% yield). MS-ESI (m/z): calcd. for $C_{26}H_{24}N_3O_5$ $[M+H]^+$ 478.17; found, 478.20.

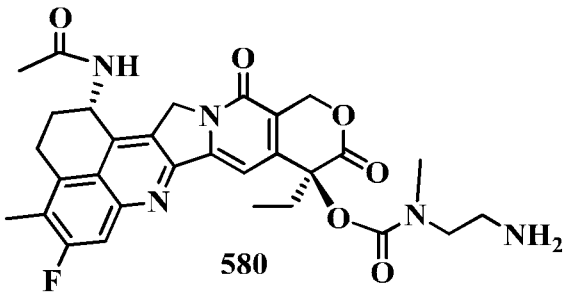
Example 592. Synthesis of (1S,9S)-1-acetamido-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**579**).



579

To a suspension of compound **578** (1.05 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), 4-nitrophenyl carbonochloridate (4.30 g, 21.6 mmol) and DMAP (2.60 g, 21.6 mmol) were added at 0 °C and then warmed to r.t. and stirred for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate. tert-Butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was then added and stirred at r.t. for 2 hours. The reaction mixture was washed with brine again, dried and concentrated. The residue was purified by a silica gel column (5% MeOH/DCM) to give a yellow solid (0.90 g, 60% yield). MS-ESI (m/z): calcd. for $C_{35}H_{40}FN_5O_8$, $[M+H]^+$ 678.73; found, 678.59.

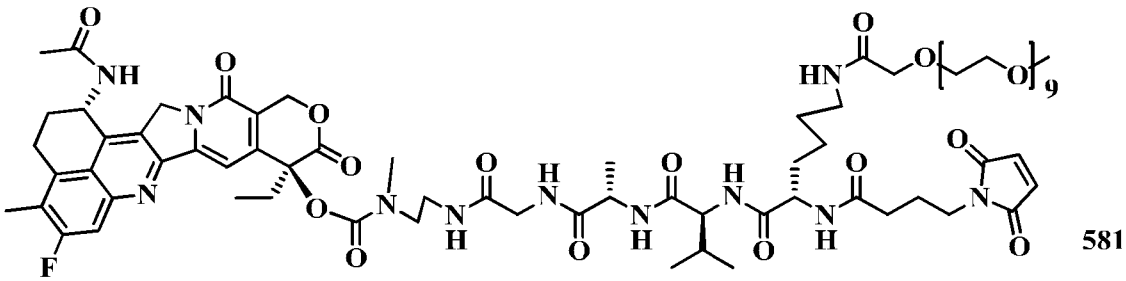
Example 593. Synthesis of (1S,9S)-1-acetamido-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**580**).



580

Compound **579** (0.90 g, 1.33 mmol) was treated with methanolic HCl (15 mL) at r.t. for 6 hours, and then concentrated to give a yellow solid (0.80 g). MS-ESI (m/z): calcd. for $C_{30}H_{32}FN_5O_6$ $[M+H]^+$ 578.61; found, 578.85.

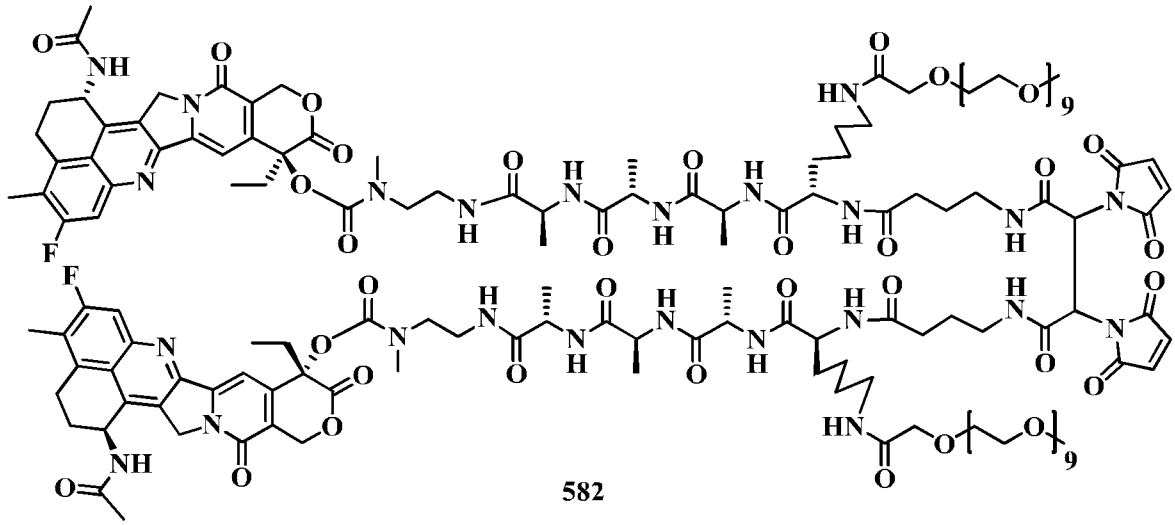
Example 594. Synthesis of (1S,9S)-1-acetamido-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ((37S,40S,43S)-37-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)-40-isopropyl-43-methyl-31,38,41,44,47-pentaoxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,39,42,45,48-pentaazapentacontan-50-yl)(methyl)carbamate (**581**).



581

To a solution of compound **580** (87 mg, 0.15 mmol) and **433** (151 mg, 0.15 mmol) in DMF (3 mL), HATU (120 mg, 0.30 mmol) and N,N-diisopropylethylamine (40 mg, 0.30 mmol) were added. The reaction was stirred at r.t. for 2 hours and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (42 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{75}H_{108}FN_{11}O_{24}$, $[M+H]^+$ 1567.74; found, 1567.99.

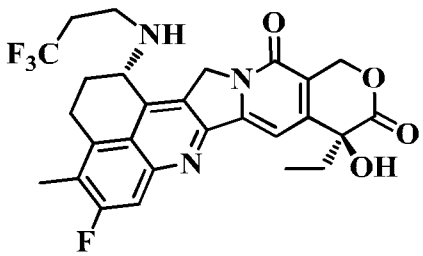
Example 595. Synthesis of bis((1S,9S)-1-acetamido-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**582**).



582

To a solution of compound **139** (105 mg, 0.05 mmol) in DMF (5 mL), HATU (60 mg, 0.15 mmol) was added and stirred for 15 min. Compound **578** (58 mg, 0.10 mmol) and N,N-diisopropylethylamine (21 mg, 0.15 mmol) were then added. The reaction was stirred at r.t. for 30 min and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (34 mg, 35% yield). MS-ESI (m/z): calcd. for $C_{152}H_{216}F_2N_{24}O_{50}$, $[M+2H]^{2+}$ 1609.74; found, 1609.78.

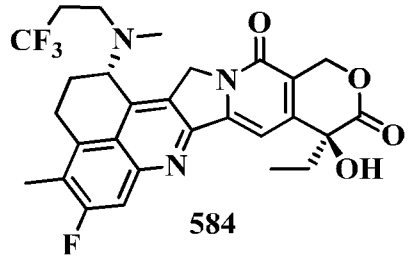
Example 596. Synthesis of (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1-((3,3,3-trifluoropropyl)amino)-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione (**583**).



583

To a solution of exatecan mesylate (4.0 g) and N,N-diisopropylethylamine (3.6 g) in DMF (60 mL), was added compound trifluoroethyl triflate (2.3 g) at 0 °C. The reaction was stirred at 0 °C for 2 hours, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (1.1 g, 22% yield). MS-ESI (m/z): calcd. for $C_{27}H_{25}F_4N_3O_4$, $[M+H]^+$ 532.17; found, 532.20.

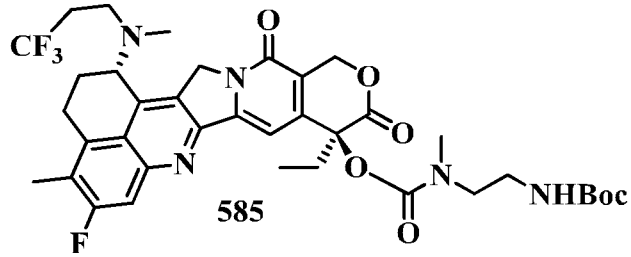
Example 597. Synthesis of (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1-(methyl(3,3,3 -trifluoropropyl)amino)-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione (**584**).



584

To a solution of compound **583** (0.30 g) in acetic acid (5 mL), were added paraformaldehyde (25 mg) and sodium cyanoborohydride (71 mg). The reaction was stirred at r.t. overnight, concentrated and purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (250 mg, 81% yield). MS-ESI (m/z): calcd. for $C_{28}H_{27}F_4N_3O_4$, $[M+H]^+$ 546.19; found, 546.20.

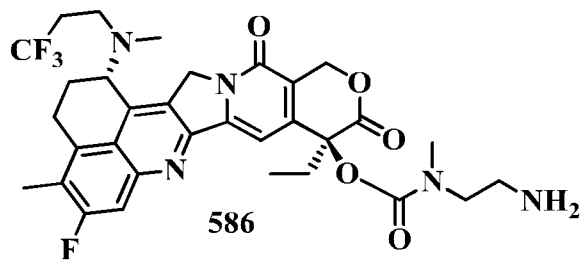
Example 598. Synthesis of (1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(methyl(3,3,3-trifluoropropyl) amino)-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**585**).



585

To a suspension of compound **584** (1.20 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), DMAP (2.6 g, 21.6 mmol) and 4-nitrophenyl carbonochloridate (4.3 g, 21.6 mmol) were added at 0 °C and then stirred at 30 °C for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate, and filtered. Tert-butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was added to the filtrate and stirred at 30 °C for 2 hours. The reaction mixture was washed with brine, dried, filtered and concentrated. The residue was purified by a silica gel column (MeOH/DCM 1:20) to give the title compound (1.02 g, 62% yield). MS-ESI (m/z): calcd. for $C_{37}H_{43}F_4N_5O_7$, $[M+H]^+$ 746.77; found, 746.64.

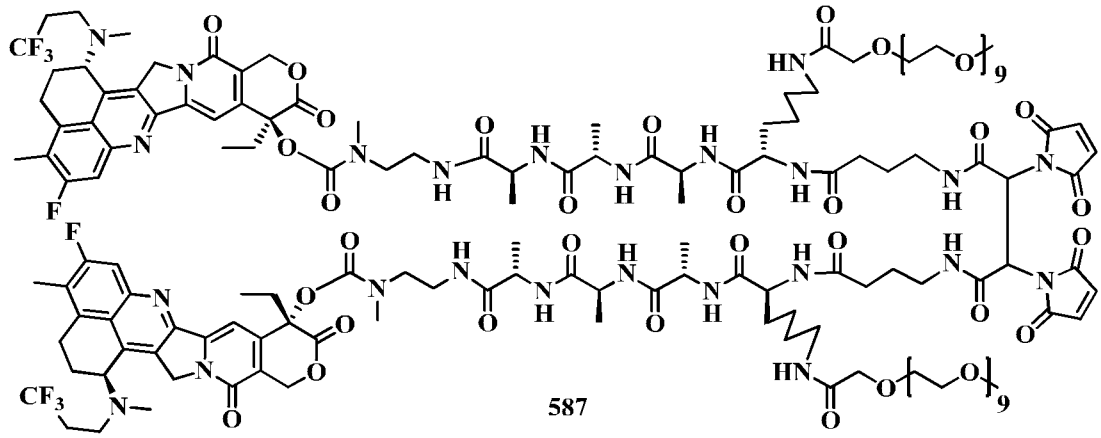
Example 599. Synthesis of (1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(methyl(3,3,3-trifluoropropyl) amino)-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**586**).



586

A solution of compound **585** (1.02 g, 1.36 mmol) in methanolic HCl (15 mL) was stirred at 30 °C for 6 hours and concentrated to give a yellow solid (1.30 g), which was used directly in the next step. MS-ESI (m/z): calcd. for $C_{32}H_{35}F_4N_5O_5$ $[M+H]^+$ 646.66; found, 646.74.

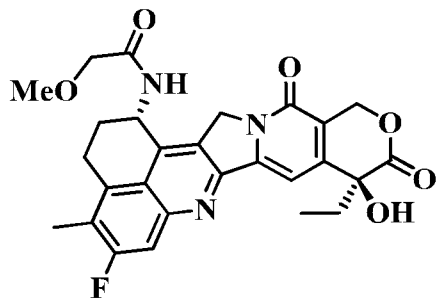
Example 600. Synthesis of bis((1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(methyl(3,3,3-trifluoropropyl)amino)-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**587**).



587

To a solution of compound **139** (105 mg, 0.05 mmol) in DMF (1 mL), HATU (60 mg, 0.15 mmol) was added and stirred for 15 min. Compound **586** (64 mg, 0.10 mmol) and N,N-diisopropylethylamine (21 mg, 0.15 mmol) were then added. The reaction was stirred at r.t. for 30 min and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (34 mg, 20% yield). MS-ESI (m/z): calcd. for $C_{156}H_{222}F_8N_{24}O_{48}$, $[M+2H]^{2+}$ 1677.80; found, 1677.56.

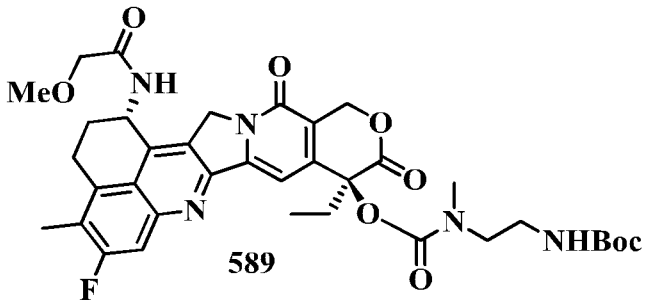
Example 601. Synthesis of N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)-2-methoxyacetamide (**588**).



588

To a solution of exatecan mesylate (50 mg, 0.11 mmol) and 2-methoxyacetic acid (21 mg, 0.23 mmol) in DMF (3 mL), NMM (25 mg, 0.23 mmol) and DMTMM (101 mg, 0.34 mmol) were added. The reaction was stirred at r.t. for 4 hours, and then directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (30 mg, 50% yield). MS-ESI (m/z): calcd. for $C_{27}H_{26}FN_3O_6$ $[M+H]^+$ 508.18; found, 508.20.

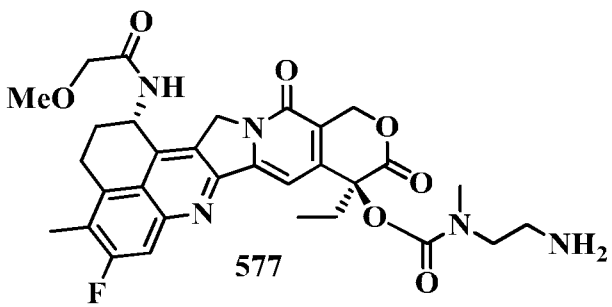
Example 602. Synthesis of (1S,9S)-9-ethyl-5-fluoro-1-(2-methoxyacetamido)-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**589**).



589

To a suspension of compound **588** (1.16 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), 4-nitrophenyl carbonochloridate (4.30 g, 21.6 mmol) and DMAP (2.60 g, 21.6 mmol) were added at 0 °C and then warmed to r.t. and stirred for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate. tert-Butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was then added and stirred at r.t. for 2 hours. The reaction mixture was washed with brine again, dried and concentrated. The residue was purified by a silica gel column (5% MeOH/DCM) to give a yellow solid (0.86 g, 55% yield). MS-ESI (m/z): calcd. for $C_{36}H_{42}FN_5O_9$, $[M+H]^+$ 708.76; found, 708.54.

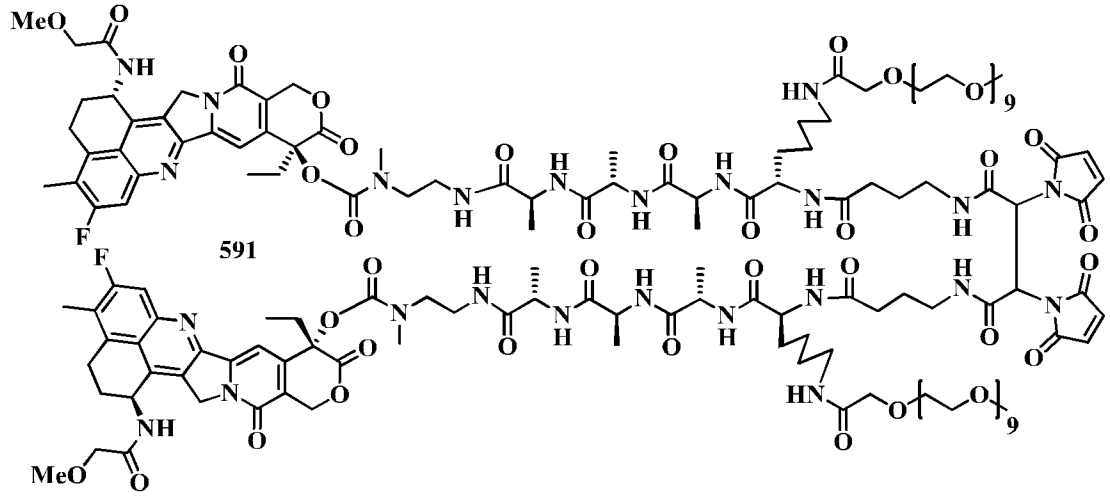
Example 603. Synthesis of (1S,9S)-9-ethyl-5-fluoro-1-(2-methoxyacetamido)-4-methyl- 10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**590**).



577

Compound **589** (0.71 g, 1.00 mmol) was treated with methanolic HCl (15 mL) at r.t. for 6 hours, and then concentrated to give a yellow solid (0.70 g). MS-ESI (m/z): calcd. for $C_{31}H_{34}FN_5O_7$ $[M+H]^+$ 608.64; found, 608.68.

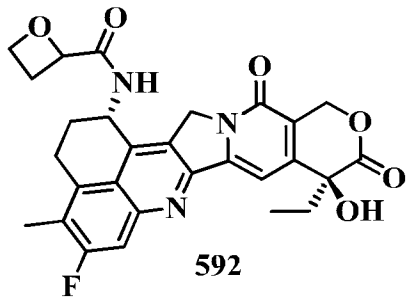
Example 604. Synthesis of bis((1S,9S)-9-ethyl-5-fluoro-1-(2-methoxyacetamido)-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**591**).



591

To a solution of compound **139** (105 mg, 0.05 mmol) in DMF (1 mL), HATU (60 mg, 0.15 mmol) was added and stirred for 15 min. Compound **590** (61 mg, 0.10 mmol) and N,N-diisopropylethylamine (21 mg, 0.15 mmol) were then added. The reaction was stirred at r.t. for 30 min and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (30 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{154}H_{220}F_2N_{24}O_{52}$, $[M+2H]^{2+}$ 1639.78; found, 1639.71.

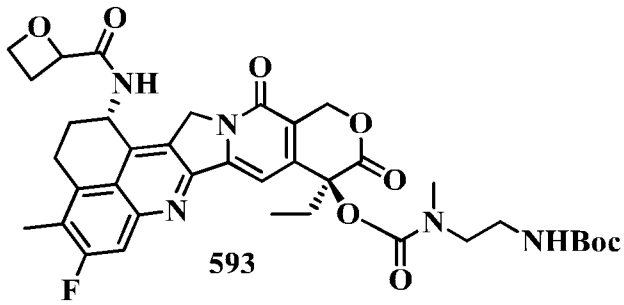
Example 605. Synthesis of N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)oxetane-2-carboxamide (**592**).



592

To a solution of exatecan mesylate (50 mg, 0.11 mmol) and oxetane-2-carboxylic acid (24 mg, 0.23 mmol) in DMF (3 mL), NMM (25 mg, 0.23 mmol) and DMTMM (101 mg, 0.34 mmol) were added. The reaction was stirred at r.t. for 4 hours, and then directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (45 mg, 75% yield).

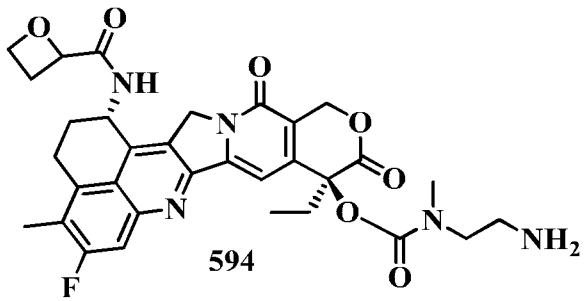
Example 606. Synthesis of (1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(oxetane-2-carboxamido)-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**593**).



593

To a suspension of compound **592** (1.14 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), 4-nitrophenyl carbonochloridate (4.30 g, 21.6 mmol) and DMAP (2.60 g, 21.6 mmol) were added at 0 °C and then warmed to r.t. and stirred for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate. tert-Butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was then added and stirred at r.t. for 2 hours. The reaction mixture was washed with brine again, dried and concentrated. The residue was purified by a silica gel column (5% MeOH/DCM) to give a yellow solid (0.97 g, 61% yield). MS-ESI (m/z): calcd. for $C_{37}H_{42}FN_5O_9$, $[M+H]^+$ 720.77; found, 720.58.

Example 607. Synthesis of (1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(oxetane-2-carboxamido)-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**594**).

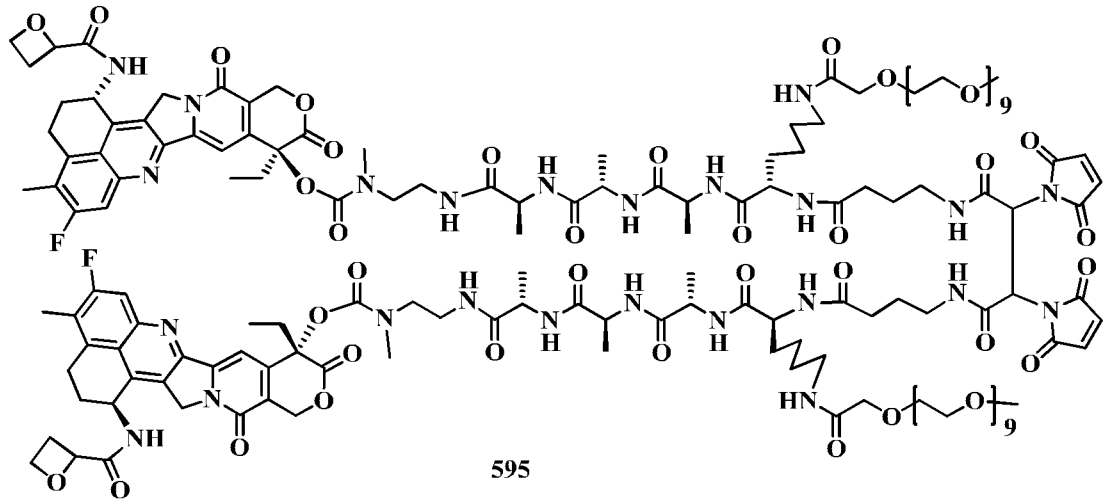


594

Compound **593** (0.72 g, 1.00 mmol) was treated with methanolic HCl (15 mL) at r.t. for 6 hours, and then concentrated to give a yellow solid (0.70 g). MS-ESI (m/z): calcd. for $C_{32}H_{34}FN_5O_7$ $[M+H]^+$ 620.65; found, 620.47.

Example 608. Synthesis of bis((1S,9S)-9-ethyl-5-fluoro-4-methyl-1-(oxetane-2-carboxamido) -10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1 -yl)succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-

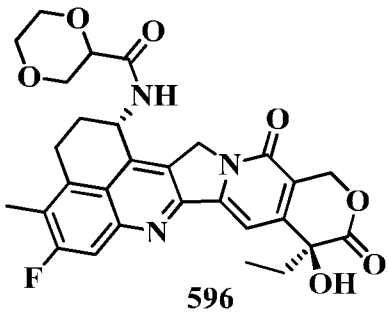
pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**595**).



595

To a solution of compound **139** (105 mg, 0.05 mmol) in DMF (1 mL), HATU (60 mg, 0.15 mmol) was added and stirred for 15 min. Compound **594** (62 mg, 0.10 mmol) and N,N-diisopropylethylamine (21 mg, 0.15 mmol) were then added. The reaction was stirred at r.t. for 30 min and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (30 mg, 18% yield). MS-ESI (m/z): calcd. for $C_{156}H_{220}F_2N_{24}O_{52}$, $[M+2H]^{2+}$ 1651.80; found, 1651.75.

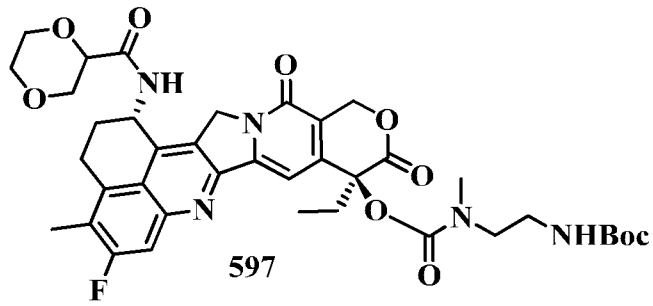
Example 609. Synthesis of N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)-1,4-dioxane-2-carboxamide (**596**).



596

To a solution of exatecan mesylate (50 mg, 0.11 mmol) and 1,4-dioxane-2-carboxylic acid (34 mg, 0.23 mmol) in DMF (3 mL), NMM (25 mg, 0.23 mmol) and DMTMM (101 mg, 0.34 mmol) were added. The reaction was stirred at r.t. for 4 hours, and then directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound as an off-white solid (22 mg, 37% yield). MS-ESI (m/z): calcd. for $C_{29}H_{28}FN_3O_7$, $[M+H]^+$ 550.18; found, 550.20.

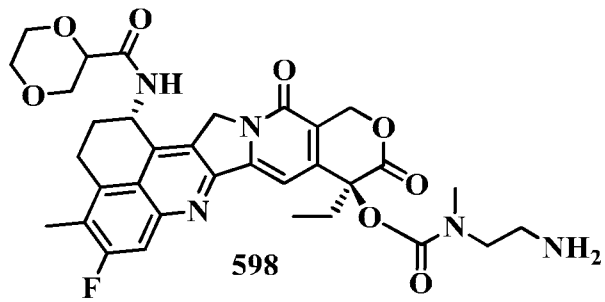
Example 610. Synthesis of (1S,9S)-1-(1,4-dioxane-2-carboxamido)-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-((tert-butoxycarbonyl)amino)ethyl)(methyl)carbamate (**597**).



597

To a suspension of compound **596** (1.14 g, 2.2 mmol) in DMF (5 mL) and dichloromethane (30 mL), 4-nitrophenyl carbonochloridate (4.30 g, 21.6 mmol) and DMAP (2.60 g, 21.6 mmol) were added at 0 °C and then warmed to r.t. and stirred for 2 hours. The reaction solution was washed with brine and dried over anhydrous sodium sulfate. tert-Butyl (2-(methylamino)ethyl)carbamate (750 mg, 4.3 mmol) was then added and stirred at r.t. for 2 hours. The reaction mixture was washed with brine again, dried and concentrated. The residue was purified by a silica gel column (5% MeOH/DCM) to give a yellow solid (0.91 g, 55% yield). MS-ESI (m/z): calcd. for $C_{37}H_{44}FN_5O_{10}$, $[M+H]^+$ 750.79; found, 750.87.

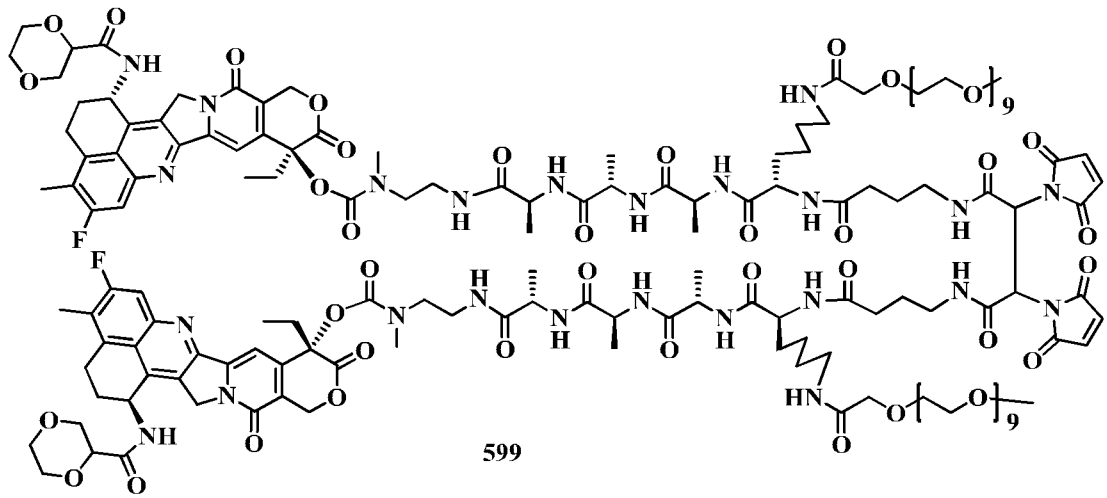
Example 611. Synthesis of (1S,9S)-1-(1,4-dioxane-2-carboxamido)-9-ethyl-5-fluoro-4-methyl -10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (2-aminoethyl)(methyl)carbamate (**598**).



598

Compound **597** (0.75 g, 1.00 mmol) was treated with methanolic HCl (15 mL) at r.t. for 6 hours, and then concentrated to give a yellow solid (0.70 g). MS-ESI (m/z): calcd. for $C_{33}H_{36}FN_5O_8$ $[M+H]^+$ 650.68; found, 650.59.

Example 612. Synthesis of bis((1S,9S)-1-(1,4-dioxane-2-carboxamido)-9-ethyl-5-fluoro-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ((37S,37'S,40S,40'S,43S,43'S,46S,46'S)-((4,4'-((2,3-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)succinyl)bis(azanediyl))bis(butanoyl))bis(azanediyl))bis(40,43,46-trimethyl-31,38,41,44,47-pentaoxo-2',5',8',11',14',17',20',23',26',29'-decaoxa-32',39',42',45',48'-pentaazapentacontane-37,50-diyl))bis(methylcarbamate) (**599**).



599

To a solution of compound **139** (105 mg, 0.05 mmol) in DMF (1 mL), HATU (60 mg, 0.15 mmol) was added and stirred for 15 min. Compound **598** (65 mg, 0.10 mmol) and N,N-diisopropylethylamine (21 mg, 0.15 mmol) were then added. The reaction was stirred at r.t. for 30 min and directly purified by preparative HPLC (acetonitrile/water containing 0.1% formic acid) to give the title compound (22 mg, 13% yield). MS-ESI (m/z): calcd. for $C_{158}H_{224}F_2N_{24}O_{54}$, $[M+2H]^{2+}$ 1681.82; found, 1681.76.

Example 613. A tridational method of conjugation of an antibody to a cytotoxin having a terminal of maleimido group.

A purified IgG antibody was incubated with a 3.2 – 4.2 molar excess of the reducing agent TCEP (Tris(2-carboxyethyl)phosphine) in PBS pH 7.2, 1 mM EDTA (Ethylenediamine tetraaceticacid) for 1 hours at 37°C. Subsequently, 6.5 -9.0 equivalents of the payload of a cytotoxin having a terminal of maleimido group from a stock solution in 10% (v/v) DMA or DMSO was added, followed by incubation at room temperature for one hour to 3 hours under gentle rotation. The conjugation reaction was optionally quenched by the addition of four molar equivalents (over the payload) of N-acetyl cysteine. After incubation, the reducing agent and the excess payload/linker complexes were removed by 2 -10 times of dialysis in PBS pH 5.0 -7.2, at 4 °C using 10,000 MWCO dialysis cassettes.

The conjugation process may result in 0.1 to 10% of aggregate formation. Macromolecular aggregates, conjugation reagents, including cysteine quenched paylaods, can be removed using ceramic hydroxyapatite Type II chromatography (CHT) as described e.g. Thompson et al., J. Control Release, 236: 100-116 (2016). The ADCs were optionally formulated in 25 mM Histidine-HCl, 7% sucrose, 0.02% polysorbate-20 or 80, pH 6.

To determine monomeric content, aggregates, and fragments, analytical size-exclusion chromatography (SEC-HPLC) was performed using 100 m (100 µL volume) of antibodies or ADCs, which were loaded into a TSKgel.RTM. G3000WXL column (Tosoh Bioscience, Tokyo, Japan). The

mobile phase was composed of 0.1 M sodium sulfate, 0.1 M sodium phosphate, and 10% isopropanol, pH 6.8. The flow rate was 1 mL/min, and each analysis was carried out for 10 - 45 minutes at room temperature. Hydrophobic interaction chromatography (HIC-HPLC) was used to assess conjugation and drug load distribution, and was performed using a butyl-non porous resin (NPR) column (4.6 mm ID x3.5 cm, 2.5 μm, Tosoh Bioscience). The mobile phase A was composed of 25 mM Tris-HCl, 1.5 M $(NH_4)_2SO_4$, pH 8.0; and the mobile phase B was composed of 25 mM Tris-HCl and 5% isopropanol, pH 8.0. 100 μL of antibodies or ADCs at a concentration of 1 mg/mL were loaded and eluted at a flow rate of 1 mL/min with a gradient of 5% B to 100% B over 10 - 30 min. Reduced reverse phase chromatography (rRP-HPLC) was used to confirm chain-specific conjugation. The antibodies and ADCs were reduced at 37 °C for 20 minutes using 42 mM dithiothreitol (DTT) in PBS (pH 7.2). 10 μg of reduced antibodies or ADCs were loaded onto a polymeric reverse phase media (PLRP-S) 1000 A column (2.1 x 50 mm) (Agilent Technologies, Santa Clara, Calif.) and eluted at 80°C at a flow rate of 1 mL/min with a gradient of 5% B to 100% B over 20 -35 minutes (mobile phase A: 0.1% trifluoroacetic acid in water; mobile phase B: 0.1% trifluoroacetic acid in acetonitrile).

Conjugation at the heavy and light chains and drug/antibody ratios (DAR) were determined by reduced liquid chromatography mass spectrometry analysis (rLCMS) performed on an Agilent 1290 series uHPLC coupled to an Agilent 6230 TOF (Agilent Technologies, Santa Clara, Calif.). 2 μg of reduced antibodies or ADCs were loaded onto a ZORBAX.RTM. rapid resolution high definition (RRHD) 300-Diphenyl column (2.1 x50 mm, 1.8 μm) (Agilent Technologies, Santa Clara, Calif.) and eluted at a flow rate of 0.5 mL/min using a step gradient of 80% B after 2.1 min (mobile phase A: 0.1% Formic acid in water and mobile phase B: 0.1% Formic acid in acetonitrile). A positive time-of-flight MS scan was acquired, and data collection and processing were carried out using MassHunter software (Agilent Technologies, Santa Clara, Calif.).

Example 614. The specific conjugation reaction.

A zinc amino complex (e.g. Zinc 2-methylpropane-1, 2-diamine chloride complex) (in 10 - 60 mM, 1.0- 5.0 eq. of an antibody used) and TCEP (in 100 mM, 2.5 -4.5 eq. of an antibody used) were added in sequence to a solution containing an antibody (such as BCMA, Her2, EGFR, CD33, Trop2, Steap1, CD56, PSMA, B7H3, Muc-1 and Her3 generated in house, 10 - 30 mg/mL, in 20 mM PBS pH 5.5 – 7.5) at 2 - 8 °C. After incubation at 2- 8 °C for 12 - 16 h (overnight), a payload/linker complex (100 - 200 mM, 2.0 – 8 .0 eq. of the antibody used) was introduced and incubated for further 2 - 4 h at 2 - 8 °C. After the incubation, cystine (100 - 200 mM, 4.0 – 8.0 eq. of the antibody) was added to the mixture to deplete the excess TCEP, cysteine (100 - 200 mM, 2.0 - 6.0 eq. of the antibody) was added to deplete the excess payload, EDTA (100 -200 mM, 4.0 – 6.0 eq. of the antibody) was added to trap zinc, and DHAA (100 -200 mM, 8.0 – 30.0 eq. of the antibody) was

added to oxidize the free thiol groups in the protein. The reaction mixture was finally purified using a de-salting column (Zeba Spin Desalting Columns, 40K MWCO), or UF/DF, or ion exchange chromatography, and drug/antibody ratio (DAR) were analyzed using HIC-HPLC or HPLC-MS. The HIC-HPLC results are exampled in Table 1 and 2.

Example 615. Reduced Molecular Weight and DAR Analysis for the Deglycosylated BCMA-Tub ADCs by LC-MS.

Sample preparation: Reduction of an ADC (e. g. (DXC009 DP) with 5mM dithiothreitol at 37°C for about 2 h, followed by a deglycosylation step with PNGase F at 37°C overnight generated six fragments as illustrated in Fig. 58 and 59. HC and LC existed as naked or conjugated forms carrying up to 3 payloads. The masses of each ADC fragments and the average DARs of the ADC as illustrated in Table 1. The following equation was used for average DAR calculation for conventional conjugated ADC.

Table 1. The summary of masses and proportions of the different ADC fragments and the average DAR measured from peak areas.

Species	Theoretical mass (Da)	Experimental mass (Da)	Delta (ppm)	Distribution (%)	Average DAR
L0	24191.6966	24191.463	-9.7	10.60	
L1	25842.6633	25842.4693	-7.5	89.40	
H0	48942.5955	48943.2271	12.9	14.21	3.86
H1	50593.5621	50594.3737	16	74.65	
H2	52244.5288	52245.128	11.5	4.65	
H3	53895.4955	53896.2266	13.6	6.50	

Average DAR = L1/(L0+L1) x 2 +H1/(H0 + H1 + H2 + H3) x 2 + H2/(H0 + H1 + H2 + H3) x 2 + H3/(H0 + H1 + H2+ H3) x 2.

The other example of DAR Analysis for the Deglycosylated Trop2 –CPT ADC by LC-MS is shown in Fig 60 and Table. 2 below.

Table 2. Summarizing masses and proportions of the different DXA002-CPT090 ADC fragments and the average DAR measured from peak intensity.

Species	Theoretical mass	Experimental mass	Delta	Distribution	Average

	(Da)	(Da)	(ppm)	(%)	DAR
L0	23334.7352	23334.6847	-2.2	8.11	4.54
L1	24761.27	24761.3385	2.8	91.89	
H0	50594.1727	50593.9633	-4.1	8.13	
H1	52020.7074	52020.9703	5.1	64.11	
H2	53447.2422	53446.9475	-5.5	12.36	
H3	54873.7769	54873.7636	-0.2	15.39	

Method conditions: UPLC system: Waters ACQUITY UPLC H-Class System; Detector: ACQUITY UPLC TUV; Absorption Wavelength: 280nm; Trap Column: ACQUITY UPLC C4 1.7 μm 2.1 x 50 mm Column; Mobile phase A: 0.1% formic acid (FA) in water, Mobile phase B: 0.1% formic acid (FA) in ACN; Performed the chromatographic separation at a flow rate of 0.4 ml/min using a linear gradient of mobile phase B (ACN with 0.1% FA) from 5% to 25% for 2 min, followed by 25% to 45% for 8 min, then 45% to 85% for 2 min.

MS conditions: MS system: Waters Xevo-G2XS Q-TOF; Ionization mode: ESI positive; Mass Range: m/z 500-4000 Da. Informatics: the data analysis using UNIFI V1.8.2.169 Software (Waters).

Example 616. DAR analysis.

Table 3. DAR was analyzed by using HIC-HPLC, and the HPLC parameters are as follows:

HPLC	Agilent 1260					
Column	Thermo HIC butyl 4.6 × 100 mm					
Phase A	PBS buffer containing $(NH_4)_2SO_4$,					
Phase B	PBS buffer					
Sample	injection volume 10 μL					
Rate	0.8 mL/min					
Wavelength	280 nm					
Gradient	Time (min)	0	35	40	42	48
	Phase A (%)	80	0	0	80	80
	Phase B (%)	20	100	100	20	20

Example 617. General preparation of formulation of the conjugates.

In a liquid formulation of 80 mg of each conjugate (with the antibody of BCMA, Her2, EGFR, CD33, Trop2, Steap1, CD56, PSMA, B7H3 and Her3 independently): C-25, C-30, C-36, C-45, C-51, C-58, C-68a, C-68b, C-68c, C-72a, C-72b, C-73, C-83, C-88, C-91, C-96, C-102, C-115, C-120, C-121, C-126, C-130, C-137, C-140, C-152, C-157, C-168, C-178, C-181a, C-181b, C-181c, C-181d, C-190, C-192, C-195, C-202, C-209, C-215, C-221, C-227, C-233, C-241, C-255, C-258, C-267, C-

277, C-283, C-284, C-290, C-295, C-300, C-306, C-309, C-310, C-312, C-319, C-322, C-326, C-328, C-330, C-332, C-334, C-335, C-336, C-337, C-338, C-361, C-362, C-372, C-373, C-374, C-375, C-384, C-385, C-390, C-396, C-399, C-400, C-402, C-406, C-409, C-416, C-417, C-427, C-432, C-437, C-441, C-450, C-455, C-462, C-464, C-470, C-478, C-481, C-484, C-487, C-488, C-494, C-498, C-499, C-500, C-501, C-504, C-508, C-511, C-514, C-518, C-519, C-521, C-527, C-531, C-532, C-537, C-542, C-543, C-544, C-546, C-550, C-553, C-560, C-561, C-562, C-565, C-570, C-573, C-577, C-581, C-582, C-587, C-591, C-595, C-599, in the 10 mL of borosilicate vial containing 240 mg of sucrose, 0.8 mg of polysorbate-80 or -20, 24 mg of sodium citrate in 4 mL of sterile water were adjusted with citric acid to pH 6.0. Then each of the conjugate solution was lyophilized at temperature from -65 °C to 0 °C, and then to RT at reduced pressure (5 ~10 torr) to form a dryness cake. The cake conjugates were stored at 2 ~ 8 °C in a stock room, and then reconstituted with 4 mL of sterile water when for further application.

Example 618. In vitro cytotoxicity evaluation of the conjugate (with the antibody of BCMA, Her2, EGFR, Trop2, Steap1, CD56, PSMA, B7H3 and Her3): C-25, C-30, C-36, C-45, C-51, C-58, C-68a, C-68b, C-68c, C-72a, C-72b, C-73, C-83, C-88, C-91, C-96, C-102, C-115, C-120, C-121, C-126, C-130, C-137, C-140, C-152, C-157, C-168, C-178, C-181a, C-181b, C-181c, C-181d, C-190, C-192, C-195, C-202, C-209, C-215, C-221, C-227, C-233, C-241, C-255, C-258, C-267, C-277, C-283, C-284, C-290, C-295, C-300, C-306, C-309, C-310, C-312, C-319, C-322, C-326, C-328, C-330, C-332, C-334, C-335, C-336, C-337, C-338, C-361, C-362, C-372, C-373, C-374, C-375, C-384, C-385, C-390, C-396, C-399, C-400, C-402, C-406, C-409, C-416, C-417, C-427, C-432, C-437, C-441, C-450, C-455, C-462, C-464, C-470, C-478, C-481, C-484, C-487, C-488, C-494, C-498, C-499, C-500, C-501, C-504, C-508, C-511, C-514, C-518, C-519, C-521, C-527, C-531, C-532, C-537, C-542, C-543, C-544, C-546, C-550, C-553, C-560, C-561, C-562, C-565, C-570, C-573, C-577, C-581, C-582, C-587, C-591, C-595, C-599, in comparison with Paclitaxel. For evaluation of Her2-ADCs, the comparison was chosen to T-DM1, and the evaluation of BCMA-ADCs was in comparison with Belantamab mc-MMAF.

The cell lines used in the cytotoxicity assays were (1). Myeloma(+) cells, NCI-H929, and MM1S were obtained from ATCC, and 8226-2A1 cell is Myeloma antigen express cells through culturing and clone-picking of ATCC's RPMI-8226; (2). EGFR (+) cells: HCC-827 is lung cancer cells, and LN229 and U87MG are human glioma tumor cell lines; (3). MUC-1 (+) cells: Colo205 is colon cancer cell line; (4). Trop2 (+) cells: MDA-MB-468 cells is a human triple negative breast cancer cell line, Calu-3 is a homo sapiens lung adenocarcinoma or a submucosal gland cell line; (5). Her2(+) cells: BT-474 is a human breast cancer cell line, NCI-N87 is a human gastric carcinoma cell line (NCI-N87 cells also express Trop2 antigens); SK-OV-3 is a human ovarian cancer cell line,

A431, a human epithelial carcinoma cell line. The cells were grown according to the provider manuals, For instance, N87 cells were grown in RPMI-1640 with 10% FBS. To run the assay, the cells (180 µl, 6000 cells) were added to each well in a 96-well plate and incubated for 24 hours at 37°C with 5% CO_2. Next, the cells were treated with test compounds (20 µl) at various concentrations in appropriate cell culture medium (total volume, 0.2 mL). The control wells contain cells and the medium but lack the test compounds. The plates were incubated for 120 hours at 37°C with 5% CO_2. MTT (5 mg/mL) was then added to the wells (20 µl) and the plates were incubated for 1.5 hr at 37°C. The medium was carefully removed and DMSO (180 µl) was added afterward. After it was shaken for 15min, the absorbance was measured at 490 nm and 570 nm with a reference filter of 620 nm. The inhibition% was calculated according to the following eq.uation: inhibition% = [1-(assay-blank)/(control-blank)] × 100. The MTT results of BCMA-ADCs are listed in Table 4.

Table 4. MTT assays of the BCMA antibody conjugates against tumor cells of NCI-H929, MM1S, JJN-3, at 6000 cells, 96 h incubation. The DAR indicated in the table 4 was DARs of the conjugate prepared via homogeneous conjugation process of the invention:

ADC Compound	DAR ratio	IC50 (nM) NCI-H929	IC50 (nM) MM1S	IC50 (nM) JJN-3
C-25	4.2	6.3	6.5	7.9
C-30	4.4	3.22	3.13	4.71
C-36	4.4	3.13	3.21	4.63
C-45	4.4	1.15	1.08	1.77
C-51	4.4	1.05	1.32	1.98
C-58a	4.6	0.78	0.95	1.56
C-68a	4.6	0.36	0.39	1.17
C-68b	4.4	0.37	0.39	1.28
C-68c	4.6	0.29	0.41	1.39
C-72a	4.4	0.43	0.52	0.98
C-72b	4.4	0.59	0.61	1.12
C-73	4.2	0.68	0.71	1.35
C-83	4.2	0.02	0.04	0.08
C-88	4.2	0.08	0.11	0.19
C-91	4.2	0.07	0.13	0.14
C-96	4.2	0.13	0.16	0.21
C-102	4.0	0.89	0.14	0.28

C-115	4.0	0.02	0.04	0.10
C-120	4.2	0.21	0.23	0.31
C-121	4.2	0.06	0.09	0.12
C-126	4.2	0.18	0.21	0.29
C-130	4.3	0.87	0.98	1.17
C-137	4.4	0.75	0.87	0.91
C-140	4.2	0.77	0.83	0.96
C-152	4.2	0.78	0.87	0.99
C-157	4.6	0.70	0.87	0.95
C-168	4.2	0.59	0.89	1.28
C-178	4.1	0.38	0.46	0.65
C-181a	4.2	0.92	0.98	1.31
C-181b	4.2	0.95	0.93	1.19
C-181c	4.4	0.65	0.80	1.02
C-181d	4.4	0.49	0.72	0.89
C-190	4.8	1.18	1.11	1.28
C-192	4.6	1.07	0.98	1.21
C-195	4.6	1.12	1.38	1.69
C-209	3.4	1.48	1.57	1.67
C-215	4.6	0.80	0.96	1.31
C-221	4.2	0.04	0.05	0.08
C-227	4.4	0.05	0.06	0.08
C-233	4.4	0.05	0.06	0.11
C-241	4.5	0.13	0.29	0.31
C-255	4.4	0.06	0.08	0.11
C-258	4.4	0.05	0.08	0.09
C-267	4.4	0.12	0.18	0.38
C-277	4.2	0.03	0.06	0.09
C-283	4.1	1.22	1.60	1.96
C-284	4.0	0.04	0.07	0.12
C-290	4.1	0.35	0.49	0.93
C-295	4.2	1.01	1.06	1.03
C-300	4.4	0.29	0.25	0.39

C-306	4.4	0.08	0.09	0.12
C-309	4.5	0.06	0.09	0.17
C-310	4.4	0.15	0.19	0.33
C-312	4.5	0.13	0.15	0.19
C-319	4.4	0.25	0.27	0.38
C-322	4.4	0.29	0.34	0.59
C-326	4.4	0.45	0.59	0.73
C-328	4.4	0.06	0.09	0.19
C-330	4.4	0.21	0.29	0.92
C-332	4.4	0.08	0.19	0.31
C-334	4.1	0.20	0.13	0.62
C-335	4.0	0.23	0.32	0.83
C-336	4.2	0.13	0.16	0.39
C-337	4.0	0.57	0.49	0.97
C-338	4.1	0.51	0.50	0.61
C-361	4.0	5.93	7.50	8.57
C-362	4.2	5.63	8.54	6.67
C-373	4.1	4.47	5.57	5.78
C-374	4.1	5.46	5.62	5.66
C-375	4.2	5.32	5.49	4.79
C-384	4.0	1.41	1.63	1.87
C-385	4.2	1.30	1.49	1.63
C-390	4.4	0.06	0.05	0.09
C-396	4.4	0.04	0.06	0.07
C-399	4.4	0.17	0.28	0.39
C-400	4.1	0.07	0.04	0.06
C-402	4.2	0.03	0.04	0.06
C-406	4.4	0.11	0.14	0.29
C-409	4.4	0.07	0.12	0.11
C-416	4.0	0.08	0.09	0.11
C-417	4.4	0.07	0.09	0.09
C-427	4.2	0.08	0.08	0.10
C-432	4.0	0.07	0.08	0.10

C-437	4.2	0.07	0.09	0.10
C-441	4.2	0.06	0.08	0.09
C-450	4.0	0.09	0.12	0.13
C-455	4.2	0.08	0.12	0.13
C-462	4.2	0.08	0.10	0.12
C-464	4.2	0.09	0.12	0.13
C-470	4.2	0.10	0.12	0.14
C-478	4.0	0.18	0.22	0.28
C-481	4.0	0.17	0.20	0.28
C-484	4.0	0.18	0.20	0.28
C-487	4.0	0.17	0.19	0.26
C-488	4.2	0.16	0.19	0.26
C-494	4.0	0.14	0.17	0.23
C-498	4.0	0.14	0.18	0.22
C-499	4.0	0.17	0.22	0.28
C-500	4.0	0.14	0.20	0.22
C-501	4.2	0.14	0.19	0.22
C-504	4.2	0.15	0.18	0.22
C-508	4.2	0.14	0.17	0.20
C-511	4.2	0.14	0.18	0.20
C-514	4.2	0.15	0.18	0.20
C-518	4.2	0.14	0.16	0.17
C-519	4.2	0.15	0.17	0.17
C-521	4.1	0.15	0.17	0.20
C-527	4.2	0.17	0.18	0.22
C-531	4.2	0.16	0.17	0.20
C-532	4.2	0.17	0.17	0.20
C-534	4.2	0.16	0.17	0.20
C-537	4.1	0.17	0.19	0.21
C-542	4.2	0.17	0.19	0.22
C-543	4.2	0.17	0.18	0.20
C-544	4.2	0.18	0.19	0.22
C-546	4.2	0.17	0.18	0.20

C-550	4.2	0.36	0.42	0.51
C-553	4.2	0.37	0.42	0.55
C-560	4.2	0.08	0.09	0.11
C-561	4.2	0.09	0.08	0.10
C-562	4.2	0.08	0.08	0.09
C-565	3.8	0.13	0.17	0.21
C-570	3.8	0.14	0.17	0.25
C-573	4.2	0.14	0.17	0.19
C-577	4.2	0.13	0.17	0.18
C-581	4.2	0.13	0.17	0.18
C-582	4.2	0.14	0.18	0.20
C-587	4.2	0.17	0.19	0.20
C-591	4.2	0.17	0.20	0.22
C-595	4.2	0.18	0.24	0.23
C-599	4.2	0.19	0.23	0.23
Belantamab mc-MMAF	4.0	0.08	0.11	0.41
Paclitaxel		2.89	3.28	4.31

Example 619. Antitumor Activity in vivo (BALB/c Nude Mice Bearing HCC-827, NCI-N87, NCi-H929, BT-474, SK-OV-3, OE-19, Calu-3, HCT-116, Mz-ChA-1, or UCC, Xenograft Tumors independently).

The in vivo efficacy of, BCMA (hu5D2) conjugates of C-68a, C-83, C-115, C-126, C-137, C-181b, C-192, C-195, C-202, C-212b, C-258, C-277, C-290, C-306, C-385, C-390, C-396, C-399, C-400, C-402, C-406, and C-417 along with Belantamabmc-MMAF against human multiple myeloma NCI-H929 cells at dose of 2 or 3 mg/Kg, or JJN-3 cells or 5 mg/Kg; B7H3 antibody conjugates of C-550, C-570-T (with traditional conjugation at DAR =4.0), C-543, C-595, C-570-H (with homogeneous conjugation of this invention), C-581, C-582, C-560, C-562, and C-561, at dose of 6 mg/Kg, single injection, along with Paclitaxel at dose of 15 mg/Kg, QWx3, against Calu-6 cells; Muc1 antibody conjugates of C-553, C-484, C-490, C-450, C-432-T (with traditional conjugation at DAR =4.0), C-501, C-519, C-432-H (with homogeneous conjugation of this invention), C-417, and C-560, at dose of 6 mg/Kg, single injection, along with Paclitaxel at dose of 15 mg/Kg, QWx3, against OE19 cells; administered intravenously, in xenograft models. Five-week-old female BALB/c Nude mice (6 animals per group) were inoculated subcutaneously in the area under the right shoulder with respective

carcinoma cells (5×10^6 cells/mouse) in 0.1 - 0.2 mL of serum-free medium. The tumors were grown for 6-8 days to an average size of 150 mm^3, or 8 - 9 days to an average size of 180 mm^3. The animals were then randomly divided into different groups (6 animals per group). The first group of mice served as the control group and was treated with the phosphate-buffered saline (PBS) vehicle. The other groups were treated with conjugates at dose indicated. Three dimensions of the tumor were measured every 3 or 4 days (twice a week) and the tumor volumes were calculated using the formula tumor volume =1/2 (length × width × height). The weight of the animals was also measured at the same time. A mouse was sacrificed when any one of the following criteria was met: (1) loss of body weight of more than 20% from pretreatment weight, (2) tumor volume larger than 1500 mm^3, (3) too sick to reach food and water, or (4) skin necrosis. A mouse was considered to be tumor-free if no tumor was palpable.

The results were plotted in Figures 58-62. All the conjugates did not cause the animal body weight loss at dose indicated. All conjugates demonstrated antitumor activity as comparison with PBS buffer.

Example 620. Analysis the DAR and the conjugation sites by UPLC-MS:

DAR measurement: Reduction (5mM dithiothreitol at 37 °C for about 2 h) of the ADC followed by a deglycosylation step (Rapid PNGase F at 50 °C for about 15min) can generate six fragments as illustrated in Figure 2 - 4 . HC and LC exist as naked or conjugated forms carrying up to 3 payloads. Chromatographic separations of these fragments with MS detection were performed with Acquity UPLC(Waters) using BEH 300 C_4 1.7μm 2.1 × 50mm column coupled with Xevo G2XS Q-TOF mass spectrometer (Waters). Performed the chromatographic separation at a flow rate of 0.25 ul/min using a linear gradient of mobile phase B (ACN with 0.1% FA) from 5% to 85% over 4min. Conducted the data acquisition with MassLynx software, and used the mass acquisition range from 500 Da to 4000 Da. Performed the data analysis using UNIFI software (Waters). The following equation was used for average DAR calculation for conventional conjugated ADC.

Average DAR = L1/(L0+L1)*2 + H1/(H0+H1+H2+H3)*2 + H2/(H0+H1+H2+H3)*2 + H3/(H0+H1+H2+H3)*2

Conjugation Site: ADC samples were denatured and reduced (6M Urea, 10mM dithiothreitol at 56 °C for about 40 min), alkylated (about 30mM Iodoacetamide, 40 min in the dark at room temperature), diluted in 50mM NH_4HCO_3 and digested with trypsin (1/50 enzyme/substrate weight ratio, 4h, 37 °C). Chromatographic separations of peptides with MS detection were performed with Acquity UPLC (Waters) using BEH C_{18} 1.7 μm 2.1×100 mm Column coupled with Xevo G2XS Q-TOF mass spectrometer (Waters). Perform the chromatographic separation at a flow rate of 0.2 ul/min using a linear gradient of mobile phase B (ACN with 0.1% FA) from 1% to 40% over 95 min.

Conducted the data acquisition with MassLynx software, and used the mass acquisition range from 100 Da to 2500 Da. Perform the data analysis using UNIFI software (Waters).

Species	Theoretical mass (Da)	Experimental mass (Da)	Delta (ppm)	Distribution (%)	Average DAR
L0	23334.7352	23334.6847	-2.2	8.11	4.54
L1	24761.27	24761.3385	2.8	91.89	
H0	50594.1727	50593.9633	-4.1	8.13	
H1	52020.7074	52020.9703	5.1	64.11	
H2	53447.2422	53446.9475	-5.5	12.36	
H3	54873.7769	54873.7636	-0.2	15.39	

Table.1 Summarizing masses and proportions of the different Trop2-CPT090 ADC fragments and the average DAR measured from peak intensity.

CLAIMS

What is claimed is:

1. A homogenous conjugation process comprises the following steps:

(a) incubating an antibody or antibody-like protein in the presence of an effective amount of a Zinc-amino chelate/complex ($Zn(NR_1R_2R_3)_{m1}^{2+}$) chelate and a reductant, in a water based buffer system to selectively reduce inter-chain disulfide bonds within the antibody or antibody-like protein to generate thiol groups;

(b) introducing an effective amount of a linker or drug/linker complex bearing thiol reactive groups to react with the thiol groups resulted from step (a);

(c) optionally adding an effective amount of an oxidant to re-oxidize unreacted thiol groups; or adding a quenching agent to cap unreacted thiol groups; and

(d) purifying resulted conjugate;

wherein the optional step (c) can be replaced by: adding an effective amount of cysteine, or a relative disulfide compound, or an azido compound to quench unreacted reductant, while generating cysteine or a thiol compound from reduction of the cystine or a disulfide compound to quench excessive conjugation linker or drug/linker complex containing a thiol reactive group, or an effective amount of cysteine or relative thiol compound can be added to quench excessive linker or linker/payload complex molecule;

wherein R_1, R_2 and R_3 are independently a C_1-C_8 alkyl; C_2-C_8 heteroalkyl, alkylcycloalkyl, or heterocycloalkyl; C_3-C_8 aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, or heteroaryl; m1 is 1, 2, 3, 4, 5, 6, 7 or 8; or N, R_1, R_2 and/or R_3 themselve can form a heterocyclic, carbocyclic, diheterocyclic, or dicarbocyclic ring; and $(NR_1R_2R_3)_{m1}$ can form a dimer, trimer, tetramer, pentamer, or hexamer wherein these polymers are covalently linked by N, R_1, R_2 and/or R_3; the zinc-amino chelate/complex, $Zn(NR_1R_2R_3)_{m1}^{2+}$, used in step (a) is 0.01 mM-1.0 mM in concentration, or 0.5-20 equivalents in moles of the protein;

wherein the reductant is an organic phosphine reductant and used at 1.0 – 20 equivalents in moles of the protein;

wherein the oxidant in step (c) is DHAA, Fe^{3+}, I_2, Cu^{2+}, Mn^{3+}, MnO_2, or a mixture of Fe^{3+}/I^-, the oxidant used in a reaction solution of step (c) is 0.02 mM—1.0 mM in concentration, or 1-100 equivalents in moles of the protein;

wherein a pH in a conjugation reaction of step (b) is between about 5.0 to 8.0;

wherein the reaction solution of step (a) comprises up to 30% of a water mixable (miscible) organic solvent selected from DMA, DMF, ethanol, methanol, acetone, acetonitrile, THF,

isopropanol, dioxane, propylene glycol, or ethylene diol as a co-solvent in the water based buffer system;

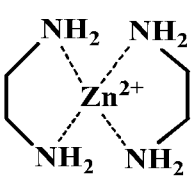
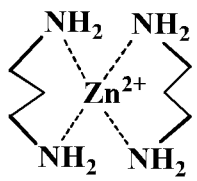
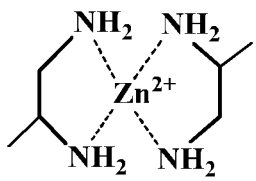
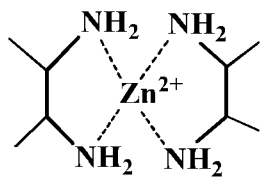
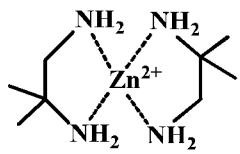
wherein a temperature in the conjugation reaction of step (b) is between about - 5 °C to 40 °C; a reaction time of step (b) is between about 15 min to about 48 hours;

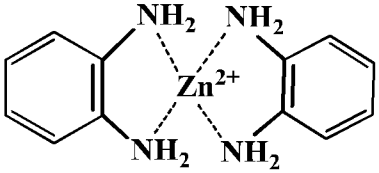
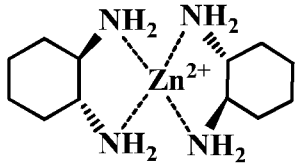
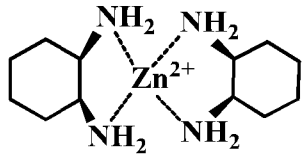
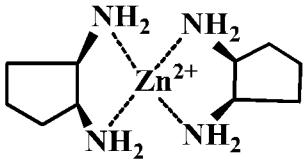
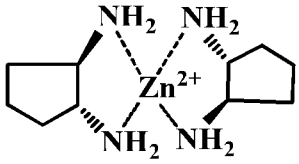
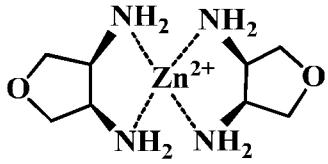
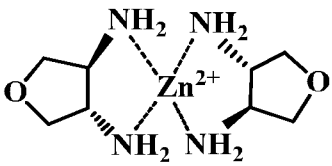
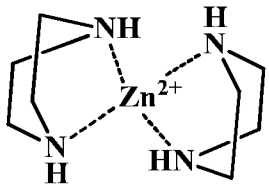
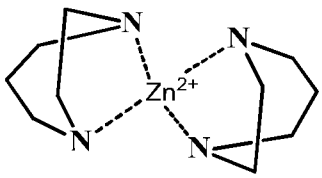
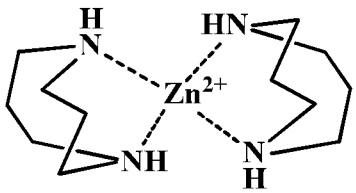
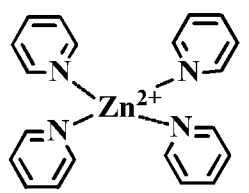
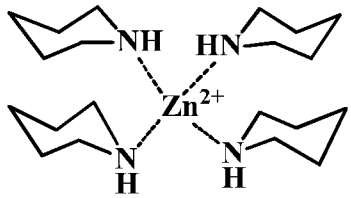
wherein the resulted conjugate is purified by gel filtration on a Sephadex G25 or Sephacryl S300 column, adsorption chromatography, ion (cation or anion) exchange chromatography or by dialysis (ultrafiltration or hyperfiltration (UF) and/or diafiltration (DF);

wherein a drug/antibody like protein (antibody) ratio (DAR) of the conjugate is measured by UV at wavelength of range 240-380 nm, by hydrophobic interaction chromatography (HIC-HPLC), Capilary electrophoresis (CE), LC-MS, CE-MS, or LC-MS/MS.

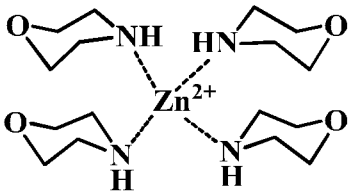
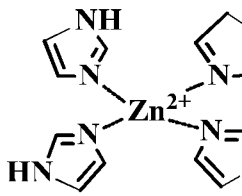
2. The process according to Claim 1, wherein

(a) the zinc cation-amino chelate is selected from the group consisting of $Zn(NH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2CH_2CH_3)_2^{2+}$, $Zn(NH_2CH(CH_3)_2)_2^{2+}$, $Zn(NH_2C(CH_3)_3)_2^{2+}$, $Zn(NH_2CH_2C(CH_3)_3)_2^{2+}$, $Zn(NH(CH_3)_2)_2^{2+}$, $Zn(NH(CH_2CH_3)_2)_2^{2+}$, $Zn(NH(CH(CH_3)_2)_2)_2^{2+}$, $Zn(NH(C(CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH(CH_2CH_3)_2)_2)_2^{2+}$, $Zn(NH(CH_2C(CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH_2C(CH_2CH_3)_3)_2)_2^{2+}$, $Zn(NH(CH_2CH_2C(CH_3)_3)_2)_2^{2+}$, $Zn(NH_2CH_2CH_2OH)_2^{2+}$, $Zn(NH(CH_2CH_2OH)_2)_2^{2+}$, $Zn(N(CH_2CH_2OH)_3)_2^{2+}$, $Zn(NH_2CH_2COOH)_2^{2+}$, $Zn(NH_2CH_2CONH_2)_2^{2+}$, $Zn(NH_2CH_2COOCH_3)_2^{2+}$, $Zn(NH_2CH_2COOCH_2CH_3)_2^{2+}$, $Zn(NH_2CH_2COOC(CH_3)_3)_2^{2+}$, $Zn(NH_2CH_2COOCH(CH_3)_2)_2^{2+}$, $Zn(NH_2CH_2CH_2COOH)_2^{2+}$, $Zn(NH(CH_2COOH)_2)_2^{2+}$, $Zn(N(CH_2CH_2COOH)_3)_2^{2+}$, $Zn(NH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2CH_2CH_3)_4^{2+}$, $Zn(NH_2CH(CH_3)_2)_4^{2+}$, $Zn(NH_2C(CH_3)_3)_4^{2+}$, $Zn(NH_2CH_2C(CH_3)_3)_4^{2+}$, $Zn(NH(CH_3)_2)_4^{2+}$, $Zn(NH(CH_2CH_3)_2)_4^{2+}$, $Zn(NH(CH(CH_3)_2)_2)_4^{2+}$, $Zn(NH(C(CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH(CH_2CH_3)_2)_2)_4^{2+}$, $Zn(NH(CH_2C(CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH_2C(CH_2CH_3)_3)_2)_4^{2+}$, $Zn(NH(CH_2CH_2C(CH_3)_3)_2)_4^{2+}$, $Zn(NH_2CH_2CH_2OH)_4^{2+}$, $Zn(NH(CH_2CH_2OH)_2)_4^{2+}$, $Zn(N(CH_2CH_2OH)_3)_4^{2+}$, $Zn(NH_2CH_2COOH)_4^{2+}$, $Zn(NH_2CH_2CONH_2)_4^{2+}$, $Zn(NH_2CH_2COOCH_3)_4^{2+}$, $Zn(NH_2CH_2COOCH_2CH_3)_4^{2+}$, $Zn(NH_2CH_2COOC(CH_3)_3)_4^{2+}$, $Zn(NH_2CH_2COOCH(CH_3)_2)_4^{2+}$, $Zn(NH_2CH_2CH_2COOH)_4^{2+}$, $Zn(NH(CH_2COOH)_2)_4^{2+}$, $Zn(N(CH_2CH_2COOH)_3)_4^{2+}$,


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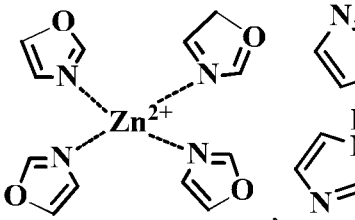
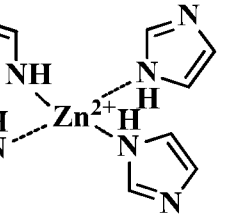
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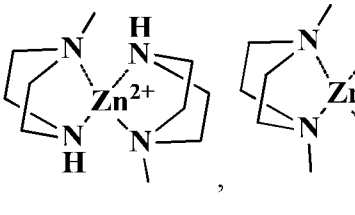
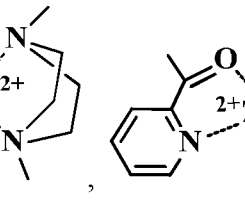
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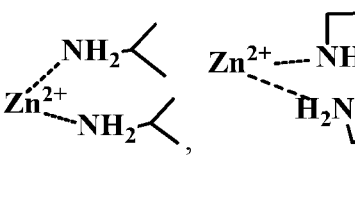
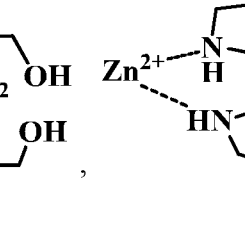
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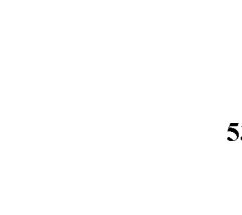
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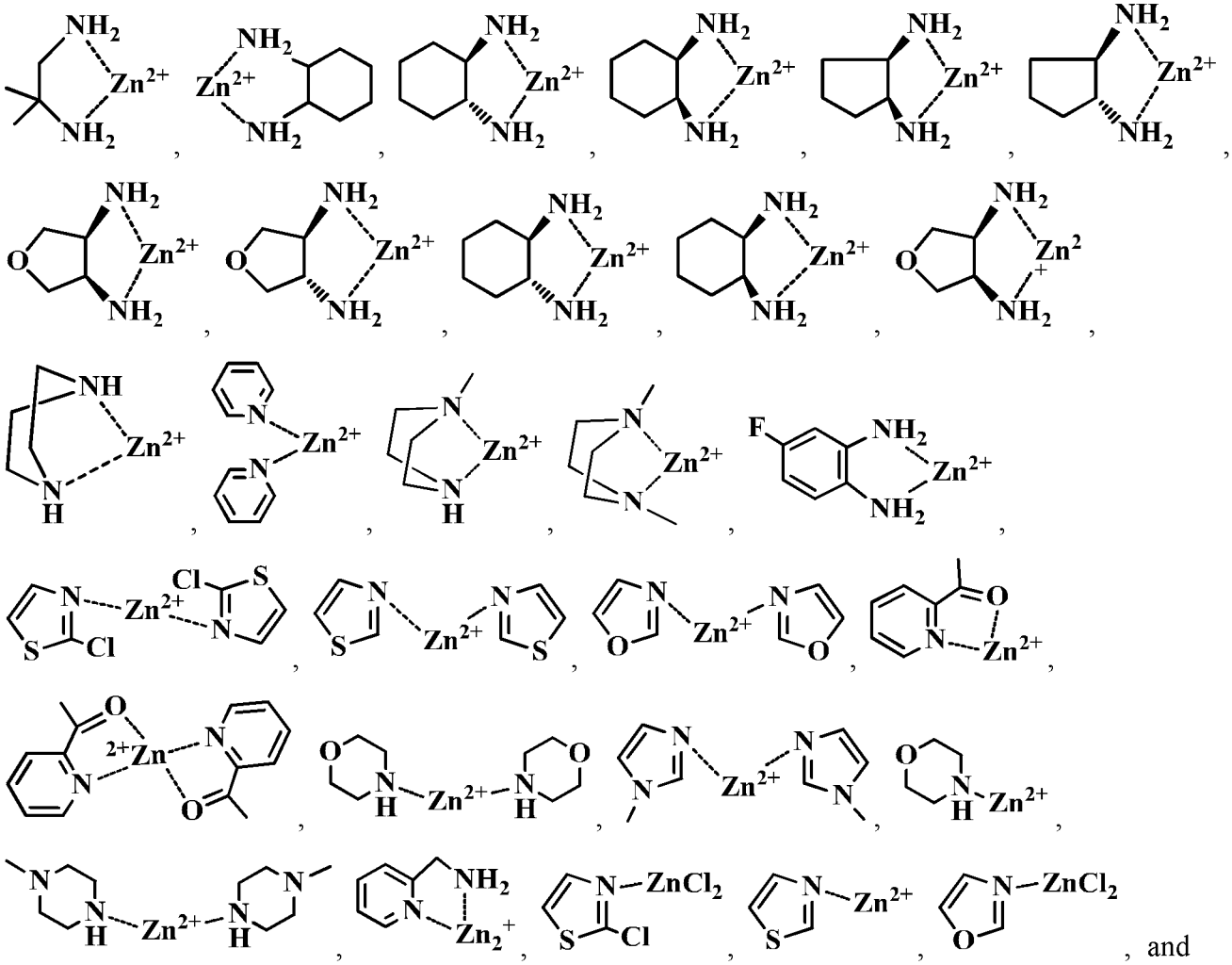
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a salt formed from any of the above cations with an anion which is selected from the group consisting of Cl^-, Br^-, I^-, SO_4^{2-}, HSO_4^-, NO_3^-, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, CO_3^{2-}, HCO_3^-, $HCOO^-$, CH_3COO^-, F_3CCOO^-, Cl_3CCOO^-, FCH_2COO^-, $ClCH_2COO^-$, F_2CHCOO^-, Cl_2CHCOO^-, BF_4^-, SO_3^{2-}, HSO_3^-, CH_3SO^{3-}, $C_6H_5CH_2SO^{3-}$, $C_6H_5SO^{3-}$, $C_6H_5COO^-$, $C_6H_5CH_2COO^-$, $C_6F_5O^-$, $C_6H_4(OH)COO^-$, $C_6H_2F_3O^-$, $C_6H_4(NO_2)O^-$, and $C_6H_2(NO_2)_3O^-$;

wherein the zinc-amino complex in the reaction solution of step (a) are 0.5 -20 equivalents in moles of the protein, and is added with a water- miscible organic solvent, selected from ethanol, methanol, propanol, propandiol, DMA, DMF, DMSO, THF, or CH_3CN.

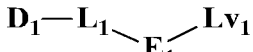
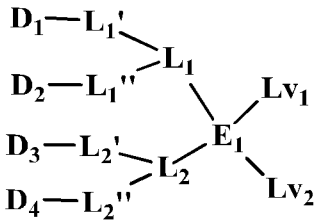
(b). wherein the organic phosphine reductant is selected from the group consisting of Tris(2-carboxyethyl)phosphine (TCEP) ($P(CH_2CH_2COOH)_3$), Tris(hydroxypropyl)phosphine ($P(CH_2CH_2CH_2OH)_3$), and the reductant used in the reaction solution of step (A) is 2.0 - 4.0 equivalents in moles of the protein.

(c). wherein the buffer system comprises PBS, Mes, Bis-Tris, Bis-Tris Propane, Pipes, Aces, Mopso, Bes, Mops, Hepes, Tes, Pipps, Dipso, Tapso, Heppso, Tris-up, Tris-HCl, Tricine, Hepps, Gly-Gly, Bicine, Taps, Hepee, Acetate, Histidine, Citrate, MES, Borate, or a combination

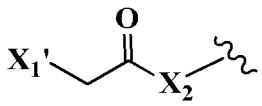
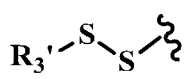
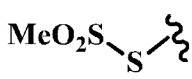
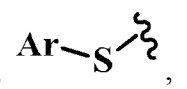
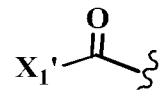
of two, three or four buffer components from above; a pH of the buffer system is 4.0 -9.0; a concentration of the buffer in the reaction solution of step (A) is 0.02 – 1.0 M.

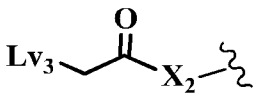
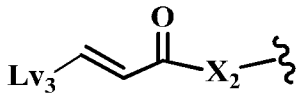
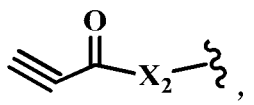
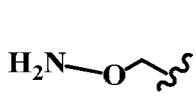
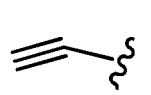
3. The process according to Claim 1, wherein the drug/linker complex has a formula (I), (II), (III) or (IV):

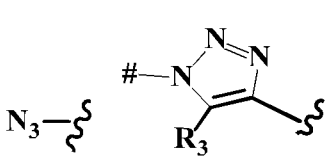
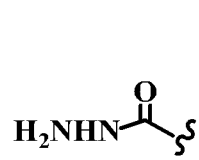
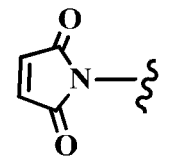
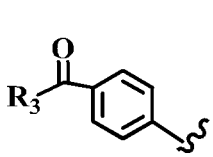
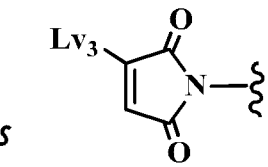
(I), or

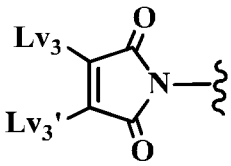
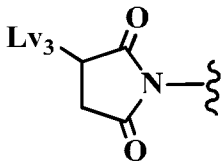
(II), or

(III), or

(IV),

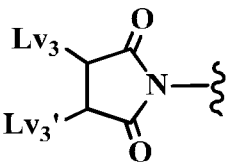
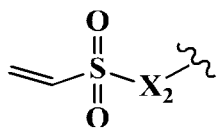
wherein: Lv_1 and Lv_2 are a thiol reaction group, and are independently or jointly selected from:

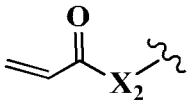
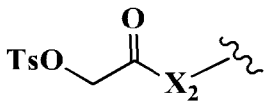

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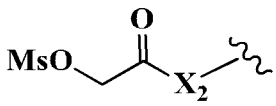
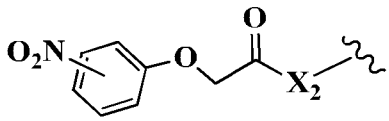

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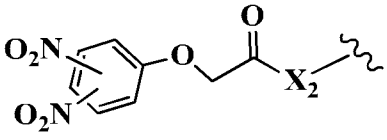
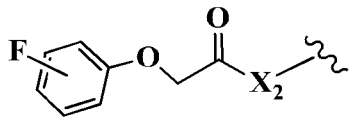
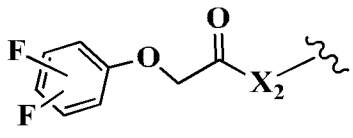
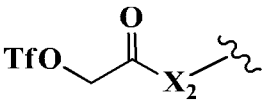
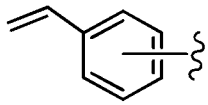
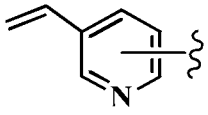
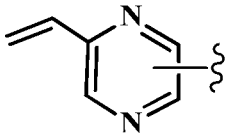
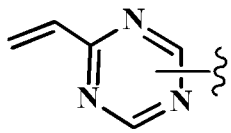
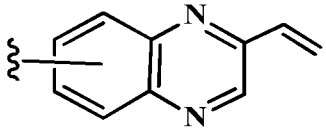
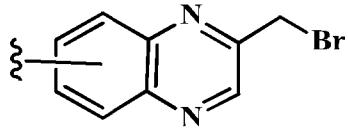
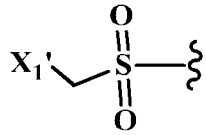
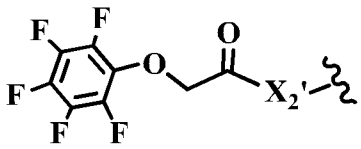
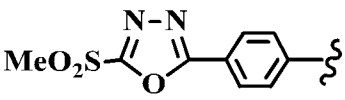
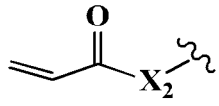
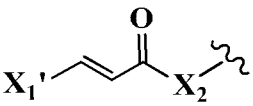
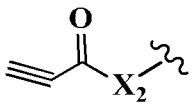
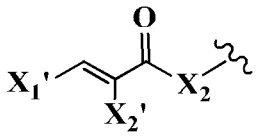
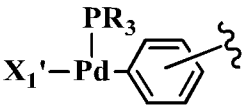
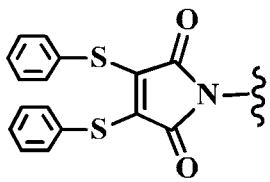
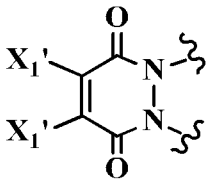
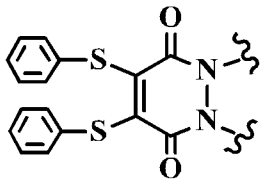
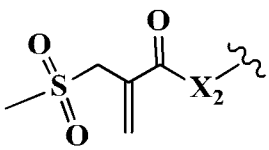
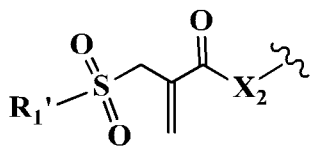
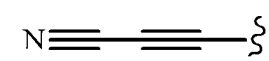
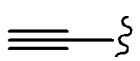

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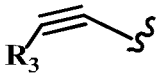
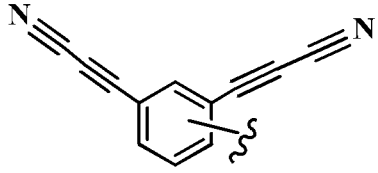
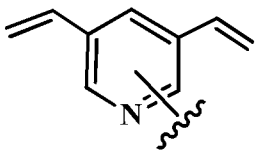
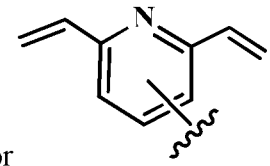
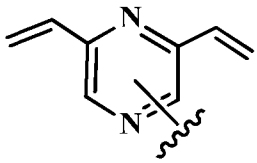
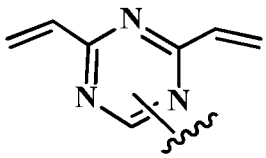
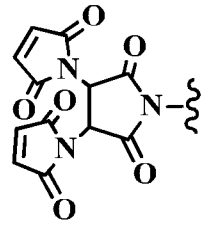
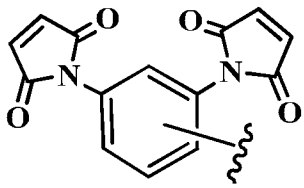
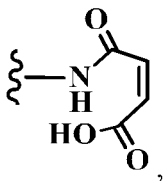
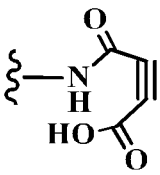
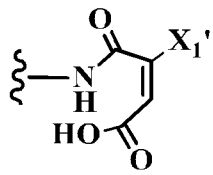
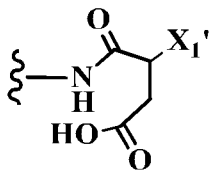
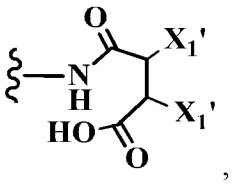
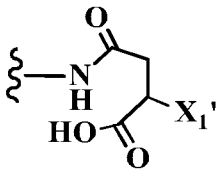
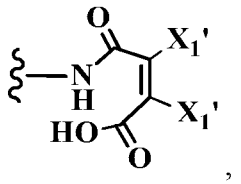
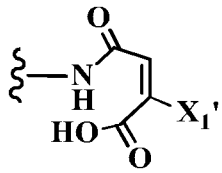
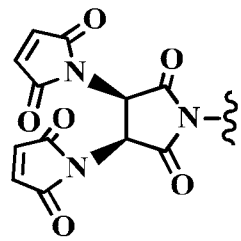
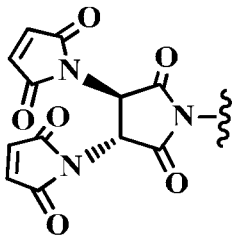
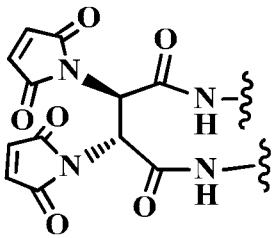
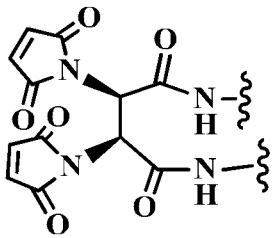
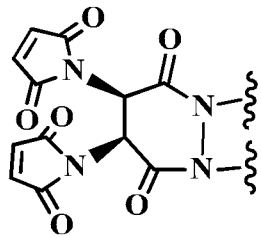
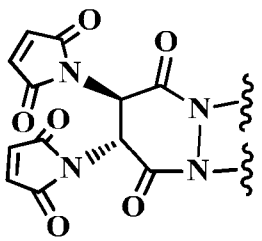
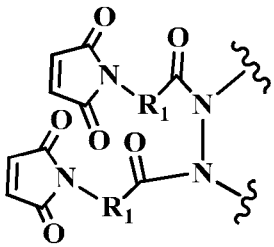
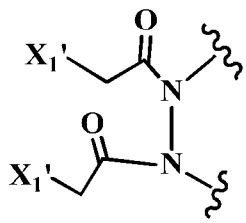
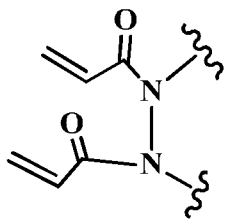
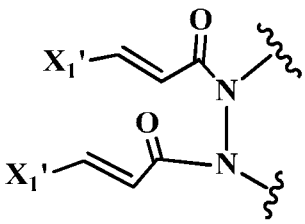
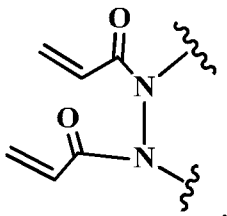
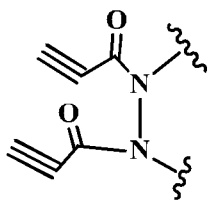
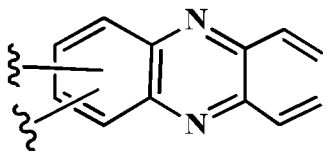
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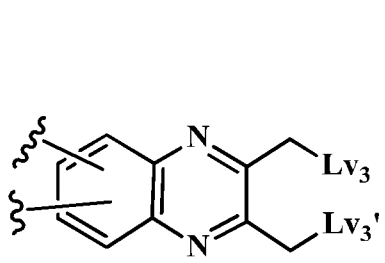
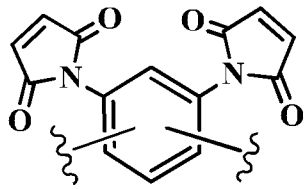
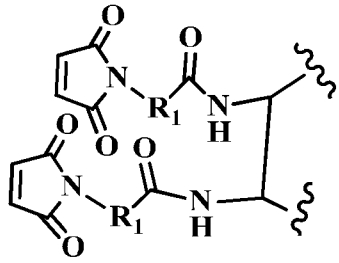
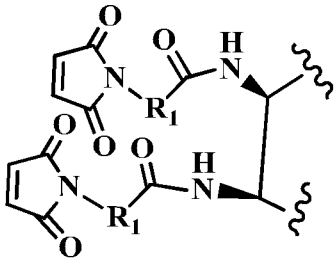
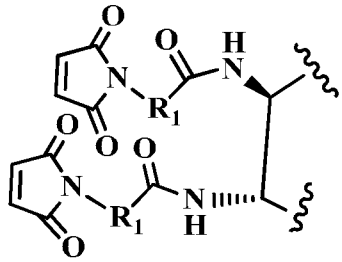
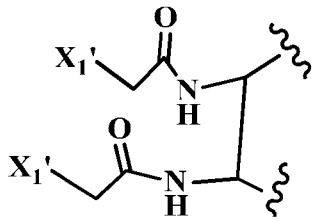
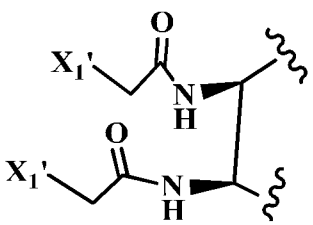
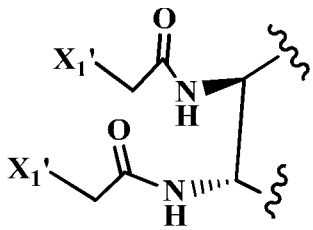
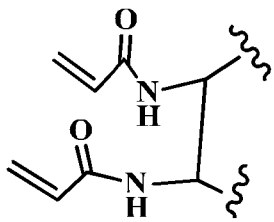
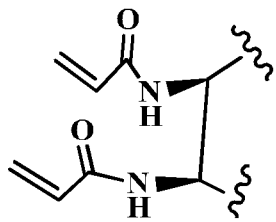
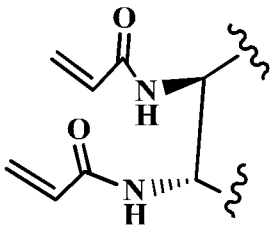
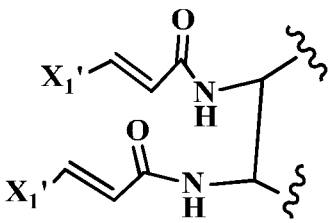
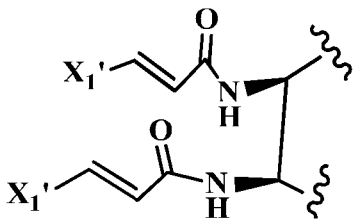
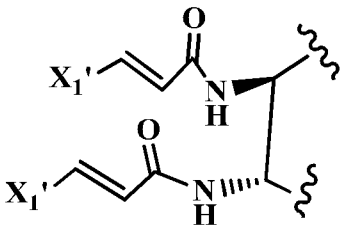
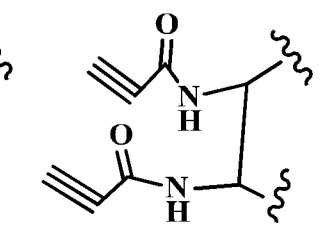
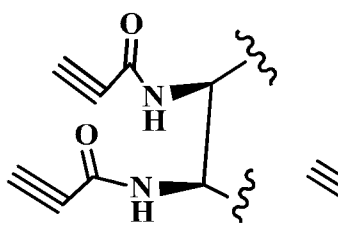
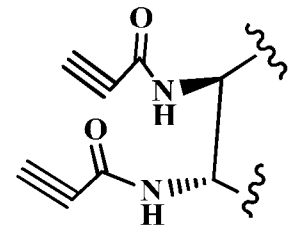
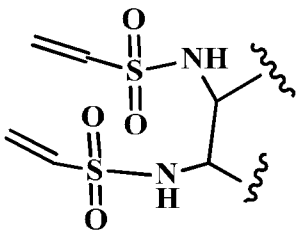
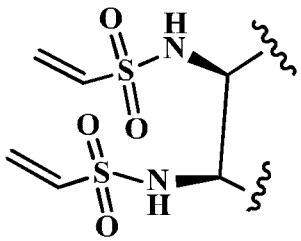
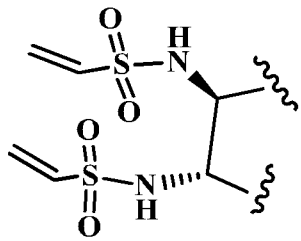
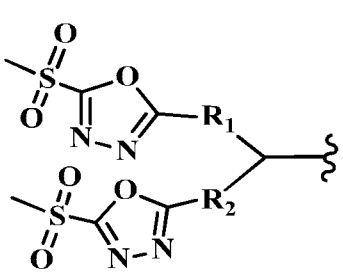
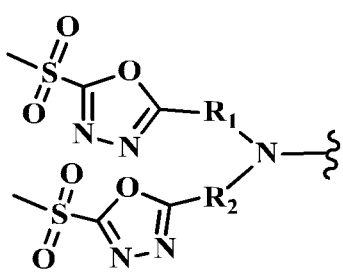
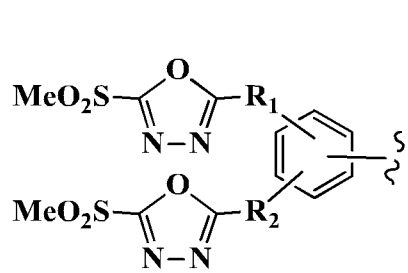
tuted succinimide;

disubstituted succinimide; -CHO aldehyde;


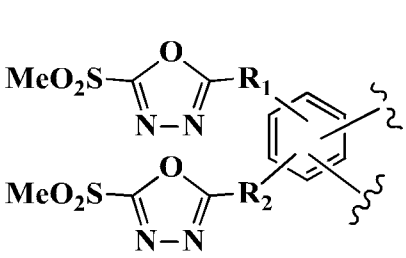
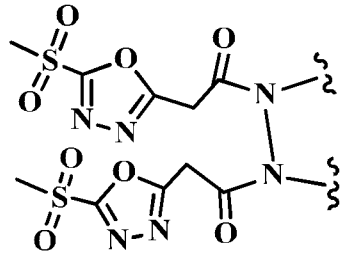
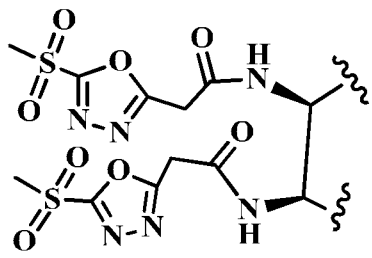
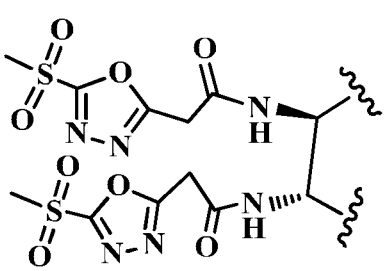
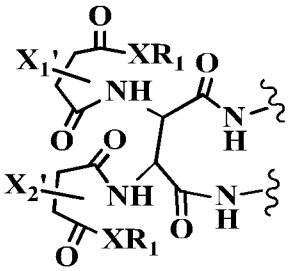
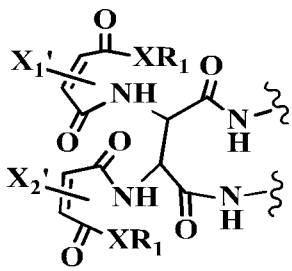
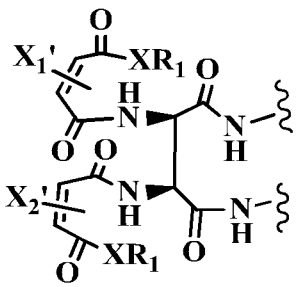
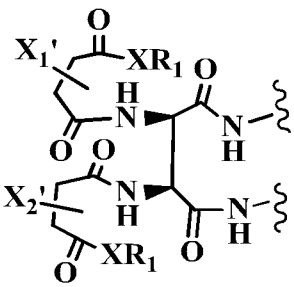
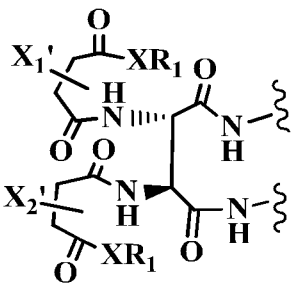
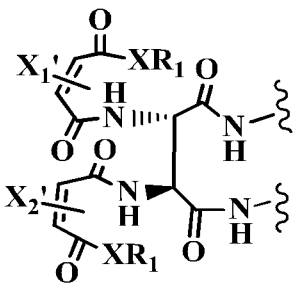
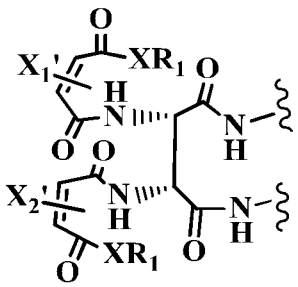
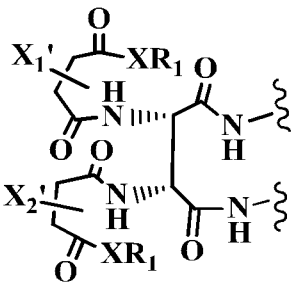
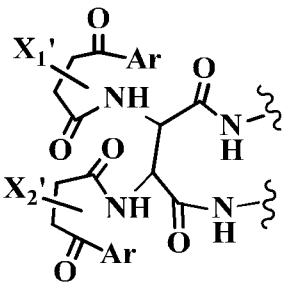
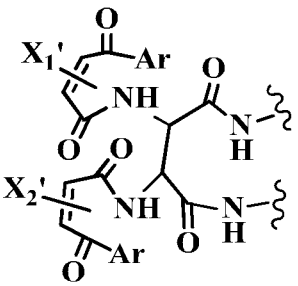
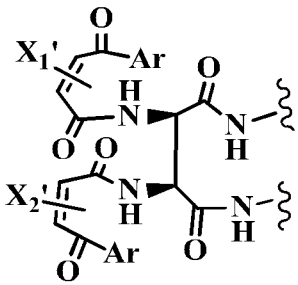
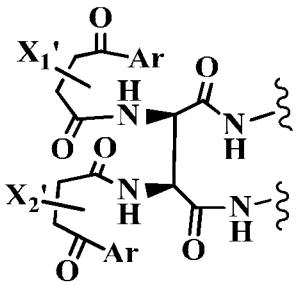
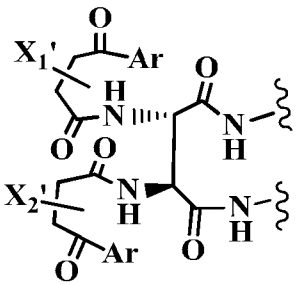
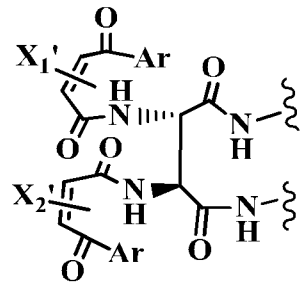
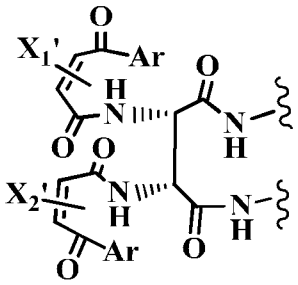
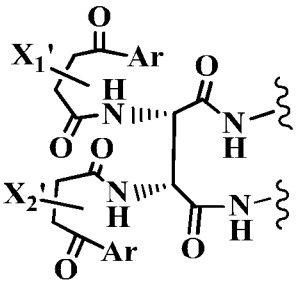
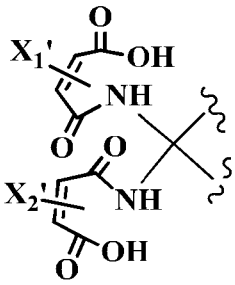
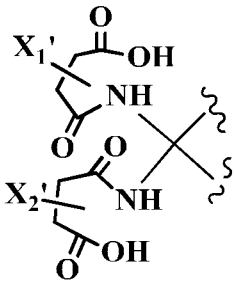
ethenesulfonyl;

acryl (acryloyl);

2-(tosyloxy)acetyl;

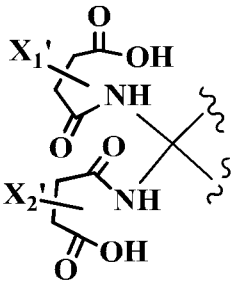
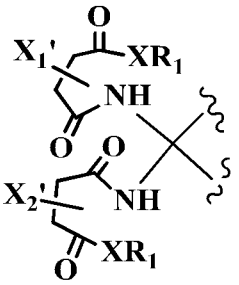
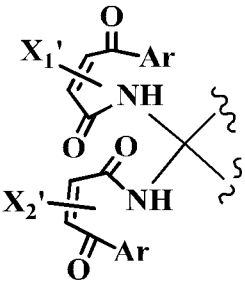
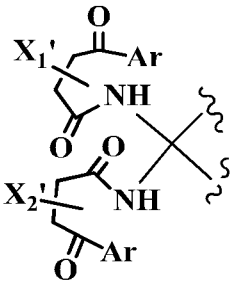
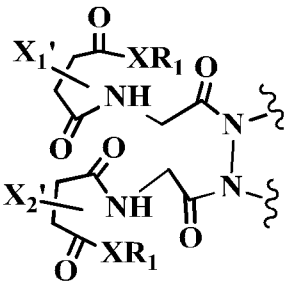
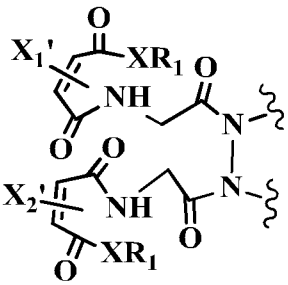
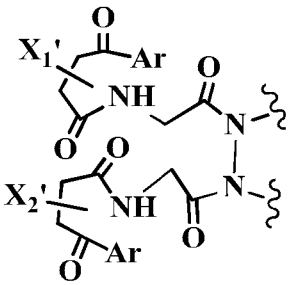
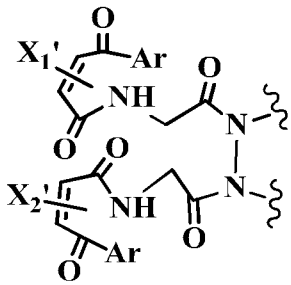
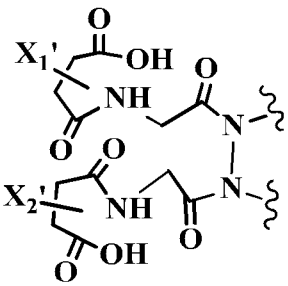
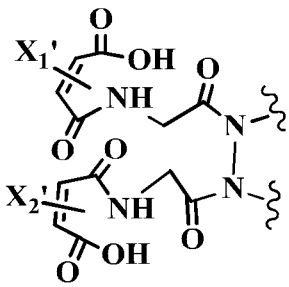
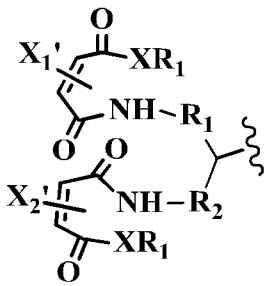
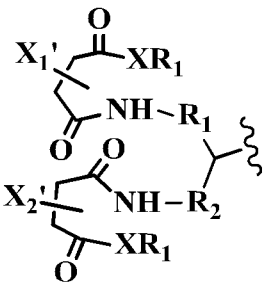
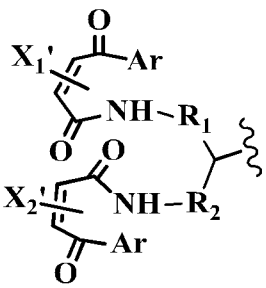
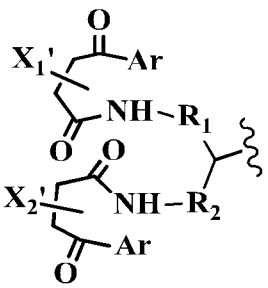
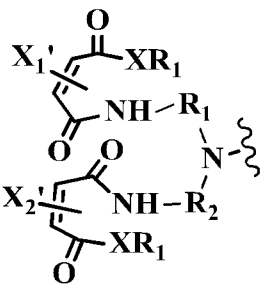
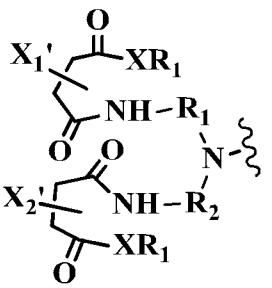
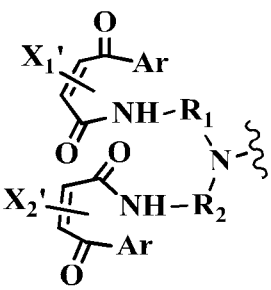
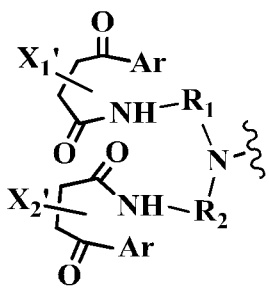
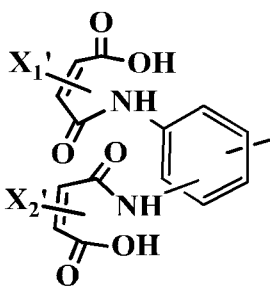
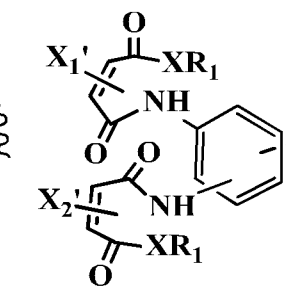
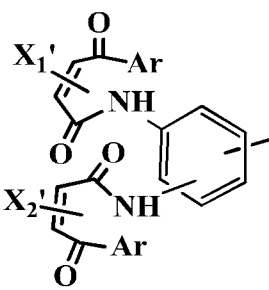
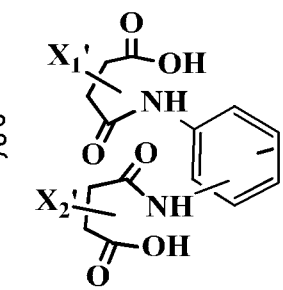
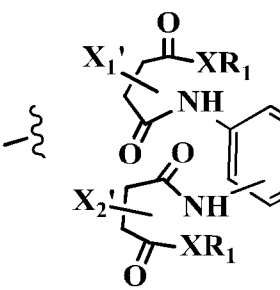
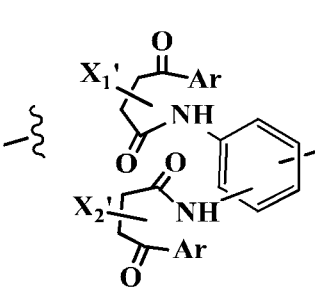

2-(mesyloxy)acetyl;

2-(nitrophenoxy)acetyl;


2-(dinitrophenoxy)acetyl;

2-(fluorophenoxy)-acetyl;

2-(difluorophenoxy)-acetyl;

2-(((trifluoromethyl)-sulfonyl)oxy)acetyl;

styrene,

vinylpyridine,

vinylpyrazine,

vinyl-1, 3, 5-triazine,

vinylquinoxaline,

,

substituted methylsulfonyl,

2-(pentafluorophenoxy)acetyl;

, methylsulfonephenyloxadiazole (ODA);

acryl,

halo acryl,

propiol,

2, 3-dihaloacryl,

Aryl-palladium complex,

bis(phenylsulfanyl)-maleimides,

bis-halide-pyridazinediones,

bis-phenylsulfanyl-pyridazinedione,

2-((methylsulfonyl)methyl)acryl,

2-((alkyl or aryl-sulfonyl)methyl)acryl,

cyanoethynyl,

ethynyl;


alkynyl,

arylenedipropiolonitrile (ADPN),

or

divinylpyridine,

divinylpyrazine,

divinyltriazine, or

3, 4-bis(maleimido)-2, 5-dioxopyrrolidine,

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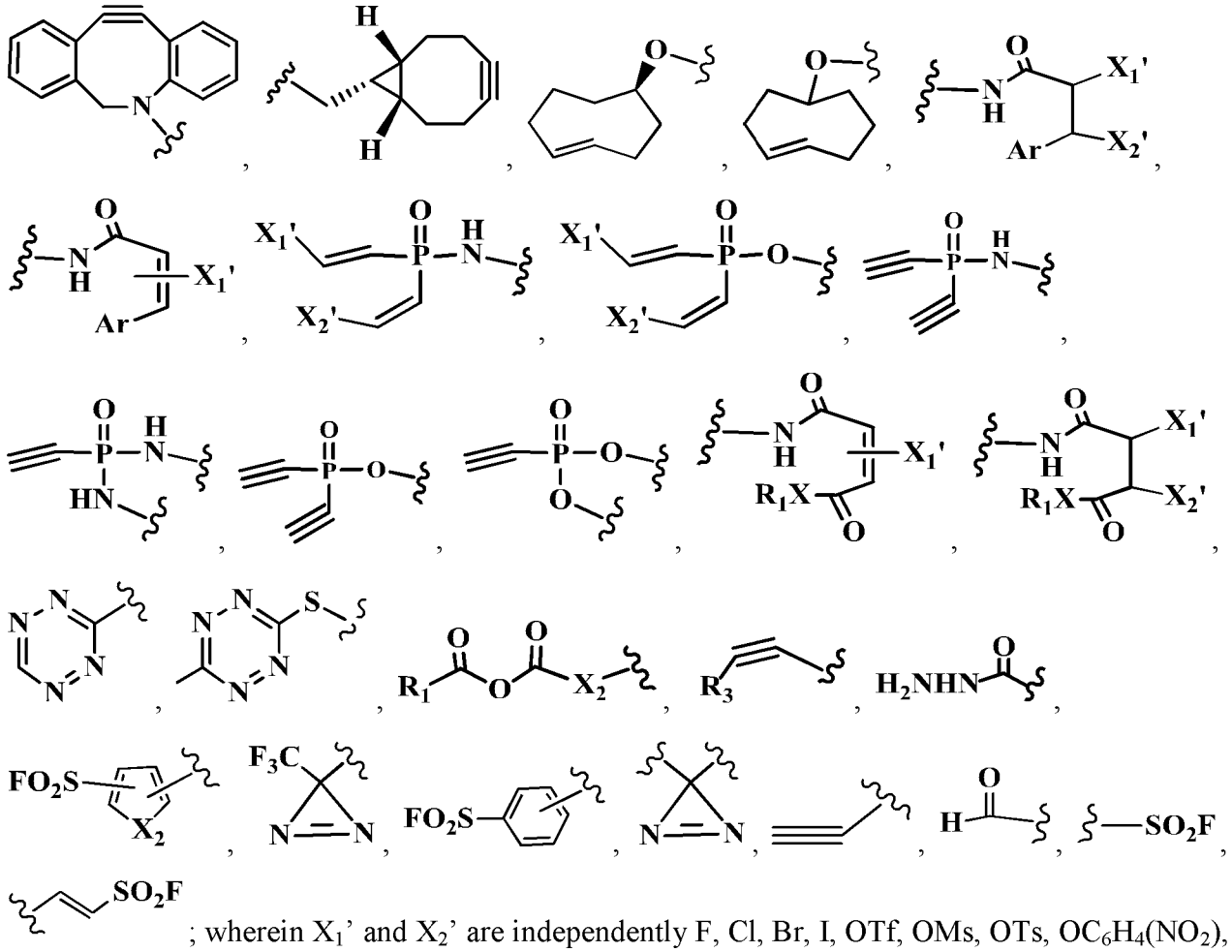

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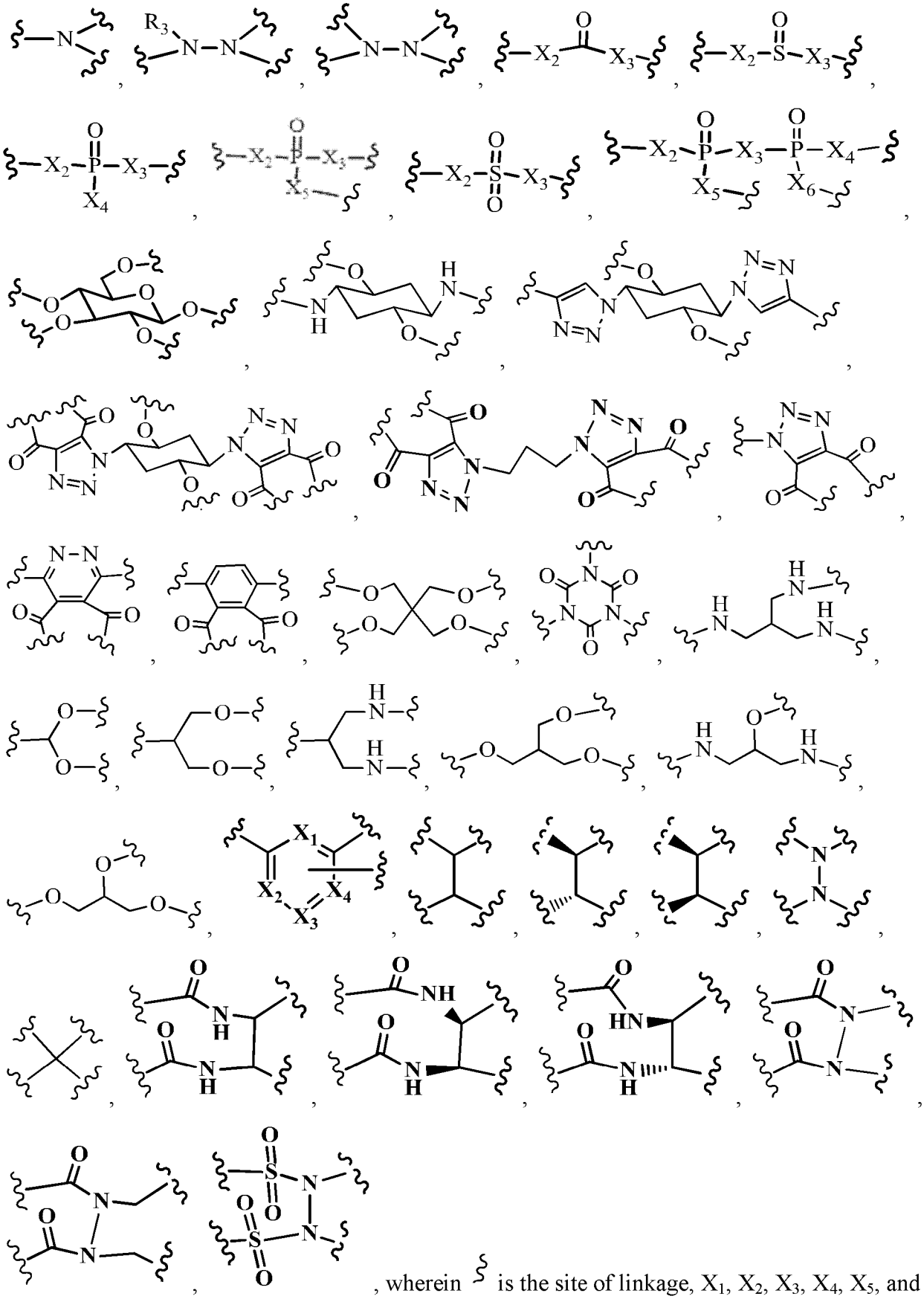
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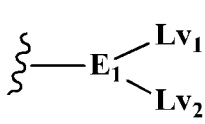
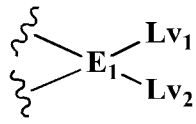
; wherein X_1' and X_2' are independently F, Cl, Br, I, OTf, OMs, OTs, $OC_6H_4(NO_2)$, $OC_6H_3(NO_2)_2$, OC_6F_5, OC_6HF_4, $OC_6H_2F_3$, or Lv_3; X_2 is O, NH, $N(R_1)$, or CH_2; R_3 and R_5 are independently H, R_1, aromatic, heteroaromatic, or aromatic group wherein one or several H atoms are replaced independently by $-R_1$, -halogen, $-OR_1$, $-SR_1$, $-NR_1R_2$, $-NO_2$, $-S(O)R_1$, $-S(O)_2R_1$, or $-COOR_1$; Lv_3 and Lv_3' are independently a leaving group selected from F, Cl, Br, I, nitrophenoxyl; N-hydroxysuccinimide (NHS); phenoxyl; benzenethiol, dinitrophenoxyl; pentafluorophenoxyl; tetrafluorophenoxyl; difluorophenoxyl; monofluorophenoxyl; pentachlorophenoxyl; triflate; imidazole; dichlorophenoxyl; tetrachlorophenoxyl; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3′-sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions or for Mitsunobu reactions;

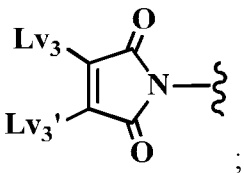
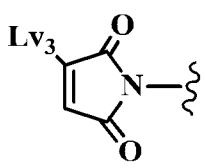
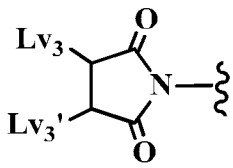
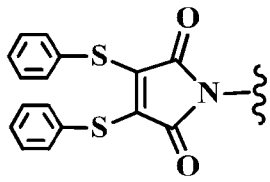
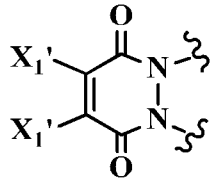
Wherein E_1 is a joint group that link two thiol reactive groups of Lv_1 and Lv_2. E_1 is selected from CH, CH_2, NH, NHNH, $N(R_3)$, $N(R_3)N(R_3)$, N=N, N-N, P, P(=O), S, Si, C_2-C_8 of alkyl, heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; a peptide containing1~4 units of aminoacids, preferably selected from aspatic acid, glutamic acid, arginine, histidine, lysine, serine, threonine,

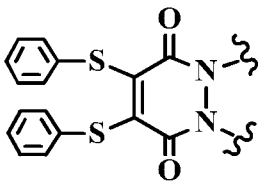
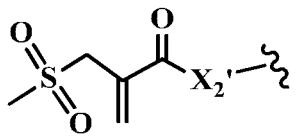
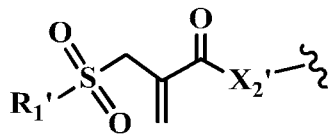
asparagine, glutamine, cysteine, selenocysteine, tyrosine, phenylalanine, glycine, proline, tryptophan, alanine; or one of the following structures:

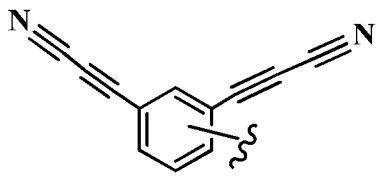
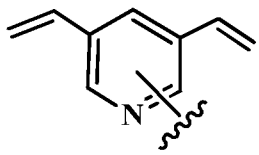
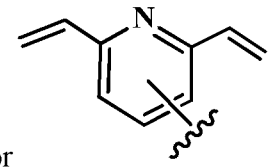


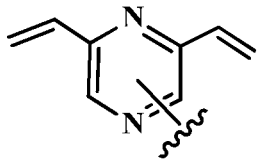
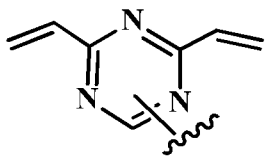
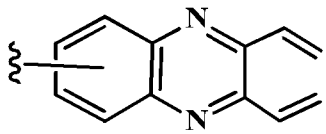
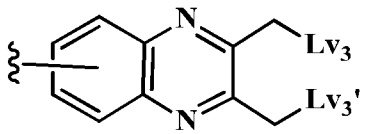
, wherein ~ is the site of linkage, X_1, X_2, X_3, X_4, X_5, and X_6 are independently selected from O, NH, S, N(R_3), CH_2; R_3 is C_1-C_6 alkyl.

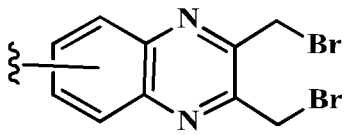
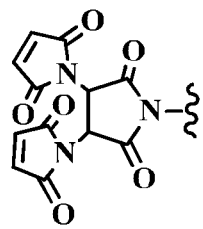
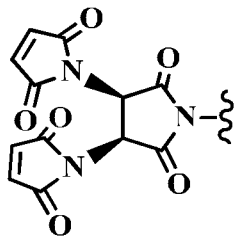
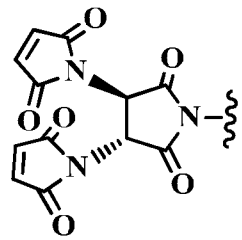
In the formula (II), formula (III) and formula (IV) wherein  and  can be selected from:

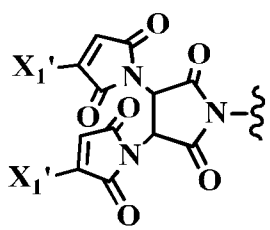
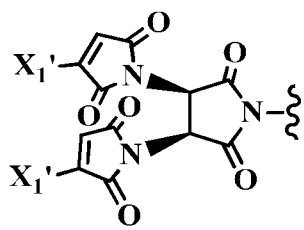
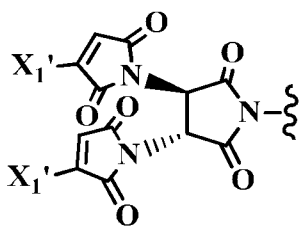
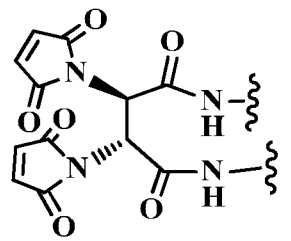
 ;  ;  ;  ,  ,

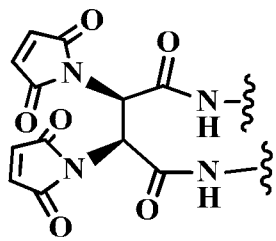
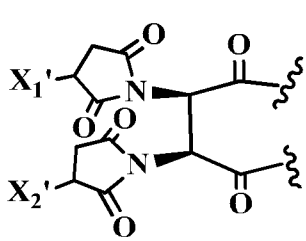
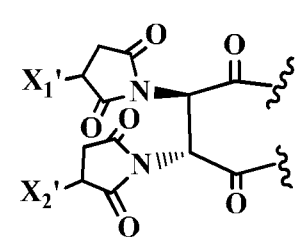
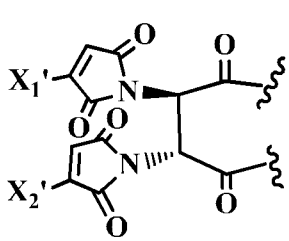
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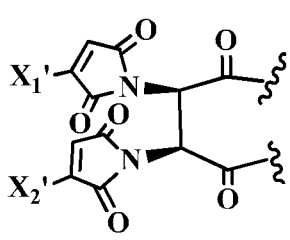
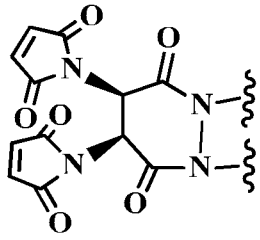
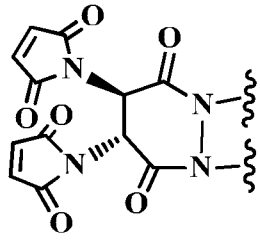
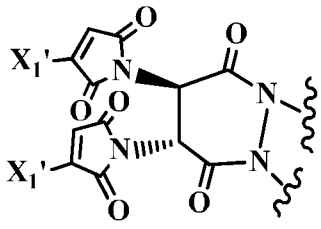
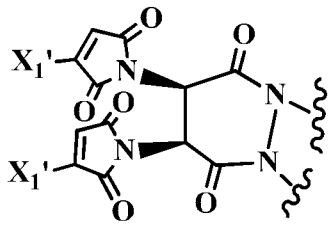
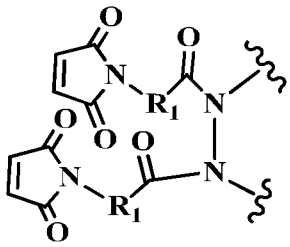
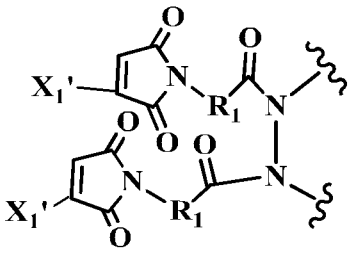
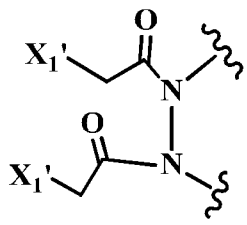
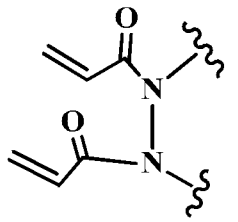
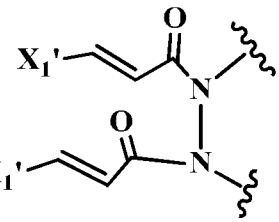
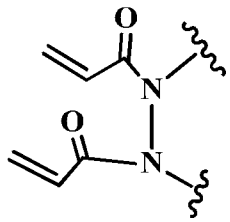
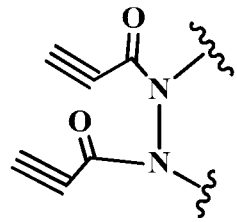
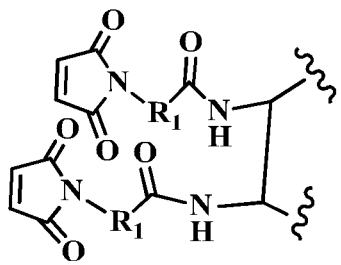
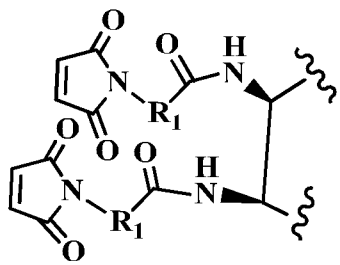
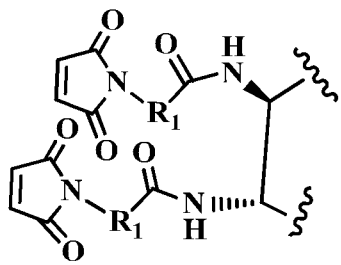
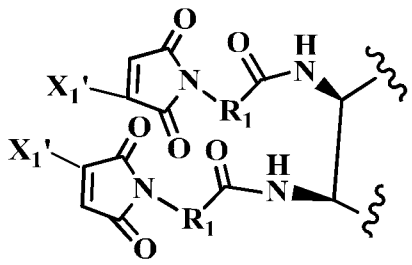
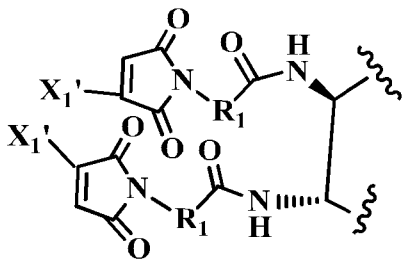
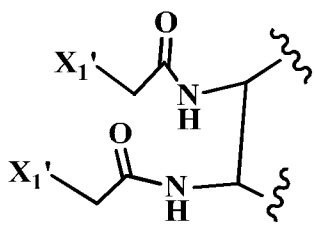
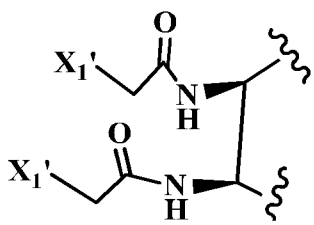
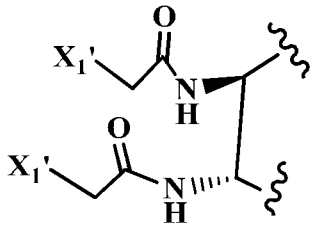
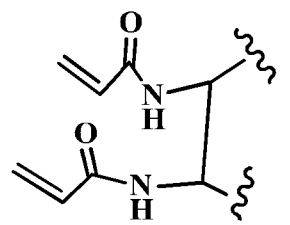
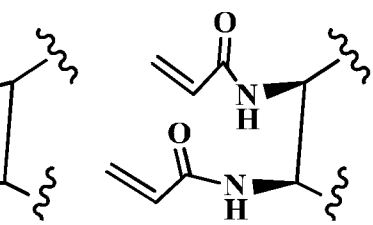
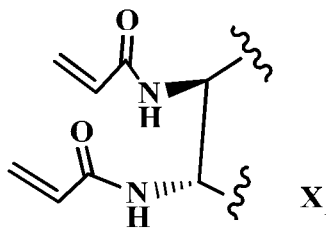
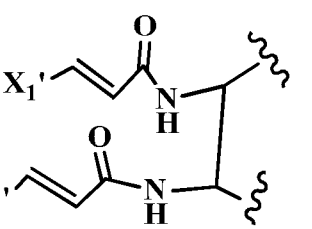
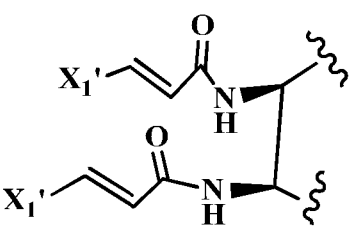
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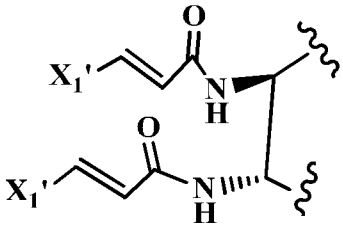
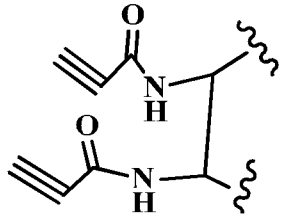
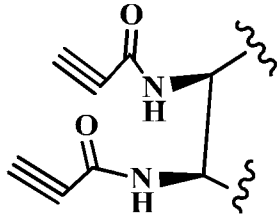
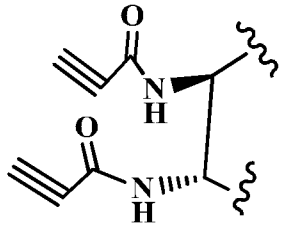
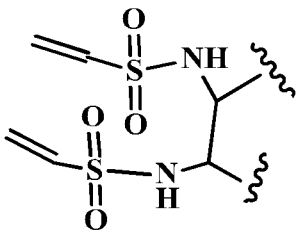
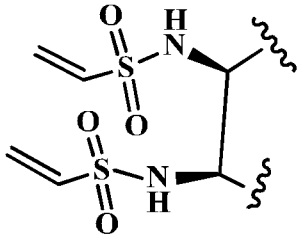
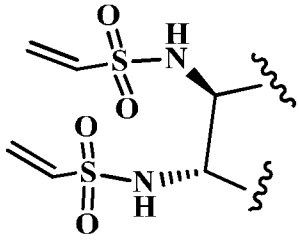
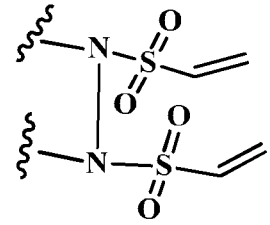
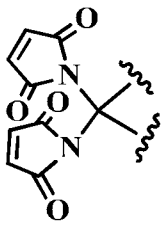
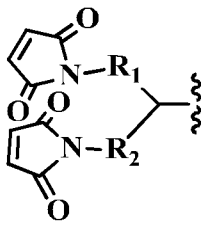
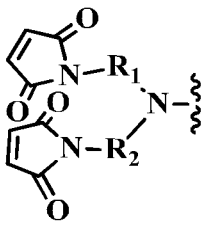
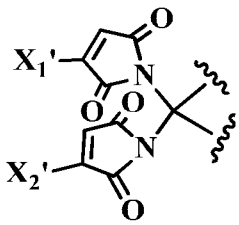
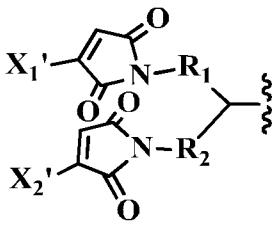
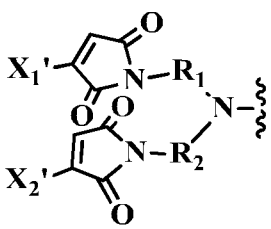
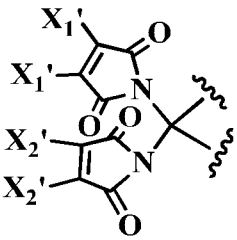
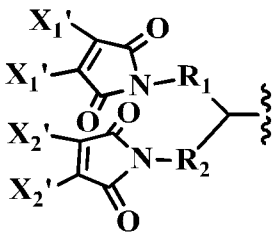
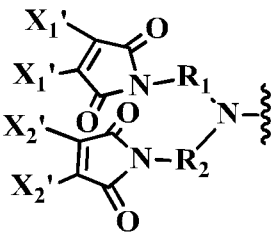
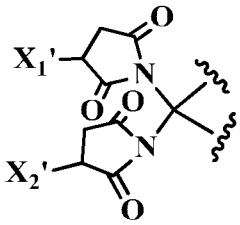
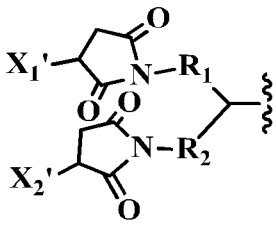
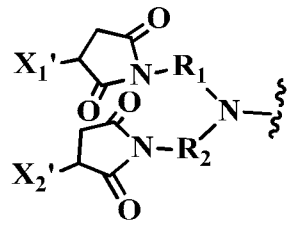
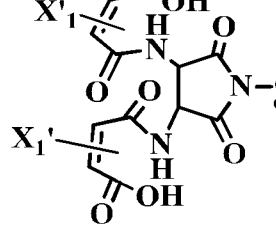
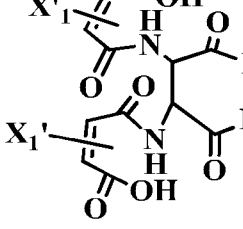
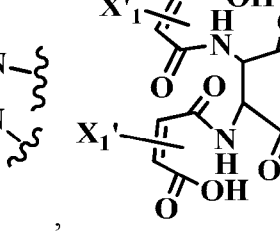
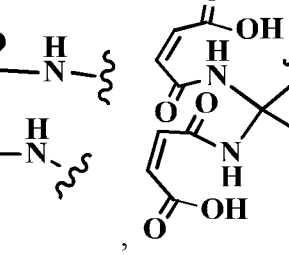
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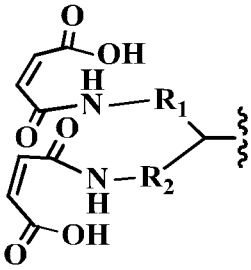
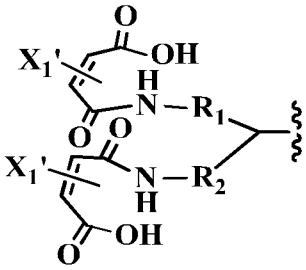
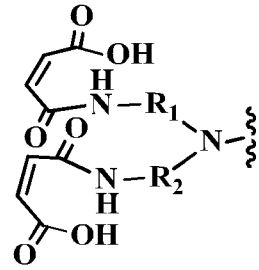
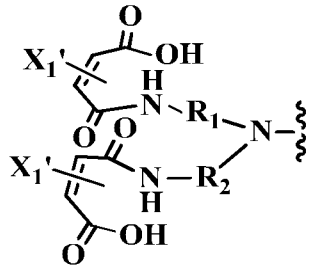
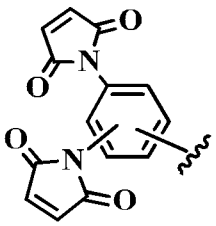
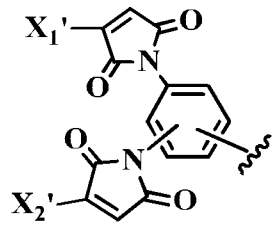
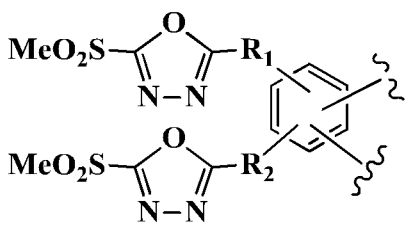
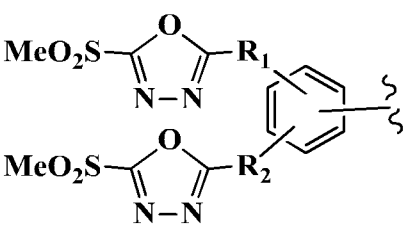
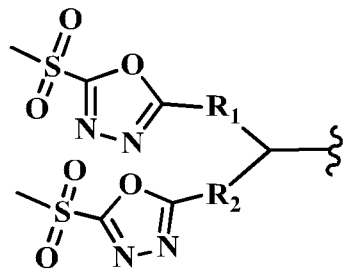
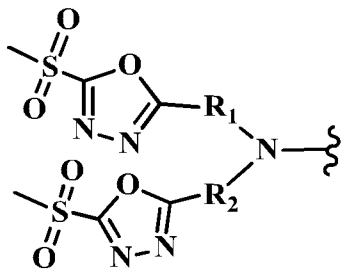
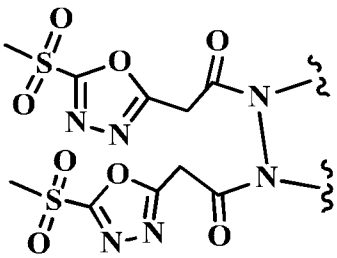
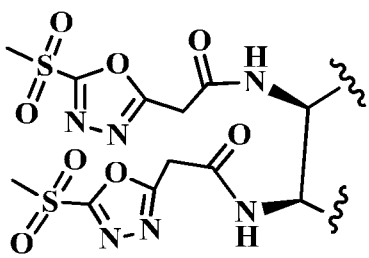
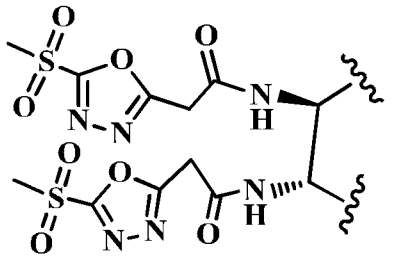
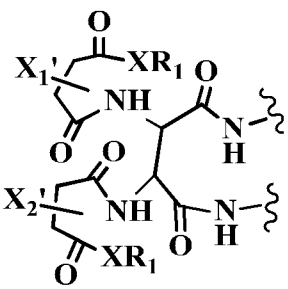
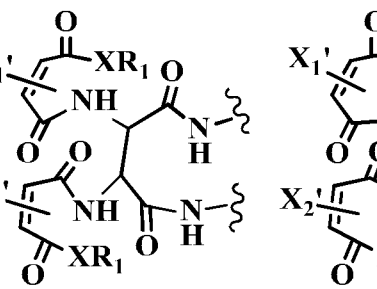
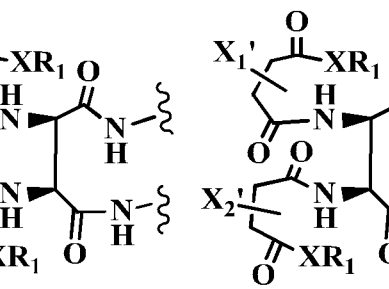
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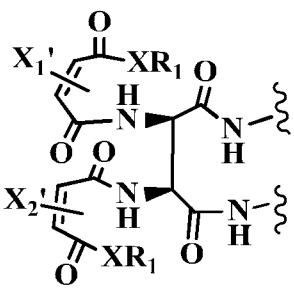
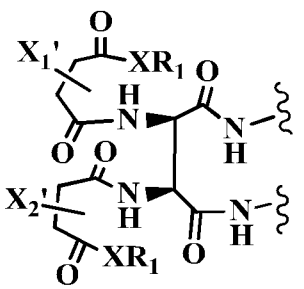
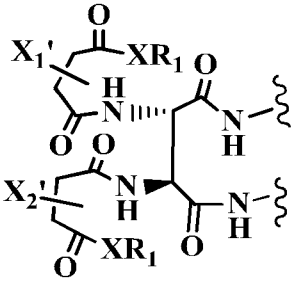
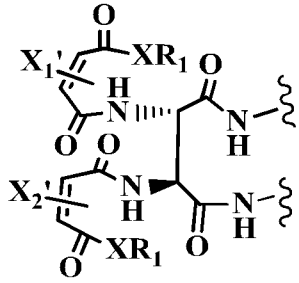
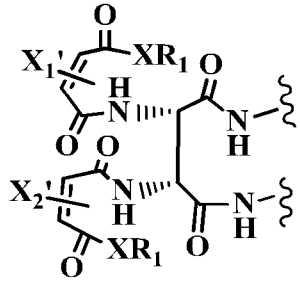
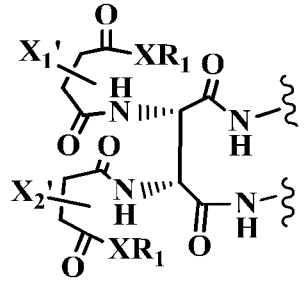
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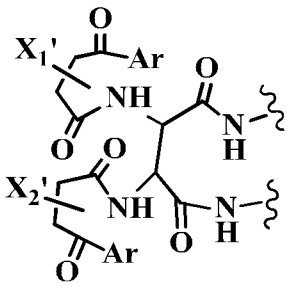
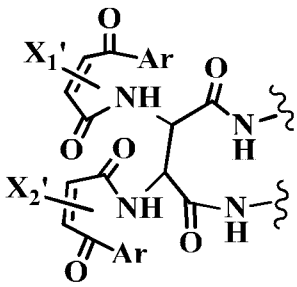
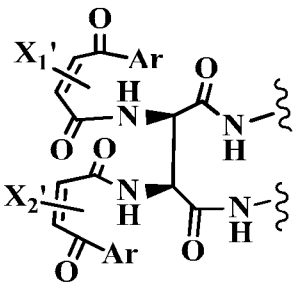
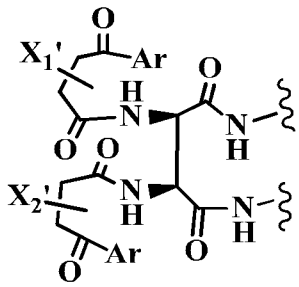
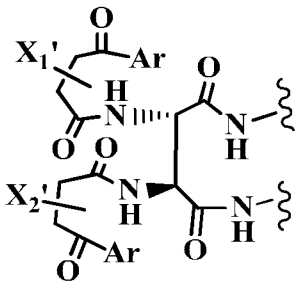
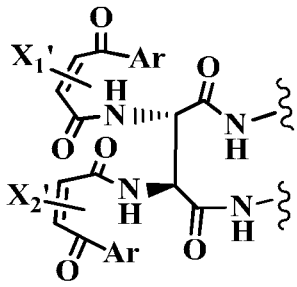
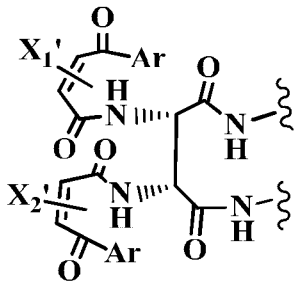
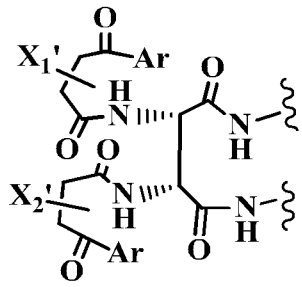
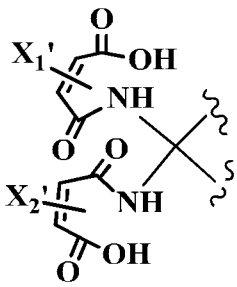
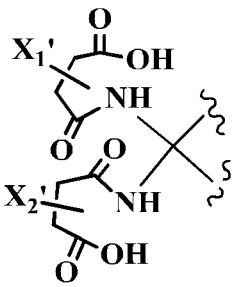
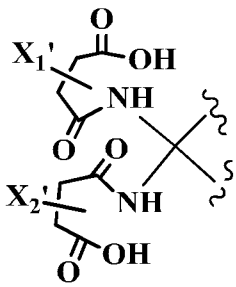
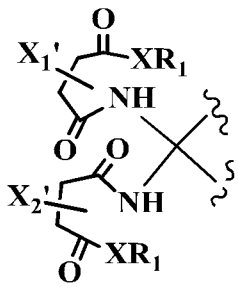
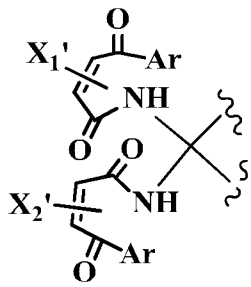
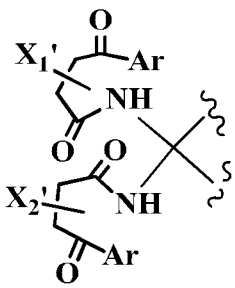
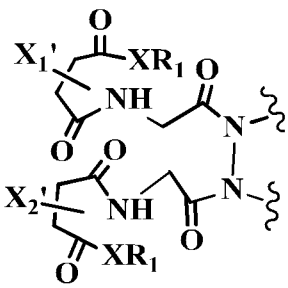
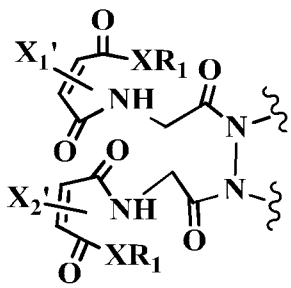
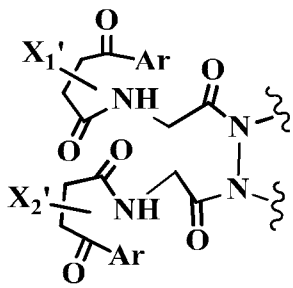
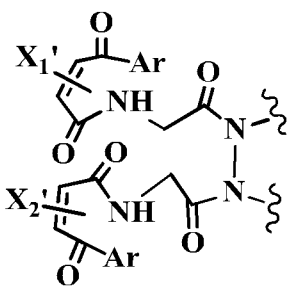
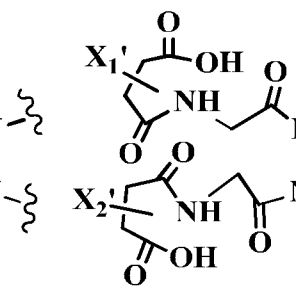
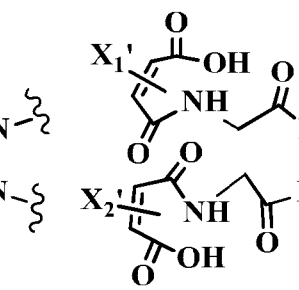
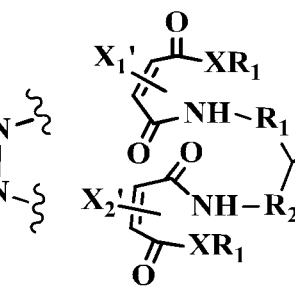
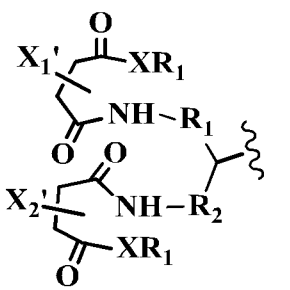
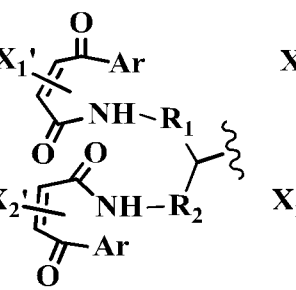
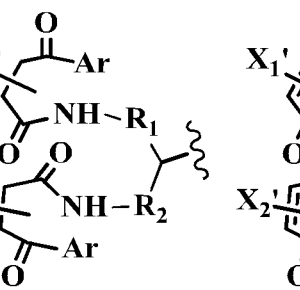
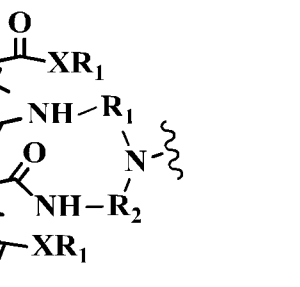
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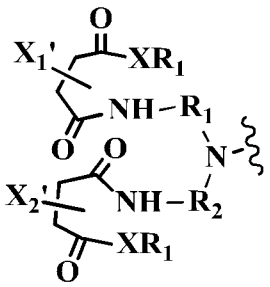
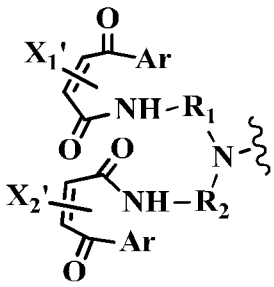
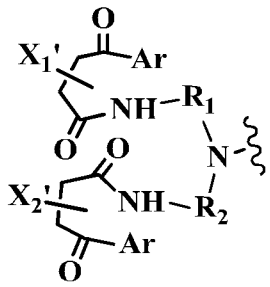
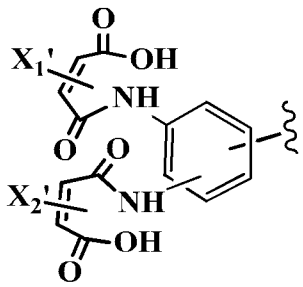
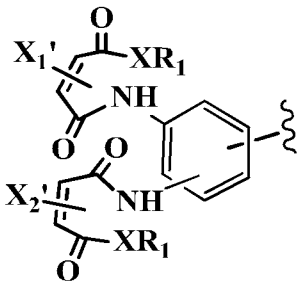
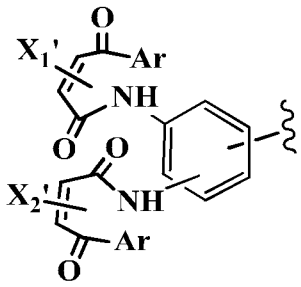
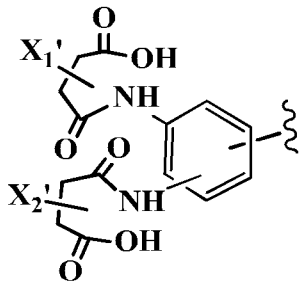
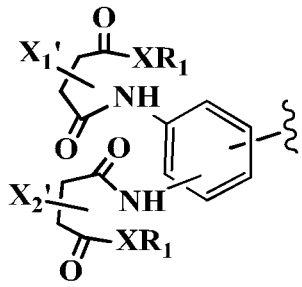
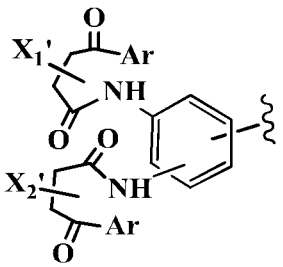
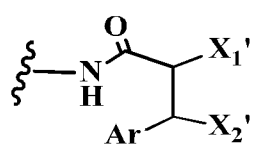
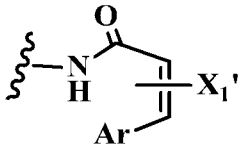
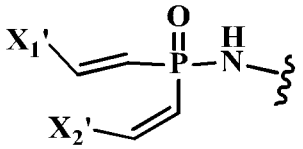
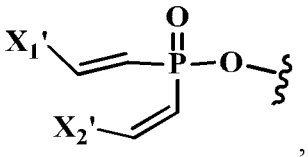
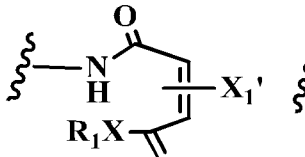
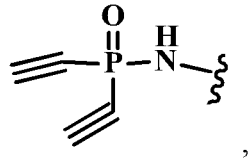
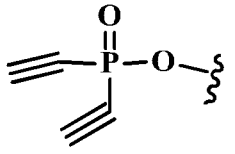
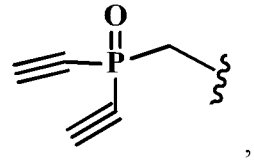

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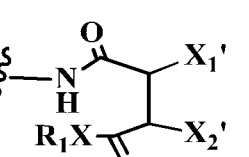
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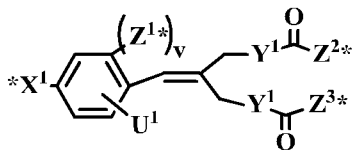
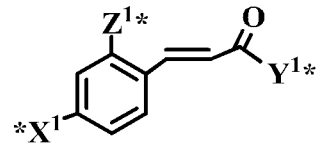
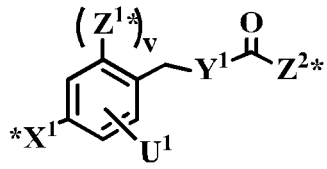
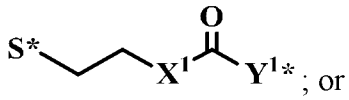
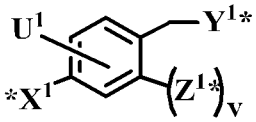
wherein Lv_3, Lv_3', X_1' and X_2'are described above; the conneting bond " — " in the middle of the two atoms means it can link either one of the two atoms;

Wherein L_1, L_2, L_1' and L_2' are, the same or different, independently selected from O, NH, S, NHNH, $N(R_3)$, $N(R_3)N(R_3')$, polyethyleneoxy unit of formula $(OCH_2CH_2)_pOR_3$, or $(OCH_2CH(CH_3))_pOR_3$, or $NH(CH_2CH_2O)_pR_3$, or $NH(CH_2CH(CH_3)O)_pR_3$, or $N[(CH_2CH_2O)_pR_3][(CH_2CH_2O)_{p'}R_3']$, or $(OCH_2CH_2)_pCOOR_3$, or $CH_2CH_2(OCH_2CH_2)_pCOOR_3$, wherein p and p' are independently an integer selected from 0 to about 1000, or combination thereof; C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; Wherein R_3 and R_3' are independently H; C_1-C_8 of alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 1-8 carbon atoms of esters, ether, amide or aminoalkylcarboxyl; or 1~8 natural or unnatural amino acids or

peptides described in the definition; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000, or combination above thereof.

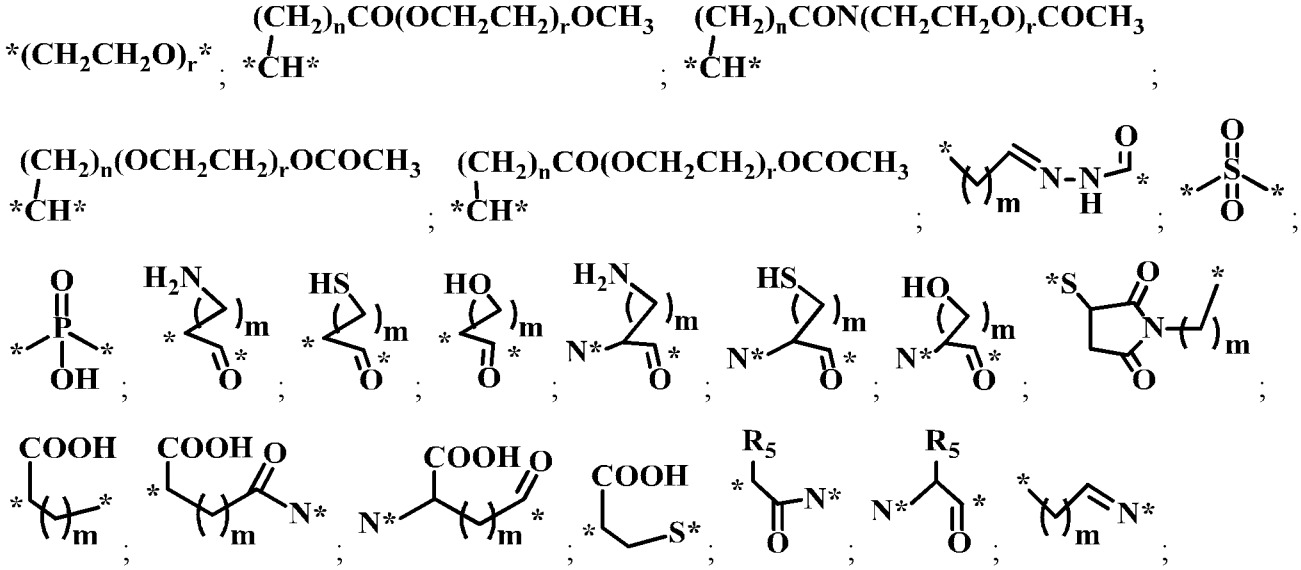
Wherein L_1, L_1', L_2, or L_2', may contain a self-immolative or a non-self-immolative component, peptidyl units, a hydrazone bond, a disulfide, an ester, an oxime, an amide, or a thioether bond. The self-immolative unit includes, but is not limited to, aromatic compounds that are electronically similar to the para-aminobenzylcarbamoyl (PAB) groups such as 2-aminoimidazol-5-methanol derivatives, heterocyclic PAB analogs, beta-glucuronide, and ortho or para-aminobenzylacetals.

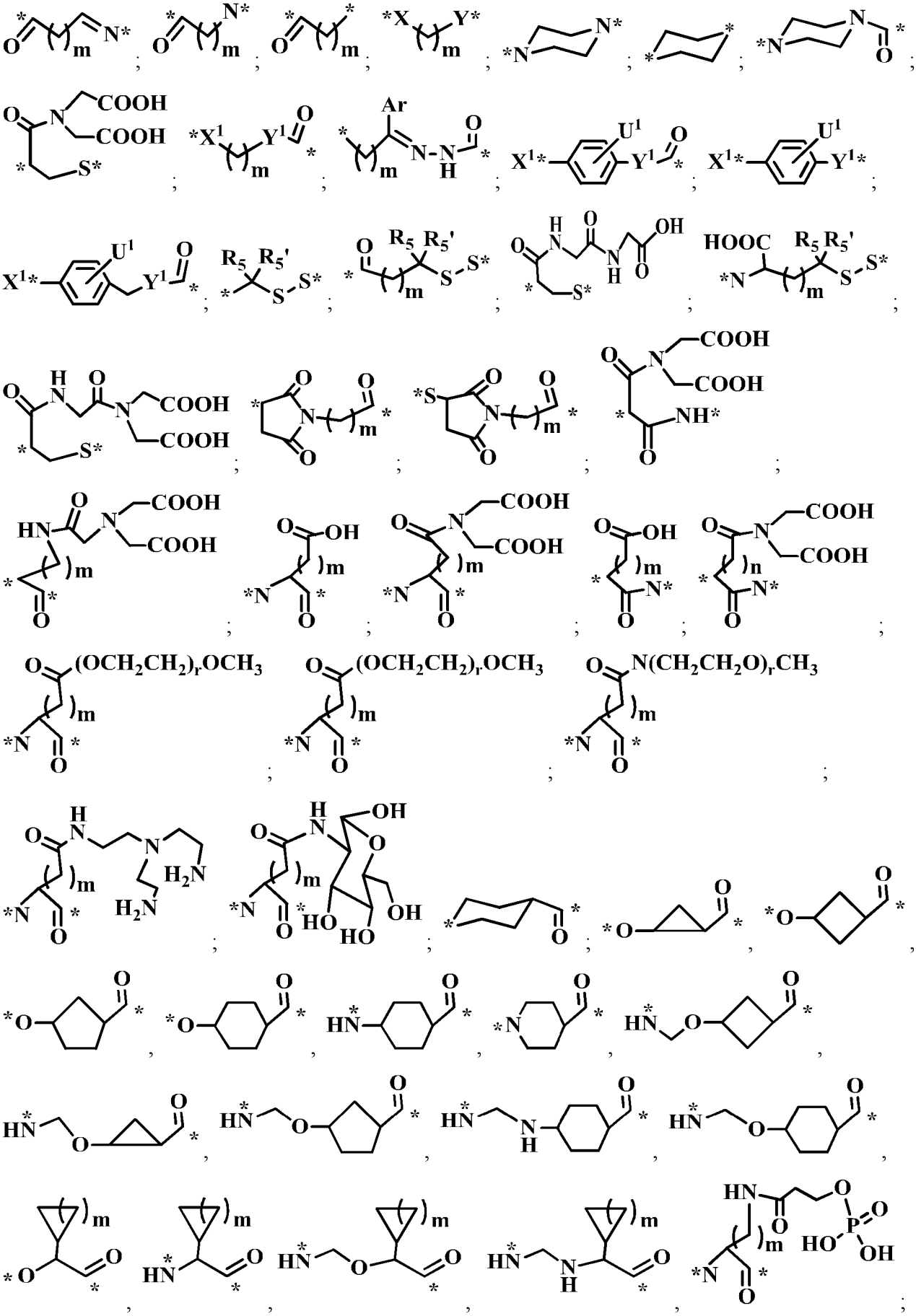
Wherein the self-immolative linker component has one of the following structures:

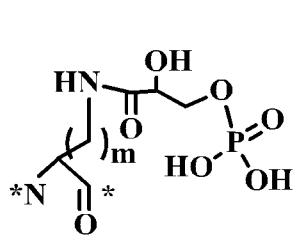
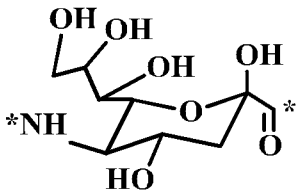
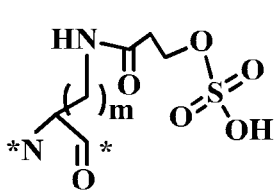
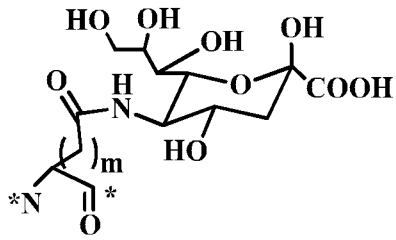
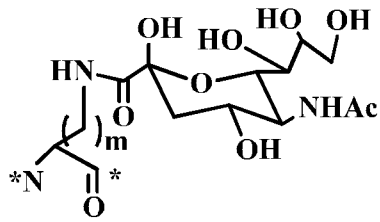
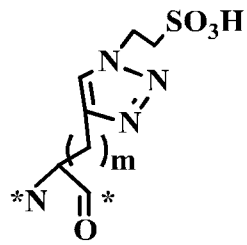
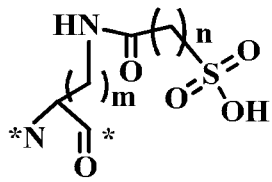
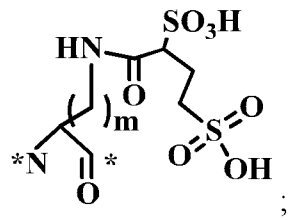
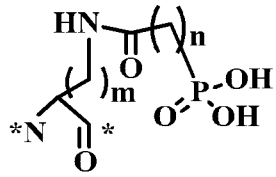






wherein the (*) atom is the point of attachment of additional spacer or releasable linker units, or the cytotoxic agent, and/or the binding molecule (CBA); X^1, Y^1, Z^2 and Z^3 are independently NH, O, or S; Z^1 is independently H, NH, O or S; v is 0 or 1; U^1 is independently H, OH, C_1~C_6 alkyl, $(OCH_2CH_2)_nF$, Cl, Br, I, OR_5, SR_5, NR_5R_5', N=NR_5, N=R_5, NR_5R_5', NO_2, SOR_5R_5', SO_2R_5, SO_3R_5, OSO_3R_5, PR_5R_5', POR_5R_5', $PO_2R_5R_5$', $OPO(OR_5)(OR_5')$, or $OCH_2PO(OR_5(OR_5')$ wherein R_5 and R_5' are as defined above; preferably R_5 and R_5' are independently selected from H, C_1~C_8 alkyl; C_2~C_8 alkenyl, alkynyl, heteroalkyl; C_3~C_8 aryl, heterocyclic, carbocyclic, cycloalkyl, heterocycloalkyl, heteroaralkyl, alkylcarbonyl; or pharmaceutical cation salts.

Wherein the non-self-immolative linker component is one of the following structures:






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Wherein the (*) atom is the point of attachment of additional spacer R_1 or releasable linkers, the cytotoxic agents, and/or the binding molecules; X^1, Y^1, U^1, R_1, R_5, R_5' are defined as above; r is 0~100; m and n are 0~6 independently.

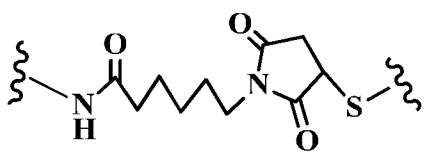
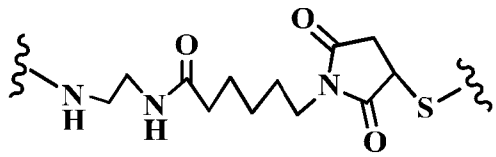
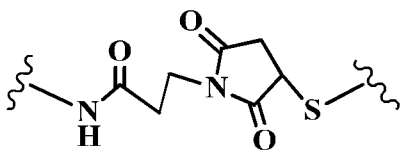
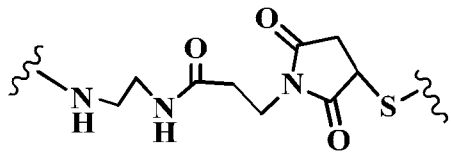
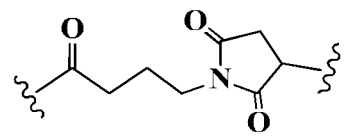
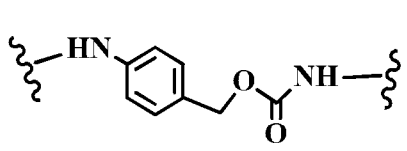
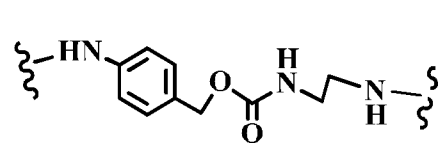
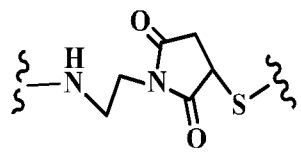
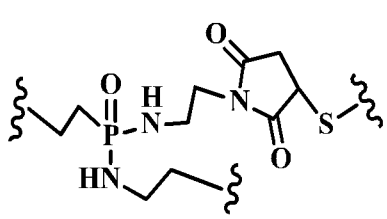
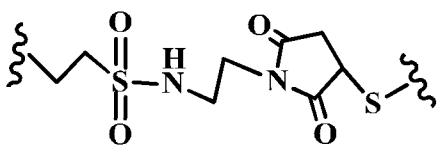
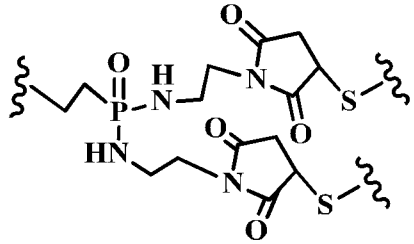
wherein L_1, L_1', L_2, or L_2' may be a releasable linker and comprises one of the following structures:

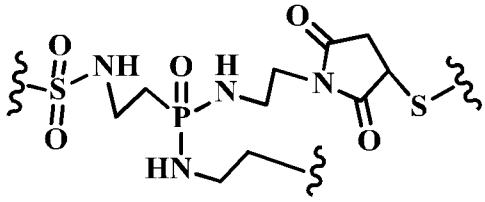
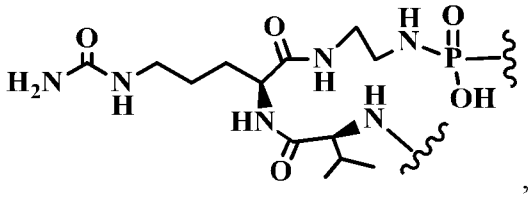
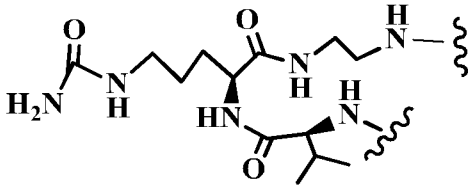
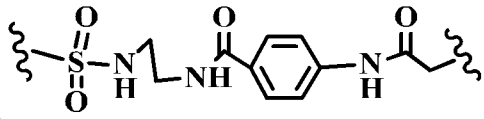
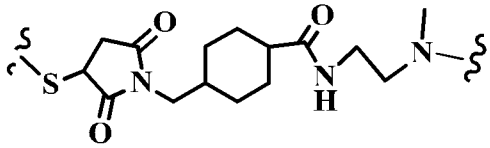
-$(CR_5R_6)_m$(Aa)r$(CR_7R_8)_n(OCH_2CH_2)_t$-, -$(CR_5R_6)_m(CR_7R_8)_n$(Aa)$_r(OCH_2CH_2)_t$-, -$(Aa)_r(CR_5R_6)_m$-$(CR_7R_8)_n(OCH_2CH_2)_t$-, -$(CR_5R_6)_m(CR_7R_8)_n(OCH_2CH_2)_r(Aa)_t$-, -$(CR_5R_6)_m(CR_7{=}CR_8)(CR_9R_{10})_n(Aa)_t$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(NR_{11}CO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(Aa)_t(NR_{11}CO)$-$(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(OCO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(OCNR_7)(Aa)_t$-$(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(CO)(Aa)_t$-$(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(NR_{11}CO)(Aa)_t$-$(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m$-$(OCO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(OCNR_7)(Aa)_t$-$(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m(CO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$(CR_5R_6)_m$-phenyl-CO-$(Aa)_t(CR_7R_8)_n$-, -$(CR_5R_6)_m$-furyl-CO$(Aa)_t(CR_7R_8)_n$-, -$(CR_5R_6)_m$-oxazolyl-CO$(Aa)_t(CR_7R_8)_n$-, -$(CR_5R_6)_m$thiazolyl-CO-$(Aa)_t(CCR_7R_8)_n$-, -$(CR_5R_6)_t$-thienyl-CO-$(CR_7R_8)_n$-, -$(CR_5R_6)_t$-imidazolyl-CO-$(CR_7R_8)_n$-, -$(CR_5R_6)_t$-morpholino-CO$(Aa)_t$-$(CR_7R_8)_n$-, -$(CR_5R_6)_t$piperazino-CO$(Aa)_t(CR_7R_8)_n$-, -$(CR_5R_6)_t$-N-methyl-piperazin-CO$(Aa)_t$-$(CR_7R_8)_n$-, -$(CR_5R)_m$-$(Aa)_t$phenyl-, -$(CR_5R_6)_m$-$(Aa)_t$furyl-, -$(CR_5R_6)_m$-oxazolyl$(Aa)_t$-, -$(CR_5R_6)_m$-thiazolyl$(Aa)_t$-, -$(CR_5R_6)_m$-thienyl-$(Aa)_t$-, -$(CR_5R_6)_m$-imidazolyl$(Aa)_t$-, -(C $R_5R_6)_m$-morpholino-$(Aa)_t$-, -$(CR_5R_6)_m$-piperazino-$(Aa)_t$-, -$(CR_5R_6)_m$-N-methylpiperazino-$(Aa)_t$-, -K$(CR_5R_6)_m$(Aa)r$(CR_7R_8)_n(OCH_2CH_2)_t$-, -K$(CR_5R_6)_m$-$(CR_7R_8)_n$-$(Aa)_r(OCH_2CH_2)_t$-, -K$(Aa)_r(CR_5R_6)_m$-$(CR_7R_8)_n(OCH_2CH_2)_t$-, -K$(CR_5R_6)_m(CR_7R_8)_n$-$(OCH_2CH_2)_r(Aa)_t$-, -K$(CR_5R_6)_m(CR_7{=}CR_8)(CR_9R_{10})_n$-$(Aa)_t(OCH_2CH_2)_r$-, -K$(CR_5R_6)_m$-

$(NR_{11}CO)(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(Aa)_t(NR_{11}CO)$-$(CR_9R_{10})_n(OCH_2$-$CH_2)_r$-, -$K(CR_5R_6)_m(OCO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(OCNR_7)(Aa)_t$-$(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(CO)(Aa)_t$-$(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(NR_{11}CO)$-$(Aa)_t$-$(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m$-$(OCO)(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(OCNR_7)$-$(Aa)_t(CR_9R_{10})_n(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m(CO)(Aa)_t(CR_9R_{10})_n$-$(OCH_2CH_2)_r$-, -$K(CR_5R_6)_m$-phenyl-$CO$-$(Aa)_t(CR_7R_8)_n$-, -$K$-$(CR_5R_6)_m$-furyl-$CO(Aa)_t$-$(CR_7R_8)_n$-, -$K(CR_5R_6)_m$-oxazolyl-$CO$-$(Aa)_t(CR_7R_8)_n$-, -$K(CR_5R_6)_m$thiazolyl-$CO(Aa)_t$-$(CR_7R_8)_n$-, -$K(CR_5R_6)_t$-thienyl-$CO(CR_7R_8)_n$-, -$K$-$(CR_5R_6)_t$imidazolyl-$CO$-$(CR_7R_8)_n$-, -$K(CR_5R_6)_t$morpholino-$CO(Aa)_t(CR_7R_8)_n$-, -$K(CR_5R_6)_t$-piperazino-$CO(Aa)_t$-$(CR_7R_8)_n$-, -$K(CR_5R_6)_t$-N-methylpiperazin$CO(Aa)_t(CR_7R_8)_n$-, -$K(CR_5R)_m(Aa)_t$-phenyl, -$K$-$(CR_5R_6)_m$-$(Aa)_t$furyl-, -$K(CR_5R_6)_m$-oxazolyl$(Aa)_t$-, -$K(CR_5R_6)_m$-thiazolyl$(Aa)_t$-, -$K$-$(CR_5R_6)_m$-thienyl-$(Aa)_t$-, -$K(CR_5R_6)_m$-imidazolyl$(Aa)_t$-, -$K(CR_5R_6)_m$-morpholino$(Aa)_t$-, -$K(CR_5R_6)_m$-piperazino-$(Aa)_t$G, -$K(CR_5R_6)_m$N-methylpiperazino-$(Aa)_t$-;

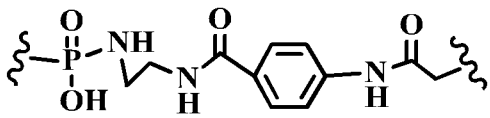
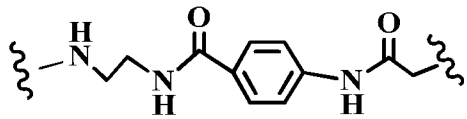
werein m, Aa, m, n, R_3, R_4, and R_5 are described above; t and r are 0 – 100 independently; R_6, R_7, and R_8 are independently chosen from H; halide; C_1~C_8 of alkyl, aryl, alkenyl, alkynyl, ether, ester, amine or amide, which optionally substituted by one or more halide, CN, NR_1R_2, CF_3, OR_1, Aryl, heterocycle, $S(O)R_1$, SO_2R_1, $-CO_2H$, $-SO_3H$, $-OR_1$, $-CO_2R_1$, $-CONR_1$, $-PO_2R_1R_2$, $-PO_3H$ or $P(O)R_1R_2R_3$; K is NR_1, -SS-, -C(=O)-, -C(=O)NH-, -C(=O)O-, -C=NH-O-, -C=N-NH-, -C(=O)NH-NH-, O, S, Se, B or C_3-C_6 heteroaromatic group.

Wherein the structures of the components of the linker L_1, L_1', L_2, or L_2', may contain:

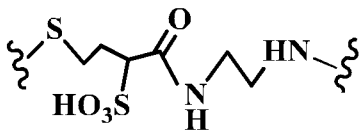
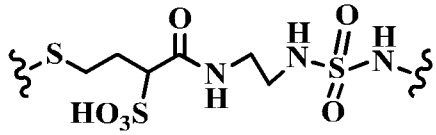

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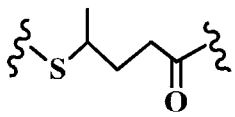
(MCC, 4-(N-maleimidomethyl)cyclohexane-1 carboxylate),

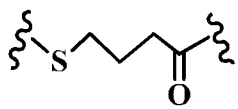
((4-acetyl)aminobenzoate),

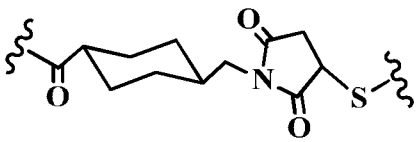
(4-thio-2-hydroxysulfonyl-butyrate, 2-sulfo-SPDB), (PAB),



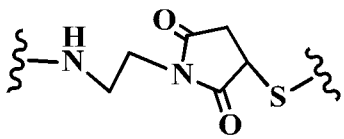
4-thio-pentanoate (SPP),



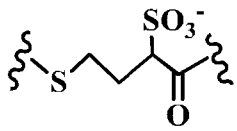
4-thio-butyrate (SPDB),



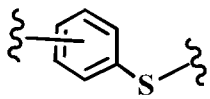
4-(N-maleimidomethyl)cyclo-hexane-1-carboxylate (MCC),



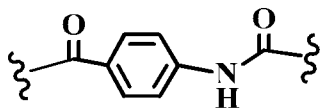
maleimidoethyl (ME),



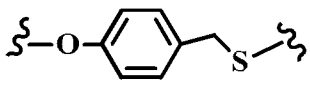
4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB),



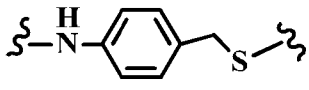
aryl-thiol (PhSS),



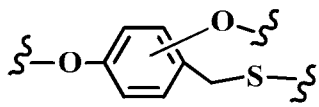
(4-acetyl)amino-benzoate (SIAB),



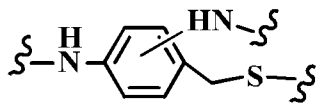
, oxylbenzylthio,



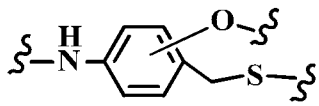
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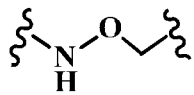
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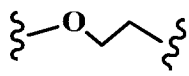
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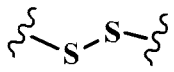


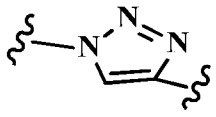
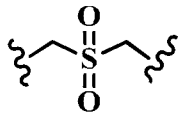
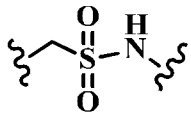
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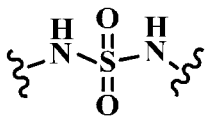
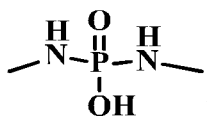
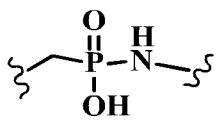


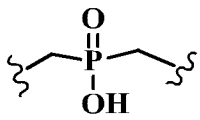
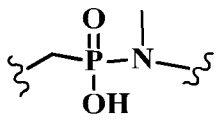
alkoxy amino (AOA),

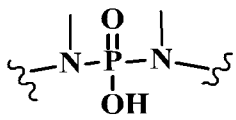
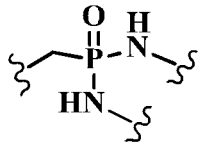


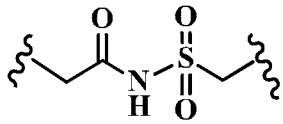
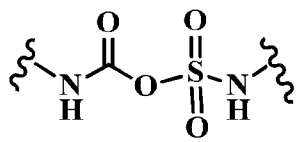
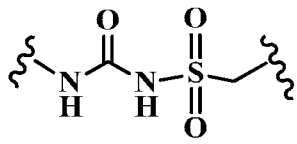
ethyleneoxy(EO),  dithio,  4-methyl-4-dithio-pentanoic (MPDP),

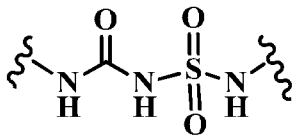
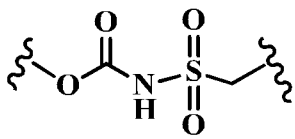
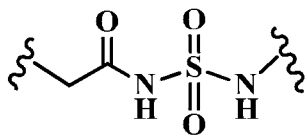
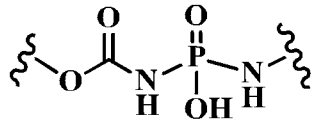
 triazole,  alkylsulfonyl,  alkylsulfonamide,

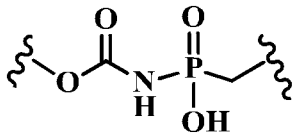
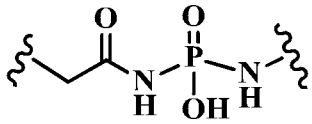
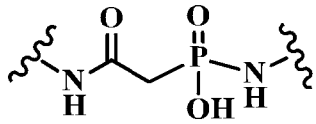
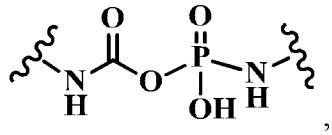
 sulfon-bisamide,  Phosphondiamide,  alkylphosphona-

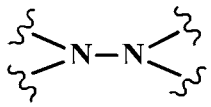
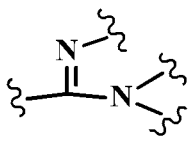
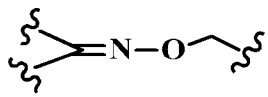
mide,  phosphinic acid,  N-methylphosphonamidic acid,

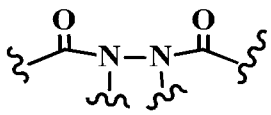
 N, N'-dimethylphosphonamidic acid,  N, N'-

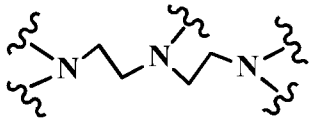
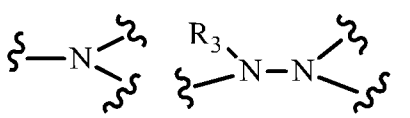
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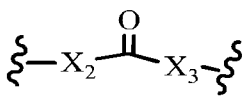
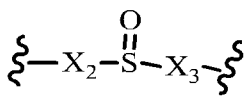
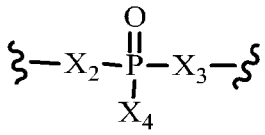
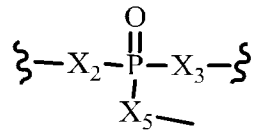
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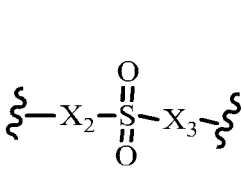
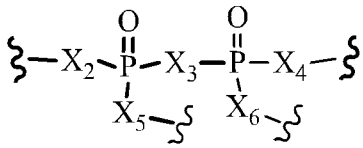
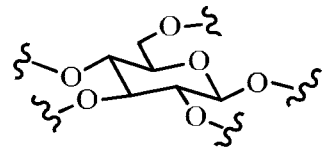
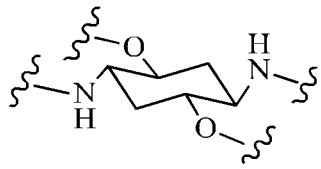
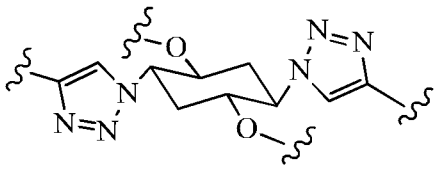
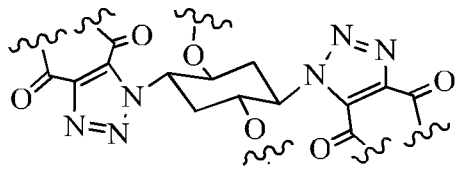
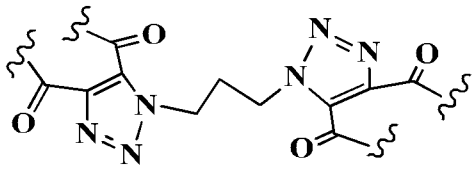
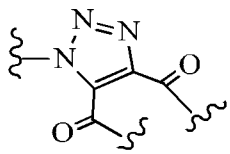
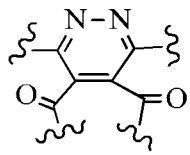
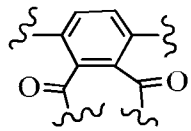
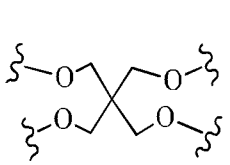
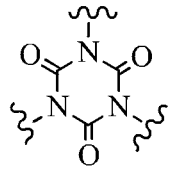
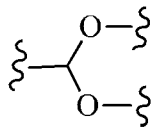
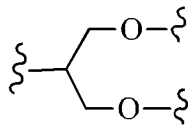
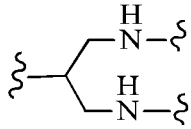
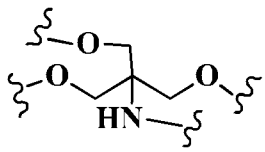
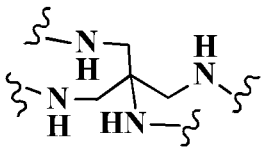
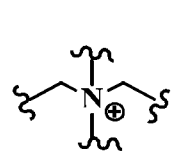
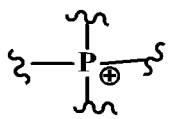
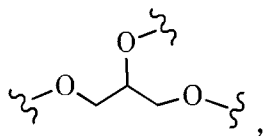
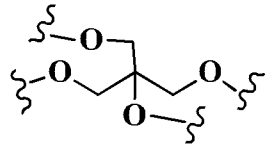
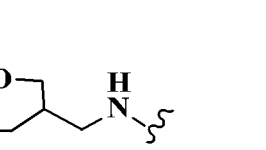
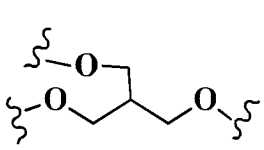
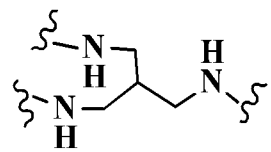
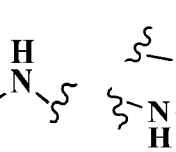
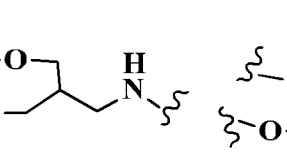
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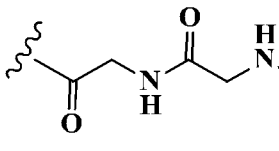
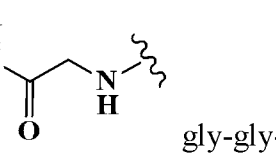
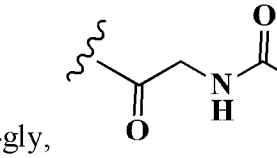
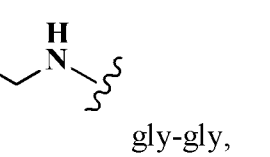
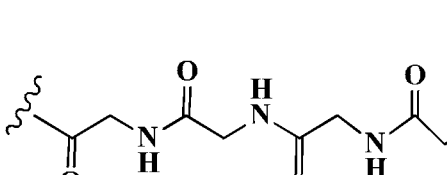
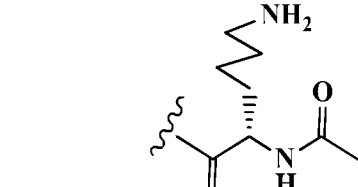
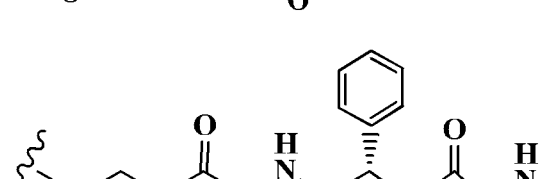
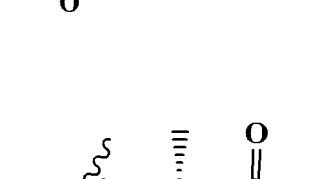
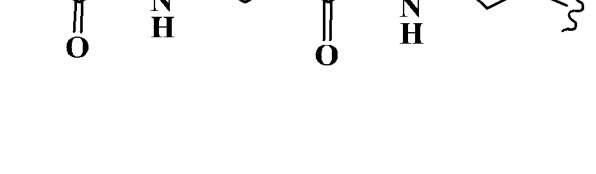
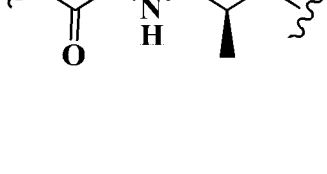
 hydrazine,  acetimidamide,  oxime,

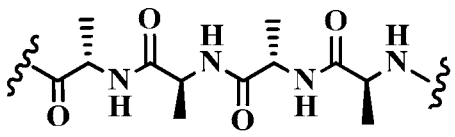
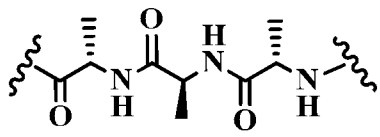
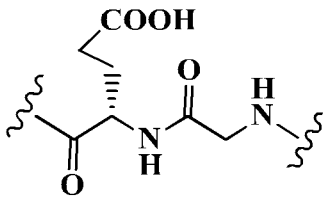
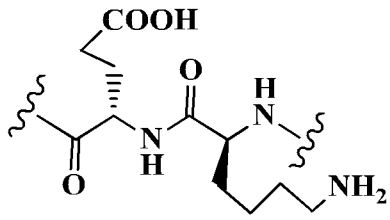
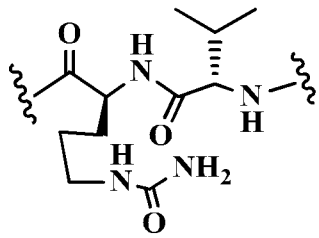
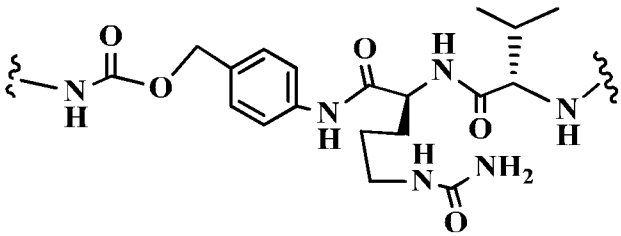
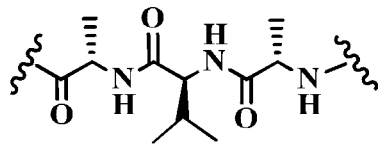
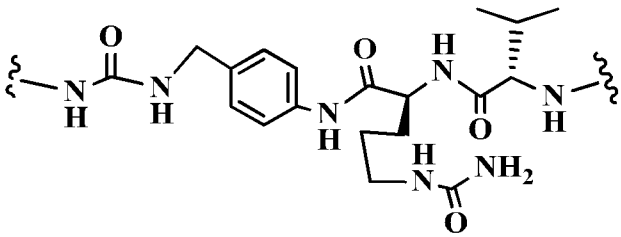
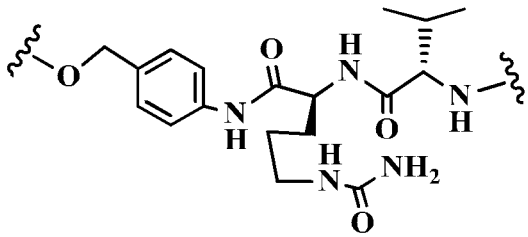
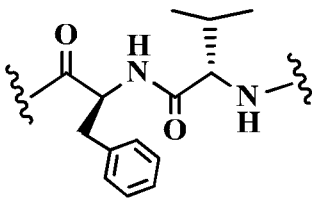
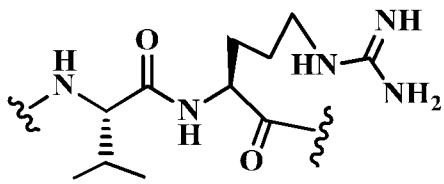
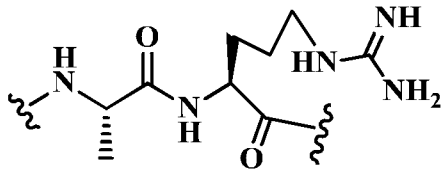
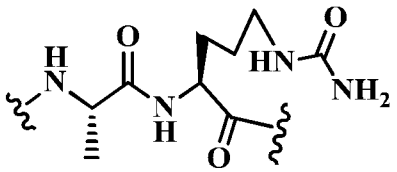
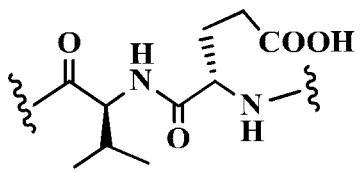
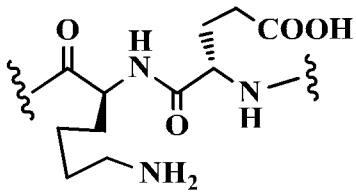
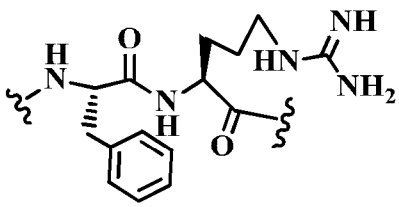
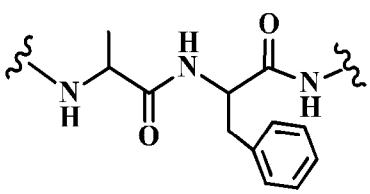
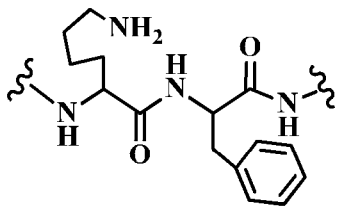
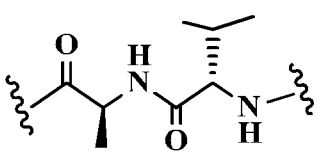
 acetylacetohydrazide,  aminoethyl-amine,

 aminoethyl-aminoethyl-amine,  ,

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gly-gly-gly,

gly-gly,

gly-gly-gly-gly,

Lys-gly,

gly-gly-phe-gly,

ala-ala,


ala-ala-ala-ala,

ala-ala-ala,

glu-gly,

glu-lys,

(VC),

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ala-val-ala,

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, (lys-phe), or a combination above thereof; wherein is the site of linkage; X_2, X_3, X_4, X_5, or X_6, are independently selected from NH; NHNH; N(R_{12}); N(R_{12})N($R_{12'}$); O; S; C_1-C_6 of alkyl; C_2-C_6 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic,

carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; CH_2OR_{12}, CH_2SR_{12}, CH_2NHR_{12}, or 1~8 amino acids; wherein R_{12} and $R_{12'}$ are independently H;C_1-C_8 of alkyl; C_2-C_8 of hetero-alkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 1-8 carbon atoms of esters, ether, or amide; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 100.

In addition, L_1, L_2, L_1, L_1', L_2', X_1, X_2, X_3, X_1', X_2' and X_3' can be independently absent.

wherein D_1 and D_2 are a cytotoxic drug, or a therapeutic drug, an immunotherapeutical short antibody-like protein, a function molecule for enhancement of binding or stabilization of the cell-binding antibody-like protein agent, a cell-surface receptor binding ligand, an antibody fragment, siRNA or DNA molecule, and are selected from:

1) Chemotherapeutic agents:

a) Alkylating agents: nitrogen mustards: chlorambucil, chlornaphazine, cyclophosphamide, da-carbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, man-nomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, predni-mustine, thiotepa, trofosfamide, uracil mustard; CC-1065 (including its synthetic analogues adozele-sin, carzelesin and bizelesin); Duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); Benzodiazepine dimers (including dimers of pyrrolobenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidino-benzodiazepines); Nitrosou-reas: (carmustine, lomustine, chlorozotocin, fotemustine, nimustine, ranimustine); Alkylsulphonates: (busulfan, treosulfan, improsulfan and piposulfan); Triazenes: (dacarbazine); Platinum containing compounds: (carboplatin, cisplatin, oxaliplatin); aziridines: benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemel-amine, trietylenephosphoramide, triethylenethio-phosphaoramide and trimethylolomel-amine];

b) Plant Alkaloids: Vinca alkaloids: (vincristine, vinblastine, vindesine, vinorelbine, navelbin); Taxoids: (paclitaxel, docetaxol) and their analogs, Maytansinoids (DM1, DM2, DM3, DM4, DM5, DM21, maytansine and ansamitocins) and their analogs, cryptophycins (particularly cryptophycin 1 and cryptophycin 8); epothilones, eleutherobin, discodermo-lide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins; pancratistatin; a sarcodictyin; spongistatin;

c) DNA Topoisomerase Inhibitors: Epipodophyllins: (9-aminocamptothecin, camptothecin, crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, reti-noic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000)); mitomycins: (mitomy-cin C);

d) Anti-metabolites: [Anti-folate: DHFR inhibitors: (methotrexate, trimetrexate, denopterin, pteropterin, aminopterin (4-aminopteroic acid) or the other folic acid analogues); IMP dehydroge-

nase Inhibitors: (mycophenolic acid, tiazofurin, ribavirin, EICAR); Ribonucleotide reductase Inhibitors: (hydroxyurea, deferoxamine)]; [Pyrimidine analogs: Uracil analogs: (ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Fluorouracil, floxuridine, ratitrexed (Tomudex)); Cytosine analogs: (cytarabine, cytosine arabinoside, fludarabine); Purine analogs: (azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine)]; folic acid replenisher, frolinic acid;

e) Hormonal therapies: Receptor antagonists: [Anti-estrogen: (megestrol, raloxifene, tamoxifen); LHRH agonists: (goscrclin, leuprolide acetate); Anti-androgens: (bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, trilostane and other androgens inhibitors)]; Retinoids/Deltoids: [Vitamin D3 analogs: (CB 1093, EB 1089 KH 1060, cholecalciferol, ergocalciferol); Photodynamic therapies: (verteporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A); Cytokines: (Interferon-alpha, Interferon-gamma, tumor necrosis factor (TNFs), human antibody-like proteins containing a TNF domain)];

f) Kinase inhibitors: BIBW 2992 (anti-EGFR/Erb2), imatinib, gefitinib, pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, axitinib, pazopanib. vandetanib, E7080 (anti-VEGFR2), mubritinib, ponatinib (AP24534), bafetinib (INNO-406), bosutinib (SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib, bevacizumab, cetuximab, Trastuzumab, Ranibizumab, Panitumumab, ispinesib; g). antibiotics, such as the enediyne antibiotics: calicheamicins:calicheamicin $\gamma 1$, $\delta 1$, $\alpha 1$ and $\beta 1$; dynemicin: dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin;

f) Polyketides (acetogenins): bullatacin and bullatacinone; gemcitabine, epoxomicins, bortezomib, thalidomide, lenalidomide, pomalidomide, tosedostat, zybrestat, PLX4032, STA-9090, Stimuvax, allovectin-7, Xegeva, Provenge, Yervoy, Isoprenylation inhibitors: Lovastatin, Dopaminergic neurotoxins: 1-methyl-4-phenylpyridinium ion, Cell cycle inhibitors: staurosporine, Actinomycins: Actinomycin D, dactinomycin, Bleomycins: bleomycin A2, bleomycin B2, peplomycin, Anthracyclines: daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin, pirarubicin, zorubicin, mtoxantrone, MDR inhibitors: verapamil, Ca^{2+}ATPase inhibitors: thapsigargin, Histone deacetylase

inhibitors: Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A; Thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, Disulfiram, Salinosporamide A.; Anti-adrenals: aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; eflornithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine, razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichlorotriethylamine; trichothecenes: T-2 toxin, verrucarin A, roridin A and anguidine; urethane, siRNA, antisense drugs, and a nucleolytic enzyme;

2) An anti-autoimmune disease agent selected from the group consisting of cyclosporine, cyclosporine A, aminocaproic acid, azathioprine, bromocriptine, chlorambucil, chloroquine, cyclophosphamide, corticosteroids: amcinonide, betamethasone, budesonide, hydrocortisone, flunisolide, fluticasone propionate, fluocortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate, DHEA, enanercept, hydroxychloroquine, infliximab, meloxicam, methotrexate, mofetil, mycophenylate, prednisone, sirolimus, and tacrolimus.

3) An anti-infectious disease agent:

a) Aminoglycosides: amikacin, astromicin, gentamicin (netilmicin, sisomicin, isepamicin), hygromycin B, kanamycin (amikacin, arbekacin, bekanamycin, dibekacin, tobramycin), neomycin (framycetin, paromomycin, ribostamycin), netilmicin, spectinomycin, streptomycin, tobramycin, verdamicin;

b) Amphenicols: azidamfenicol, chloramphenicol, florfenicol, thiamphenicol;

c) Ansamycins: geldanamycin, herbimycin;

d) Carbapenems: biapenem, doripenem, ertapenem, imipenem/cilastatin, meropenem, panipenem;

e) Cephems: carbacephem (loracarbef), cefacetrile, cefaclor, cefradine, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalexin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, cephamycin (cefoxitin, cefotetan, cefmetazole), oxacephem (flomoxef, latamoxef);

f) Glycopeptides: bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin;

g) Glycylcyclines;

h). β-Lactamase inhibitors: penam (sulbactam, tazobactam), clavam (clavulanic acid);

i). Lincosamides: clindamycin, lincomycin;

j) Lipopeptides: daptomycin, A54145, calcium-dependent antibiotics (CDA);

k) Macrolides: azithromycin, cethromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, josamycin, ketolide (telithromycin, cethromycin), midecamycin, miocamycin, oleandomycin, rifamycins (rifampicin, rifampin, rifabutin, rifapentine), rokitamycin, roxithromycin, spectinomycin, spiramycin, tacrolimus (FK506), troleandomycin, telithromycin;

l) Monobactams: aztreonam, tigemonam;

m) Oxazolidinones: linezolid;

n) Penicillins: amoxicillin, ampicillin (pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin), azidocillin, azlocillin, benzylpenicillin, benzathine benzylpenicillin, benzathine phenoxymethyl-penicillin, clometocillin, procaine benzylpenicillin, carbenicillin (carindacillin), cloxacillin, dicloxacillin, epicillin, flucloxacillin, mecillinam (pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin, penamecillin, penicillin, pheneticillin, phenoxymethylpenicillin, piperacillin, propicillin, sulbenicillin, temocillin, ticarcillin;

o) Polypeptides: bacitracin, colistin, polymyxin B;

p) Quinolones: alatrofloxacin, balofloxacin, ciprofloxacin, clinafloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, floxin, garenoxacin, gatifloxacin, gemifloxacin, grepafloxacin, kano trovafloxacin, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, orbifloxacin, ofloxacin, pefloxacin, trovafloxacin, grepafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin;

q) Streptogramins: pristinamycin, quinupristin/dalfopristin);

r) Sulfonamides: mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (co-trimoxazole);

s) Steroid antibacterials;

t) Tetracyclines: doxycycline, chlortetracycline, clomocycline, demeclocycline, lymecycline, meclocycline, metacycline, minocycline, oxytetracycline, penimepicycline, rolitetracycline, tetracycline, glycylcyclines;

u) antibiotics: annonacin, arsphenamine, bactoprenol inhibitors (Bacitracin), DADAL/AR inhibitors (cycloserine), dictyostatin, discodermolide, eleutherobin, epothilone, ethambutol, etoposide, faropenem, fusidic acid, furazolidone, isoniazid, laulimalide, metronidazole, mupirocin, mycolac-

tone, NAM synthesis inhibitors: fosfomycin, nitrofurantoin, paclitaxel, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampicin (rifampin), tazobactam tinidazole, uvaricin;

4) Anti-viral drugs:

a) Entry/fusion inhibitors: aplaviroc, maraviroc, vicriviroc, gp41 (enfuvirtide), PRO 140, CD4 (ibalizumab);

b) Integrase inhibitors: raltegravir, elvitegravir, globoidnan A;

c) Maturation inhibitors: bevirimat, vivecon;

d) Neuraminidase inhibitors: oseltamivir, zanamivir, peramivir;

e). Nucleosides &nucleotides: abacavir, aciclovir, adefovir, amdoxovir, apricitabine, brivudine, cidofovir, clevudine, dexelvucitabine, didanosine (ddI), elvucitabine, emtricitabine (FTC), entecavir, famciclovir, fluorouracil (5-FU), 3'-fluoro-substituted 2',3'-dideoxynucleoside analogues (3'-fluoro-2', 3'-dideoxythymidine (FLT) and 3'-fluoro-2', 3'-dideoxyguanosine (FLG), fomivirsen, ganciclovir, idoxuridine, lamivudine (3TC), l-nucleosides (β-l-thymidine and β-l-2'-deoxycytidine), penciclovir, racivir, ribavirin, stampidine, stavudine (d4T), taribavirin (viramidine), telbivudine, tenofovir, trifluridine valaciclovir, valganciclovir, zalcitabine (ddC), zidovudine (AZT);

f) Non-nucleosides: amantadine, ateviridine, capravirine, diarylpyrimidines (etravirine, rilpivirine), delavirdine, docosanol, emivirine, efavirenz, foscarnet (phosphonoformic acid), imiquimod, interferon alfa, loviride, lodenosine, methisazone, nevirapine, NOV-205, peginterferon alfa, podophyllotoxin, rifampicin, rimantadine, resiquimod (R-848), tromantadine;

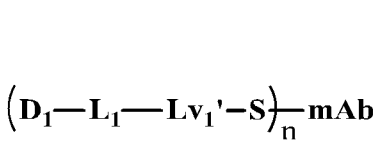
g) Protease inhibitors: amprenavir, atazanavir, boceprevir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, pleconaril, ritonavir, saquinavir, telaprevir (VX-950), tipranavir;

h) anti-virus drugs: abzyme, arbidol, calanolide a, ceragenin, cyanovirin-n, diarylpyrimidines, epigallocatechin gallate (EGCG), foscarnet, griffithsin, taribavirin (viramidine), hydroxyurea, KP-1461, miltefosine, pleconaril, portmanteau inhibitors, ribavirin, seliciclib;

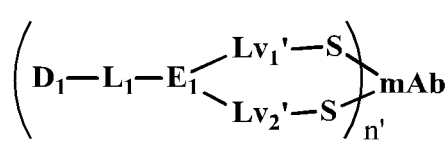
5) radioisotopes (radionuclides): ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi;

6) a pharmaceutically acceptable salt, acid or derivative of any of the above drugs.

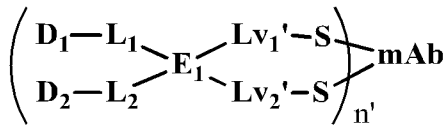
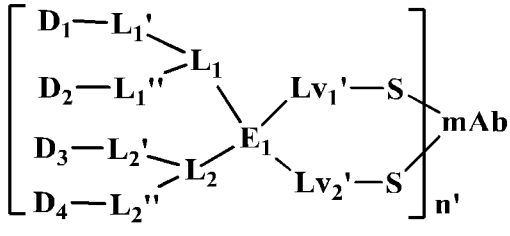
4. The process according to Claim 1, a conjugate of formula (VI), (VII), (VIII) or (IX) as represented below is generated from the rection wherein the reduced thiol groups in the antibody or antibody-like protein in step (a) simultaneously or sequentially react with the drug/linker complex of formula (I), (II), (III) or (IV) accordingly:



(VI),

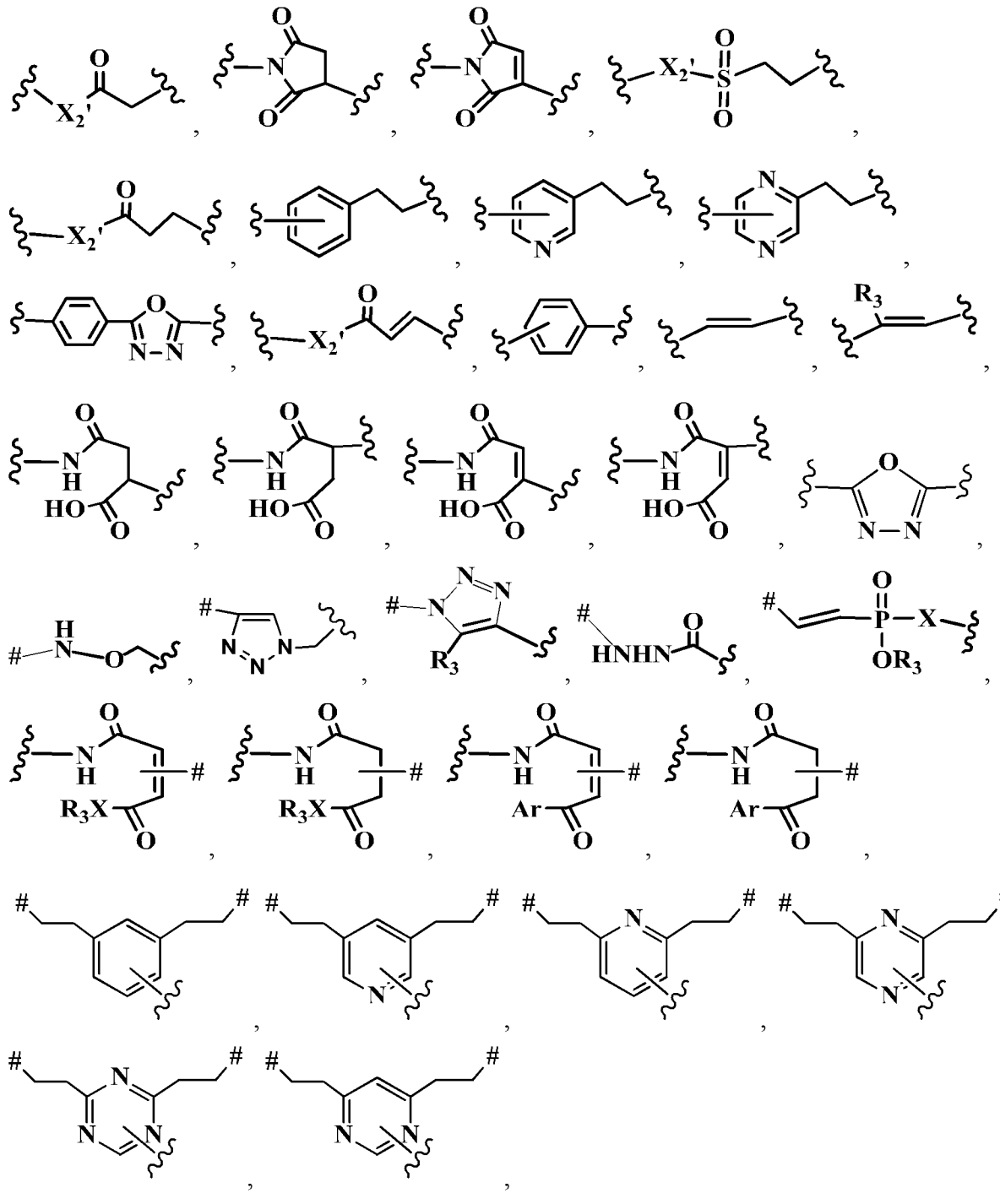


(VII),

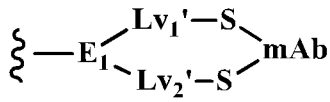
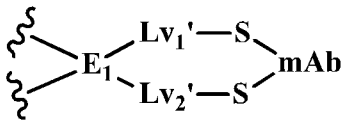

(VIII),

(IX),

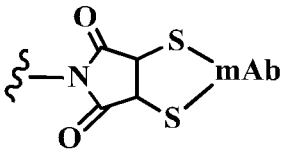
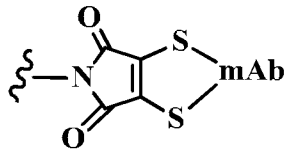
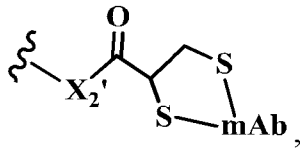
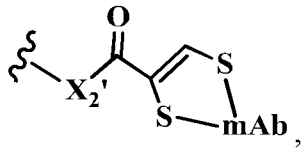
wherein n is 1 – 20; n' is 1-10; D_1, D_2, L_1, L_1', L_2, L_2', and E_1 are the same as described in claim 3; S (sulfur) is generated from the reduction of disulfide bonds in the antibody or antibody-like protein; mAb is the antibody or antibody-like protein;

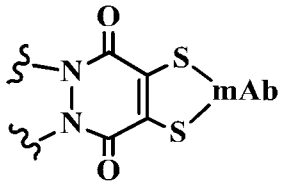
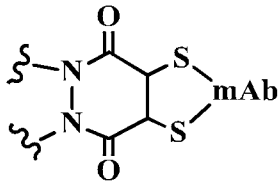
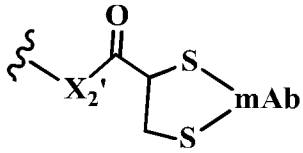
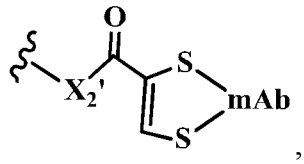
wherein Lv_1' and Lv_2' independently have one of the following structures:

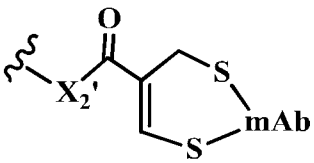
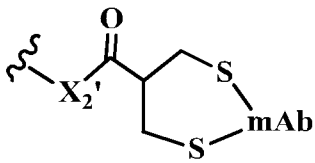
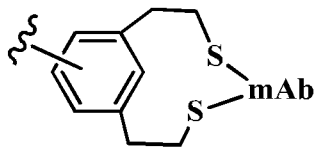


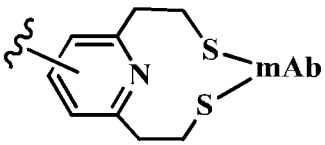
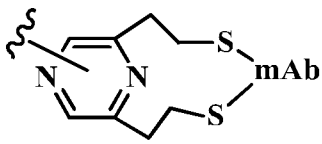
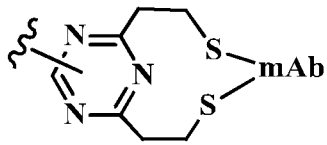
wherein Ar, X_2' and R_3 are defined the same as in Claim 3;

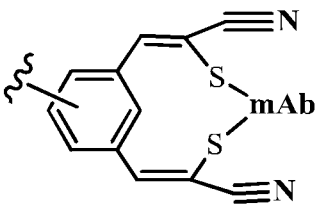
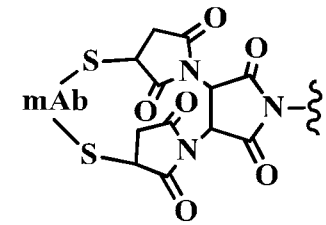
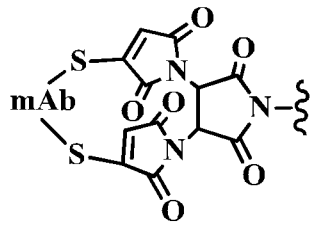
wherein in the formula (VII), (VII) and (IX), the fragments of,  and  are independently selected from:

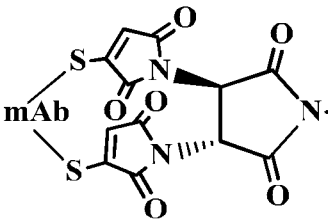
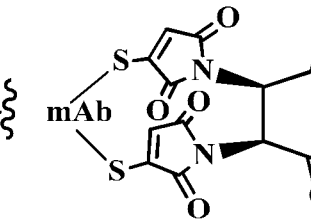
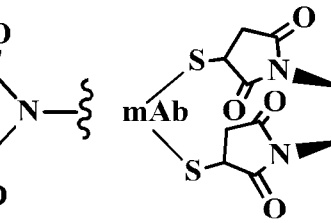
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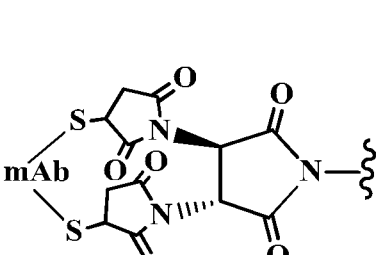
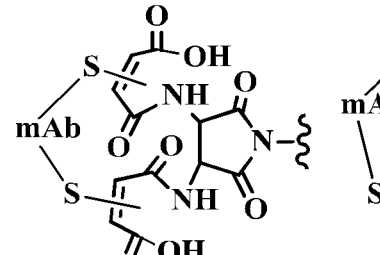
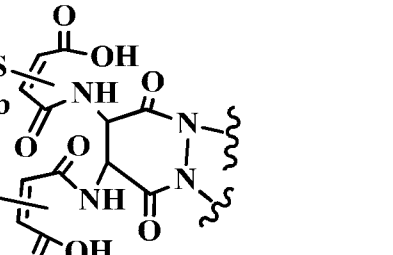
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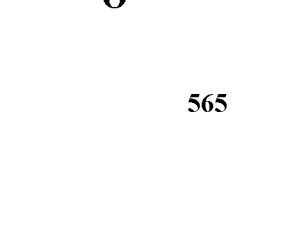
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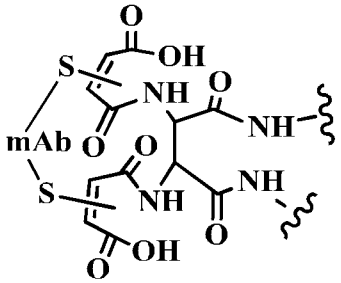
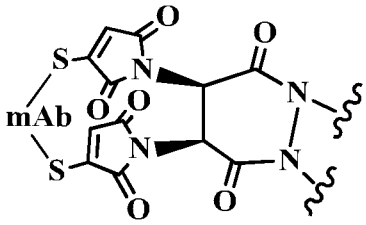
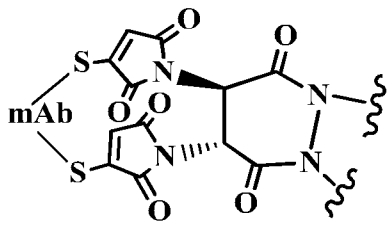
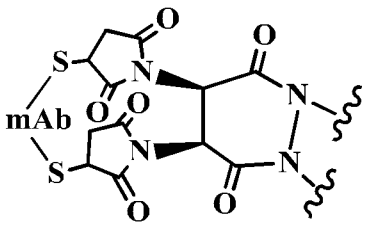
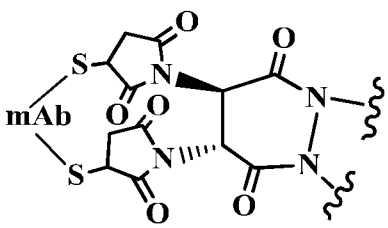
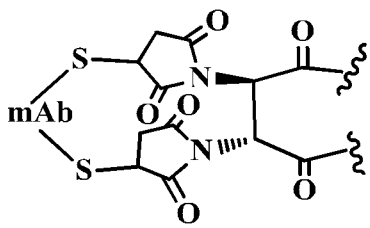
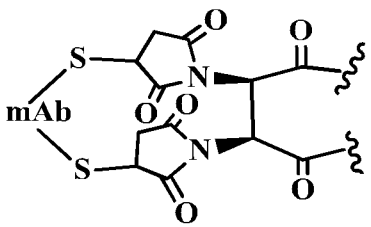
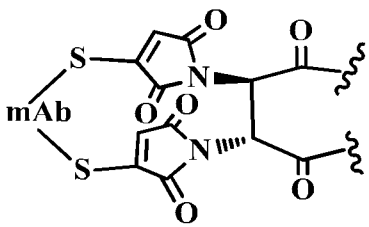
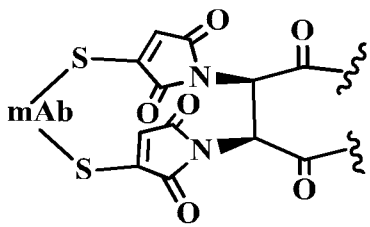
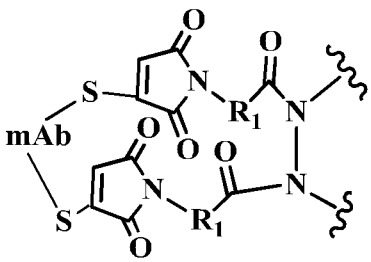
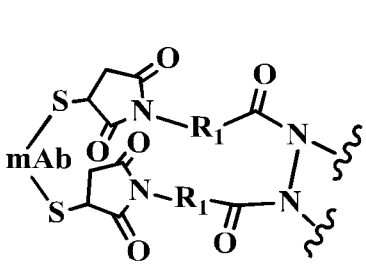
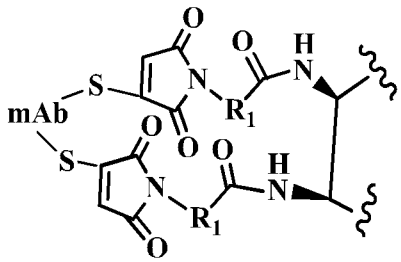
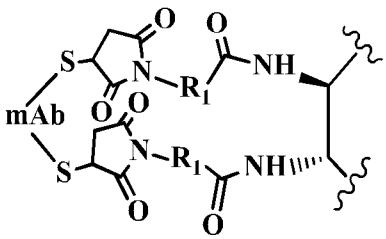
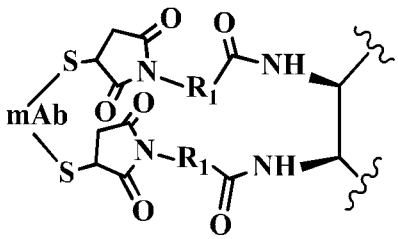
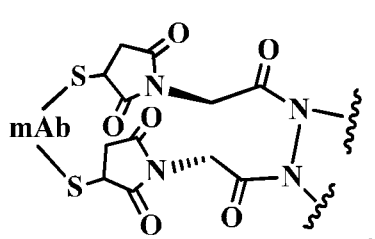
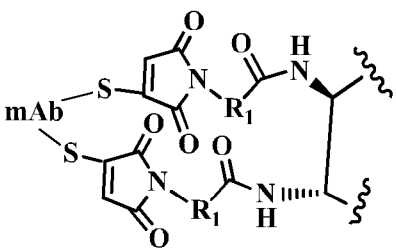
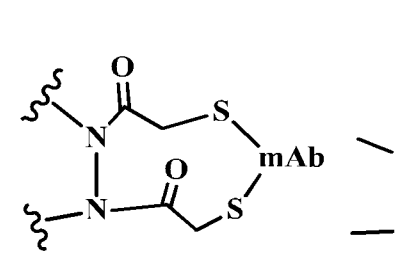
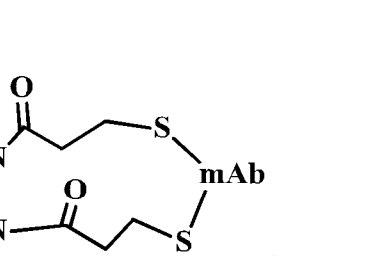
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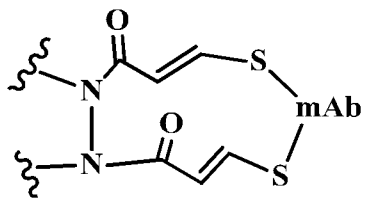
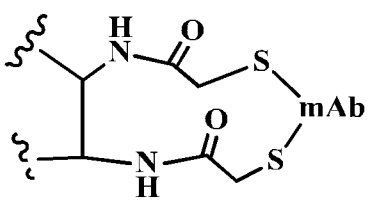
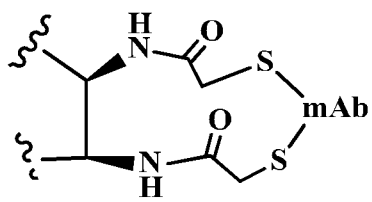
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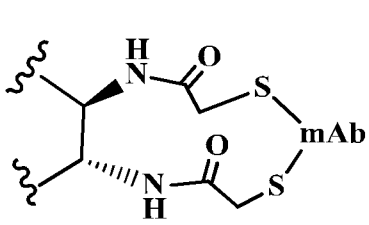
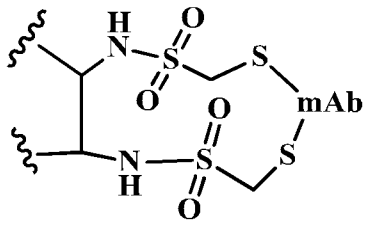
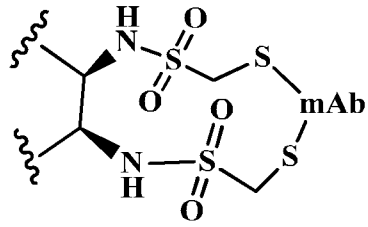
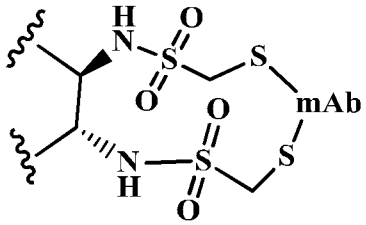
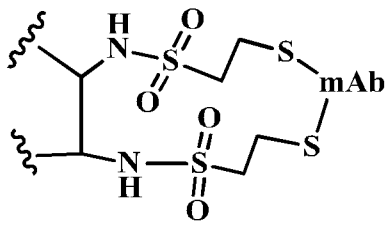
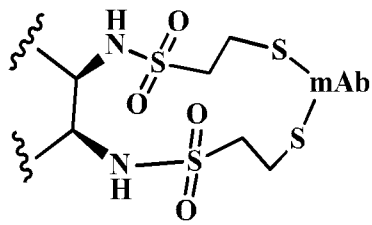
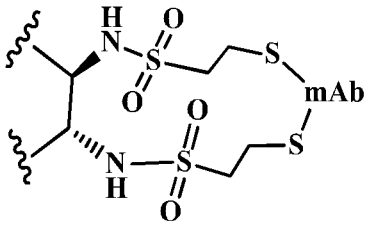
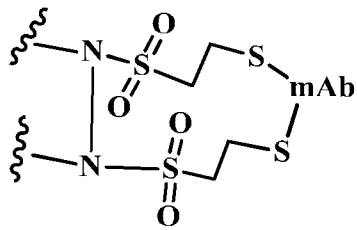
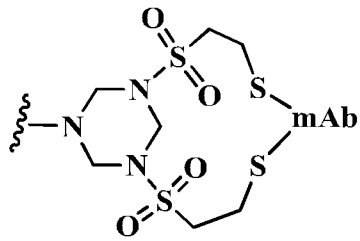
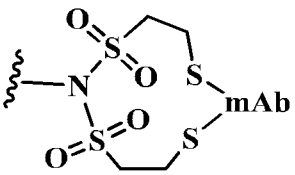
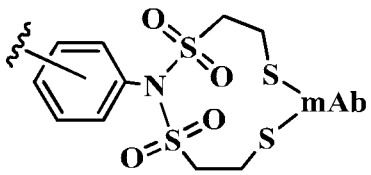
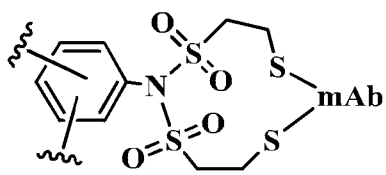
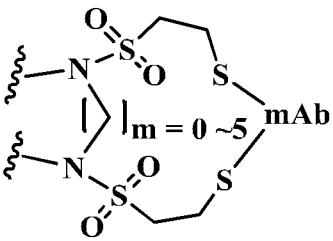
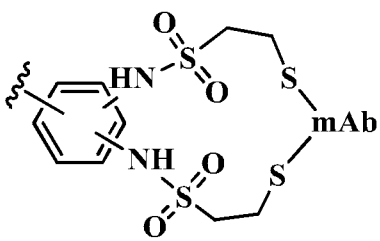
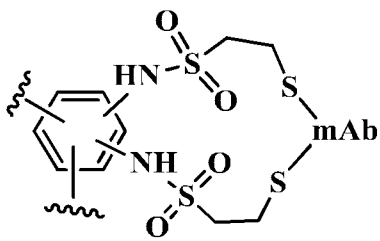
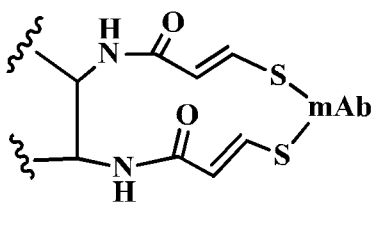
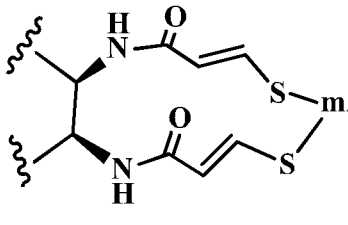
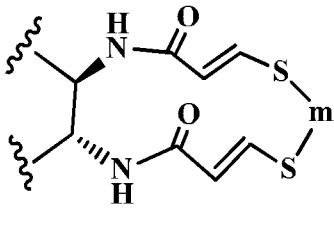
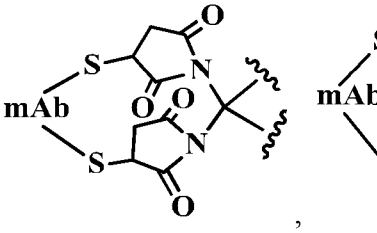
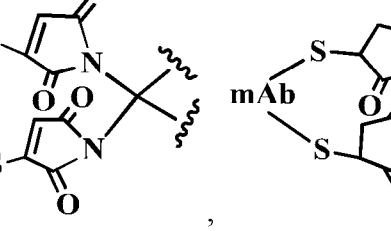
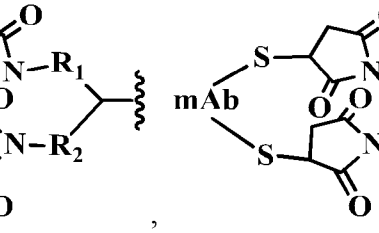
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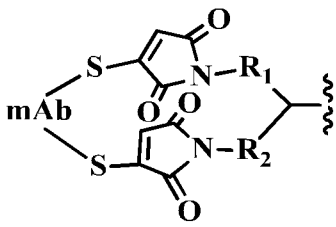
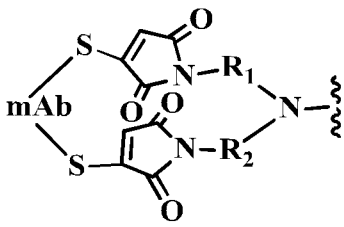
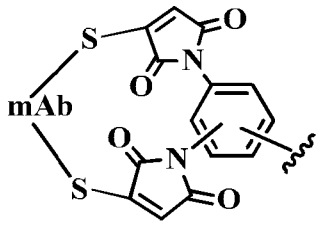
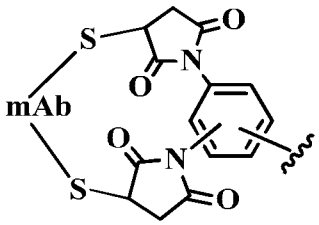
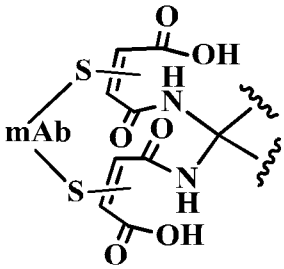
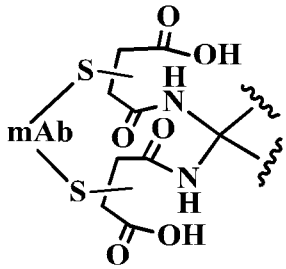
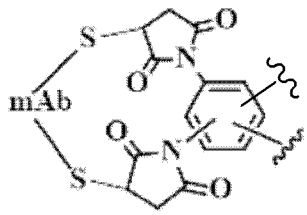
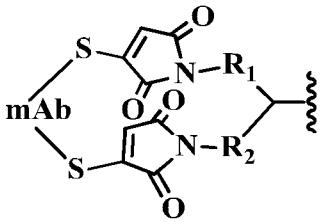
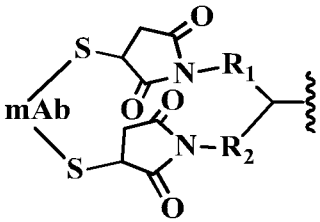
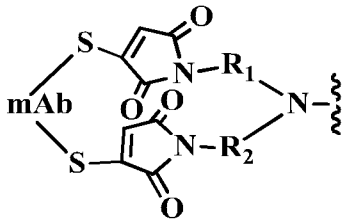
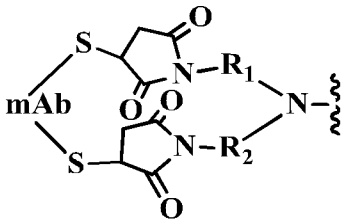
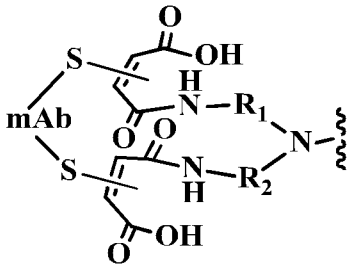
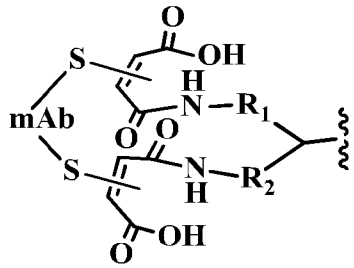
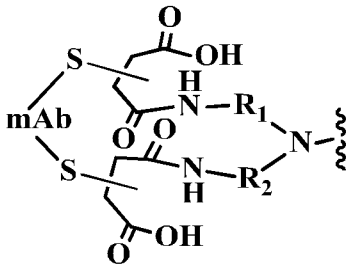
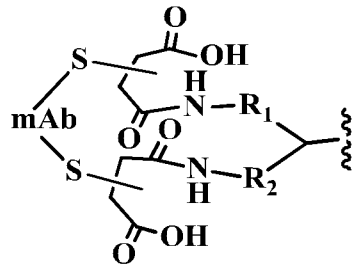
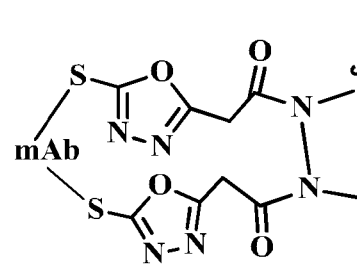
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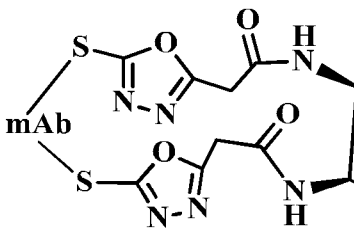
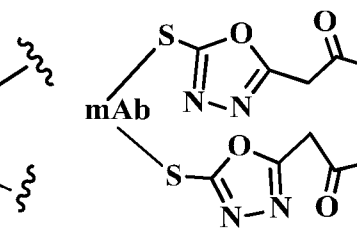
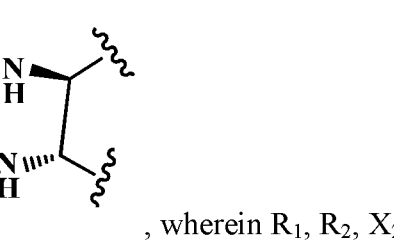
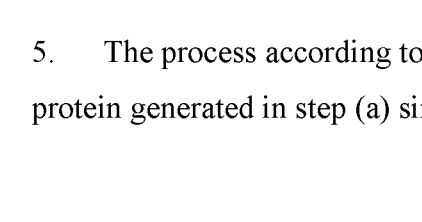
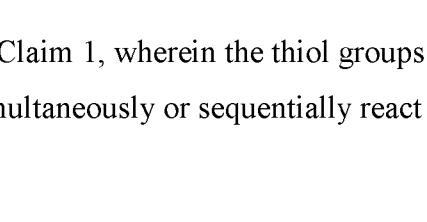
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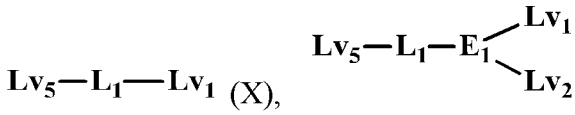

















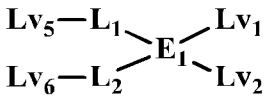
, wherein R_1, R_2, X_2 are defined the same above; mAb is an antibody.

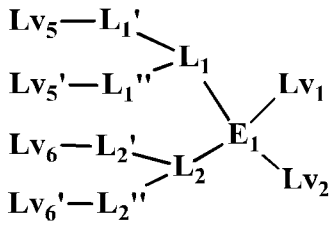
5. The process according to Claim 1, wherein the thiol groups in the antibody or antibody-like protein generated in step (a) simultaneously or sequentially react with a condensation compound

having structural formula (X), (XI), (XII) or (XIII) as illustrated below and with a drug, D_1 and/or D_2 independently, to form the conjugate of formula (VI), (VII), (VIII) or (IX):

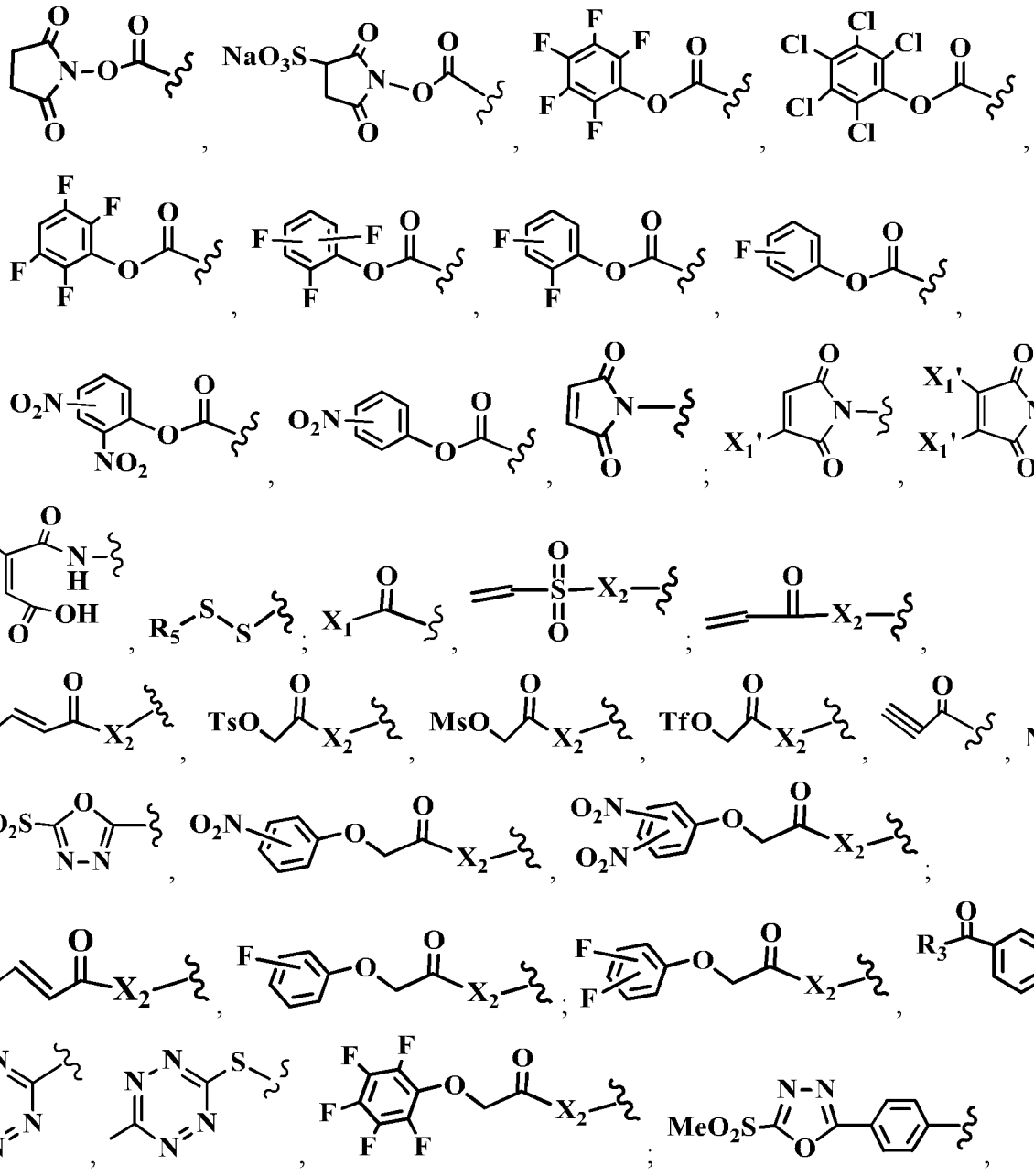

(X),

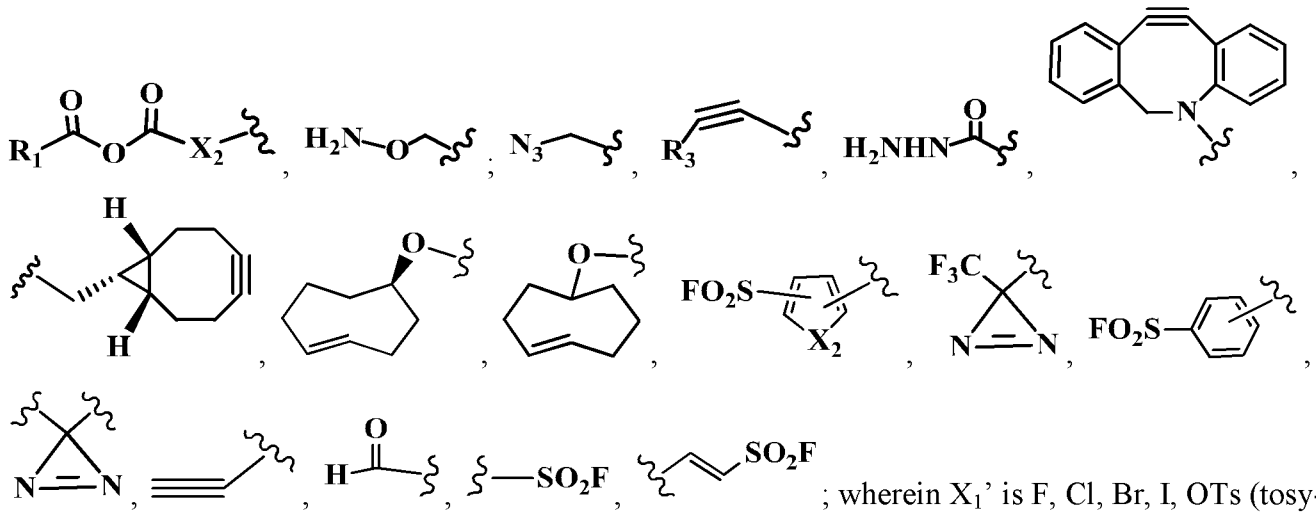
(XI),


(XII),


(XIII),

Wherein L_1, L_1', L_2, L_2', E_1, Lv_1, and Lv_2 are defined the same above in Claim 3; wherein Lv_5 and Lv_6 are independently selected from

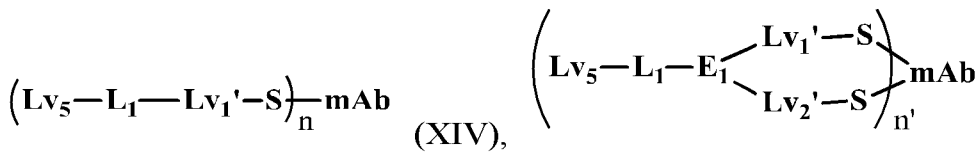




; wherein X_1' is F, Cl, Br, I, OTs (tosylate), OTf (triflate), OMs (mesylate), $OC_6H_4(NO_2)$, $OC_6H_3(NO_2)_2$, OC_6F_5, OC_6HF_4, or Lv_3; X_2' is O, NH, N(R_1), or CH_2; R_3 and R_5 are independently H, R_1, aromatic, heteroaromatic, or aromatic group wherein one or several H atoms are replaced independently by -R_1, -halogen, -OR_1, -SR_1, -NR_1R_2, -NO_2, -$S(O)R_1$, -$S(O)_2R_1$, or -$COOR_1$; Lv_3 and Lv_3' are independently a leaving group selected from F, Cl, Br, I, nitrophenoxyl; N-hydroxysuccinimide (NHS); phenoxyl; benzenethiol, dinitrophenoxyl; pentafluorophenoxyl; tetrafluorophenoxyl; difluorophenoxyl; monofluorophenoxyl; pentachlorophenoxyl; triflate; imidazole; dichlorophenoxyl; tetrachlorophenoxyl; 1-hydroxybenzotriazole; tosylate; mesylate; 2-ethyl-5-phenylisoxazolium-3′-sulfonate, anhydrides formed its self, or formed with the other anhydride, e.g. acetyl anhydride, formyl anhydride; or an intermediate molecule generated with a condensation reagent for peptide coupling reactions or for Mitsunobu reactions; wherein the fuction groups Lv_5 and/or Lv_6 can be also reacted with a thiol in a cytotoxic drug as long as the reaction are at least one fold faster or slower than the reaction between Lv_1 or Lv_2 and a thiol in an antibody-like protein, in particular, in an antibody.

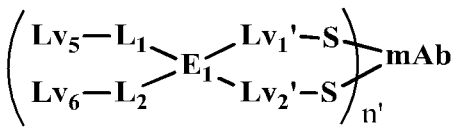
wherein Lv_5 and/or Lv_6 can be also reactive with a thiol in a cytotoxic drug as long as the reaction are at least one fold faster or slower than the reaction between Lv_1 or Lv_2 and the thiol groups in the antibody.

6. The process according to Claims 4 and 5, an antibody/linker complex molecule of formula (XIV), (XV), (XVI) or (XVII) illustrated below, is generated from a reaction of the reduced thiol groups in the antibody or antibody-like protein with formula (X), (XI), (XII), or (XIII) accordingly, and followed by reaction with the drug independently to form the conjugate of formula (VI), (VII), (VIII) or (IX).

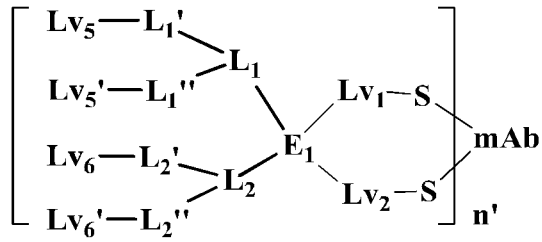


(XIV),

(XV),

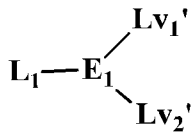
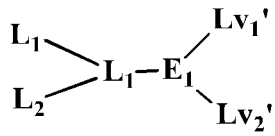
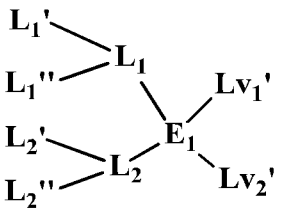


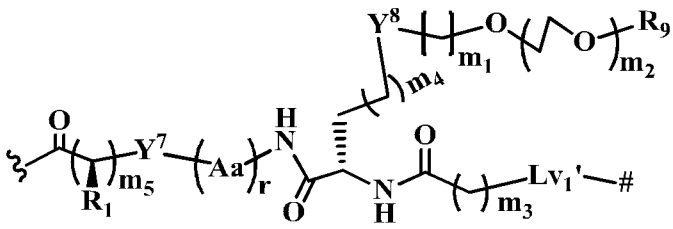
(XVI),



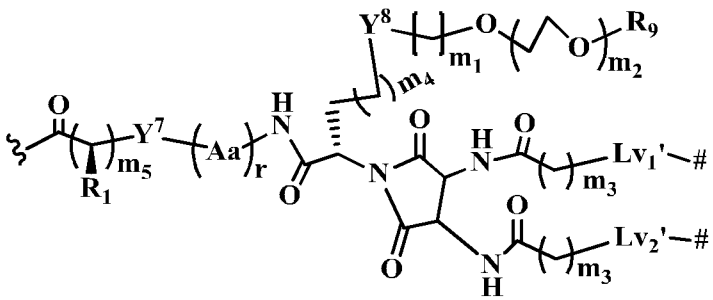
(XVII),

wherein Lv_5, Lv_6, L_1, L_2, L_1', L_2', E_1, Lv_1' Lv_2', mAb, n and n' are described are the same in claims 3 and 7.

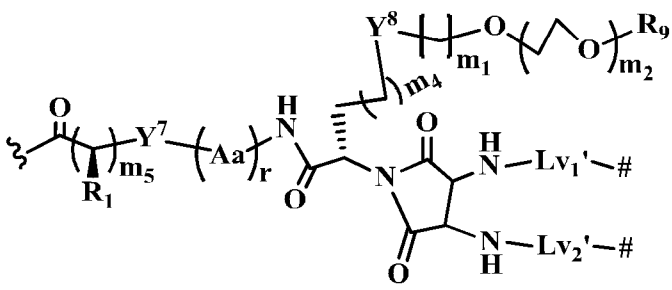
7. The conjugate of formula (VI), (VII), (VIII) or (IX) according to Claim 4, wherein the components of in formula (VI),  in formula (VII),  in formula (VIII), and  in formula (IX), are selected from the following structures accordingly:



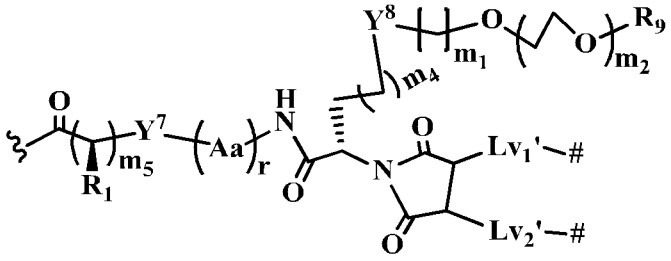
(I-a),



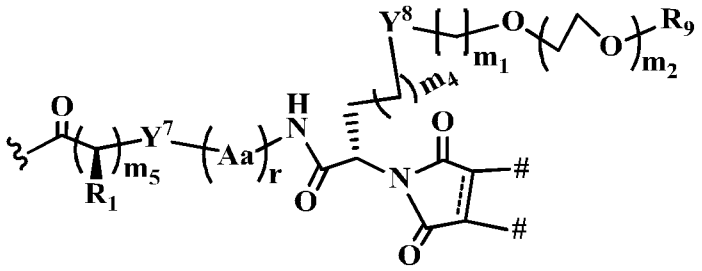
(I-b),



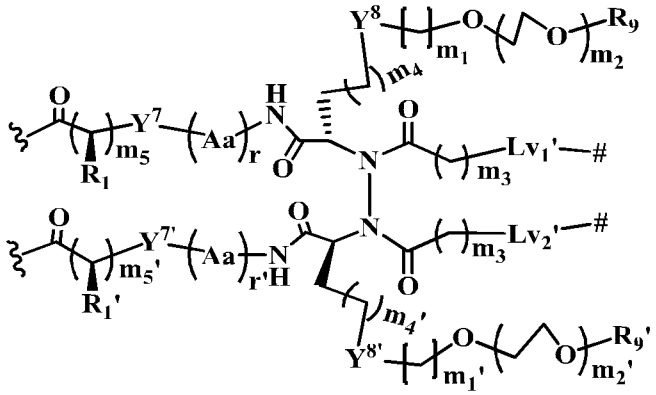
(I-c),



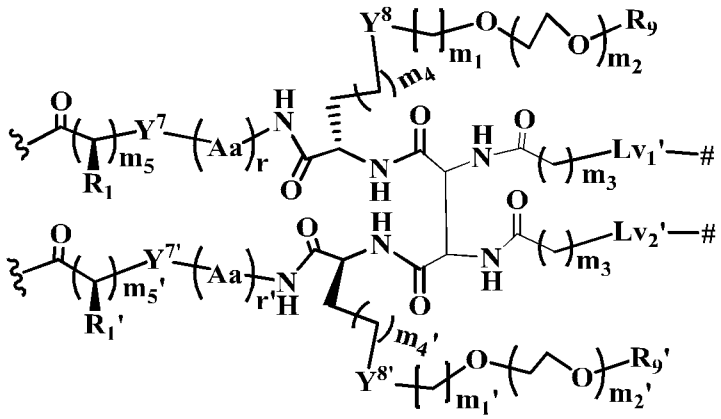
(I-d),



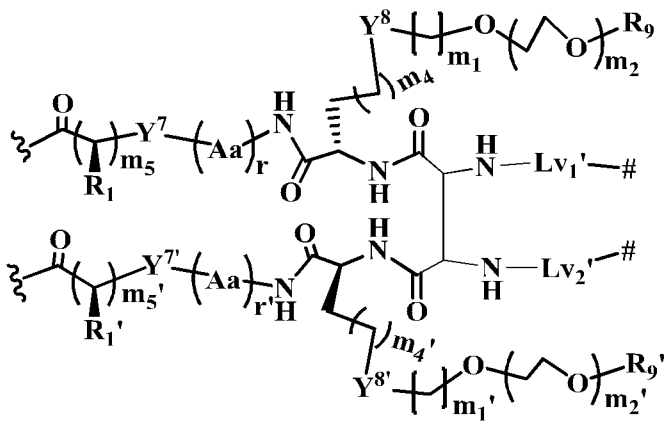
(I-e),



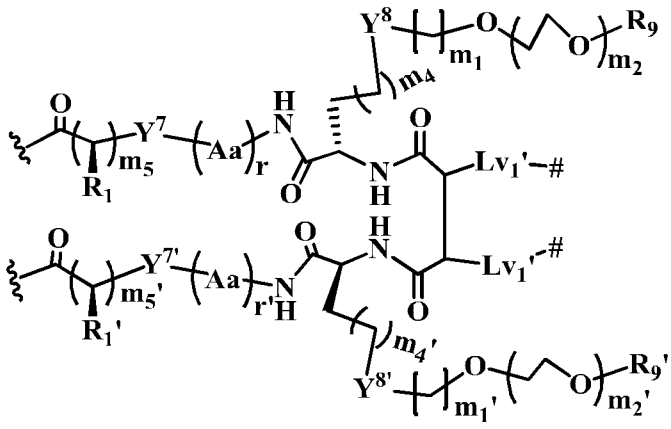
(I-f),



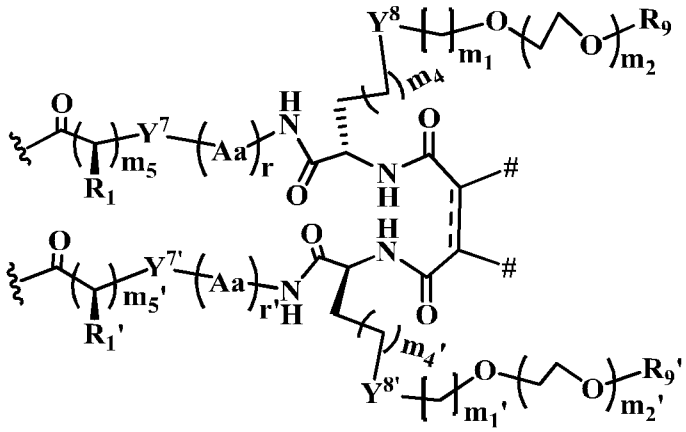
(I-g),



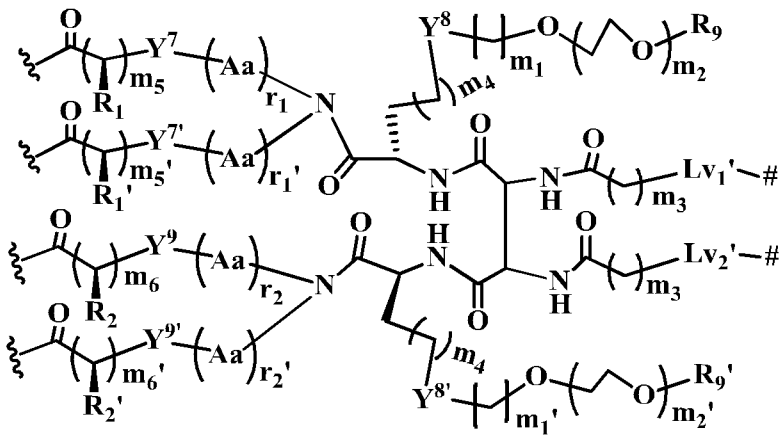
(I-h),



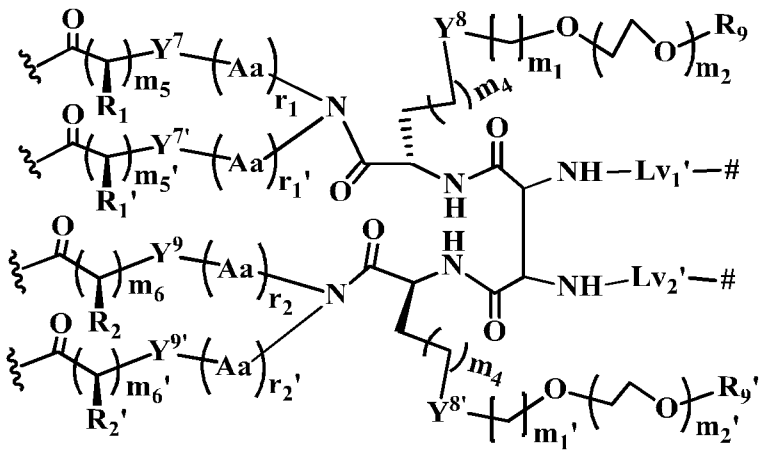
(I-i),



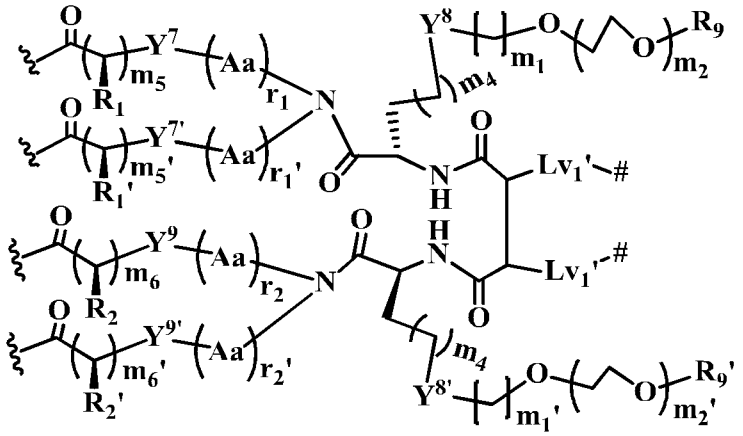
(I-j),



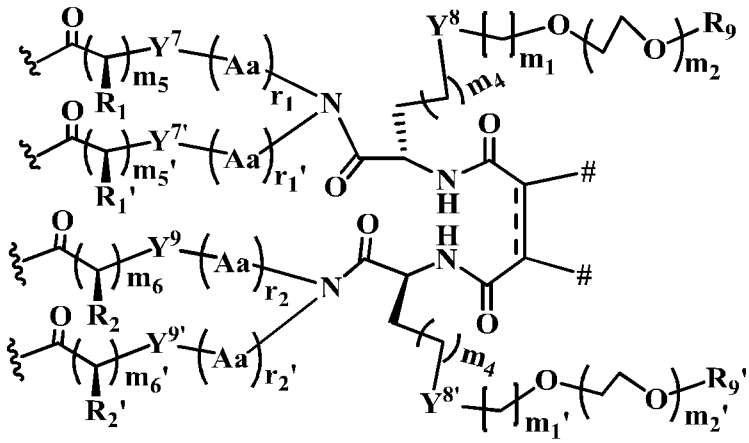
(I-k),



(I-l),



(I-m),



(I-n),

Wherein " ≀ " is a site that links a drug or a site of linker L_1, L_1', L_2, or L_2'; "#" is a site that links a S (thiol) of an antibody; Aa is L-or D- natural or unnatural amino acids;

Wherein R_1, R_1', R_2, and R_2' are independently selected from H, C_1-C_8 alkyl, OH, CH_2OH, CH_2CH_2OH, NH_2, SH, SCH_3, CH_2COOH, CH_2CH_2COOH, $CH_2CH_2CH_2CH_2NH_2$, C_6H_5, $CH_2C_6H_5$, $CH_2C_6H_4OH$, $CH(OH)CH_3$, $CH_2C(O)NH_2$, $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2NHC(=NH)NH_2$;

Wherein r, r_1, r_1', r_2, and r_2' are independently selected from is 0-12; when r is not 0, $(Aa)_r$ is the same or different amino acids or peptide units;

Wherein m_1 and m_1' are independently 1 - 18; m_2 and m_2' are independently 1-100; m_3 and m_3' are independently 1 - 8; m_4 and m_4' are independently 0 - 8; m_5 and m_5' are independently 1 - 8;

Wherein Y^7 and $Y^{7'}$ are independently NH, OCH_2NH, NHC(=O), NHNH, C(=O)NH, $N(R_1)$, SO_2, P(O)(OH), $NHS(O)_2$, $NHS(O)_2NH$, $NHS(O)_2NHC(O)$, $NHS(O)_2NHC(O)O$, $NHS(O)_2NHC(O)NH$, NHP(O)(OH), NHP(O)(OH)NH, OP(O)(OH)O, NHP(O)(OH)O, OP(O)(OH)NH, S, O, OP(O)(OH)OP(O)(OH)NH, NHP(O)(OH)OP(O)(OH)NH, NHP(O)(OH)OP(O)(OH)O, OCH_2CH_2O, OCH_2CH_2NH, $N(CH_2CH_2)_2N$, NHC_6H_4NH, CH_2;

Y^8 and $Y^{8'}$ are independently NHC(=O), $NHS(O_2)$, NH(SO), $NHS(O_2)NH$,

NHP(O)(OH)NH or C(O)NH;

Y^9 and $Y^{9'}$ are independently H, (O=)CR_1, (O=)$CNHR_1$, R_1COOH, $R_1(COCH_2NH)_{m2}H$, $R_1(Aa)_r$ or $R_1(COCH_2NCH_3)_{m2}H$, wherein R_1 is defined above.

8. The process according to Claim 4, wherein the drug D_1, D_2, D_3 and D_4 are a chromophore molecule that is capable of absorbing UV light, florescent light, IR light, near IR light, or visual light; a chromatophore molecule of a class or subclass of xanthophores, erythrophores, iridophores, leucophores, melanophores, and cyanophores; a class or subclass of fluorophore molecules which are fluorescent chemical compounds re-emitting light upon light; a class or subclass of visual phototransduction molecules; a class or subclass of photophore molecules; a class or subclass of luminescence molecules; or a class or subclass of luciferin compounds;

the chromophore molecule is selected from, non-protein organic fluorophores: Xanthene derivatives (including fluorescein, rhodamine, Oregon green, eosin, and Texas red); Cyanine derivatives: (including cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, and merocyanine); Squaraine derivatives and ring-substituted squaraines, including Seta, SeTau, and Square dyes; Naphthalene derivatives (including dansyl and prodan derivatives); Coumarin derivatives; Oxadiazole derivatives (pyridyloxazole, nitrobenzoxadiazole and benzoxadiazole); Anthracene derivatives (anthraquinones, including DRAQ5, DRAQ7 and CyTRAK Orange); Pyrene derivatives (including cascade blue); Oxazine derivatives (including Nile red, Nile blue, cresyl violet, oxazine 170); Acridine derivatives (including proflavin, acridine orange, acridine yellow); Arylmethine derivatives (including auramine, crystal violet, malachite green); Tetrapyrrole derivatives (including porphin, phthalocyanine, bilirubin);

the chromophore molecule can be also selected from analogs and derivatives of the following fluorophore compounds: CF dye, DRAQ and CyTRAK probes, BODIPY, Alexa Fluor, DyLight Fluor, Atto and Tracy, FluoProbes, Abberior Dyes, DY and MegaStokes Dyes, Sulfo Cy dyes, Hi-Lyte Fluor, Seta, SeTau and Square Dyes, Quasar and Cal Fluor dyes, SureLight Dyes (APC, RPEPerCP, Phycobilisomes), APC, APCXL, RPE, BPE, Allophycocyanin (APC), Aminocoumarin, APC-Cy7 conjugates, BODIPY-FL, Cascade Blue, Cy2, Cy3, Cy3.5, Cy3B, Cy5, Cy5.5, Cy7, Fluorescein, FluorX, Hydroxycoumarin, IR-783, Lissamine Rhodamine B, Lucifer yellow, Methoxycoumarin, NBD, Pacific Blue, Pacific Orange, PE-Cy5 conjugates, PE-Cy7 conjugates, PerCP, R-Phycoerythrin (PE), Red 613, Seta-555-Azide, Seta-555-DBCO, Seta-555-NHS, Seta-580-NHS, Seta-680-NHS, Seta-780-NHS, Seta-APC-780, Seta-PerCP-680, Seta-R-PE-670, SeTau-380-NHS, SeTau-405-Maleimide, SeTau-405-NHS, SeTau-425-NHS, SeTau-647-NHS, Texas Red, TRITC, TruRed, X-Rhodamine, 7-AAD (7-aminoactinomycin D, CG-selective), Acridine

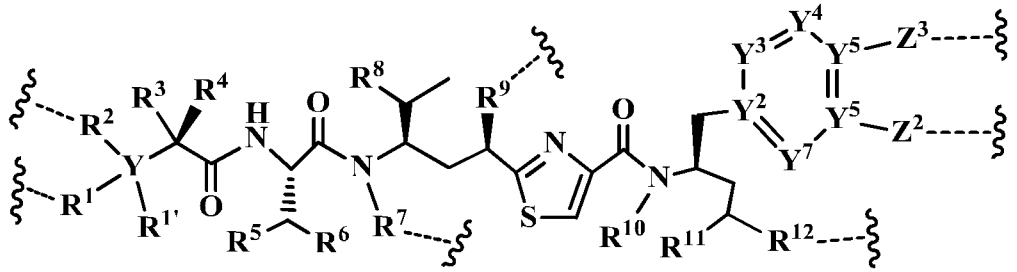
Orange, Chromomycin A3, CyTRAK Orange (Biostatus, red excitation dark), DAPI, DRAQ5, DRAQ7, Ethidium Bromide, Hoechst33258, Hoechst33342, LDS 751, Mithramycin, PropidiumIodide (PI), SYTOX Blue, SYTOX Green, SYTOX Orange, Thiazole Orange, TO-PRO: Cyanine Monomer, TOTO-1, TO-PRO-1, TOTO-3, TO-PRO-3, YOSeta-1, YOYO-1, DCFH (2',7'-Dichorodihydro-fluorescein, oxidized form), DHR (Dihydrorhodamine 123, oxidized form, light catalyzes oxidation), Fluo-3 (AM ester. pH > 6), Fluo-4 (AM ester. pH 7.2), Indo-1 (AM ester, low/high calcium (Ca^{2+})), and SNARF (pH 6/9).

9. The process according to Claim 3 or 4, wherein the drug D_1, D_2, D_3 and D_4 are independently a polyalkylene glycol selected from poly(ethylene glycols) (PEGs), poly(propylene glycol) and copolymers of ethylene oxide and propylene oxide; the polyalkylene glycol has a molecular weight of from about 10 Daltons to about 200 kDa.

10. The process according to Claim 3 or 4, wherein the drug D_1, D_2, D_3 and D_4 are selected from a tubulysin and its analogs, a maytansinoid and its analogs, a taxanoid (taxane) and its analogs, a CC-1065 and its analogs, a daunorubicin or doxorubicin and its analogs, an amatoxin and its analogs, a benzodiazepine dimer (including dimers of pyrrolobenzodiazepine (PBD), tomaymycin, anthramycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidinobenzodiazepines) and their analogs, a calicheamicin or an enediyne antibiotic and its analogs, an actinomycin and its analogs, an azaserine and its analogs, a bleomycin and its analogs, an epirubicin and its analogs, a tamoxifen and its analogs, an idarubicin and its analogs, a dolastatin and its analogs, an auristatin (including monomethyl auristatin E (MMAE), MMAF, auristatin PYE, auristatin TP, Auristatins 2-AQ, 6-AQ, EB (AEB), and EFP (AEFP)) and its analogs, a combretastatin, a duocarmycin and its analogs, a camptothecin and its analogs, a geldanamycin and its analogs, a methotrexate and its analogs, a thiotepa and its analogs, a vindesine and its analogs, a vincristine and its analogs, a hemiasterlin and its analogs, a nazumamide and its analogs, a spliceostatin, a pladienolide, a microginin and its analogs, a radiosumin and its analogs, an alterobactin and its analogs, a microsclerodermin and its analogs, a theonellamide and its analogs, an esperamicin and its analogs, PNU-159682 and its analogs, a protein kinase inhibitor, a MEK inhibitor, a KSP inhibitor, a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor, an immunotoxin, a cell receptor agonist, a cell stimulating molecule or intracellular signalling molecule, one, two or more DNA, RNA, mRNA, small interfering RNA (siRNA), microRNA (miRNA), and PIWI interacting RNAs (piRNA), and stereoisomers, isosteres, analogs, or derivatives thereof;

wherein:

(a) Tubulysin and its analogs have the following formula (V):



((V)

or a derivative with one or more isotopes; or a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof,

wherein ~~~ is a linkage site that either one or two of them can link to L_1 and/or L_2 independently; when two of " ~~~ " link to both L_1 and L_2, R^1 and R^2, or Z^2 and Z^3 are preferably the dual linkage sites;

wherein R^1, $R^{1'}$, R^2, R^3, and R^4 are independently H, C_1~C_8 alkyl; C_2~C_8 heteroalkyl, or heterocyclic; C_3~C_8 aryl, Ar-alkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, carbocyclic, or alkylcarbonyl; or R^1R^2, R^1R^3, R^2R^3, R^3R^4, or R^5R^6 form a 3~7 membered carbocyclic, cycloalkyl, heterocyclic, heterocycloalkyl, aromatic or heteroaromatic ring system; R^1 and R^2 can be independently absent when they link to L_1 or L_2 independently or simultaneously, Y^1 is N or CH;

wherein R^5, R^6, R^8, R^{10} and R^{11} are independently H, or C_1~C_4 alkyl or heteroalkyl;

wherein R^7 is independently H, R^{14}, $-R^{14}C(=O)X^1R^{15}$; or $-R^{14}X^1R^{15}$; X^1 is O, S, S-S, NH, CH_2 or NR^{14};

wherein R^9 is selected from H, OH, =O, $-OR^{14}$, $-OC(=O)R^{14}$, $-OC(=O)NHR^{14}$, $-OC(=O)NR^{14}R^{15}$, $OP(=O)(OR^{14})_2$, $-OC(=O)NR^{14}R^{15}$, or $OR^{14}OP(=O)(OR^{15})_2$; when R^9 links L_1 or L_2, R^9 is , -O-, -OC(=O)NH- or $-OC(=O)N(R^{14})$-;

wherein R^{11} is independently H, R^{14}, $-R^{14}C(=O)R^{15}$, $-R^{14}C(=O)X^2R^{15}$, wherein X^2 is -O-, -S-, -NH-, or $-N(R^{14})$-;

wherein R^{12} is -COOH, -COSH, $-CONH_2$, $CONHNH_2$, $CONHNHR^{15}$, $-CONH(R^{15})$, $-COOR^{15}$, $-R^{15}COR^{16}$, $-R^{15}COOR^{16}$, $-R^{15}C(O)NH_2$, $-R^{15}C(O)NHR^{16}$, $-COSR^{15}$, $R^{15}S(=O)_2R^{16}$, $-R^{15}P(=O)(OR^{17})_2$, $-R^{15}OP(=O)(OR^{17})_2$, $-COOCH_2OP(=O)(OR^{17})_2$, $-COX^2SO_2R^{17}$, $-COOR^{15}X^2R^{16}$, tetrazole, imidazole, or triazole, where X^2 is -O-, -S-, -NH-, $-N(R^{15})$-, $-O-R^{15}$-, $-S-R^{15}$-, CH_2 or $-NHR^{15}$-; when R^{12} links L_1 or L_2, R^{12} is $-C(O)O$-, $-C(O)NH$-, $-C(=O)NHS(O)_2R^{15}$- or $-C(=O)N(R^{15})$-;

$R^{13}$and R^{14} are independently C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl;

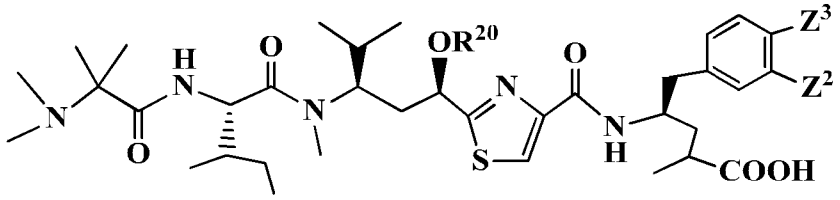
Z^2 and Z^3 are independently H, O, S, NH, $N(R^{15})$, NHNH, -OH, -SH, $-NH_2$, NH, $NHNH_2$, $-NH(R^{15})$, $-OR^{15}$, CO, $-COX^2$, $-COX^2R^{16}$, R^{17}, F, Cl, Br, I, SR^{16}, $NR^{16}R^{17}$, $N=NR^{16}$, $N=R^{16}$, NO_2, $SOR^{16}R^{17}$, SO_2R^{16}, SO_3R^{16}, OSO_3R^{16}, $PR^{16}R^{17}$, $POR^{16}R^{17}$, $PO_2R^{16}R^{17}$, $OP(O)(OR^{17})_2$,

$OCH_2OP(O)(OR^{17})_2$, $OC(O)R^{17}$, $OC(O)OP(O)(OR^{17})_2$, $PO(OR^{16})(OR^{17})$, $OP(O)(OR^{17})OP(O)(OR^{17})_2$, $OC(O)NHR^{17}$;-O-(C_4-C_{12} glycoside), -N-(C_4-C_{12} glycoside); C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl, or 2- 8 carbon atoms of esters, ether, or amide; or peptides containing 1-8 amino acids $(NH(Aa)_{1\sim8}$, or $CO(Aa)_{1\sim8}$ (which are respectively N-terminal or C-terminal 1 - 8 the same or different amino acids)), or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000, or combination of above groups thereof; X^2 is O, S, S-S, NH, CH_2, OH, SH, NH_2, CHR^{15} or NR^{15};

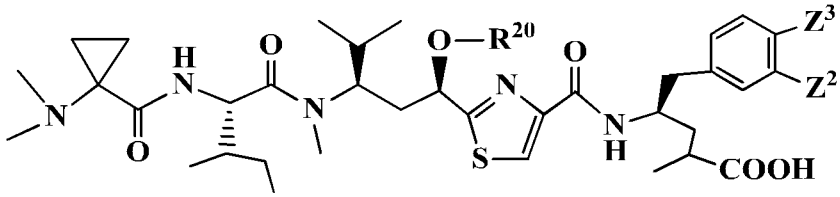
R^{15}, R^{16} and R^{17} are independently H, C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl, alkylcarbonyl, or Na^+, K^+, Cs^+, Li^+, Ca^{2+}, Mg^+, Zn^{2+}, $N^+(R^1)(R^2)(R^3)(R^4)$, $HN^+(C_2H_5OH)_3$ salt;

Y^1 and Y^2 are independently N or CH; q is 0 or 1; when q=0, Y^3 does not exist, Y^4, Y^5, Y^6 and Y^7 are independently CH, N, NH, O, S, or N (R1), thus Y^2, Y^4, Y^5, Y^6 and Y^7form a heteroaromatic ring of furan, pyrrole thiophene, thiazole, oxazole and imidazole, pyrazole, triazole, tetrazole, thiadiazole; when q=1, Y^3, Y^4, Y^5, Y^6 and Y^7 are independently CH or N, thus Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 form aromatic ring of benzene, pyridine, pyridazine, pyrimidine, pyrazine, triazine, tetrazine, pentazine;

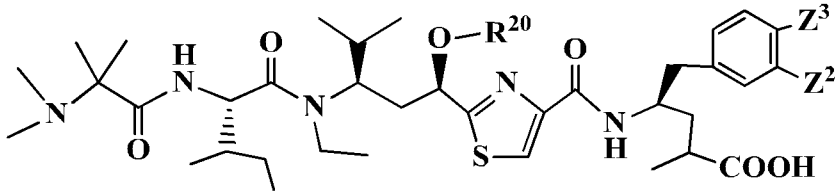
Wherein the tubulysin analogs have the structures shown below:



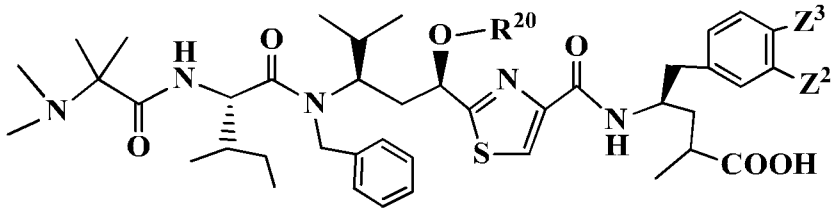
V-01,



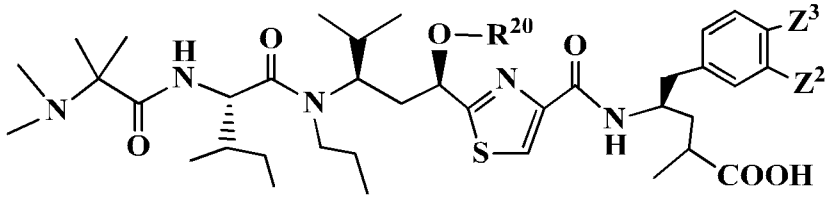
V-02,

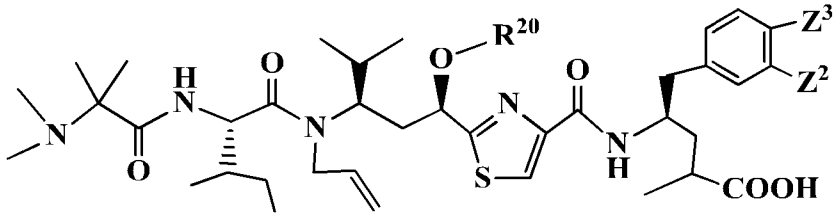


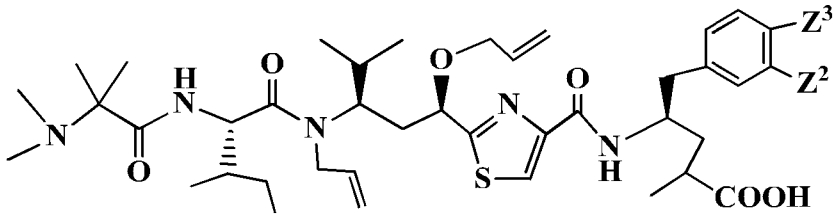
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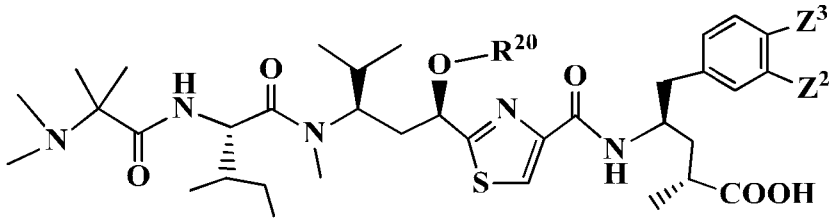


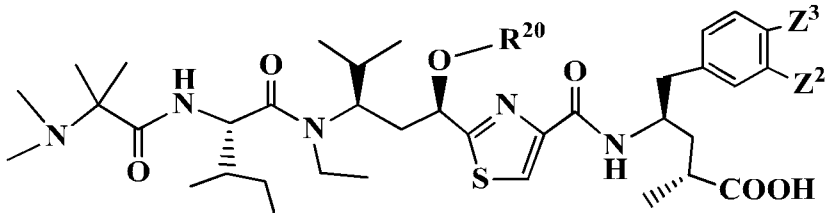
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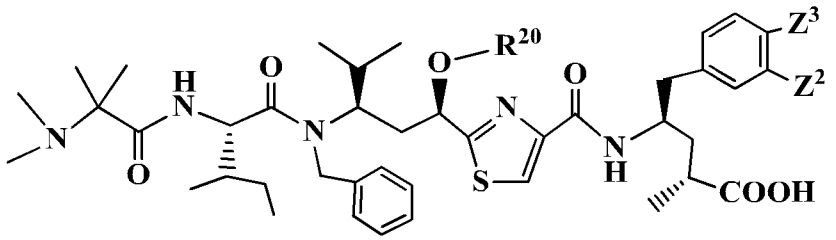

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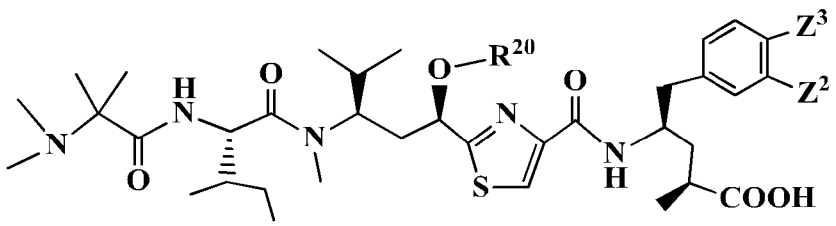

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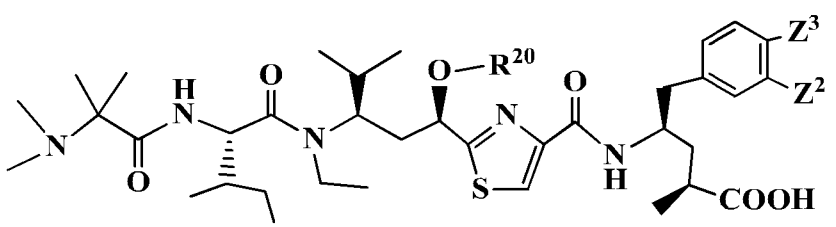

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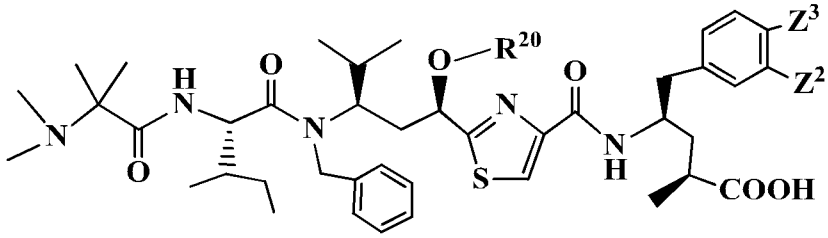

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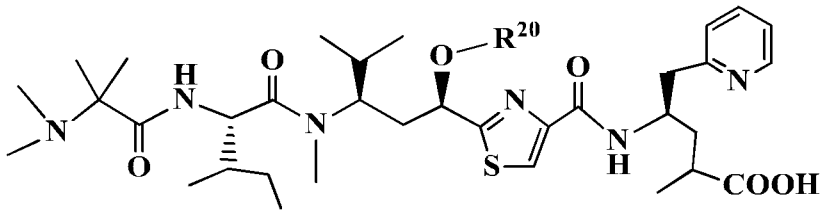

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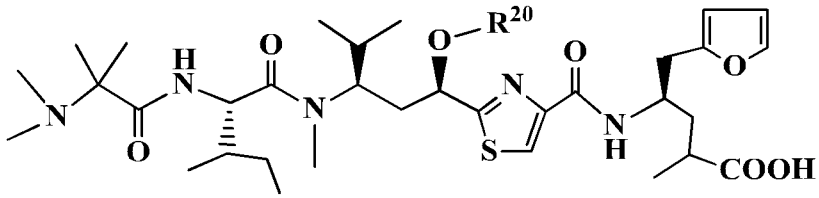

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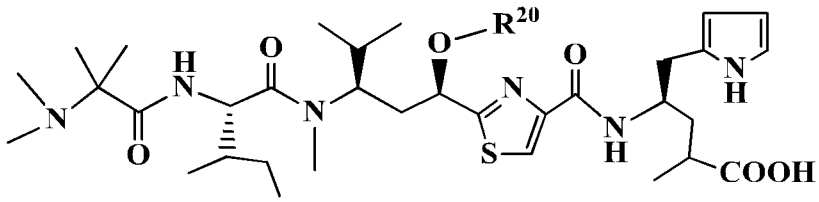

V-11,

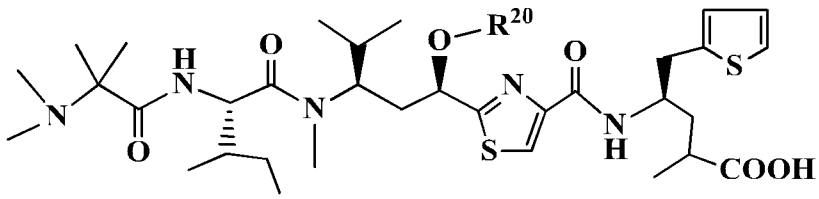

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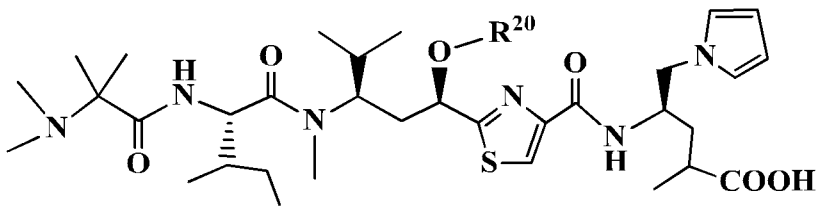

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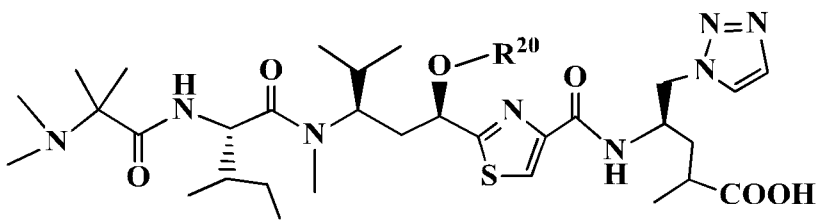

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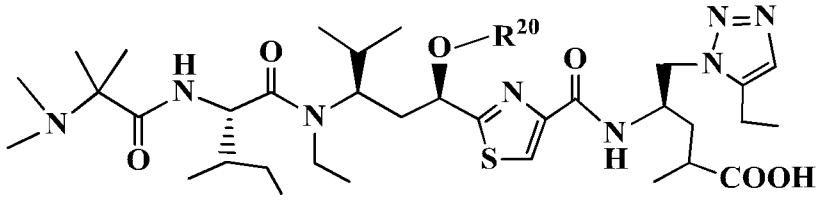

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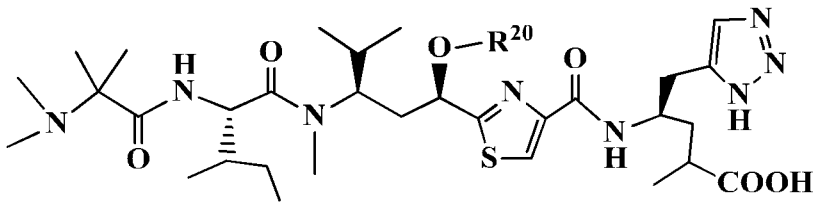

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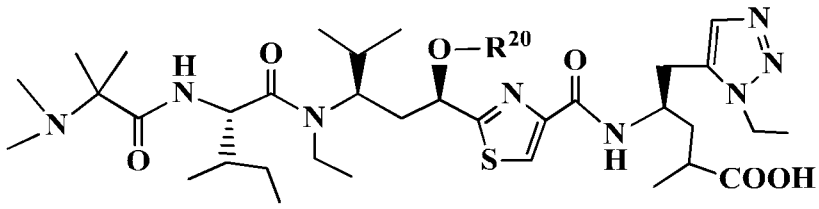

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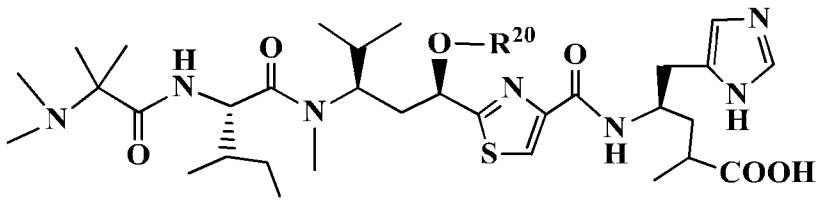

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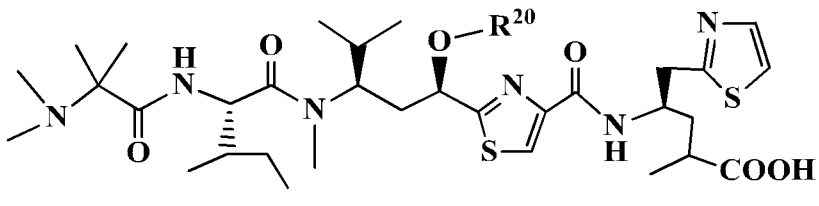

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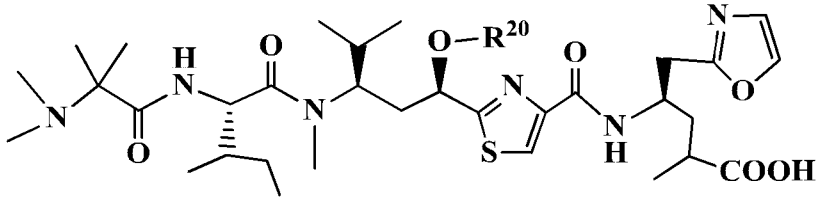

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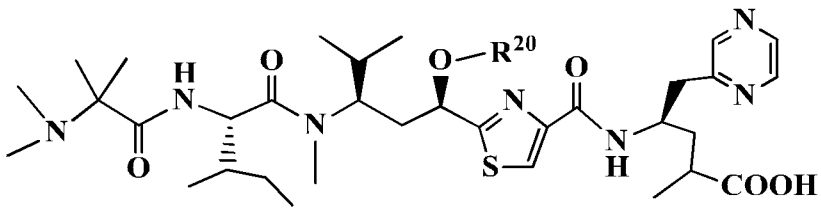

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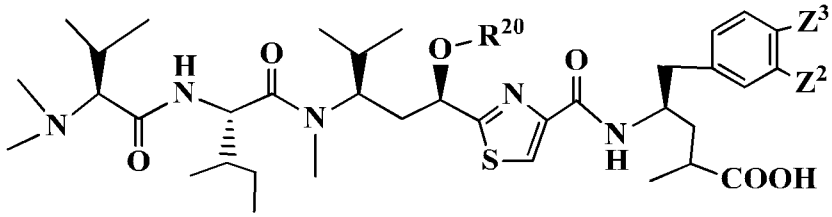

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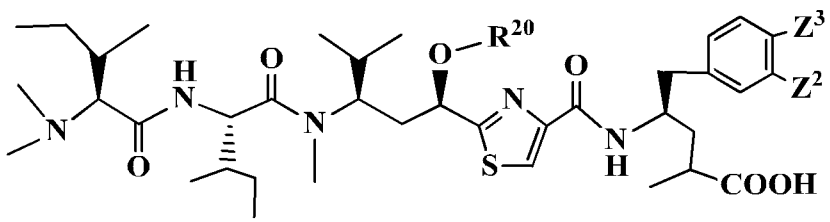

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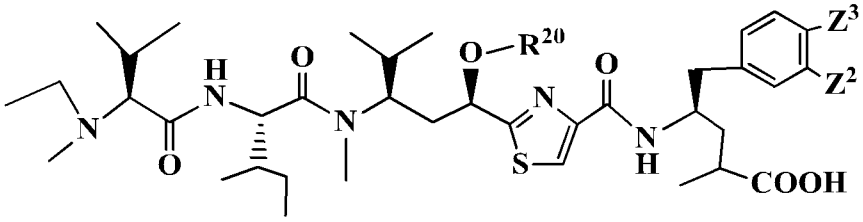

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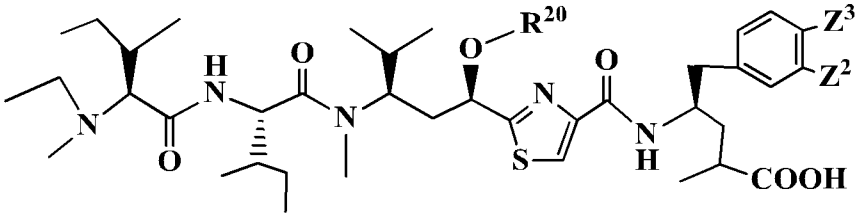

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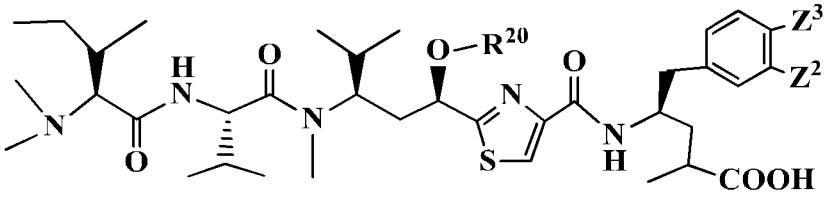

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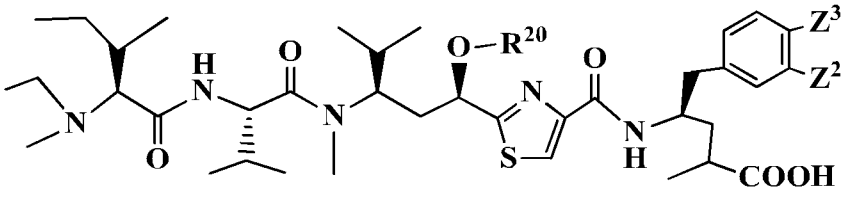

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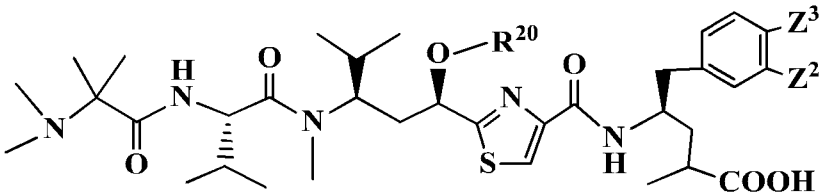

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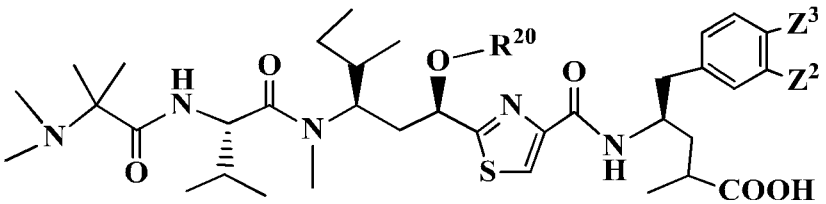

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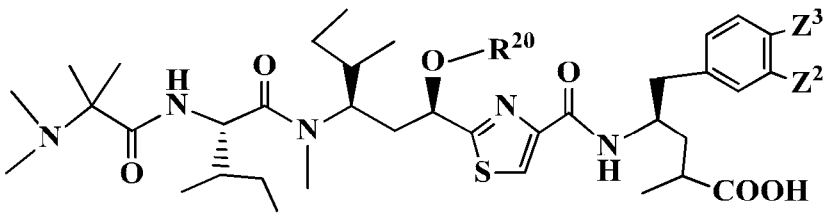

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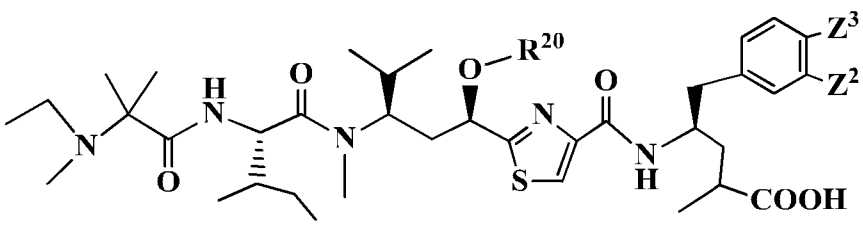

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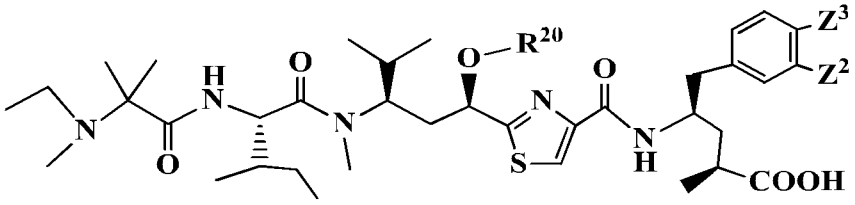

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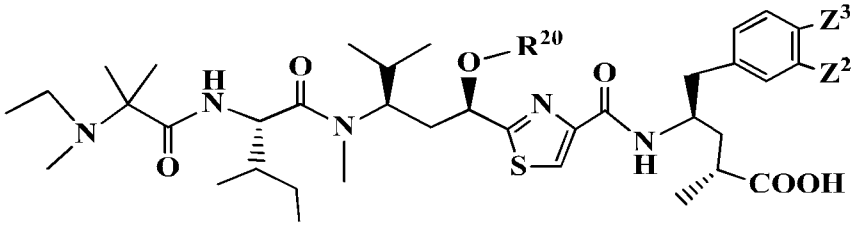

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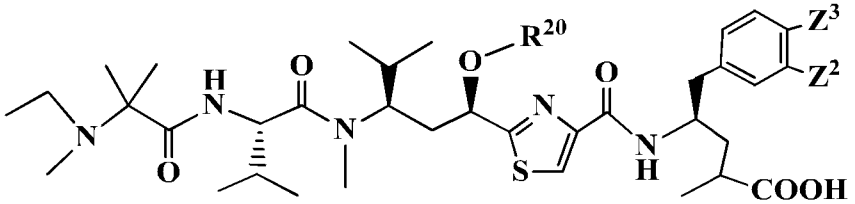

V-34,

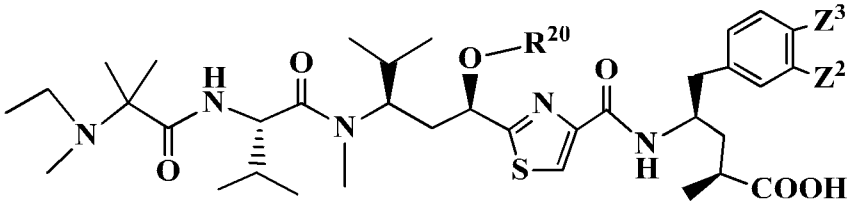

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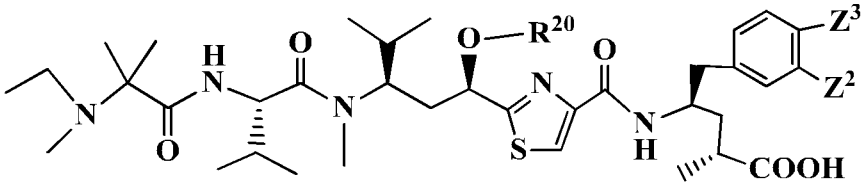

V-36,

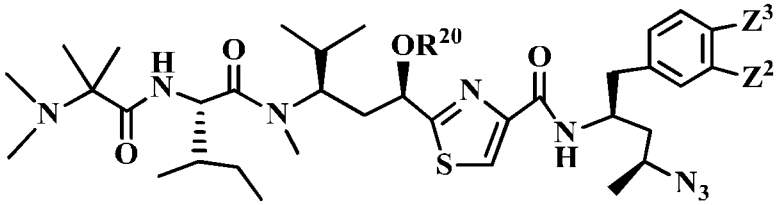

V-37,

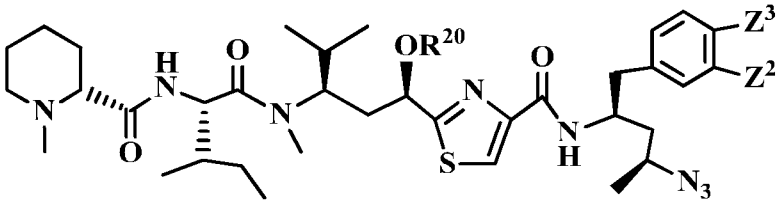

V-38,

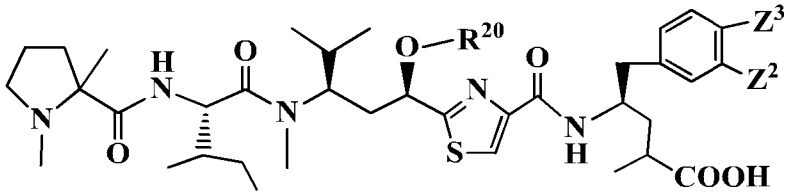

V-39,

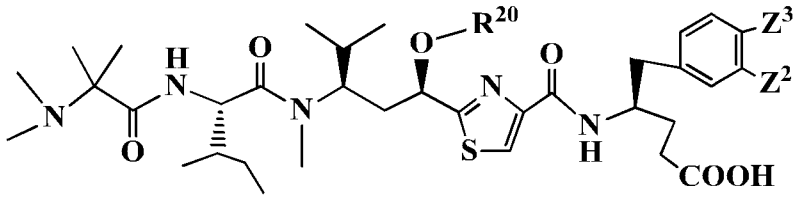

V-40,

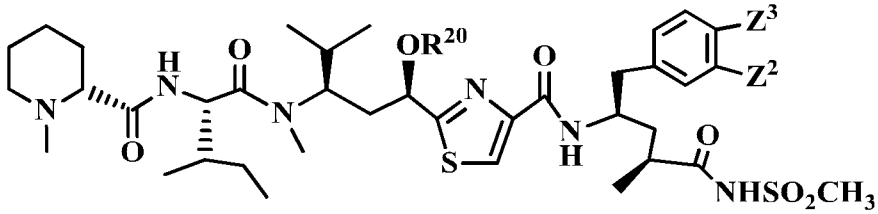

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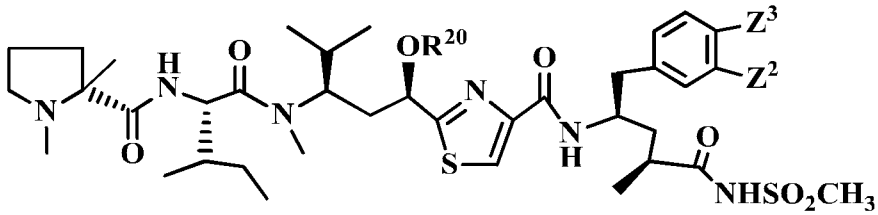

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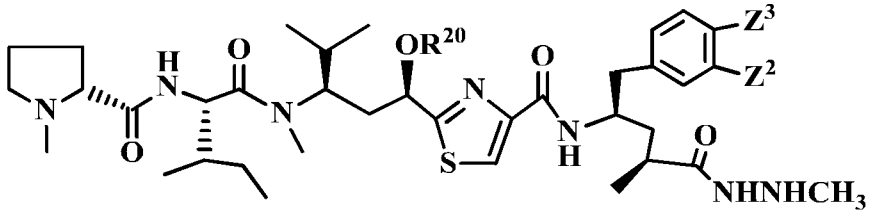

IV-43,

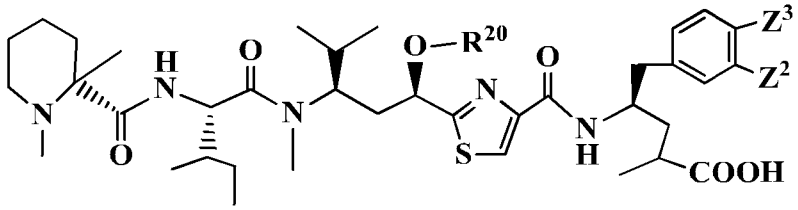

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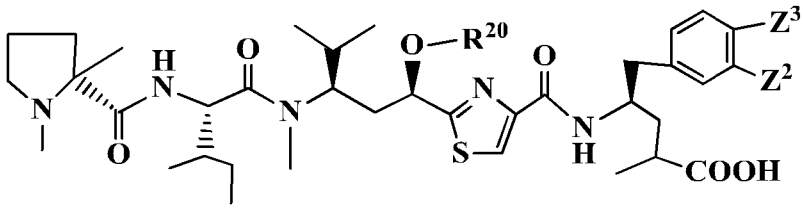

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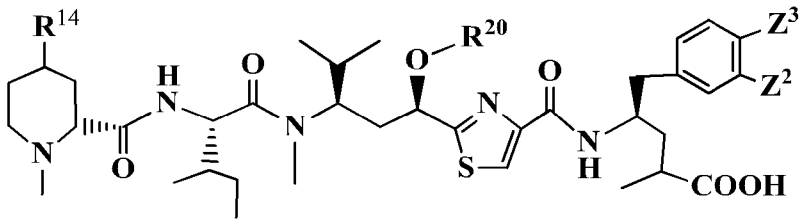

V-46,

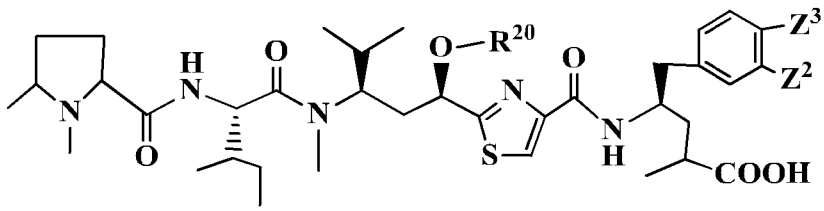

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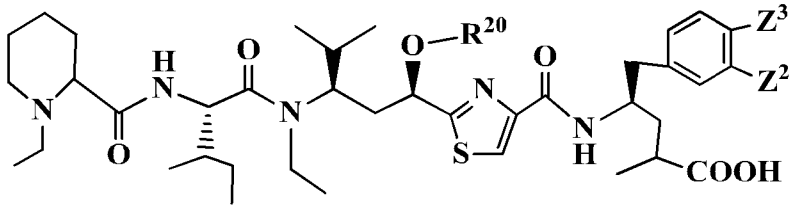

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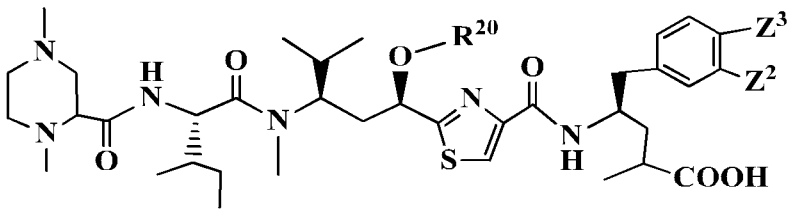

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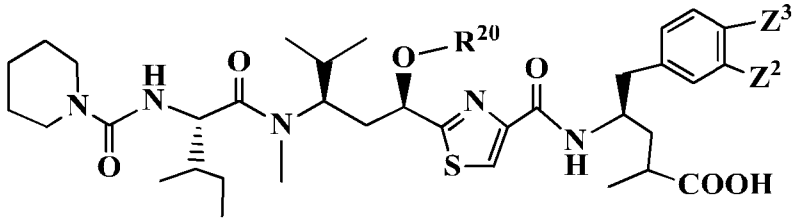

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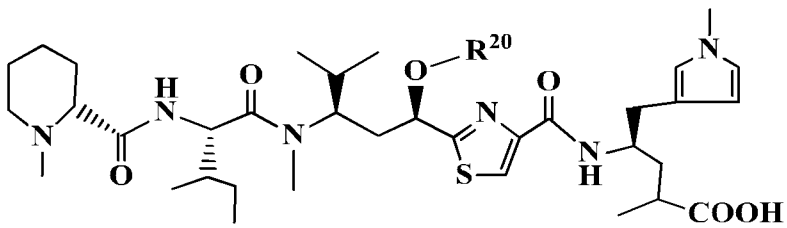

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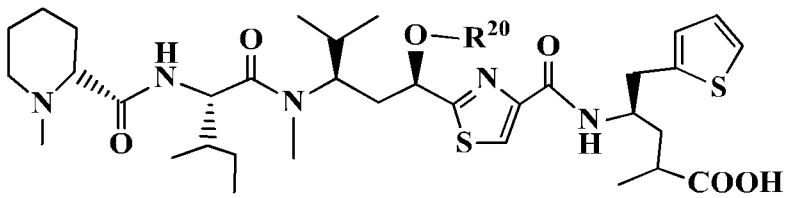

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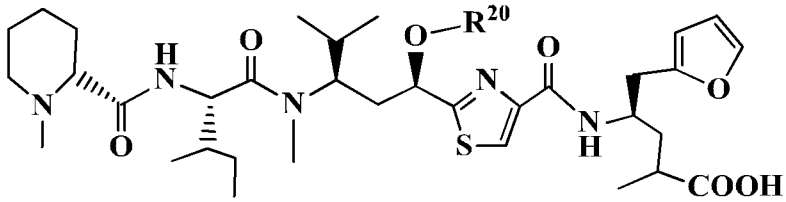

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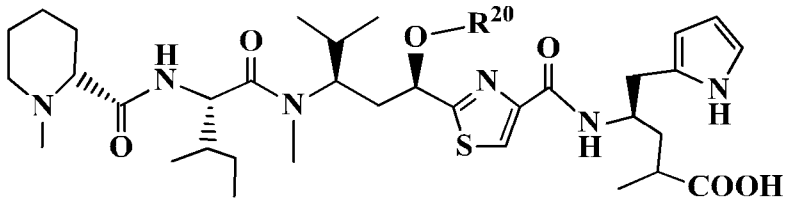

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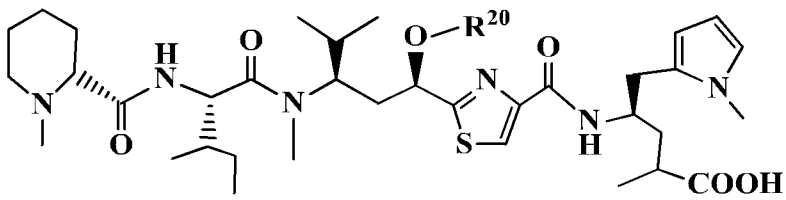

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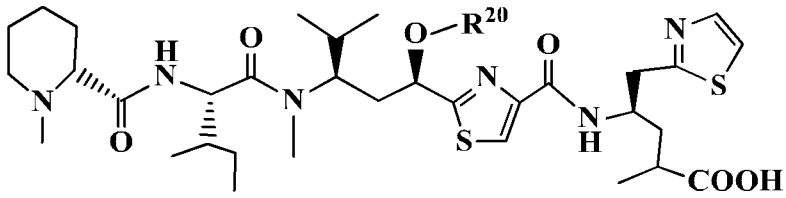

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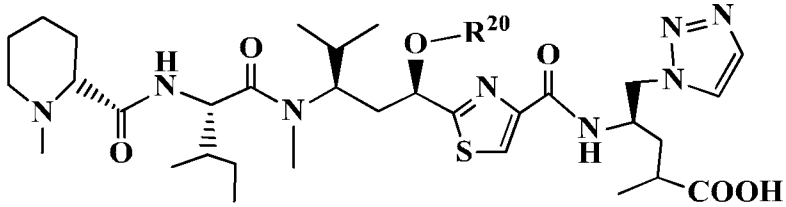

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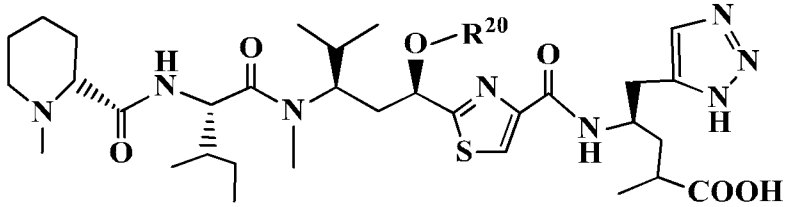

V-58,

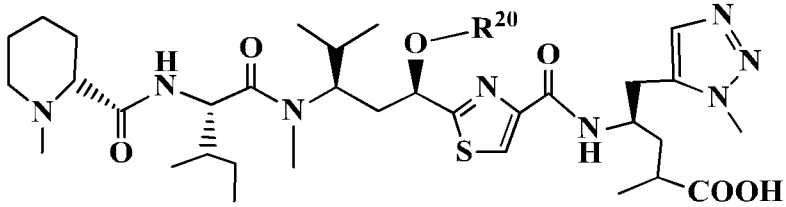

V-59,

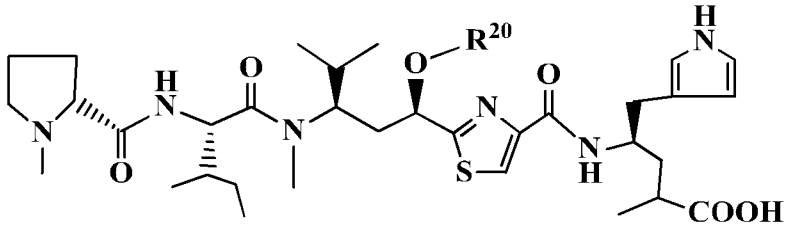

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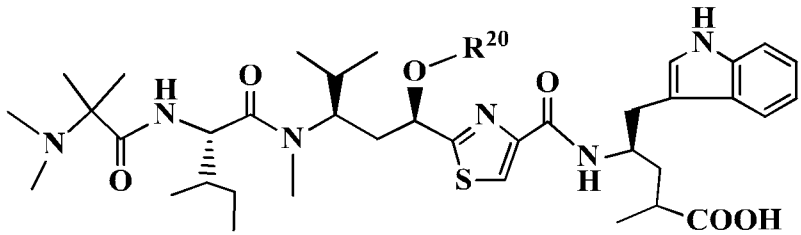

V-61,

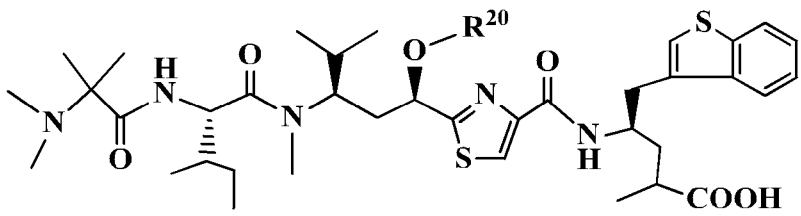

V-62,

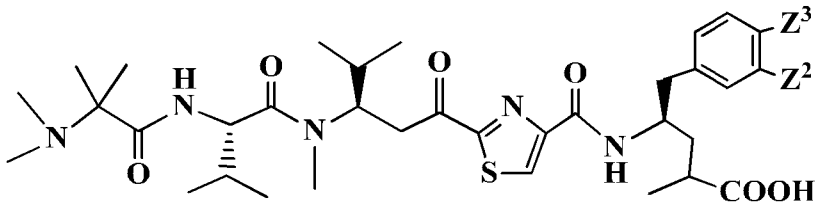

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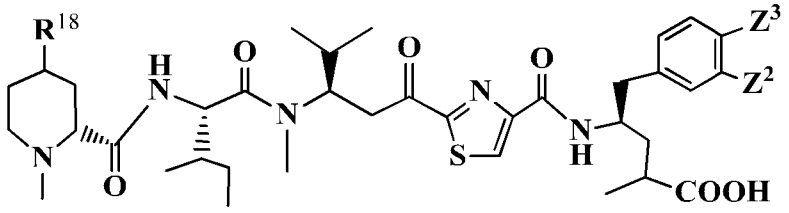

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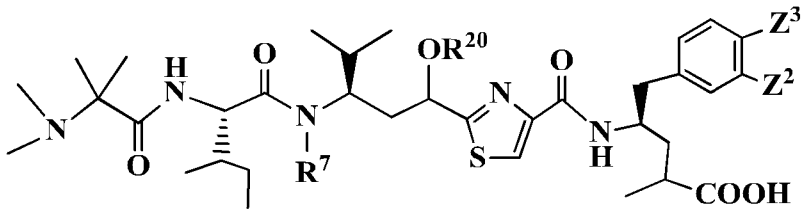

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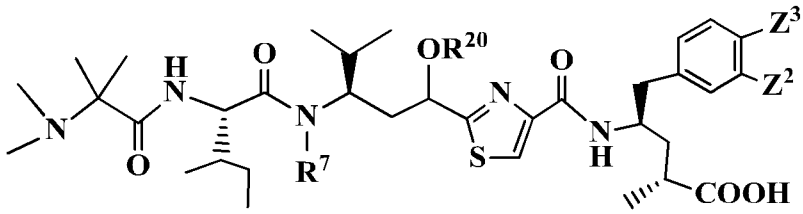

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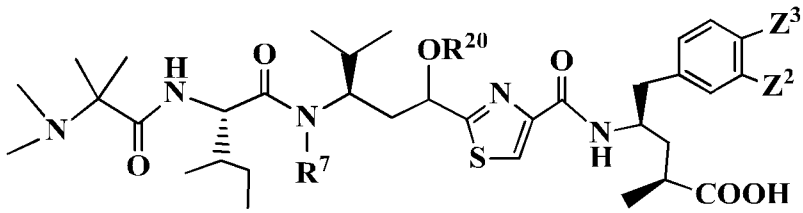

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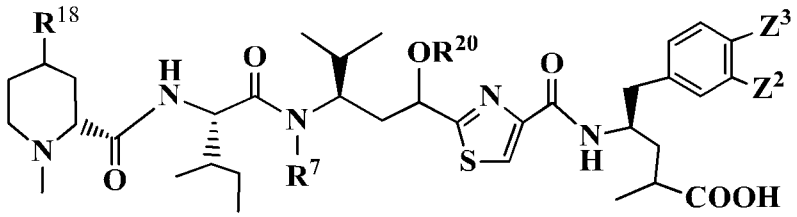

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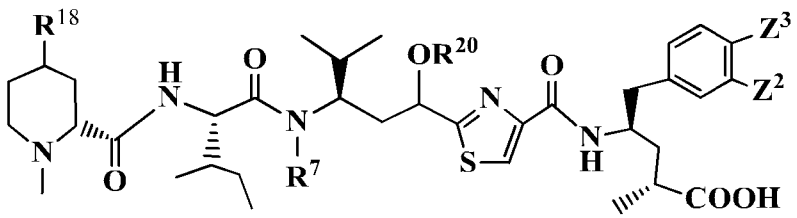

V-69,

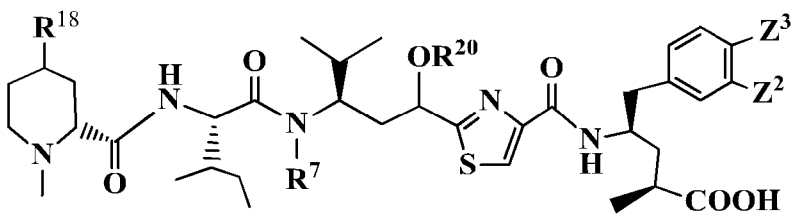

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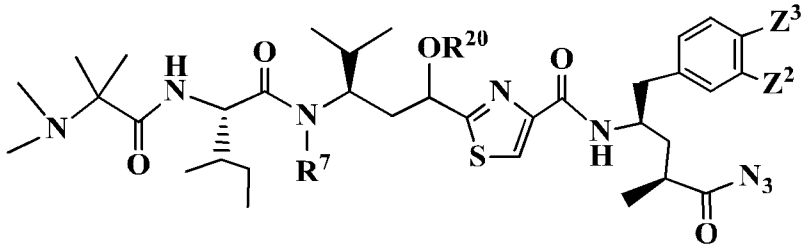

V-71,


V-72,

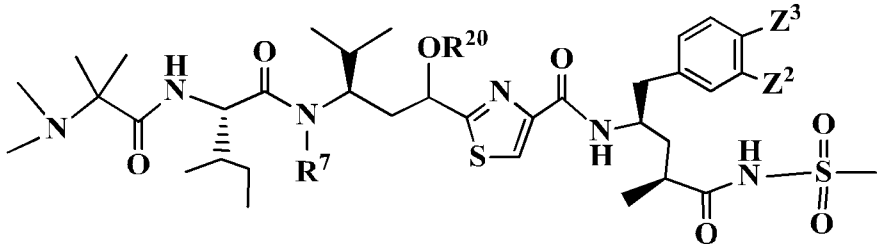

V-73,


V-74,

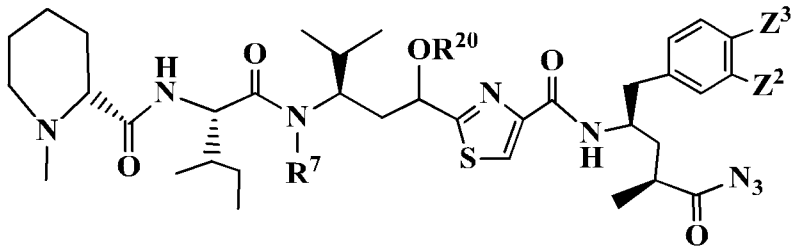

V-75,



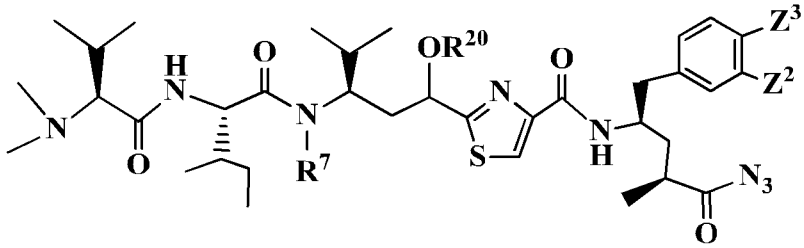
V-76,



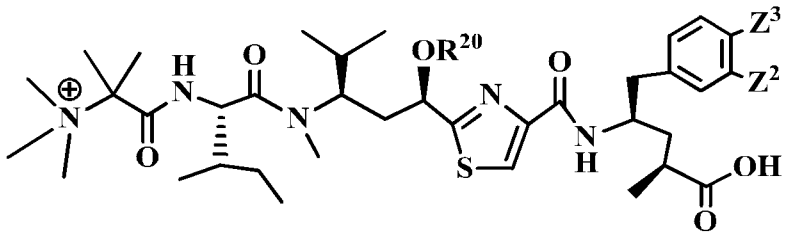
V-77,



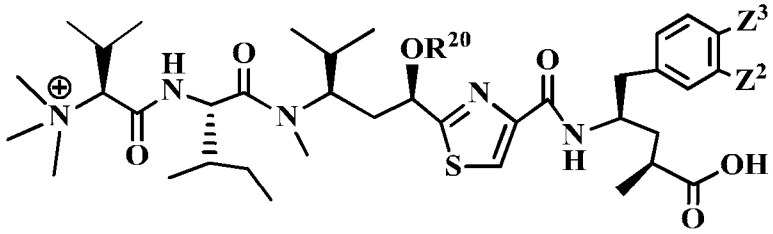
V-78,



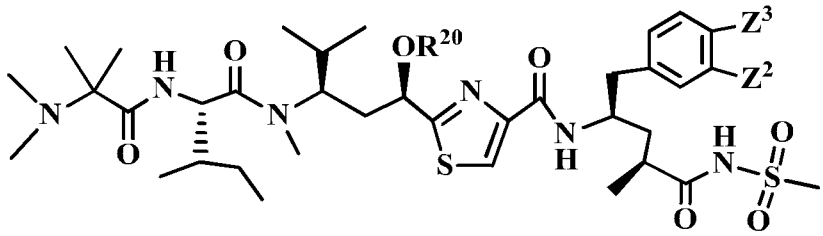
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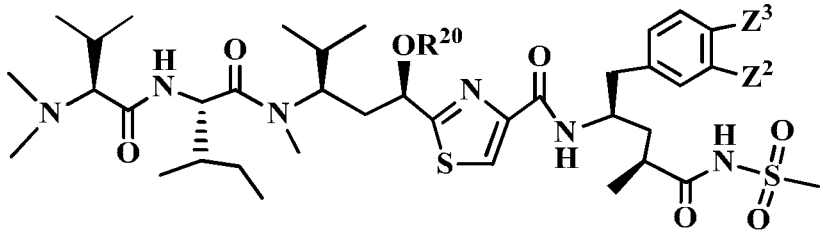
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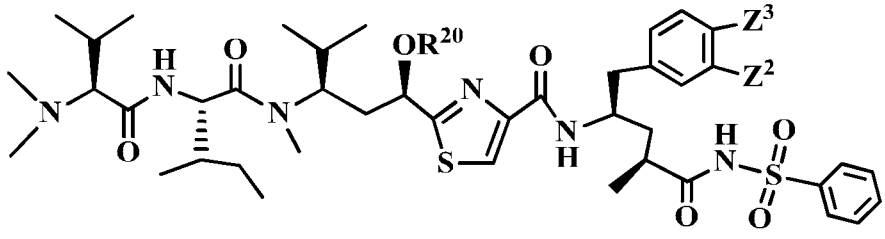


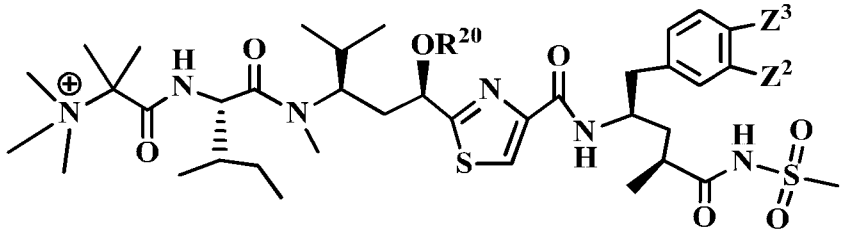
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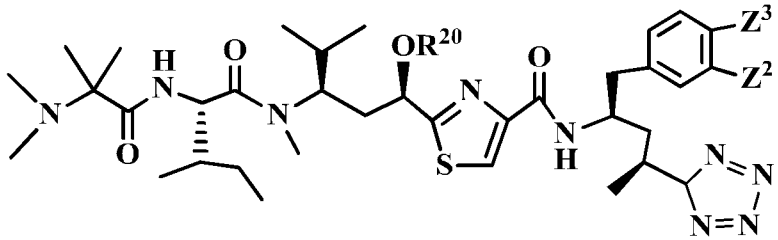


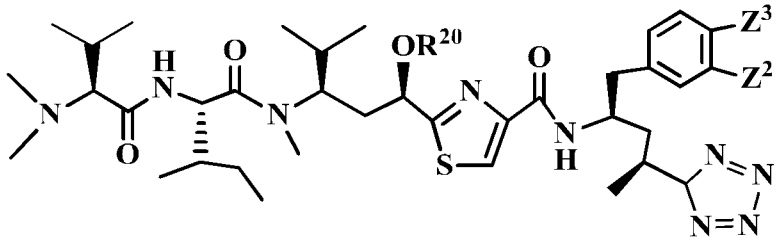
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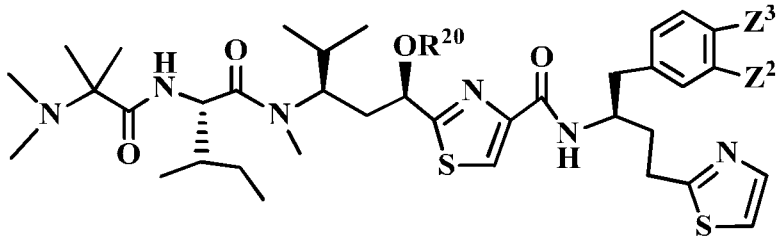

V-83,

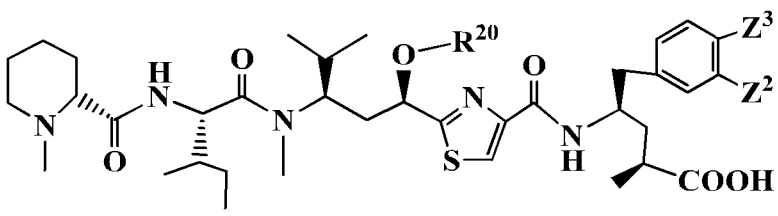

V-84,

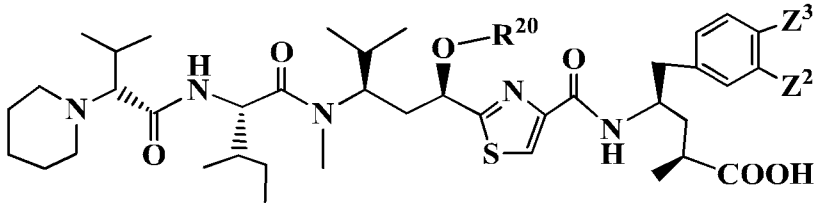

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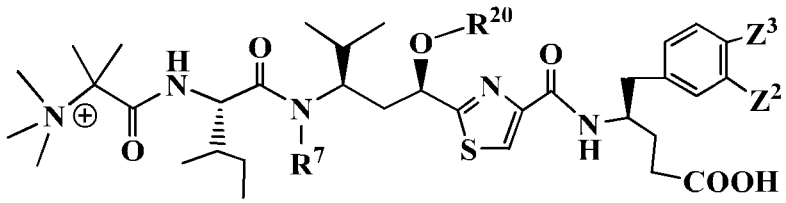

V-86,

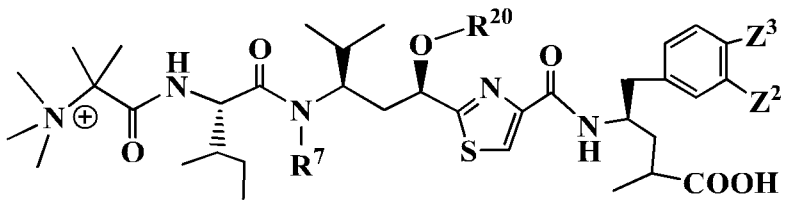

V-87,

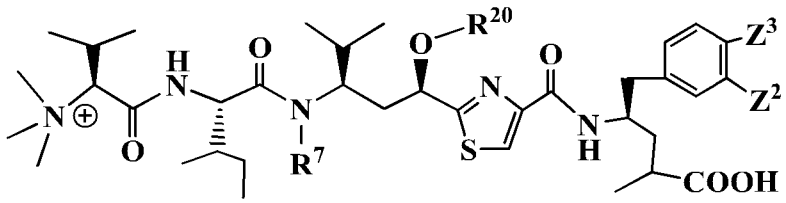

V-88,

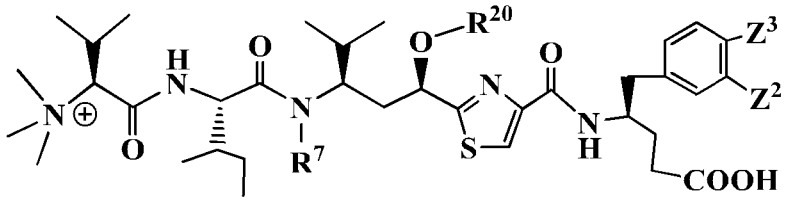

V-89,


V-90,


V-91,


V-92,

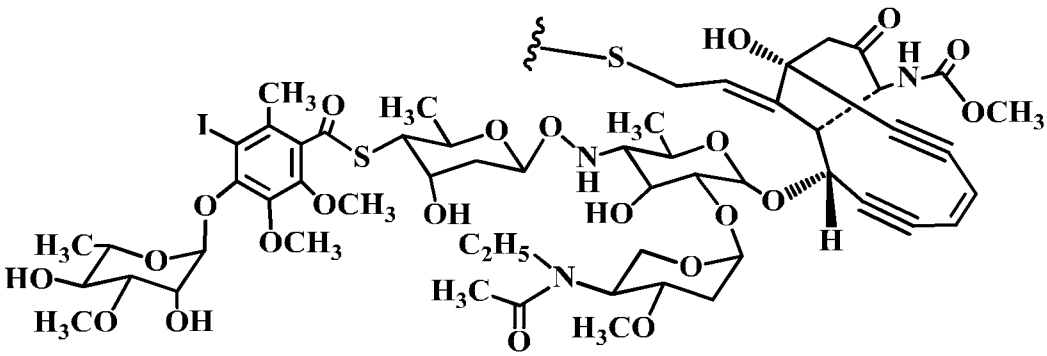

V-93,


V-94,

wherein R^{20} is H; C_1-C_8 of linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-$C(O)OR^{17}$), carbamate (-$C(O)NR^{17}R^{18}$); or 1-8 carbon atoms of carboxylate, esters, ether, or amide; or 1~8 amino acids; or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 0 to about 1000; or R^{20} is absent and the oxygene forms a ketone, or combination above thereof; $Z^3$and Z^3 are independently H, OH, NH_2, O, NH, COOH, COO, C(O), C(O), C(O)NH, $C(O)NH_2$, R^{18}, $OCH_2OP(O)(OR^{18})_2$, $OC(O)OP(O)(OR^{18})_2$, $OPO(OR^{18})_2$, $NHPO(OR^{18})_2$, $OP(O)(OR^{18})OP(O)(OR^{18})_2$, $OC(O)R^{18}$, $OC(O)NHR^{18}$, $OSO_2(OR^{18})$, O-(C_4-C_{12}-glycoside), of linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-$C(O)OR^{17}$), carbamate (-$C(O)NR^{17}R^{18}$); $R^{17}$and R^{18} are independently H, linear or branched alkyl or heteroalkyl; C_2-C_8 of linear or branched alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 linear or branched of

aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; carbonate (-C(O)OR^{17}), carbamate (-C(O)$NR^{17}R^{18}$); $R^{19}$is H, OH, NH_2, $OSO_2(OR^{18})$, $XCH_2OP(O)(OR^{18})_2$, $XPO(OR^{18})_2$, $XC(O)OP(O)(OR^{18})_2$, $XC(O)R^{18}$, $XC(O)NHR^{18}$, C_1~C_8 alkyl or carboylate; C_2~C_8 alkenyl, alkynyl, alkylcycloalkyl, heterocycloalkyl; C_3~C_8 aryl or alkylcarbonyl; or pharmaceutical salts; X isO, S, NH, NHNH, or CH_2; R^7 is H, R^{14}, -$R^{14}C(=O)X^1R^{15}$; or -$R^{14}X^1R^{15}$; X^1 is O, S, S-S, NH, CH_2 or NR^{14}, wherein R^{14} and R^{15} are independently C_1~C_8 alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heteroalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl; wherein the linkage sites, " ∿∿ " in formula IV-01- IV-94 are the same indication according to Claim 4;

(b) the calicheamicins and their related enediyne antibiotics have the following formula:

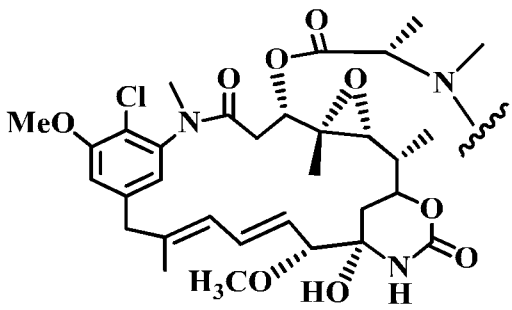


(Ia),

or a derivative with one or more isotopes, or a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof,

wherein ∿∿ is a site linked to L_1 or L_2;

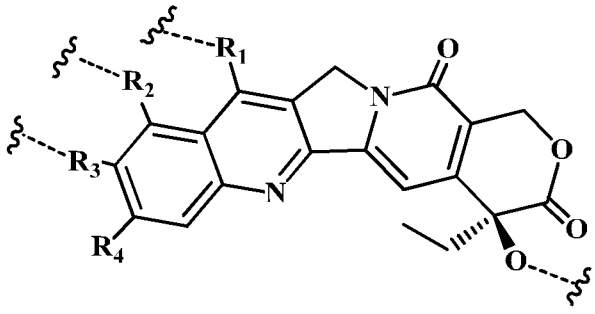
(c) the Maytansines or their derivatives maytansinoids have the following formula:



(Ib),

wherein ∿∿ is a site linked to L_1 or L_2;

(d) the camptothecin (CPTs) and its derivatives have the following formula:

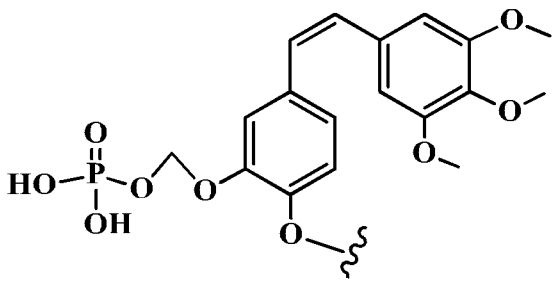


(Ic)

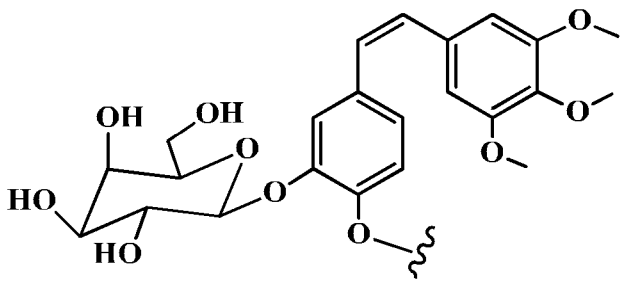
or an isotope of one or more chemical elements, or a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof;

wherein R_1, R_2 and R_4 are independently selected from H, F, Cl, Br, CN, NO_2, C_1~C_8 alkyl; O-C_1~C_8 alkyl; NH-C_1~C_8 alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heterocyclic, carbocyclic, cycloalkyl, heteroalkylcycloalkyl, alkylcarbonyl, heteroaryl; or 2-8 carbon atoms of esters, ether, amide, carbonate, urea, or carbamate; R_3 is H, OH, NH_2, C_1~C_8 alkyl; O-C_1~C_8 alkyl; NH-C_1~C_8 alkyl; C_2-C_8 of heteroalkyl, alkylcycloalkyl, heterocycloalkyl; or 2-8 carbon atoms of esters, ether, amide, carbonate, urea, or carbamate; or R_1R_2, R_2R_3 and R_3R_4 independently form a 5~7 membered carbocyclic, heterocyclic, heterocycloalkyl, aromatic or heteroaromatic ring system; ∿∿ is the site in the molecule that can be linked to L_1, L_1', L_2 or L_2'.

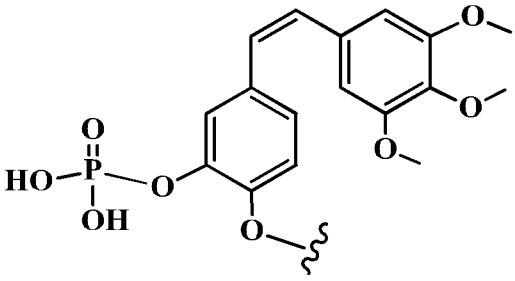
(e) the Combretastatins have the following formula:



CA-01,



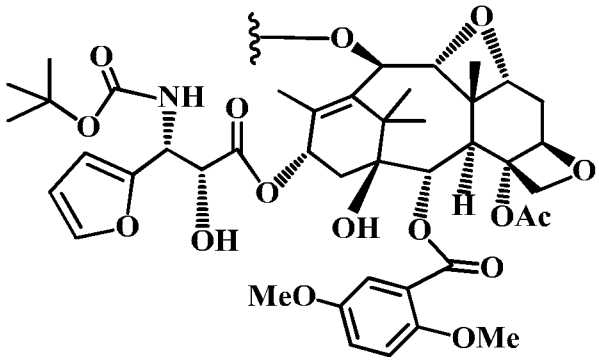
CA-02 (CA4-βGal),



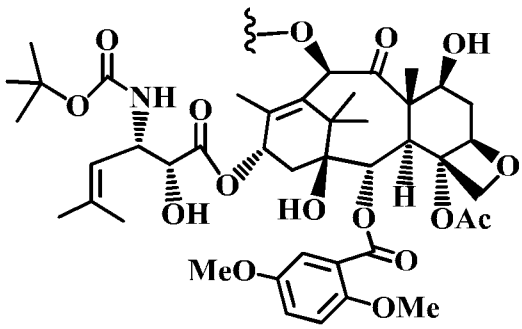
CA-03,

~~~ is a site in the molecule that can be linked to L_1, L_1', L_2 or L_2';

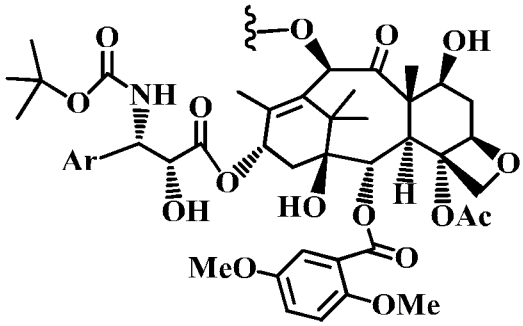
(f) the Taxanes have the following formula:



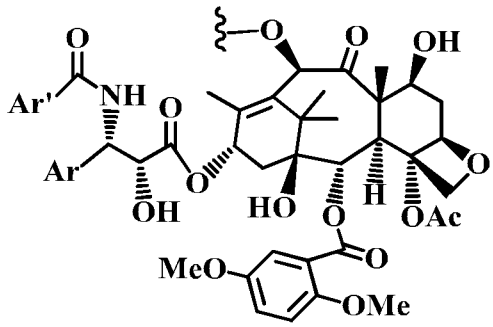
(Id-01),



(Id-02),



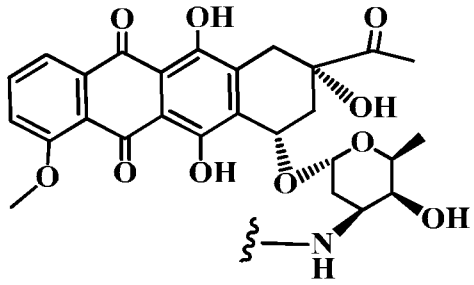
(Id-03),



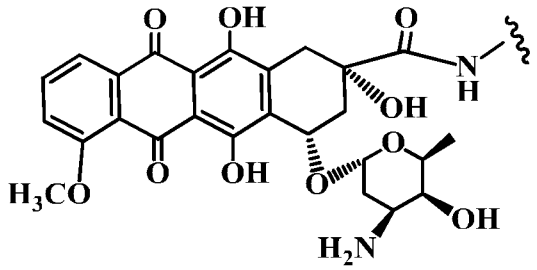
(Id-04),
~~~

Wherein ~~~ is a site in the molecule that can be linked to L_1, L_1', L_2 or L_2'; Ar and Ar' are independently aryl or heteroaryl;

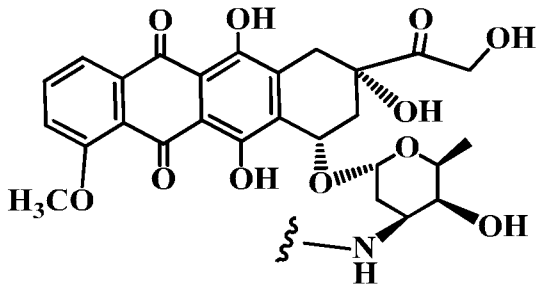
(g) the anthracyclines have the following formula:



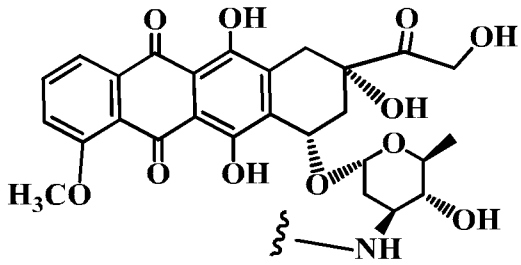
(Ie-01), Daunorubicin analog,



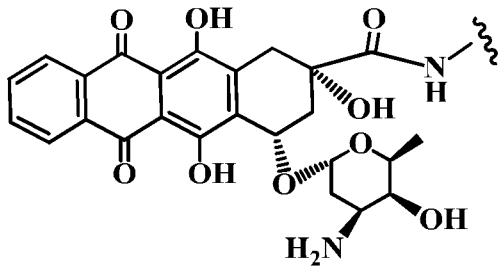
(Ie-02), Daunorubicin analog,



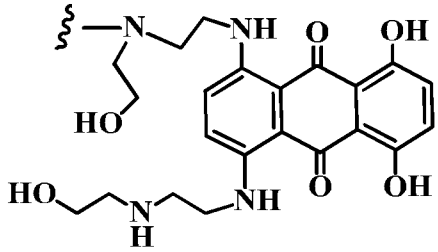
(Ie-03), Doxorubicin analog,



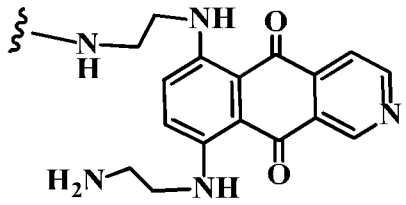
(Ie-04), Epirubicin analog,



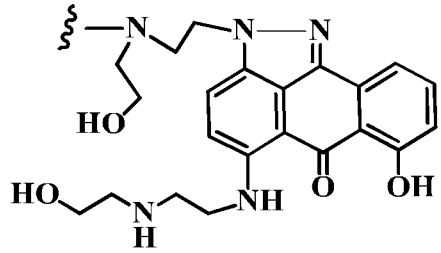
(Ie-05), Idarubicin analog,



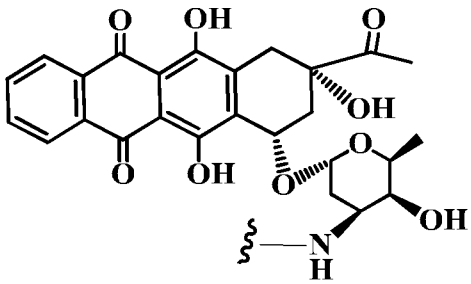
(Ie-06), Mitoxantrone analog,



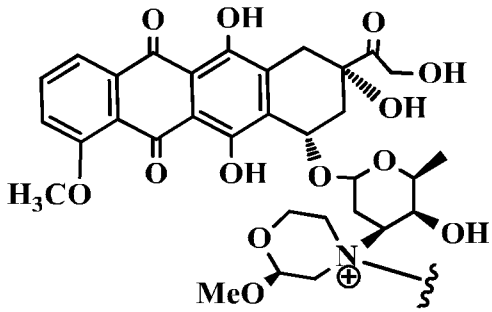
(Ie-07), Pixantrone analog,



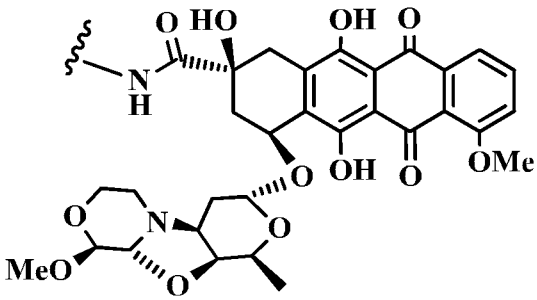
(Ie-08), Losoxantrone analog,



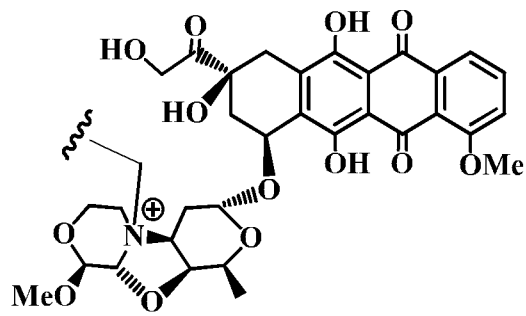
(Ie-09),



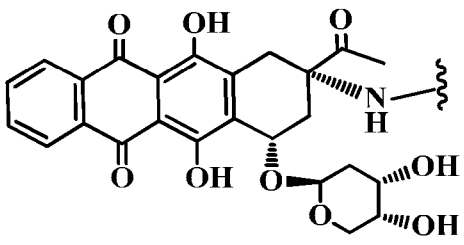
(Ie-10),



(Ie-11),



(Ie-12),

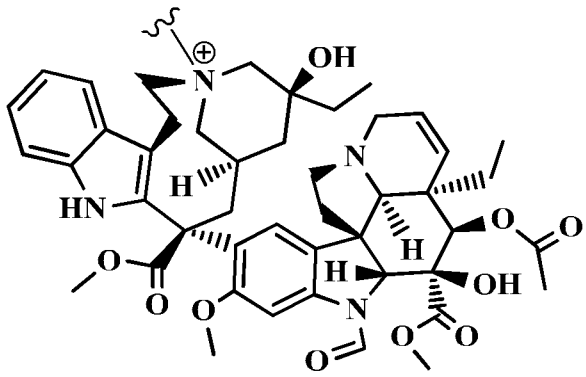


(Ie-13), Amrubicin analog,

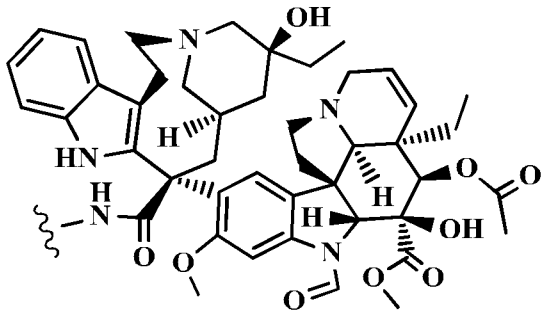
Wherein ~~~ is a site in the molecule that can be linked to L_1, L_1', L_2 and/or L_2';

(h) the Vinca alkaloids are selected from vinblastine, vincristine, vindesine, leurosine, vinorelbine, catharanthine, vindoline, vincaminol, vineridine, minovincine, methoxyminovincine,

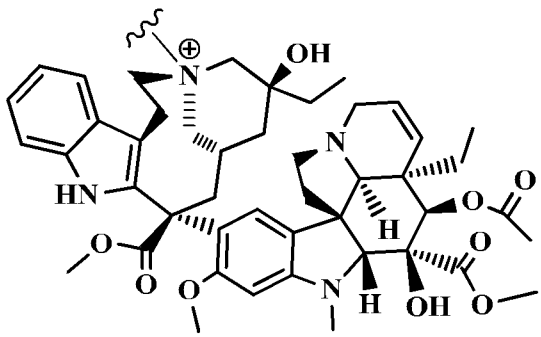
minovincinine, vincadifformine, desoxyvincaminol, vincamajine, vincamine, vinpocetine, and vinburnine and have the following formula:



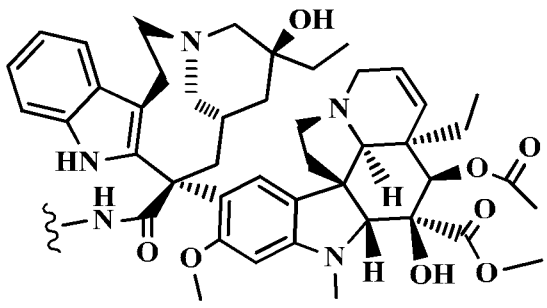
(If-01), vincristine (leurocristine),



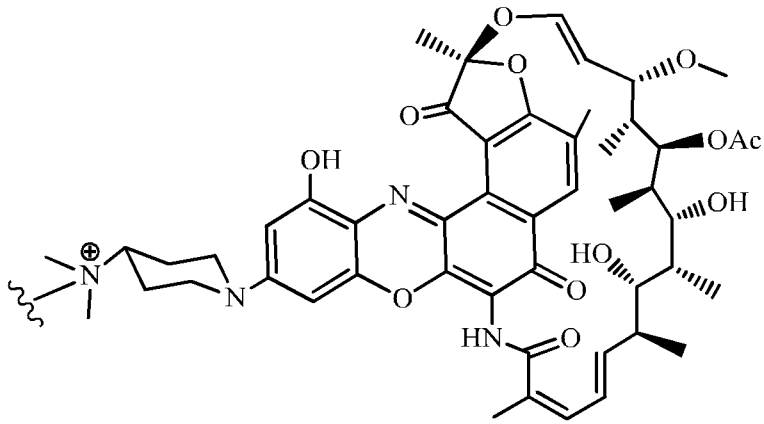
(If-02), vincristine (leurocristine),



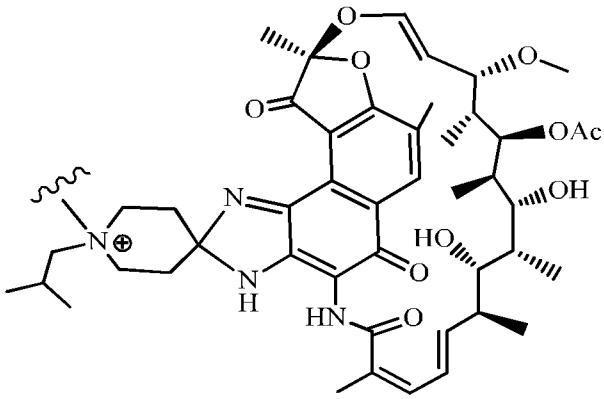
(If-03), vinblastine,



(If-04), vinblastine;



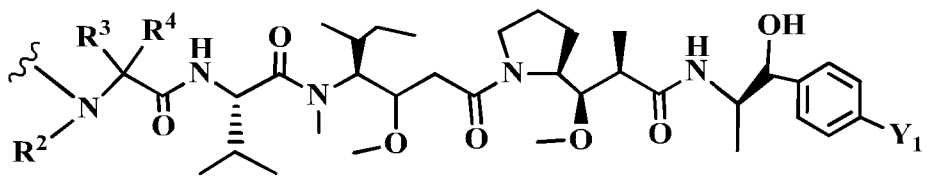
(If-05), Rifabutin analog,



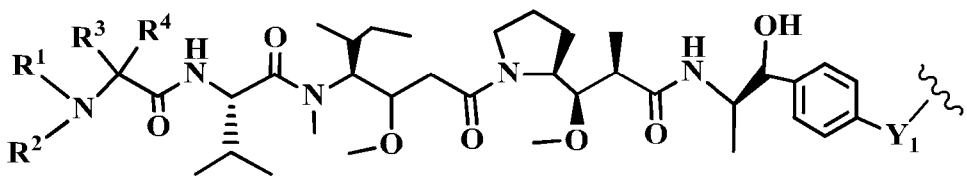
(If-06), rifabutin analog,

wherein ~~~ is a site linked to L_1, L_1', L_2 and/or L_2';

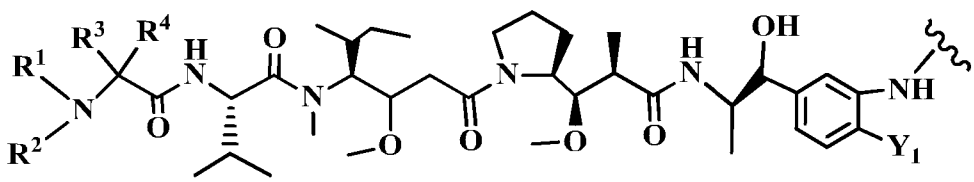
(i) the Dolastatins and their peptidic analogs and derivatives are selected from dolastatin 10, auristatin E (AE), auristatin EB (AEB), auristatin EFP (AEFP), MMAD (Monomethyl Auristatin D or monomethyl dolastatin 10), MMAF (Monomethyl Auristatin F or N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine), MMAE (Monomethyl Auristatin E or N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine), 5-benzoylvaleric acid-AE ester (AEVB), Auristatin F phenylene diamine (AFP) and have the following formula:



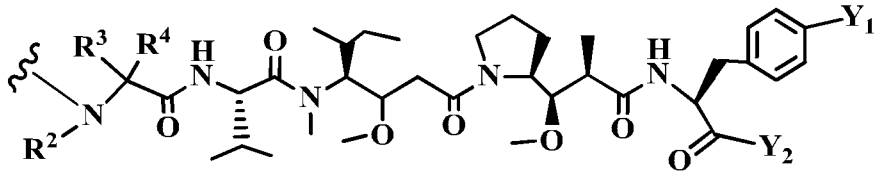
(Ih-01),



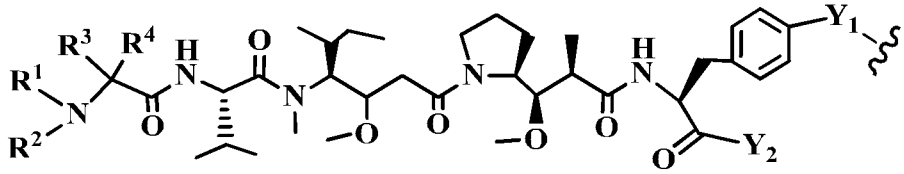
(Ih-02),



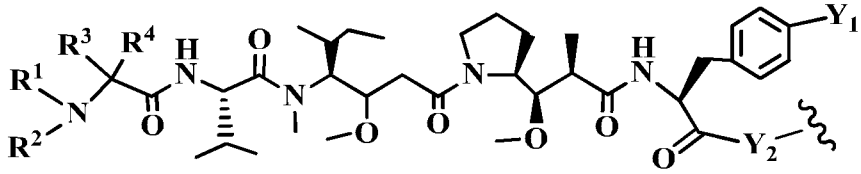
(Ih-03),



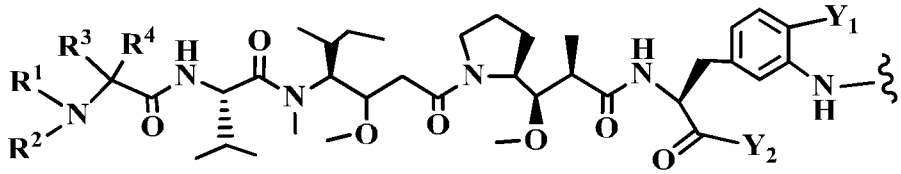
(Ih-04),



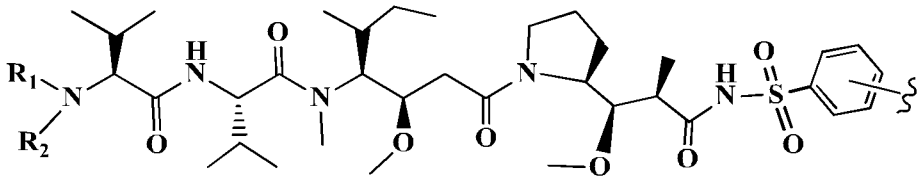
(Ih-05),



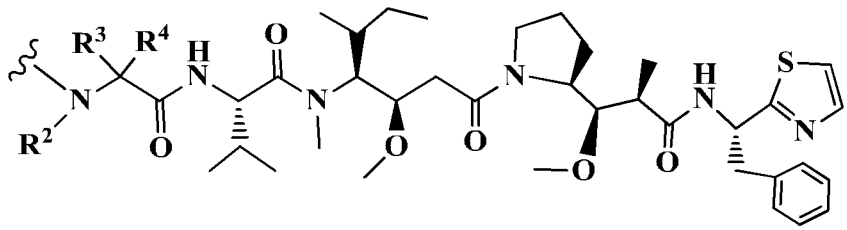
(Ih-06),



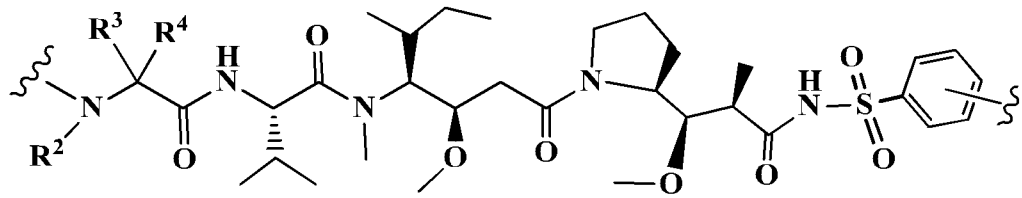
(Ih-07),



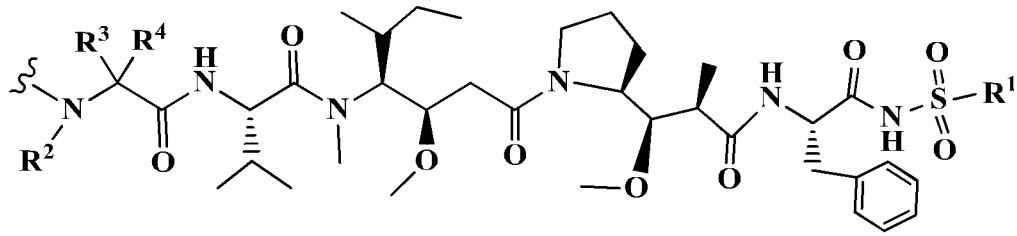
(Ih-08)



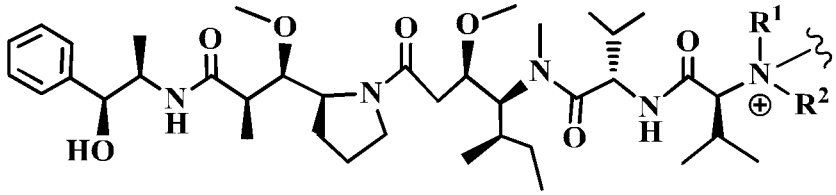
(Ih-09),



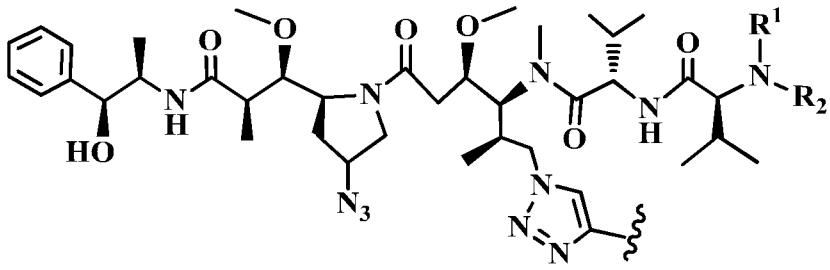
(Ih-10),



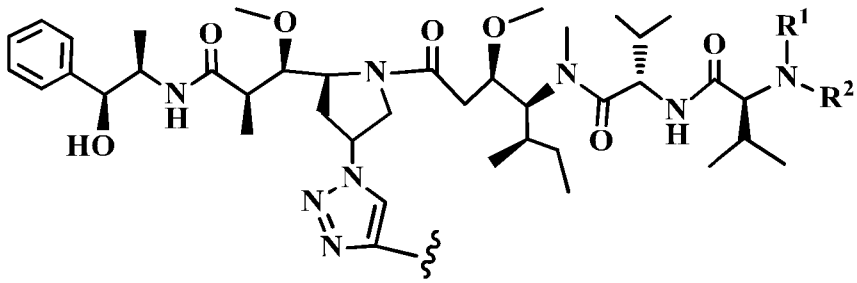
(Ih-11),



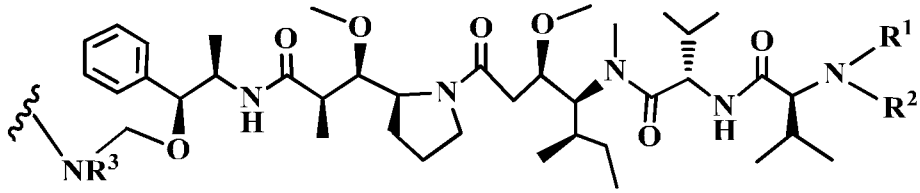
(Ih-12),



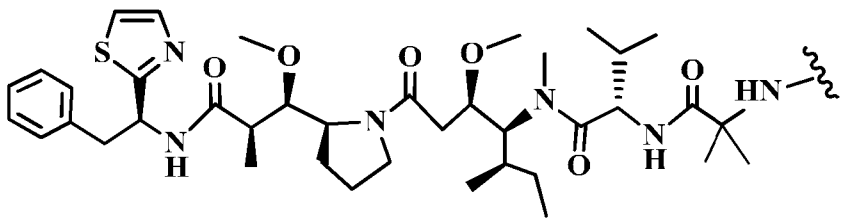
(Ih-13),



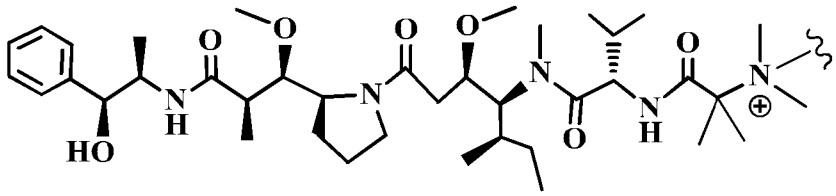
(Ih-14),



(Ih-15),



(Ih-16),



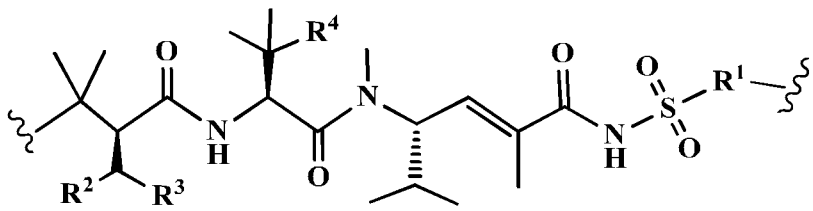
(Ih-17),

or a derivative with one or more isotopes; or a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof;

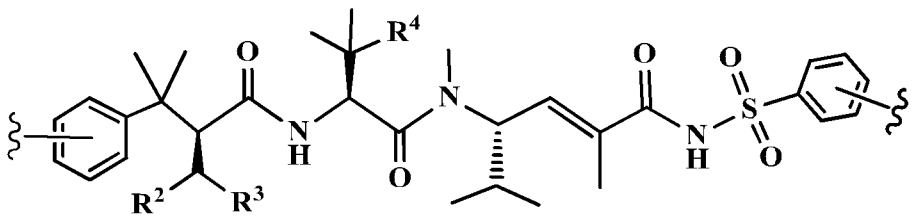
wherein R^1, R^2, R^3, R^4 and R^5 are independently H; C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester, ether, amide, amines, heterocycloalkyl, or acyloxylamines; or peptides containing 1-8 aminoacids, or polyethyleneoxy unit having formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 1000. The two Rs: R^1R^2, R^2R^3, R^1R^3 or R^3R^4 together can form 3~8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group; Y_1 and Y_2 are independently O, NH, NHNH, NR_5, S, C(O)O,

C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_2), C(O)NHNHC(O) and C(O)NR_1 when linked to the connecting site " ∿∿ " (that links to L_1, L_1', L_2 and/or L_2' independently); or OH, NH_2, $NHNH_2$, NHR_5, SH, C(O)OH, $C(O)NH_2$, $OC(O)NH_2$, OC(O)OH, $NHC(O)NH_2$, NHC(O)SH, OC(O)NH(R_1), N(R_1)C(O)NH(R_2), C(O)NHNHC(O)OH and C(O)NHR_1 when not linked to the connecting site " ∿∿ "; R_{12} is OH, NH_2, NHR_1, $NHNH_2$, NHNHCOOH, O-R_1-COOH, NH-R_1-COOH, NH-$(Aa)_n$COOH, $O(CH_2CH_2O)_pCH_2CH_2OH$, $O(CH_2CH_2O)_pCH_2CH_2NH_2$, $NH(CH_2CH_2O)_pCH_2CH_2NH_2$, NR_1R_1', NHOH, $NHOR_1$, $O(CH_2CH_2O)_pCH_2CH_2COOH$, $NH(CH_2CH_2O)_pCH_2CH_2COOH$, NH-Ar-COOH, NH-Ar-$NH_2$, $O(CH_2CH_2O)_pCH_2CH_2NH$-SO_3H, $NH(CH_2CH_2O)_pCH_2CH_2NHSO_3H$, R_1-$NHSO_3H$, NH-R_1-$NHSO_3H$, $O(CH_2CH_2O)_pCH_2$.$CH_2NHPO_3H_2$, $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, OR_1, R_1-$NHPO_3H_2$, R_1-OPO_3H_2, $O(CH_2CH_2O)_pCH_2CH_2OPO_3H_2$, OR_1-$NHPO_3H_2$, NH-R_1-$NHPO_3H_2$, $NH(CH_2CH_2NH)_pCH_2$.CH_2NH_2, $NH(CH_2CH_2S)_pCH_2CH_2NH_2$, $NH(CH_2CH_2NH)_pCH_2CH_2OH$, $NH(CH_2CH_2S)_pCH_2$.CH_2OH, NH-R_1-NH_2, or $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, wherein Aa is 1-8 the same or different aminoacids; p is 1 -1000; R_1, R_2, R_3, R_4, R_5, R_5', Z_1, Z_2, and n are defined the same above.;

(j) the Hemiasterlin and its analogues have the following formula:



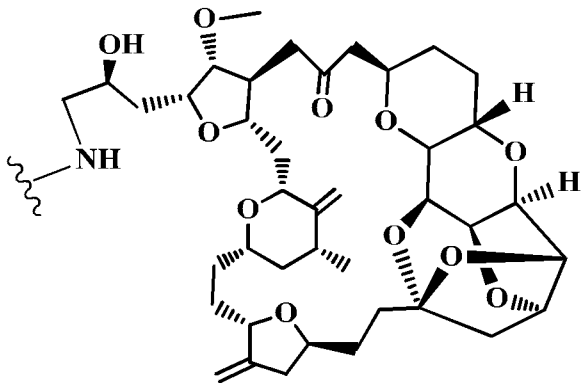
(Hs-01)



(Hs-02)

wherein R^1, R^2, R^3, R^4 and R^5 are independently H; C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester, ether, amide, amine, heterocycloalkyl, or acyloxylamine; or peptide containing 1-8 aminoacids, or polyethyleneoxy unit having formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 5000; the R^2 and R^3 can form 3-8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group;

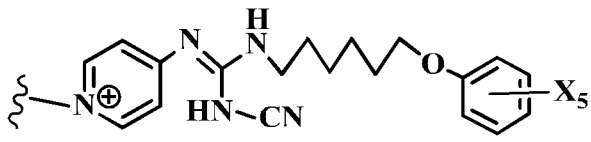
(k) the Eribulin has the following formula:



Eb01,

wherein ~~~ is a linkage site that links to L_1, L_1', L_2 and/or L_2' independently;

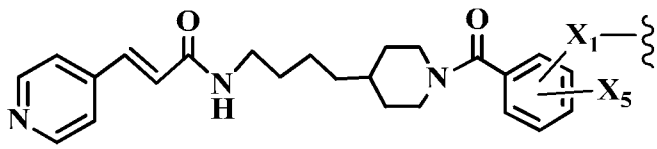
(l) the Inhibitor of nicotinamide phosphoribosyltransferases (NAMPT) have the following formula, NP01, NP02, NP03, NP04, NP05, NP06, NP07, NP08, and NP09:



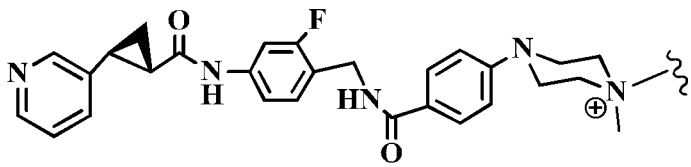
NP01,



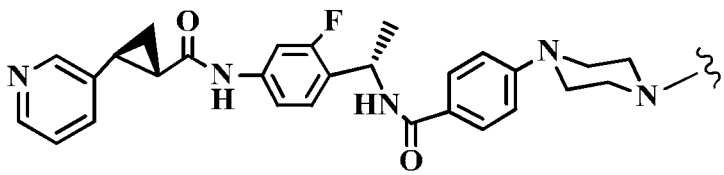
NP02,



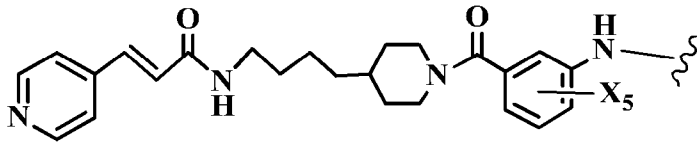
NP03,



NP04,



NP05,



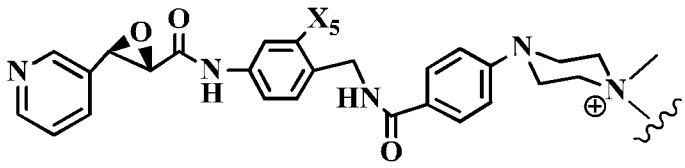
NP06,



NP07,



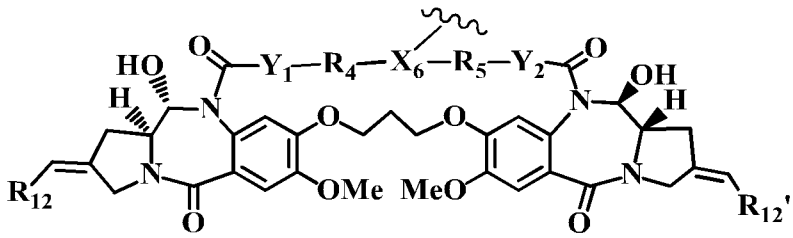
NP08,



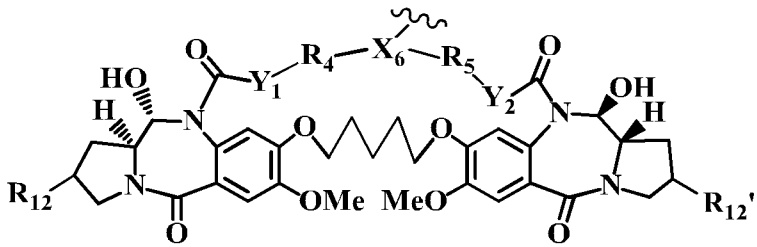
NP09,

or a derivative with one or more isotopes, or a pharmaceutically acceptable sals, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof; wherein " ∿∿ " is the same above; X_5 is F, Cl, Br, I, OH, OR_1, R_1, OPO_3H_2, OSO_3H, NHR_1, $OCOR_1$, or $NHCOR_1$;

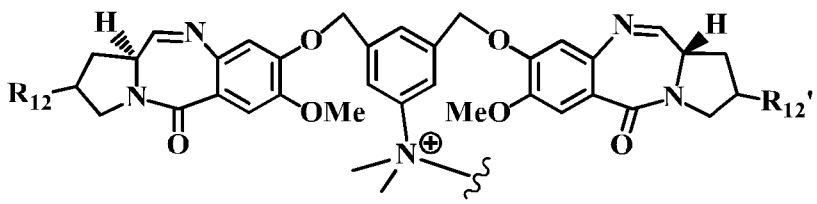
(m) the benzodiazepine dimer and its analogs have the following formulae, PB01, PB02, PB03, PB04, PB05, PB06, PB07, PB08, PB09, PB10, PB11, PB12, PB13, PB14, PB15, PB16, PB17, PB18, PB19, PB20, PB21, PB22, PB23, PB24, PB25, PB26, PB27 and PB28:



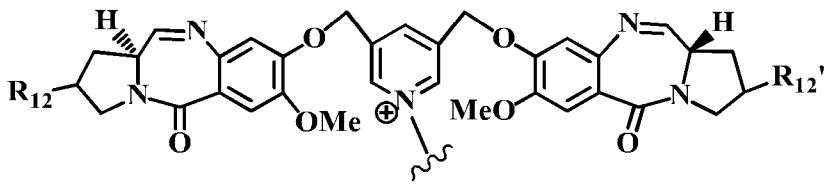
PB01,



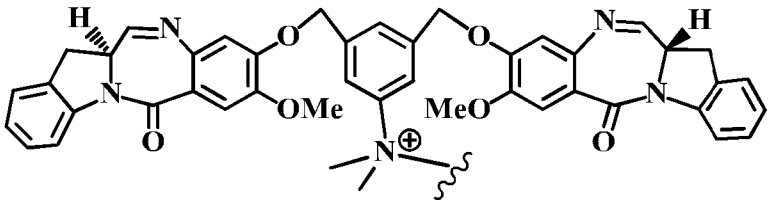
PB02,



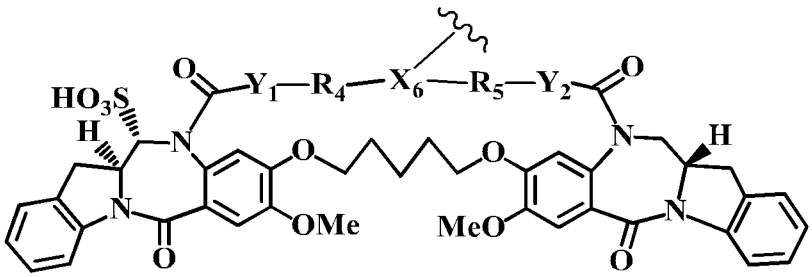
PB03,



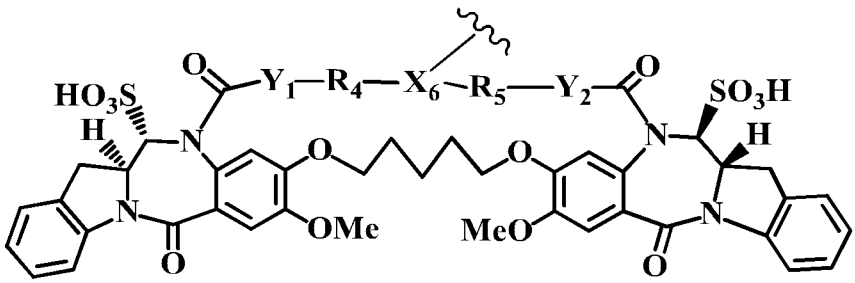
PB04,



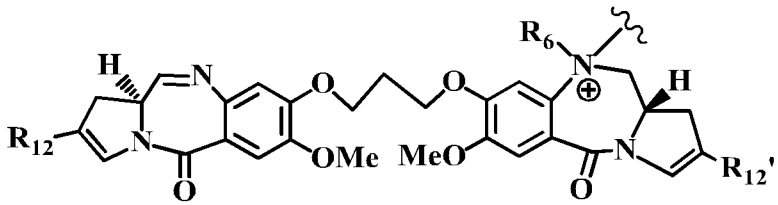
PB05,



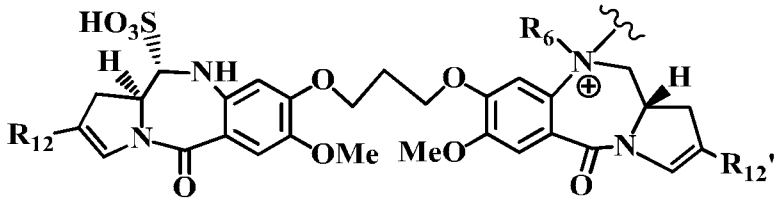
PB06,



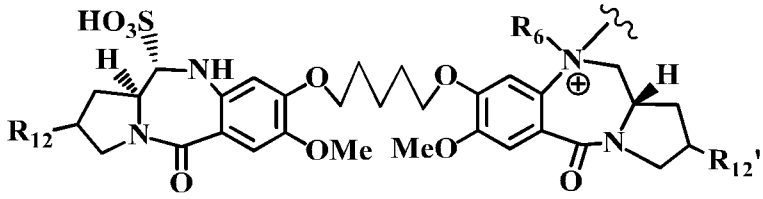
PB07,



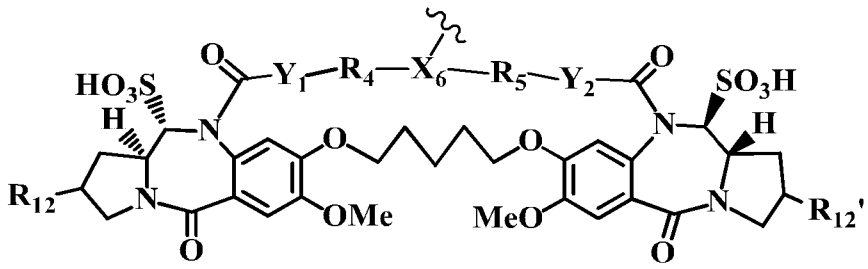
PB08,



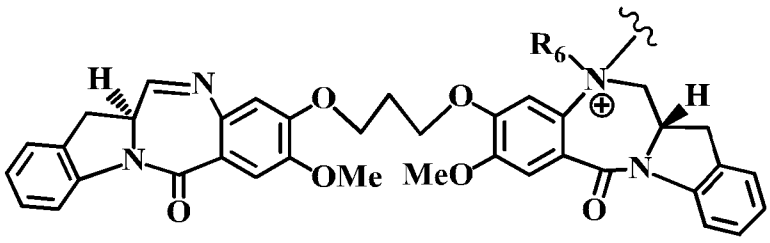
PB09,



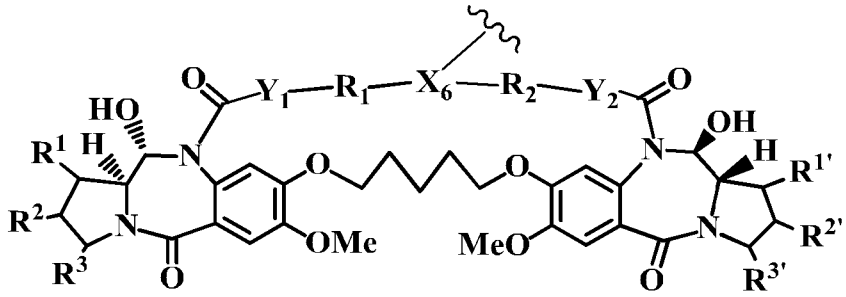
PB10,



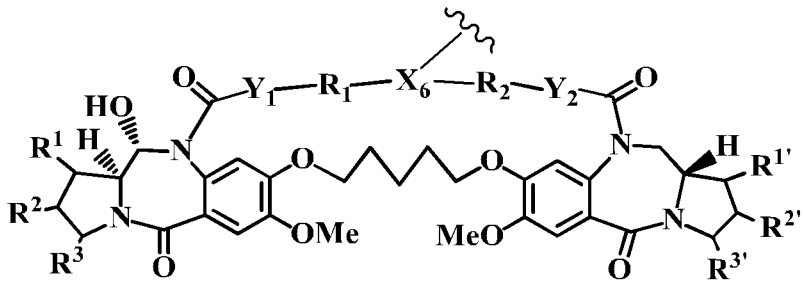
PB11,



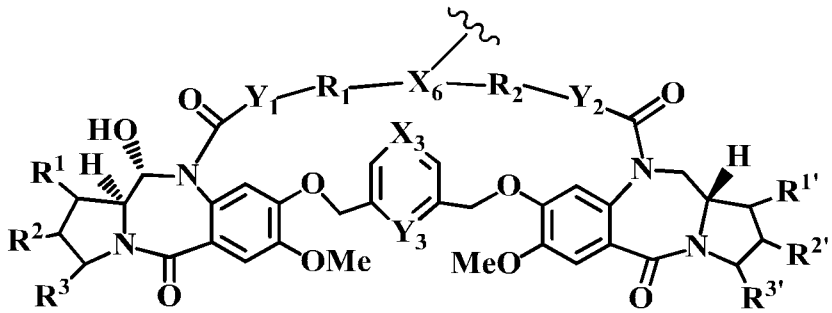
PB12,



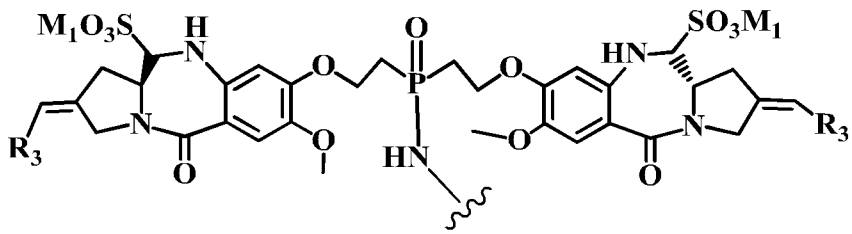
PB13,



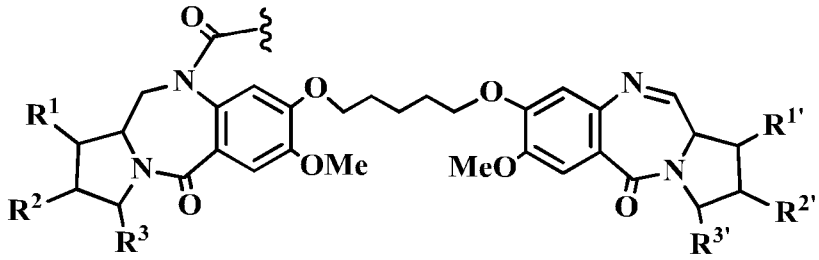
PB14,



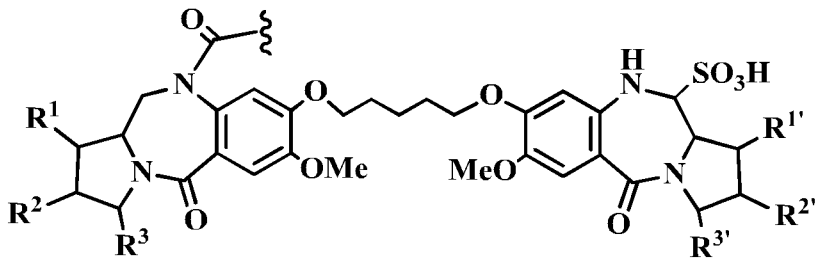
PB15,



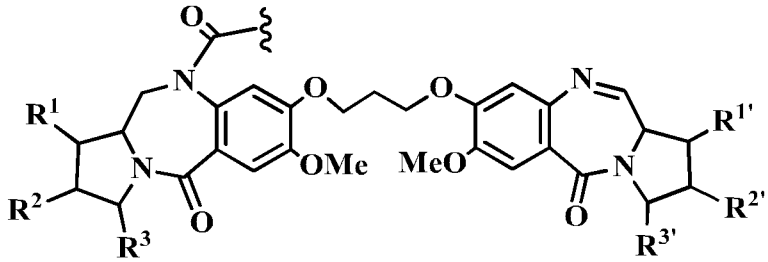
PB16,



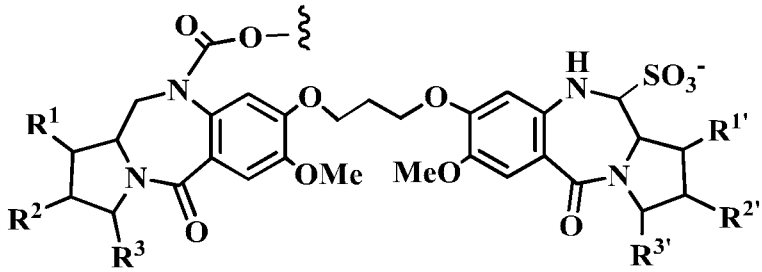
PB17,



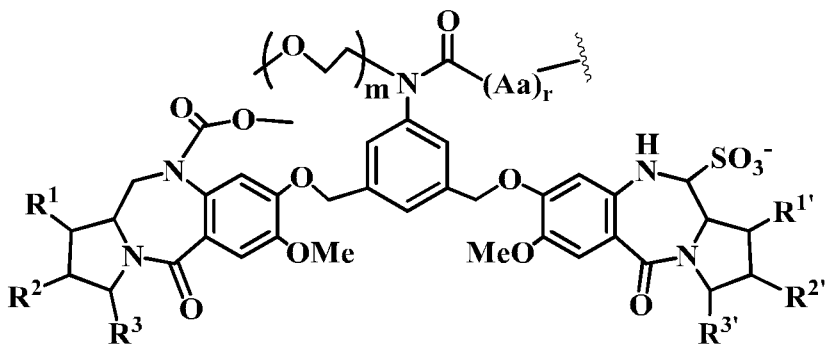
PB18,



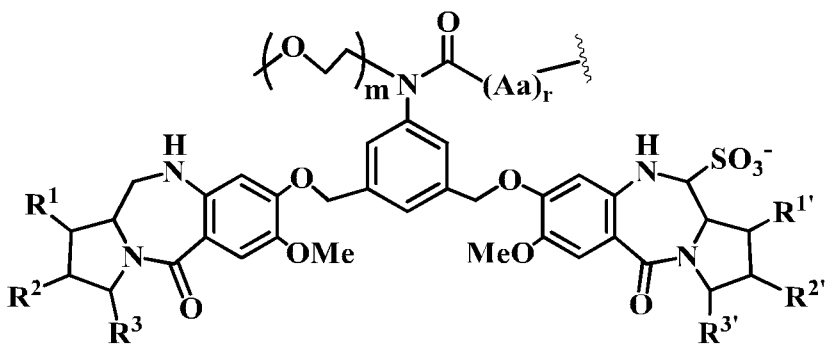
PB19,



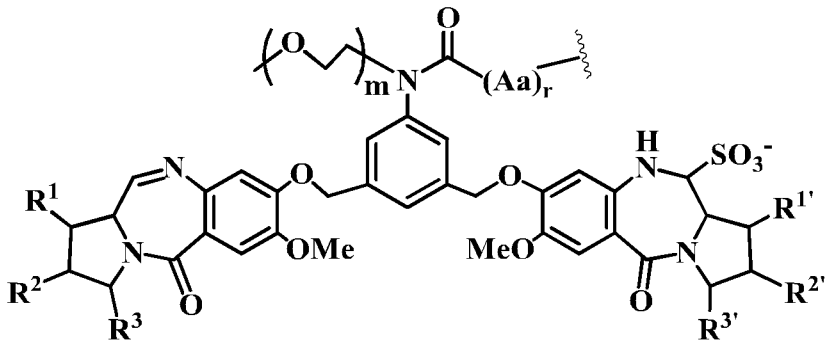
PB20,

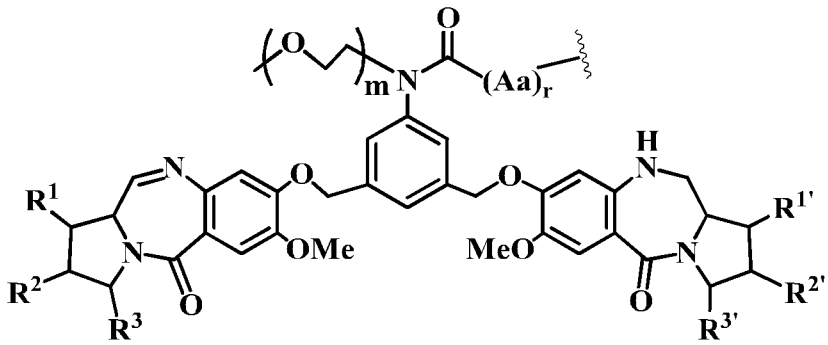


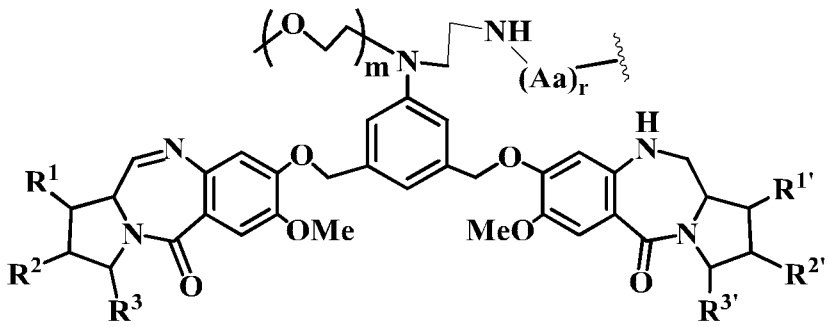
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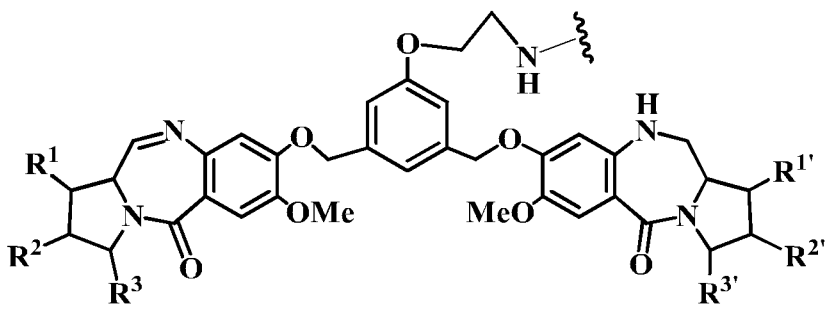


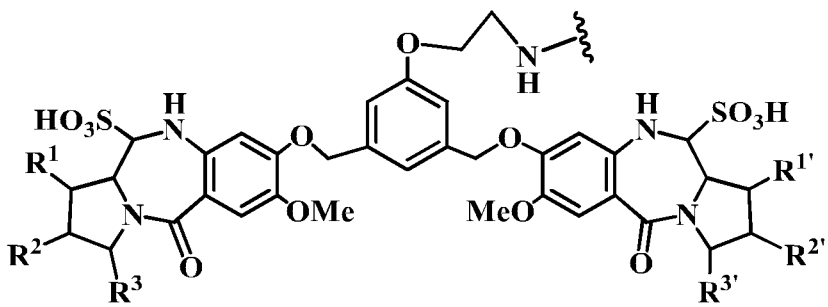
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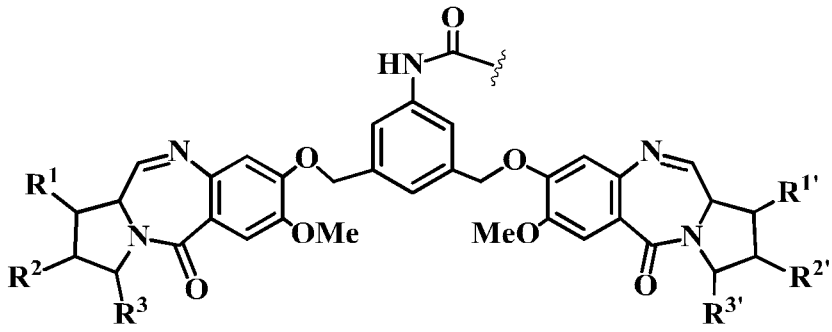

PB23,


PB24,


PB25,


PB26,

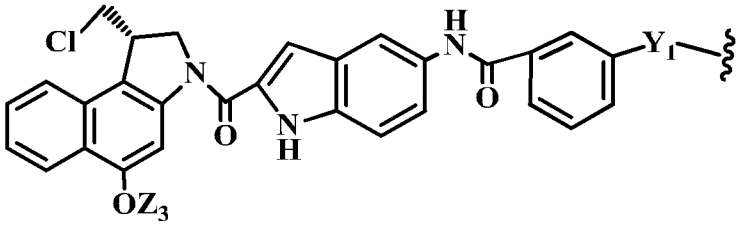

PB27,



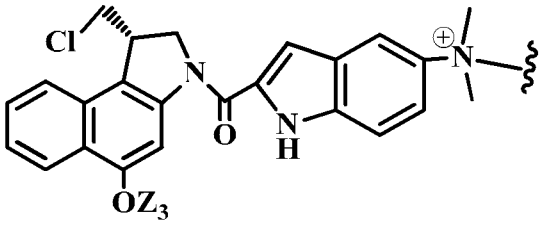
PB28,

or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein X_1, X_2, Y_1, Y_2, Z_1, Z_2, and n are defined the same above; Preferabably X_1, X_2, Y_1 and Y_2 are independently O, N, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH, C(O)NHNHC(O) and C(O)NR_1; R^1, R^2, R^3, $R^{1'}$, $R^{2'}$, and $R^{3'}$ are independently H; F; Cl; =O; =S; OH; SH; C_1-C_8 linear or branched alkyl, aryl, alkenyl, heteroaryl, heteroalkyl, alkylcycloalkyl, ester (COOR_5 or –OC(O)R_5), ether (OR_5), amide (CONR_5), carbamate (OCONR_5), amines (NHR_5, NR_5R_5'), heterocycloalkyl, or acyloxylamines (-C(O)NHOH, -ONHC(O)R_5); or peptides containing 1-20 natural or unnatural aminoacids, or polyethyleneoxy unit of formula $(OCH_2CH_2)_p$ or $(OCH_2CH(CH_3))_p$, wherein p is an integer from 1 to about 5000. The two Rs: R^1R^2, R^2R^3, R^1R^3, $R^{1'}R^{2'}$, $R^{2'}R^{3'}$, or $R^{1'}R^{3'}$ can independently form 3~8 member cyclic ring of alkyl, aryl, heteroaryl, heteroalkyl, or alkylcycloalkyl group; X_3 and Y_3 are independently N, NH, CH_2 or CR_5, or one of X_3 and Y_3 can be absent; wherein R_1, and R_2 are C_1-C_8 linear or branched alkyl, heteroalkyl; C_3-C_8 aryl, heteroaryl, alkylcycloalkyl, acyloxyl, alkylaryl, alkylaryloxyl, alkylarylamino, alkylarylthiol; or 1-6 the same or different sequence of aminao acid/peptides (Ar)r, r =1 -6; wherein R_4, R_5, R_5', R_6, R_{12} and R_{12}' are independently H, OH, NH_2, NH(CH_3), $NHNH_2$, COOH, SH, OZ_3, SZ_3, F, Cl, or C_1-C_8 linear or branched alkyl, aryl, heteroaryl, heteroalkyl, alkylcycloalkyl, acyloxylamines; Z_3 is H, OP(O)(OM_1)(OM_2), $OCH_2OP(O)(OM_1)(OM_2)$, OSO_3M_1, or O-glycoside (glucoside, galactoside, mannoside, glucuronoside/glucuronide, alloside, fructoside, etc.), NH-glycoside, S-glycoside or CH_2-glycoside; M_1 and M_2 are independently H, Na, K, Ca, Mg, NH_4, $NR_1R_2R_3$; X_6 is CH, N, P(O)NH, P(O)NR_1, CHC(O)NH, C_3-C_8 aryl, heteroaryl, alkylcycloalkyl, acyloxyl, alkylaryl, alkylaryloxyl, alkylarylamino, or an Aa (amino acid, is preferably selected from Lys, Phe, Asp, Glu, Ser, Thr, His, Cys, Tyr, Trp, Gln, Asn, Arg); "⋯⋯" is either single bond or double bond; "∿∿" is defined the same above;

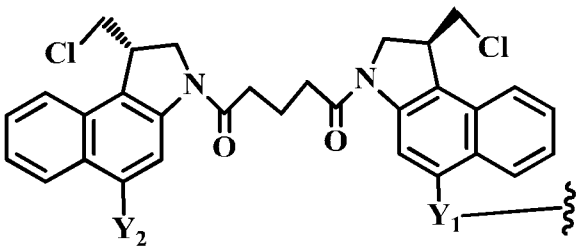
(n) the CC-1065 analog and doucarmycin analogs have the following formula, CC01, CC02, CC03, CC04, CC05, CC06 and CC07:



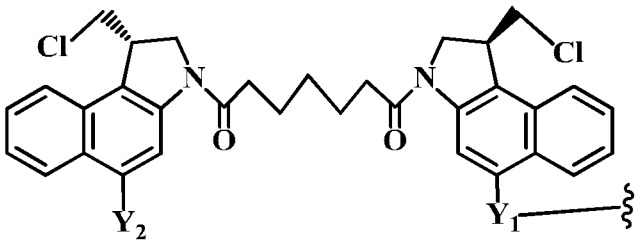
CC01,



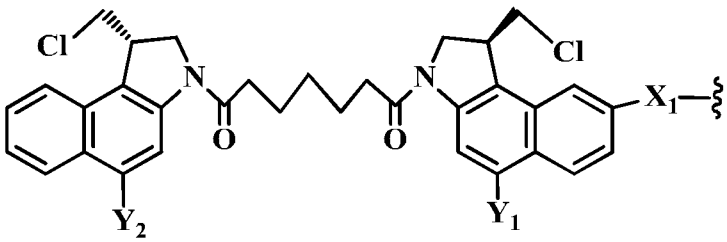
CC02,



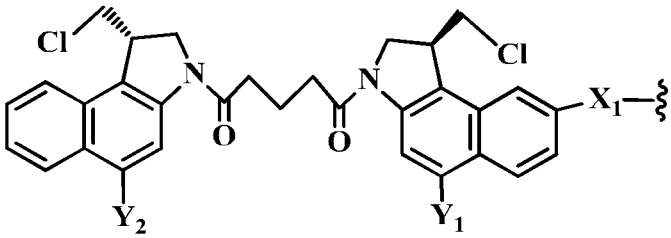
CC03,



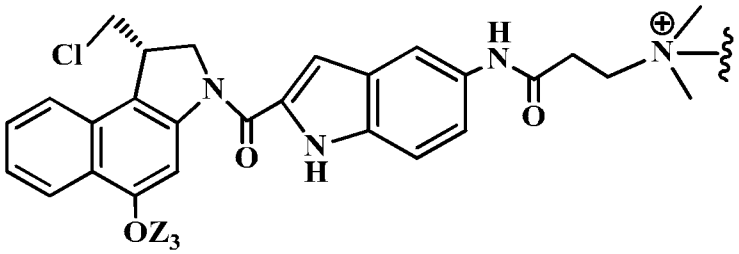
CC04,



CC05,



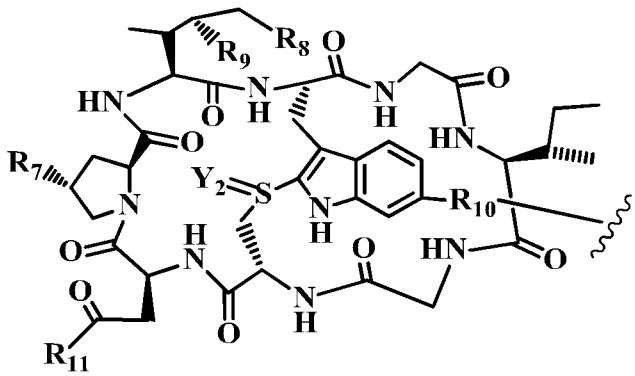
CC06,



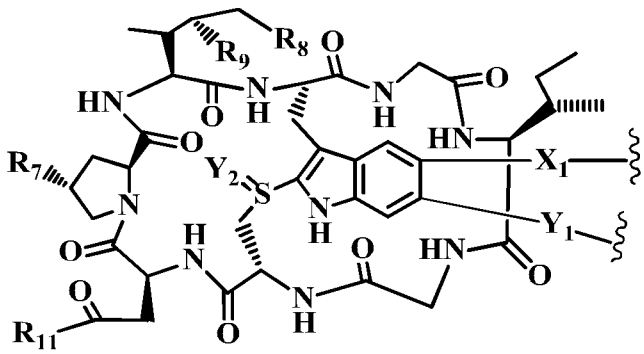
CC07,

wherein X_1, X_2, Y_1 and Y_2 are independently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_2), C(O)NHNHC(O) or C(O)NR_1 when linked to a connecting site " ∿∿ "; or OH, NH_2, $NHNH_2$, NHR_1, SH, C(O)OH, $C(O)NH_2$, $OC(O)NH_2$, OC(O)OH, $NHC(O)NH_2$, NHC(O)SH, OC(O)NH(R_1), N(R_1)C(O)NH(R_2), C(O)NHNHC(O)OH or C(O)NR_1 when not linked to the connecting site " ∿∿ "; Z_3 is H, $PO(OM_1)(OM_2)$, SO_3M_1, $CH_2PO(OM_1)(OM_2)$, $CH_3N(CH_2CH_2)_2NC(O)$-, $O(CH_2CH_2)_2NC(O)$-, R_1, or glycoside; wherein R_1, R_2, R_3, M_1, M_2, and n are the same as defined above;

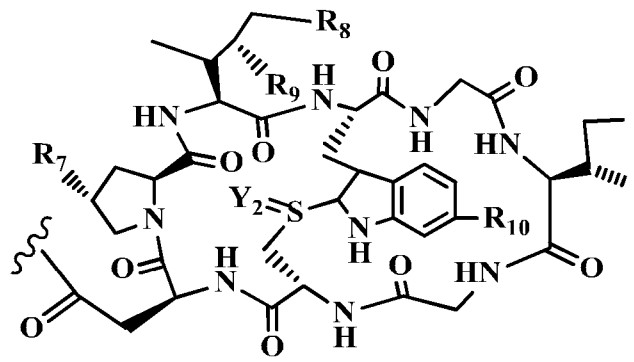
(o) the amatoxin and its analogs have the following formula, Am01, Am02, Am03 and Am04:



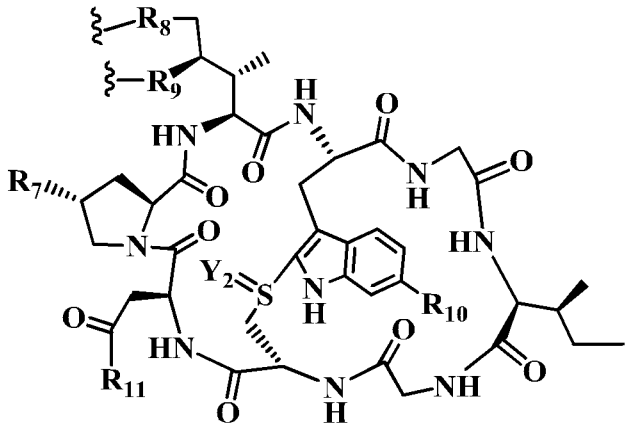
Am01,



Am02,



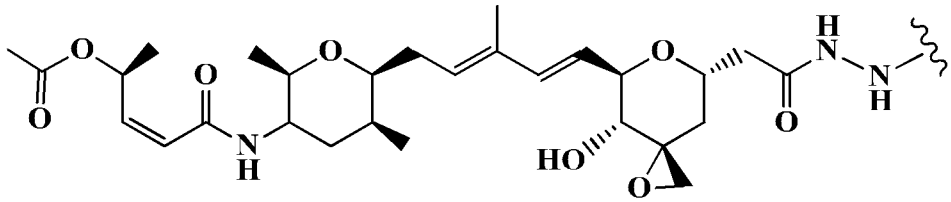
Am03,



Am04

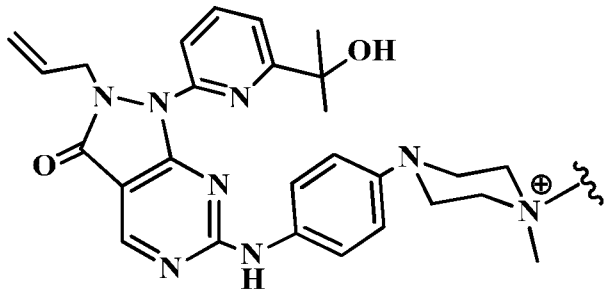
or an isotope of one or more chemical elements, or pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein X_1, and Y_1 are independently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, $OC(O)N(R_1)$, $N(R_1)C(O)N(R_1)$, CH_2, CHNH, CH_2O, C(O)NHNHC(O) and $C(O)NR_1$; R_7, R_8, and R_9 are independently H, OH, OR_1, NH_2, NHR_1, C_1-C_6 alkyl, or absent; Y_2 is O, O_2, NR_1, NH, or absent; R_{10} is CH_2, O, NH, NR_1, NHC(O), NHC(O)NH, NHC(O)O, OC(O)O, C(O), OC(O), $OC(O)(NR_1)$, $(NR_1)C(O)(NR_1)$, $C(O)R_1$ or absent; R_{11} is OH, NH_2, NHR_1, $NHNH_2$, NHNHCOOH, O-R_1-COOH, NH-R_1-COOH, NH-$(Aa)_r$COOH, $O(CH_2CH_2O)_pCH_2CH_2OH$, $O(CH_2CH_2O)_pCH_2CH_2NH_2$, $NH(CH_2CH_2O)_pCH_2CH_2NH_2$, NR_1R_2, $O(CH_2CH_2O)_pCH_2CH_2$-COOH, $NH(CH_2CH_2O)_pCH_2CH_2COOH$, NH-Ar-COOH, NH-Ar-$NH_2$, $O(CH_2CH_2O)_pCH_2CH_2$-$NHSO_3H$, $NH(CH_2CH_2O)_pCH_2CH_2NHSO_3H$, R_1-$NHSO_3H$, NH-R_1-$NHSO_3H$, $O(CH_2CH_2O)_p$-$CH_2CH_2NHPO_3H_2$, $NH(CH_2CH_2O)_pCH_2CH_2NHPO_3H_2$, OR_1, R_1-$NHPO_3H_2$, R_1-OPO_3H_2, $O(CH_2CH_2O)_pCH_2CH_2OPO_3H_2$, OR_1-$NHPO_3H_2$, NH-R_1-$NHPO_3H_2$, or $NH(CH_2CH_2O)_pCH_2$-$CH_2NHPO_3H_2$, wherein $(Aa)_r$ is 1-8 aminoacids; n and m_1 are independently 1-20; p is 1 -5000; R_1, R_2 and Ar, are the same defined through out the application; " ∿∿ " is defined the same above;

(p) the Spliceostatins and pladienolides are spliceostatin A, FR901464, and (2S, 3Z)-5-{[(2R, 3R, 5S, 6S)-6-{(2E, 4E)-5-[(3R, 4R, 5R, 7S)-7-(2-hydrazinyl-2-oxoethyl)-4-hydroxy-1, 6-dioxaspiro[2.5]oct-5-yl]-3-methylpenta-2, 4-dien-1-y- l}-2,5-dimethyltetrahydro-2H-pyran-3-yl]amino}-5-oxopent-3-en-2-yl acetate, Pladienolide B, Pladienolide D, and E7107 having a core structure of Sp-01:

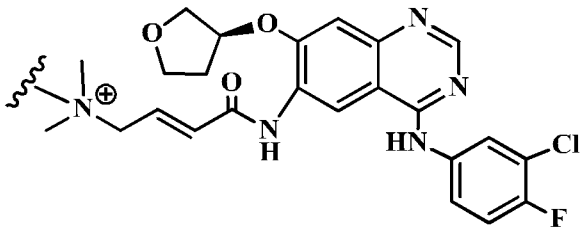


(Sp-01),

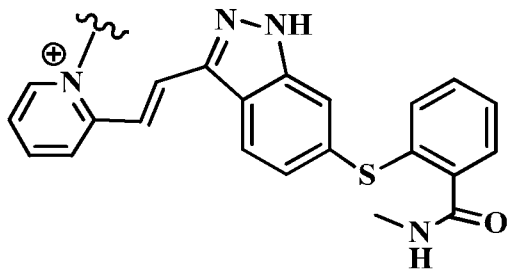
(q) the protein kinase inhibitors are selected from Adavosertib, Afatinib, Axitinib, Bafetinib, Bosutinib, Cobimetinib, Crizotinib, Cabozantinib, Dasatinib, Entrectinib, Erdafitinib, Erlotinib, Erlotinib, Fostamatinib, Gefitinib, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Pazopanib, Pegaptanib, Ponatinib, Rebastinib, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, SU6656, Tofacitinib, Vandetanib, Vemurafenib, Entrectinib, Palbociclib, Ribociclib, Abemaciclib, Dacomitinib, Neratinib, Rociletinib (CO-1686), Osimertinib, AZD3759, and Nazartinib (EGF816), having the following formula, PK01 - PK40:



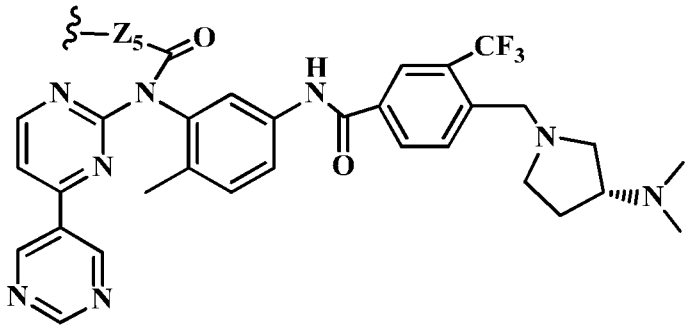
PK01, Adavosertib,



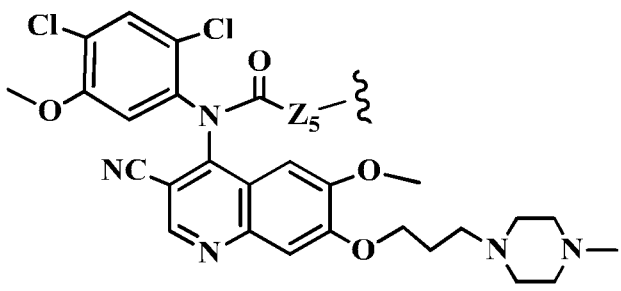
PK02, Afatinib,



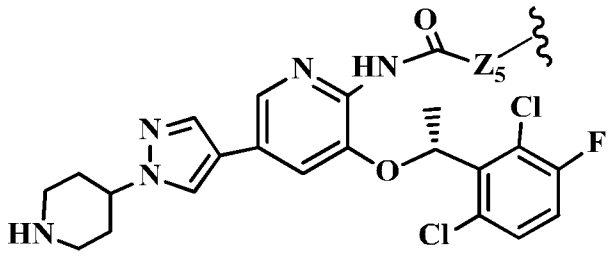
PK03, Axitinib,



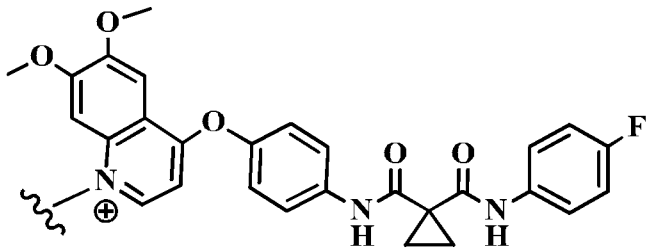
PK04, Bafetinib



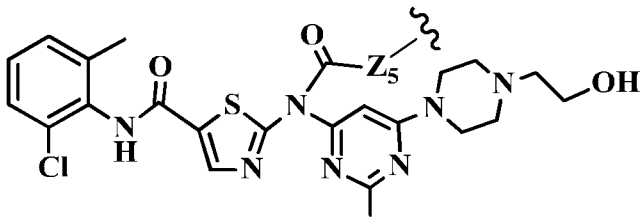
PK05, Bosutinib,



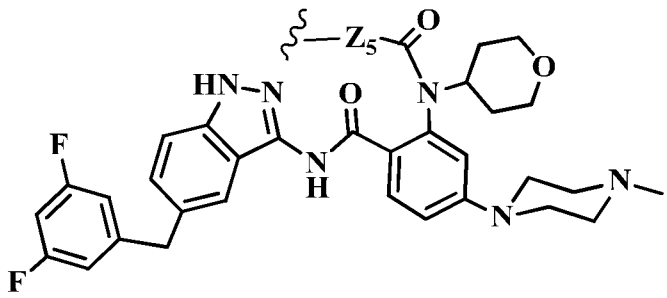
PK06, Crizotinib,



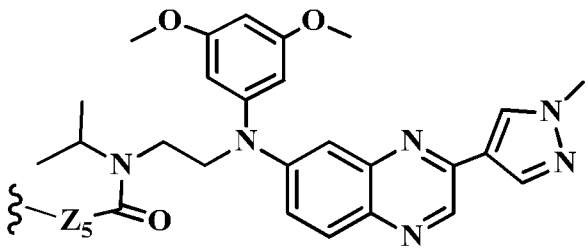
PK07, Cabozantinib,



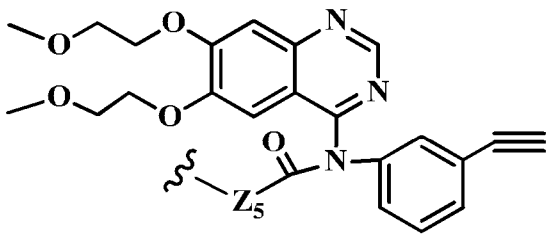
PK08, Dasatinib,



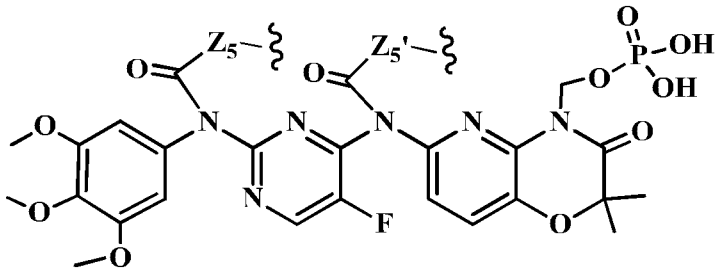
PK09, Entrectinib,



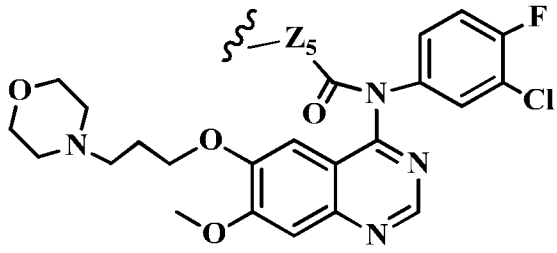
PK10, Erdafitinib,



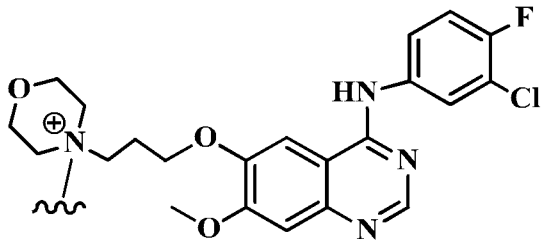
PK11, Erlotinib,



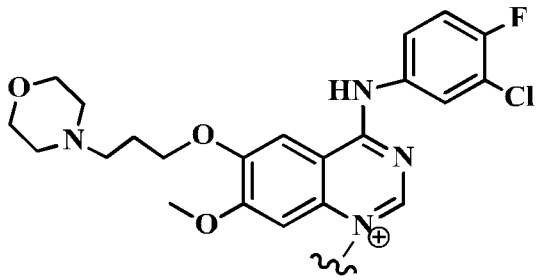
PK12, Fostamatinib,



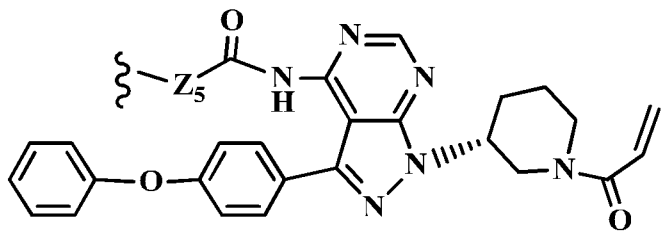
PK13, Gefitinib,



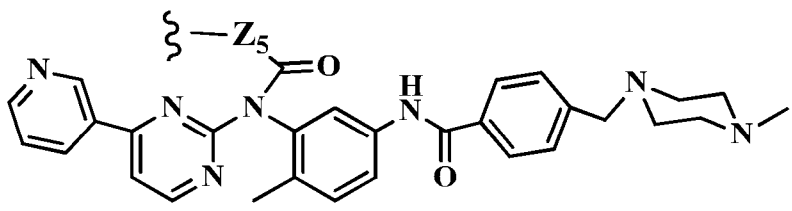
PK14, Gefitinib,



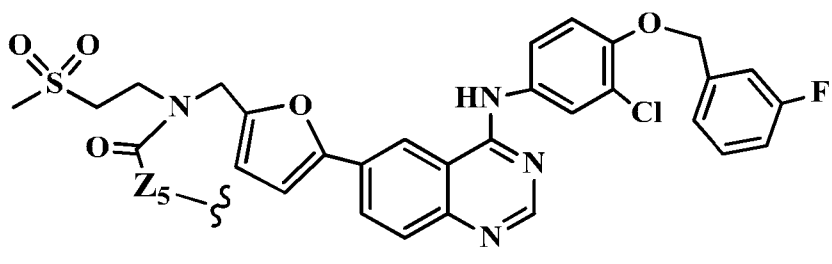
PK15, Gefitinib,



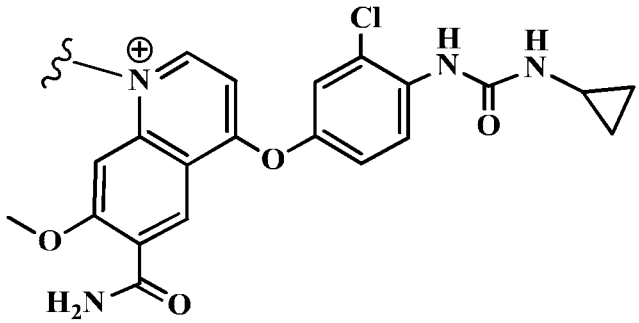
PK16, Ibrutinib,



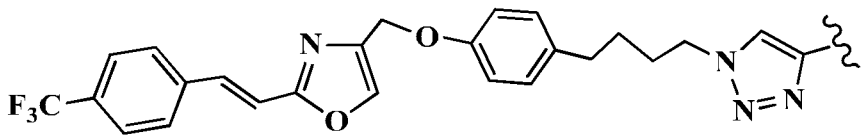
PK17, Imatinib,



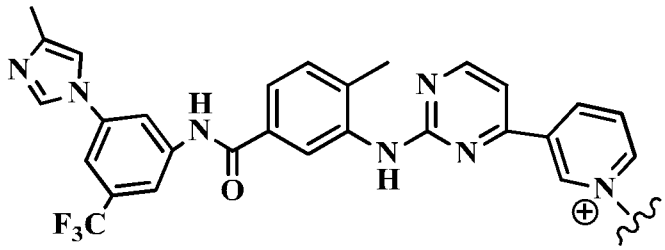
PK18, Lapatinib,



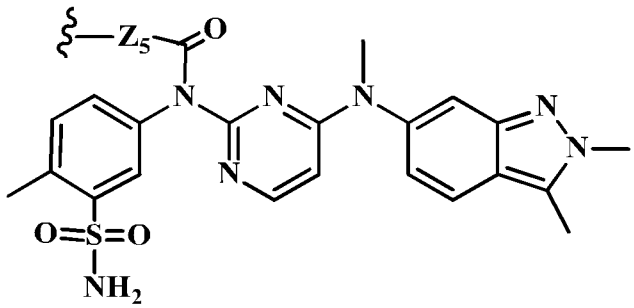
PK19, Lenvatinib,



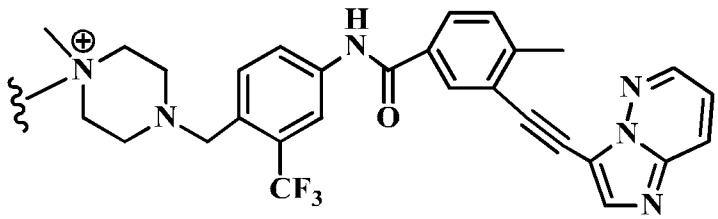
PK20, Mubritinib,



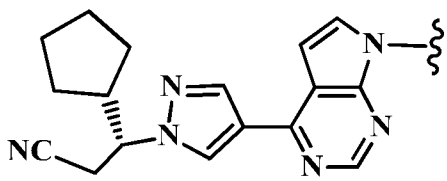
PK21, Nilotinib,



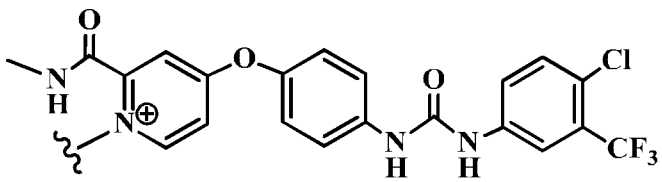
PK22, Pazopanib,



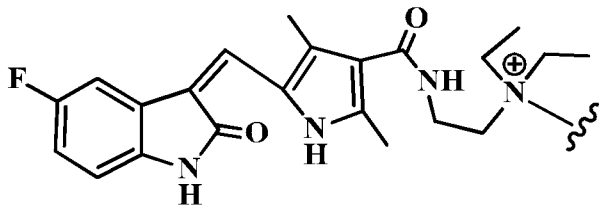
PK23, Ponatinib,



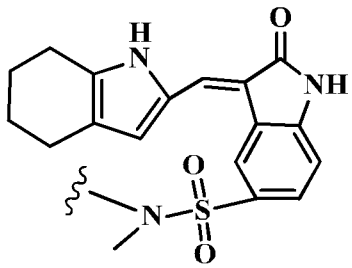
PK24, Ruxolitinib,



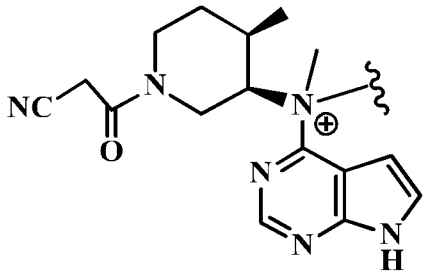
PK25, Sorafenib,



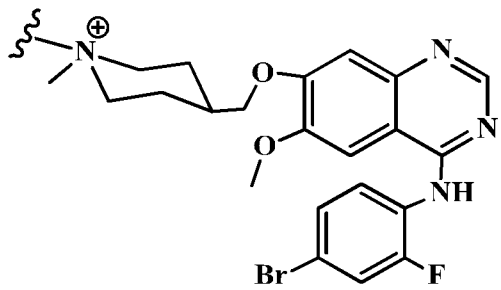
PK26, Sunitinib,



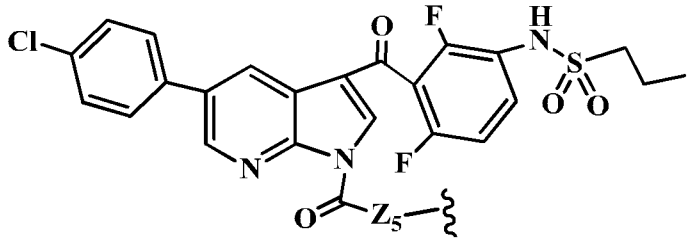
PK27, SU6656,



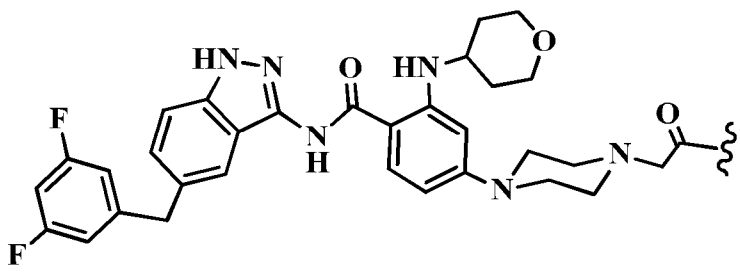
PK28, Tofacitinib,



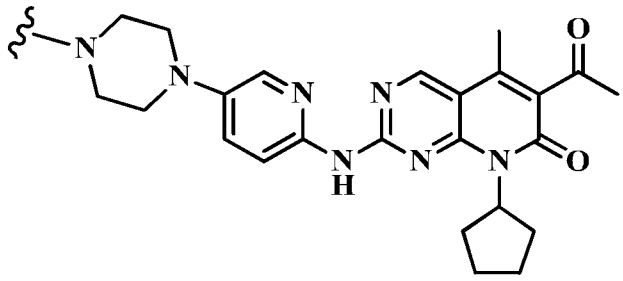
PK29, Vandetanib,



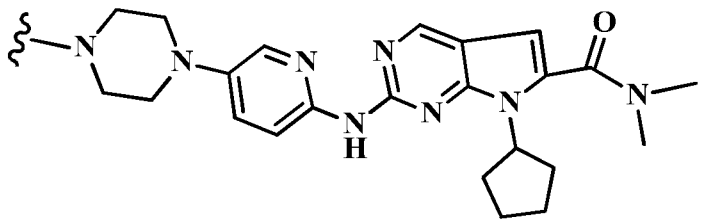
PK30, Vemurafenib;



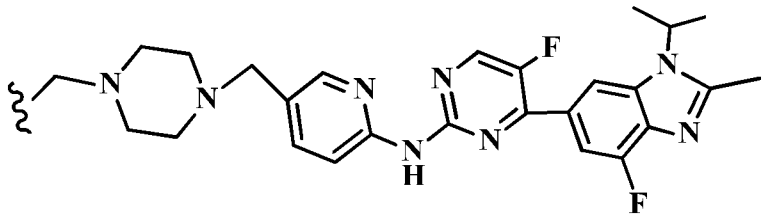
PK 31, Entrectinib;



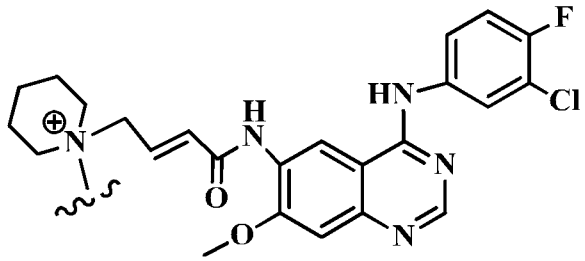
PK-32, Palbociclib,



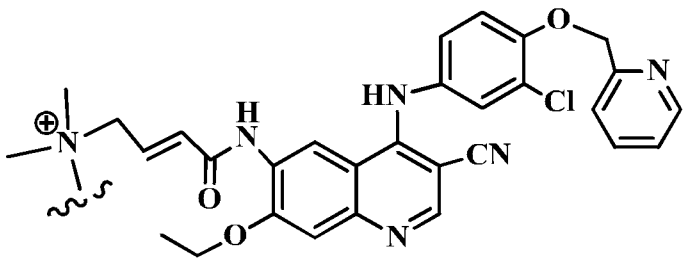
PK-33, Ribociclib,



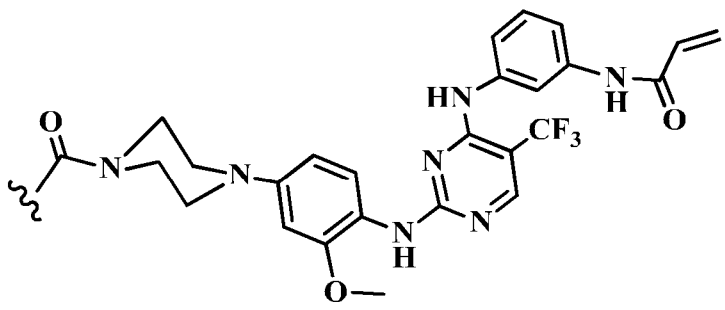
PK-34, Abemaciclib,



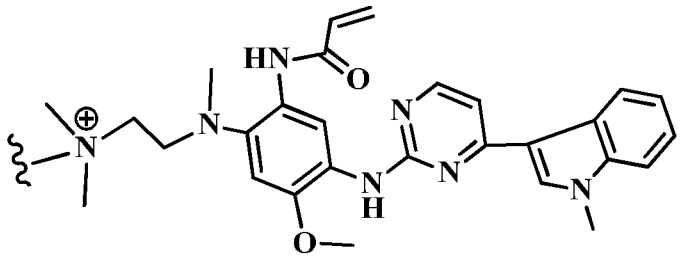
PK-35, Dacomitinib,



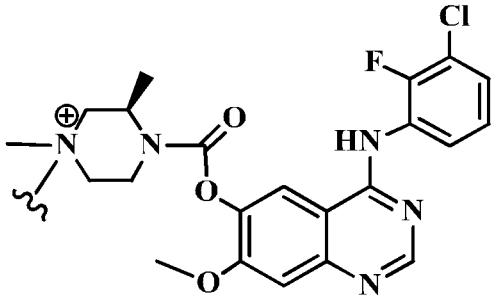
PK-36, Neratinib,



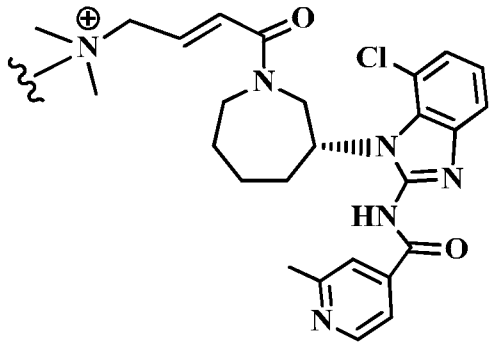
PK-37, Rociletinib (CO-1686),



PK-38, Osimertinib,



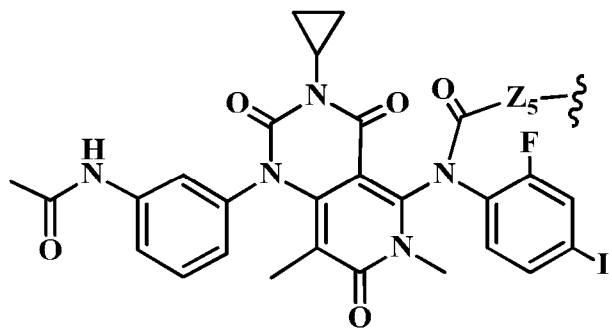
PK-39, AZD3759,



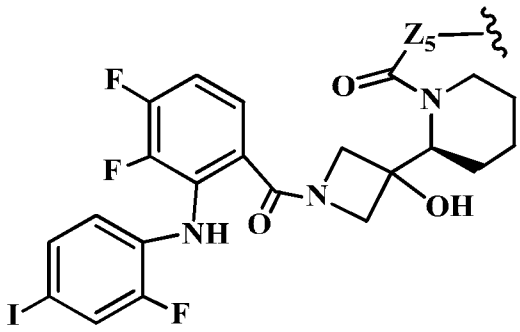
PK-40, Nazartinib (EGF816),

wherein Z_5 and Z_5' are indepently O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_2), C(O)NHNHC(O) or C(O)NR_1; R_1, R_2 and Ar, are defined the same above;

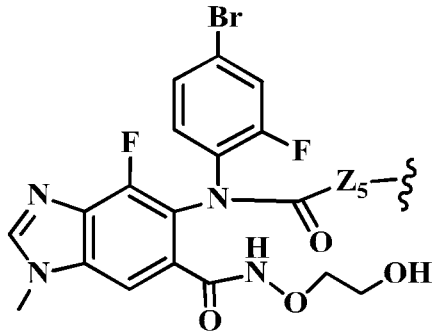
(r) the MEK inhibitors are selected from PD0325901, selumetinib (AZD6244), cobimetinib (XL518), refametinib, trametinib (GSK1120212), pimasertib, Binimetinib (MEK162), AZD8330, RO4987655, RO5126766, WX-554, E6201, GDC-0623, PD-325901 and TAK-733, and have the following formula:



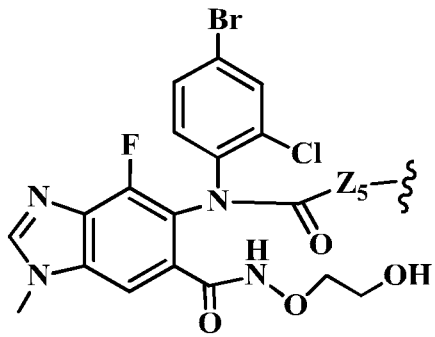
MEK01, Trametinib,



MEK02, Cobimetinib,



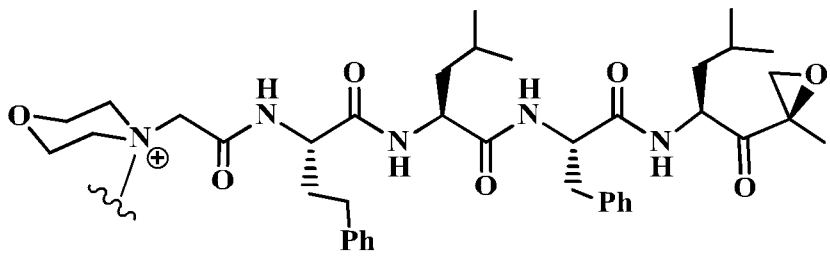
MEK03, Binimetinib,



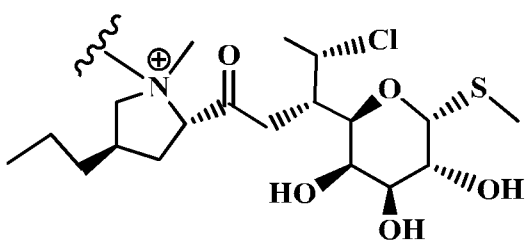
MEK04, selumetinib,

wherein Z_5 is selected from O, NH, NHNH, NR_5, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)O, NHC(O)NH, NHC(O)S, $OC(O)N(R_1)$, $N(R_1)C(O)N(R_2)$, C(O)NHNHC(O) and $C(O)NR_1$; R_1 and R_2 are the same as defined above;

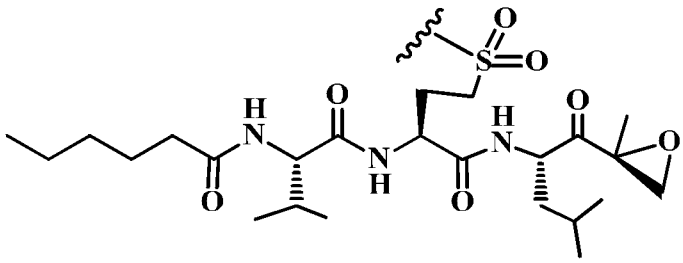
(s) the proteinase inhibitors are selected from Carfilzomib, Clindamycin, Retapamulin, Indibulin, having the following formulae:



PI01, Carfilzomib,

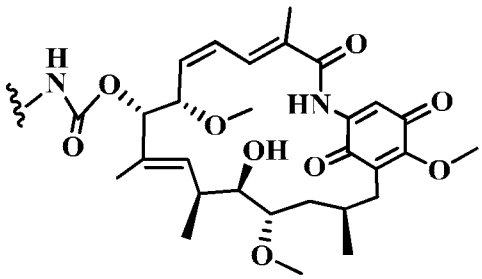


PI02, Clindamycin,



PI03, Carmaphycin analog;

(t) the geldanamycins or their derivatives have the following formula:



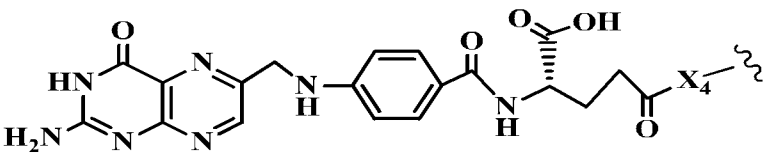
(**t1**), wherein ~~~ is a site defined above;

(u) the immunotoxin are selected from Diphtheria toxin (DT), Cholera toxin (CT), Trichosanthin (TCS), Dianthin, Pseudomonas exotoxin A (ETA), Erythrogenic toxins, Diphtheria toxin, AB toxins, Type III exotoxins, proaerolysin, and topsalysin;

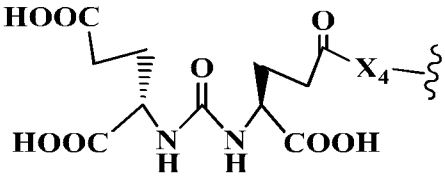
(v) the cell receptor agonist or stimulating molecule are selected from: Folate derivatives; Glutamic acid urea derivatives; Somatostatin and its analogs (selected from the group consisting of octreotide (Sandostatin) and lanreotide (Somatuline)); Aromatic sulfonamides; Pituitary adenylate cyclase activating peptides (PACAP) (PAC1); Vasoactive intestinal peptides (VIP/PACAP) (VPAC1, VPAC2); Melanocyte-stimulating hormones (α-MSH); Cholecystokinins (CCK) /gastrin receptor agonists; Bombesins (selected from the group consisting of Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2)/gastrin-releasing peptide (GRP); Neurotensin receptor ligands (NTR1, NTR2, NTR3); Substance P (NK1 receptor) ligands; Neuropeptide Y (Y1–Y6); Homing Peptides include RGD (Arg-Gly-Asp), NGR (Asn-Gly-Arg), the dimeric and multimeric cyclic RGD peptides (selected from cRGDfV), TAASGVRSMH and LTLRWVGLMS (Chondroitin sulfate proteoglycan NG2 receptor ligands) and F3 peptides; Cell Penetrating Peptides (CPPs); Peptide Hormones selected from the group consisting of luteinizing hormone-releasing hormone (LHRH) agonists and antagonists, and gonadotropin-releasing hormone (GnRH) agonist selected from the group consisting of buserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(OtBu)-Leu-Arg-Pro-NHEt), Gonadorelin (Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), Goserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(OtBu)-Leu-Arg-Pro-AzGly-NH_2), Histrelin (Pyr-His-Trp-Ser-Tyr-D-His(N-benzyl)-Leu-Arg-Pro-NHEt), leuprolide (Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt), Nafarelin (Pyr-His-Trp-Ser-Tyr-2Nal-Leu-Arg-Pro-Gly-NH_2), Triptorelin (Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), Nafarelin, Deslorelin, Abarelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-(N-Me)Tyr-D-Asn-Leu-isopropylLys-Pro-DAla-NH_2), Cetro-

relix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2), Degarelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-4-aminoPhe(L-hydroorotyl)-D-4-aminoPhe(carba-moyl)-Leu-isopropylLys-Pro-D-Ala-NH_2), and Ganirelix (Ac-D-2Nal-D-4-chloroPhe-D-3-(3-pyridyl)Ala-Ser-Tyr-D-(N9, N10-diethyl)-homoArg-Leu-(N9, N10-diethyl)-homoArg-Pro-D-Ala-NH_2); Pattern Recognition Receptor (PRRs), selected from the group consisting of Toll-like receptors' (TLRs) ligands, C-type lectins and Nodlike Receptors' (NLRs) ligands; Calcitonin receptor agonists; integrin receptors' and their receptor subtypes' (selected from the group consisting of$\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$, $\alpha_6\beta_4$, $\alpha_7\beta_1$, $\alpha_L\beta_2$, $\alpha_{IIb}\beta_3$) agonists (selected from the group consisting of GRGDSPK, cyclo(RGDfV) (L1) and its derives [cyclo(-N(Me)R-GDfV), cyclo(R-Sar-DfV), cyclo(RG-N(Me)D-fV), cyclo(RGD-N(Me)f-V), cyclo(RGDf-N(Me)V-)(Cilengitide)]; Anticalin (a derivative of Lipocalins); Adnectins (10th FN3 (Fibronectin)); Designed Ankyrin Repeat Proteins (DARPins); Avimers; EGF receptors, or VEGF receptors' agonists;

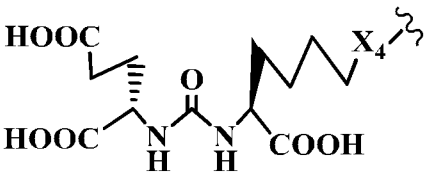
the cell receptor agonist is selected from the following: LB01 (Folate), LB02 (PMSA ligand), LB03 (PMSA ligand), LB04 (PMSA ligand), LB05 (Somatostatin), LB06 (Somatostatin), LB07 (Octreotide, a Somatostatin analog), LB08 (Lanreotide, a Somatostatin analog), LB09 (Vapreotide (Sanvar), a Somatostatin analog), LB10 (CAIX ligand), LB11 (CAIX ligand), LB12 (Gastrin releasing peptide receptor (GRPr), MBA), LB13 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH), LB14 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand), LB15 (GnRH antagonist, Abarelix), LB16 (cobalamin, vitamin B12 analog), LB17 (cobalamin, vitamin B12 analog), LB18 (for $\alpha_v\beta_3$ integrin receptor, cyclic RGD pentapeptide), LB19 (heterobivalent peptide ligand for VEGF receptor), LB20 (Neuromedin B), LB21 (bombesin for a G-protein coupled receptor), LB22 (TLR_2 for a Toll-like receptor,), LB23 (for an androgen receptor), LB24 (Cilengitide/cyclo(-RGDfV-) for an α_v integrin receptor, LB23 (Fludrocortisone), LB25 (Rifabutin analog), LB26 (Rifabutin analog), LB27 (Rifabutin analog), LB28 (Fludrocortisone), LB29 (Dexamethasone), LB30 (fluticasone propionate), LB31 (Beclometasone dipropionate), LB32 (Triamcinolone acetonide), LB33 (Prednisone), LB34 (Prednisolone), LB35 (Methylprednisolone), LB36 (Betamethasone), LB37 (Irinotecan analog), LB38 (Crizotinib analog), LB39 (Bortezomib analog), LB40 (Carfilzomib analog), LB41 (Carfilzomib analog), LB42 (Leuprolide analog), LB43 (Triptorelin analog), LB44 (Clindamycin), LB45 (Liraglutide analog), LB46 (Semaglutide analog), LB47 (Retapamulin analog), LB48 (Indibulin analog), LB49 (Vinblastine analog), LB50 (Lixisenatide analog), LB51 (Osimertinib analog), LB52 (a nucleoside analog), LB53 (Erlotinib analog) or LB54 (Lapatinib analog) having following structures:



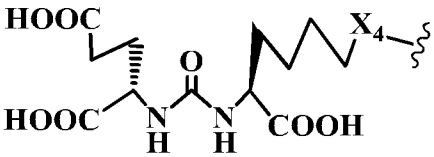
LB01 (Folate conjugate),



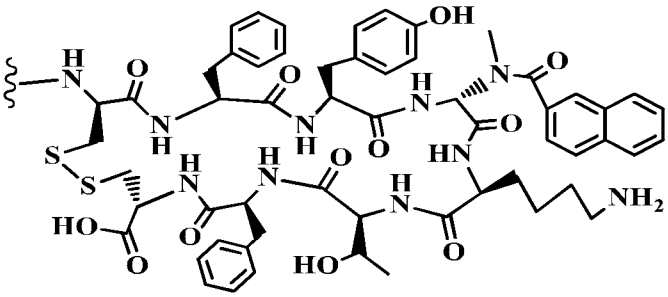
LB02 (PMSA ligand conjugate),



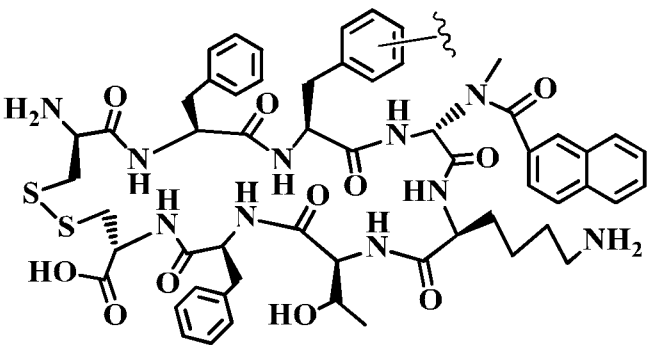
LB03 (PMSA ligand conjugate),



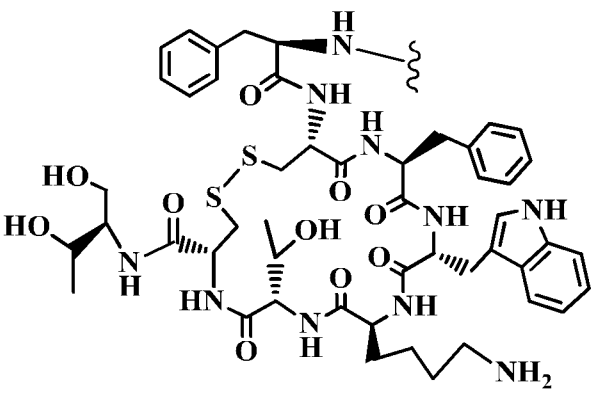
LB04 (PMSA ligand),



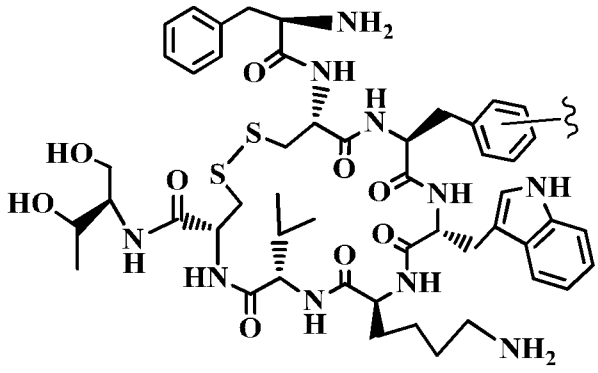
LB05 (Somatostatin),



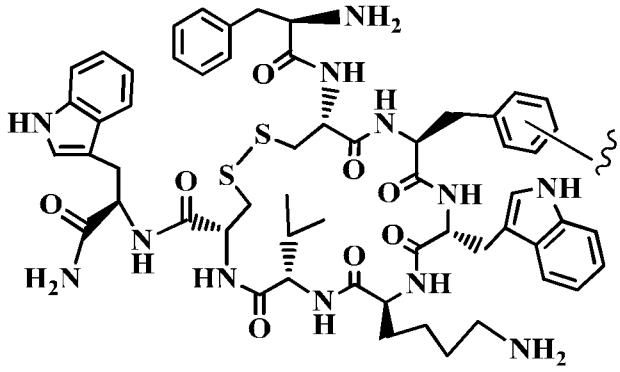
LB06 (Somatostatin),



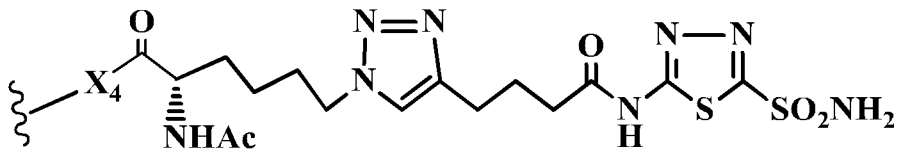
LB07 (Octreotide, a Somatostatin analog),



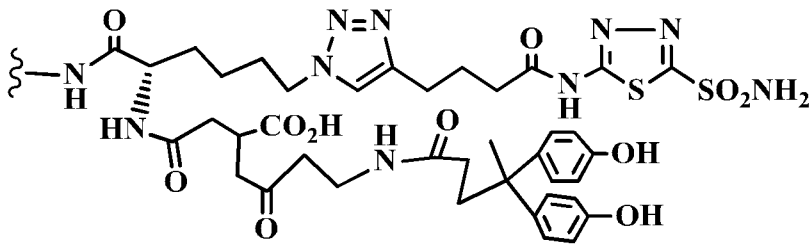
LB08 (Lanreotide, a Somatostatin analog),



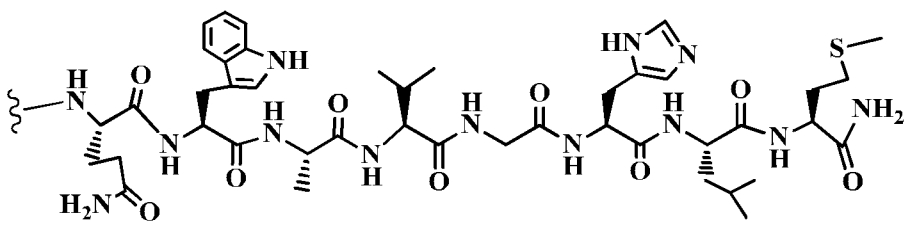
LB09 (Vapreotide (Sanvar), a Somatostatin analog),



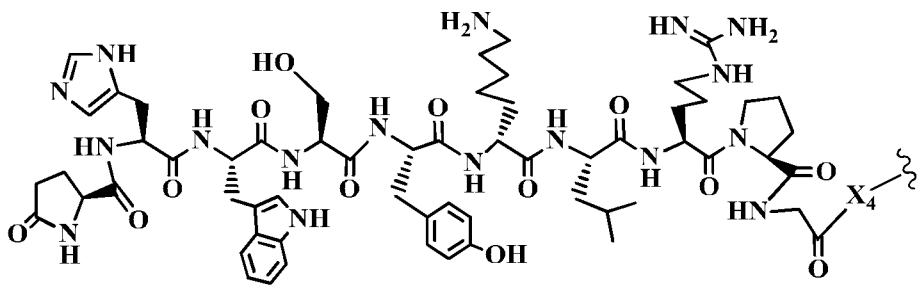
LB10 (CAIX ligand),



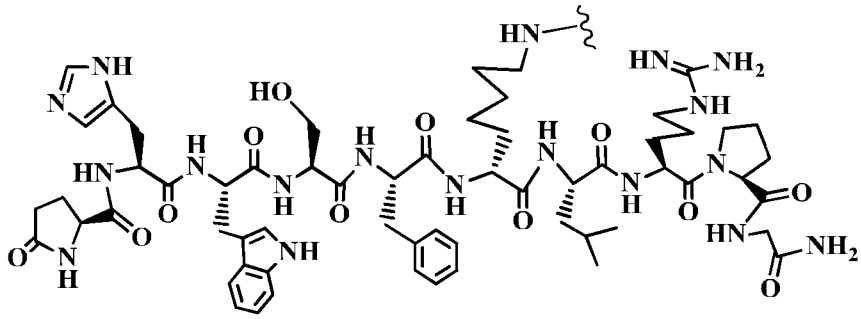
LB11 (CAIX ligand),



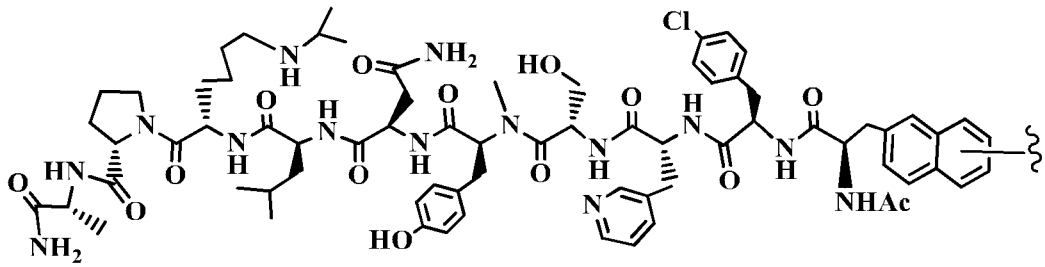
LB12 (Gastrin releasing peptide receptor (GRPr), MBA),



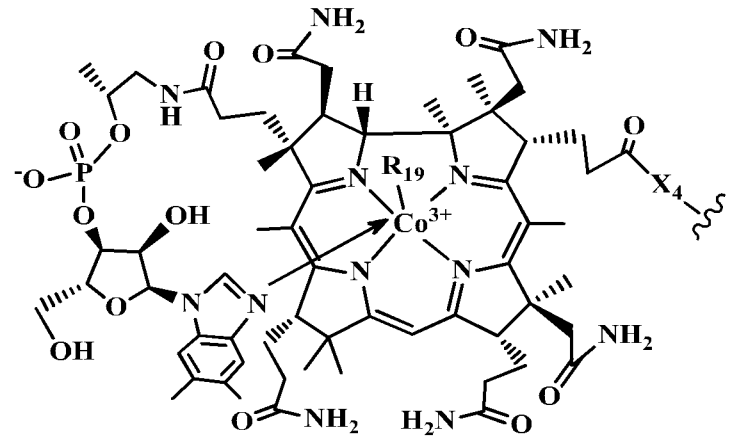
LB13 (luteinizing hormone-releasing hormone (LH-RH) ligand and GnRH),



LB14 (luteinizing hormone-releasing hormone (LH-RH) and GnRH ligand),

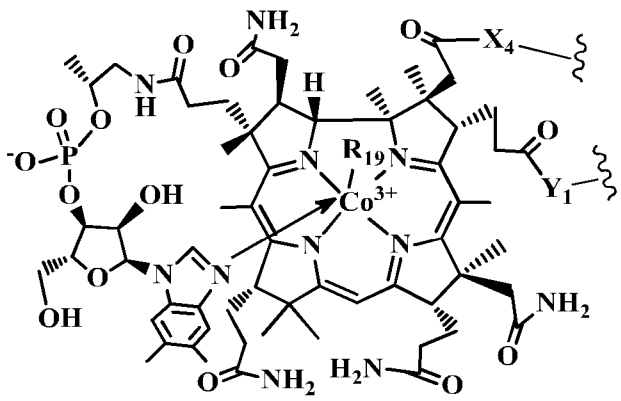


LB15 (GnRH antagonist, Abarelix),

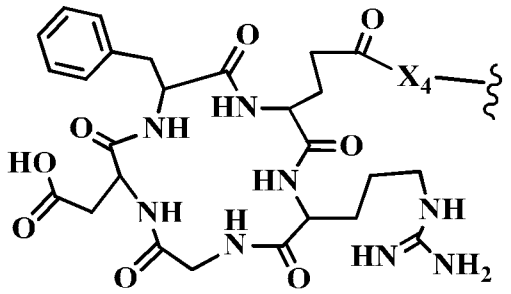


R_{19} is 5'deoxyadenosyl, Me, OH, or CN;

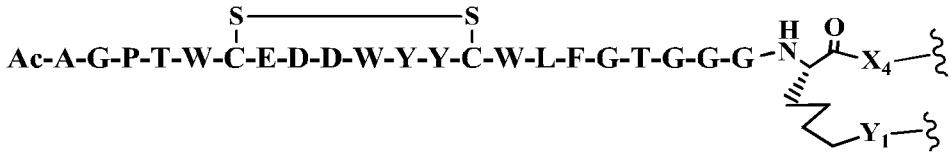
LB16 (cobalamin, vitamin B12 analog),



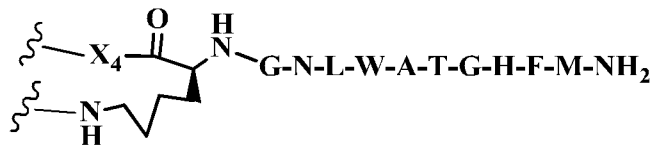
R_{19} is 5'deoxyadenosyl, Me, OH, or CN; LB17 (cobalamin, vitamin B12 analog),



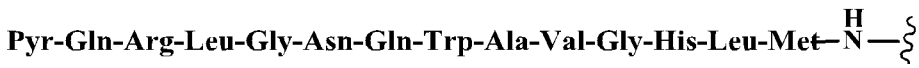
LB18 (for $\alpha_v\beta_3$ integrin receptor, cyclic RGD pentapeptide),



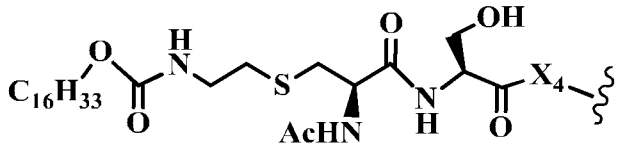
LB19 (hetero-bivalent peptide ligand conjugate for VEGF receptor),



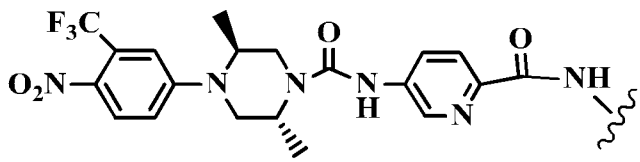
LB20 (Neuromedin B),



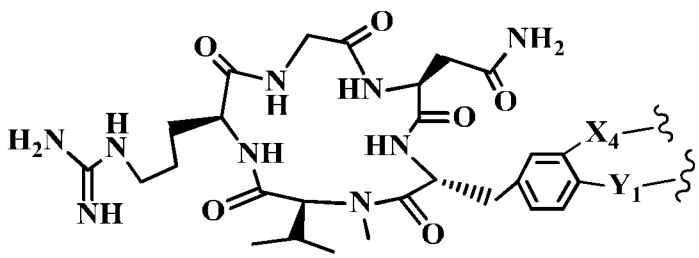
LB21 (bombesin conjugate for a G-protein coupled receptor),



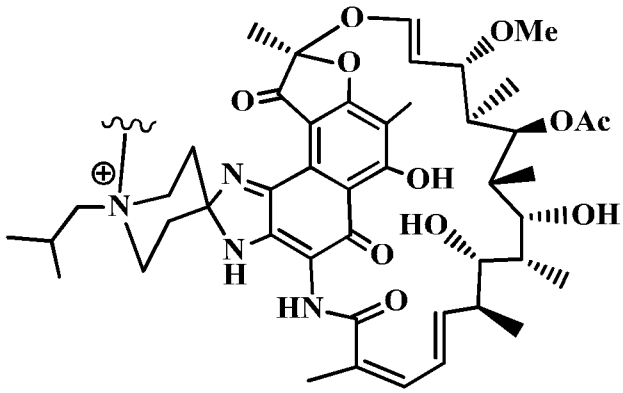
LB22 (TLR_2 conjugate for a Toll-like receptor),



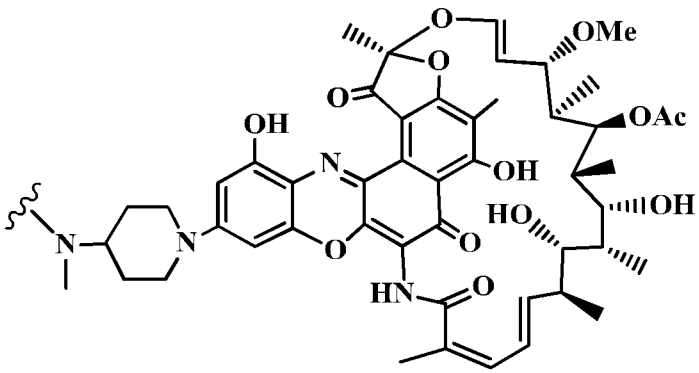
LB23 (an androgen receptor),



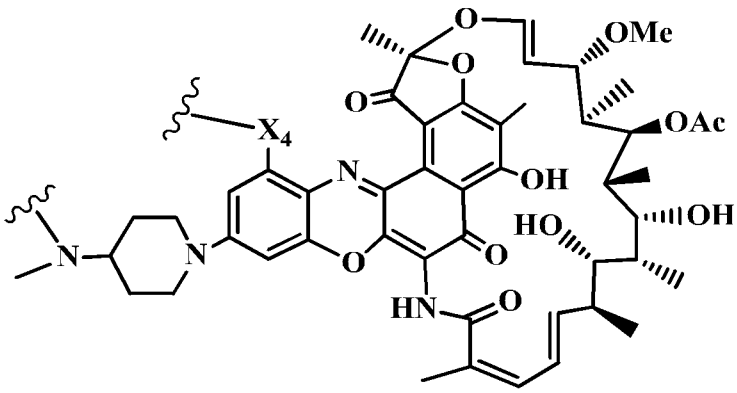
LB24 (Cilengitide/cyclo(-RGDfV-) conjugate for an α_v integrin receptor)



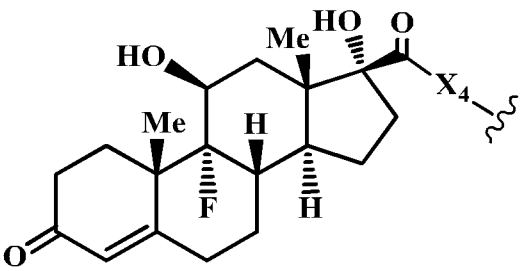
LB25 (Rifabutin analog),



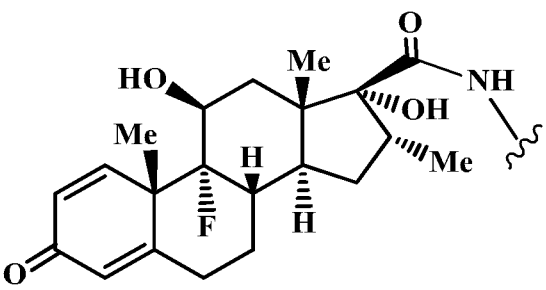
LB26 (Rifabutin analog),



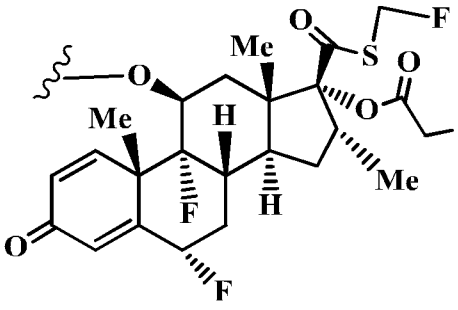
LB27 (Rifabutin analog),



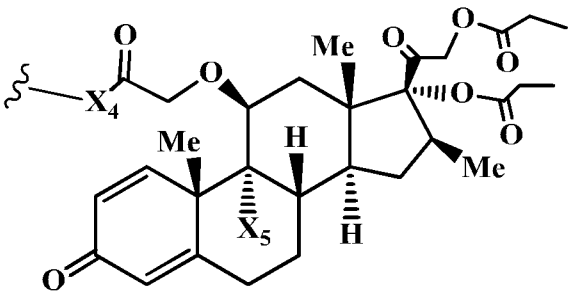
LB28 (Fludrocortisone),



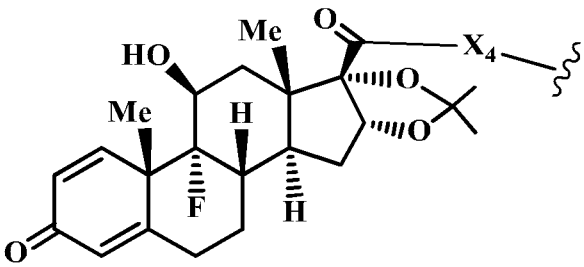
LB29 (Dexamethasone),



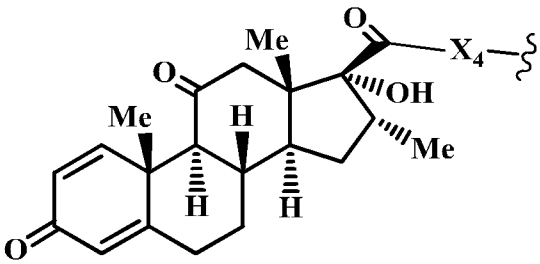
LB30 (fluticasone propionate),



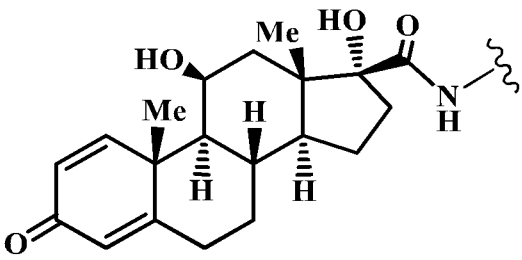
LB31 (Beclometasone dipropionate),



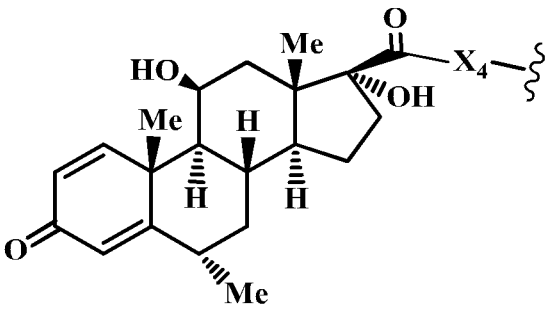
LB32 (Triamcinolone acetonide),



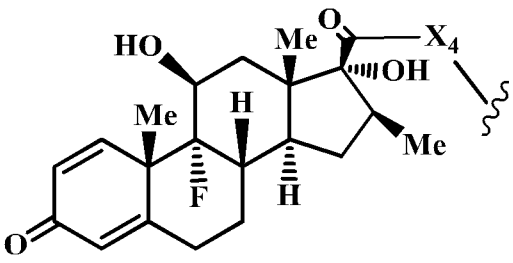
LB33 (Prednisone),



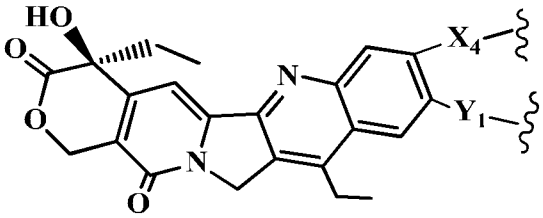
LB34 (Prednisolone),



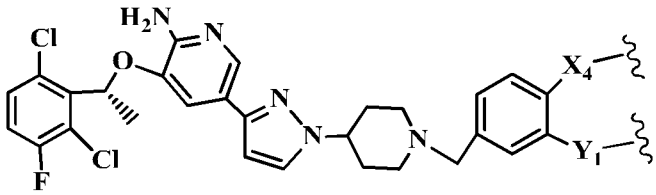
LB35 (Methylprednisolone),



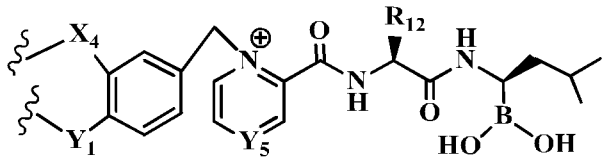
LB36 (Betamethasone),



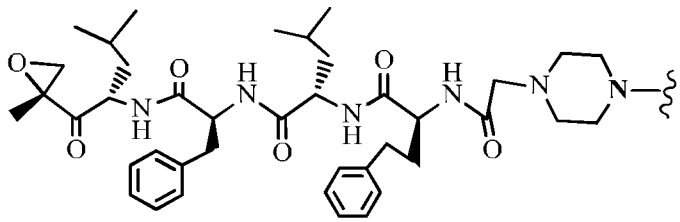
LB37 (Irinotecan analog),



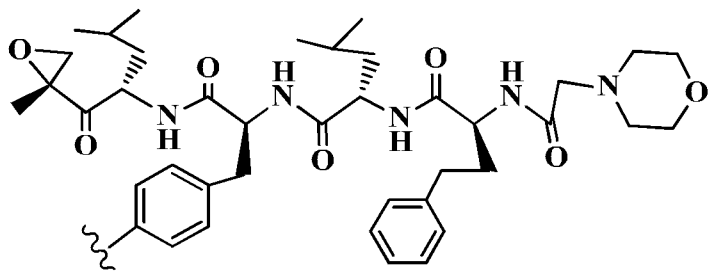
LB38 (Crizotinib analog),



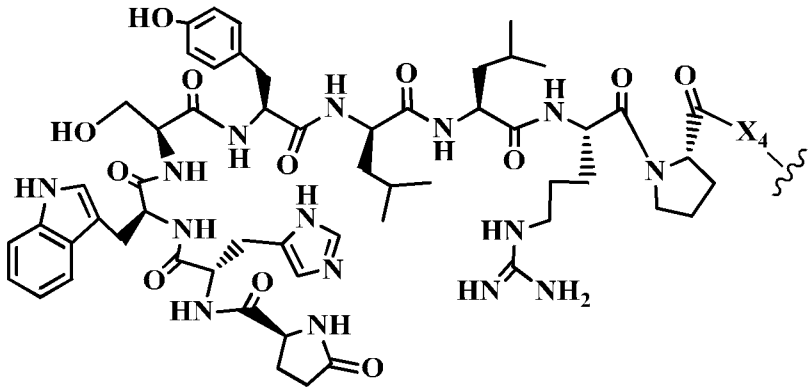
LB39 (Bortezomib analog), wherein Y_5, is N, CH, C(Cl), $C(CH_3)$, or $C(COOR_1)$; R_{12} is H, C_1-C_6 Alkyl, or C_3-C_8 Ar;



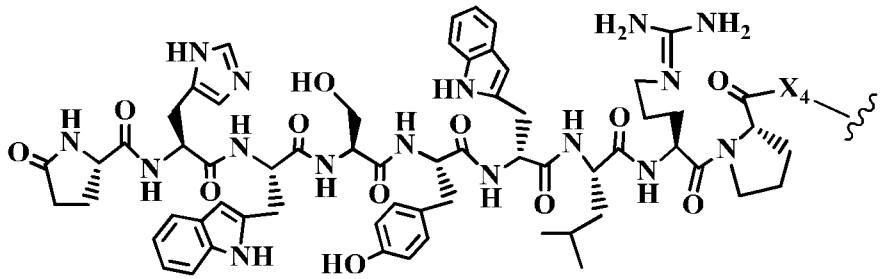
LB40 (Carfilzomib analog),



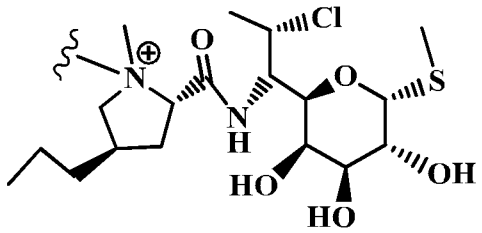
LB41 (Carfilzomib analog),



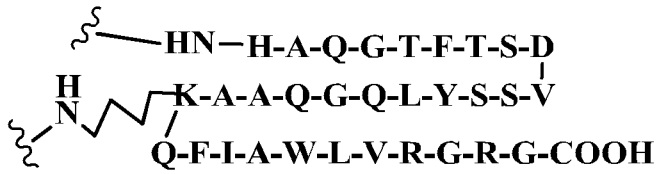
LB42 (Leuprolide analog),



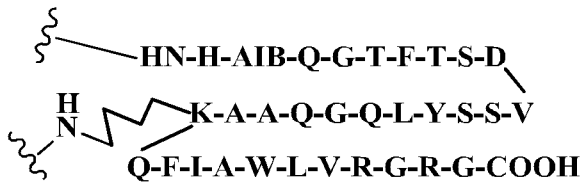
LB43 (Triptorelin analog),



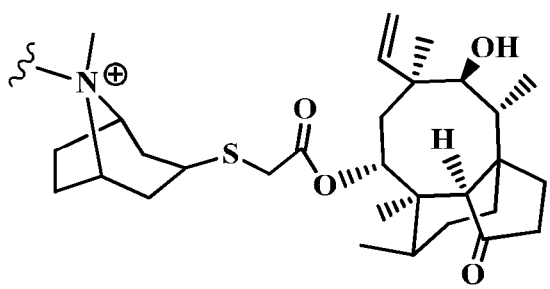
LB44 (Clindamycin),



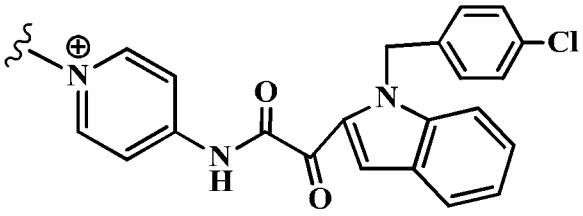
LB45 (Liraglutide analog),



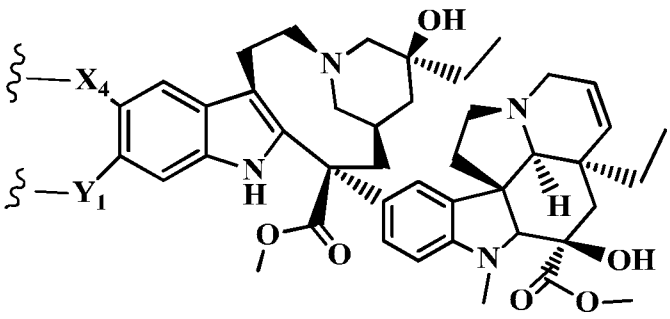
LB46 (Semaglutide analog),



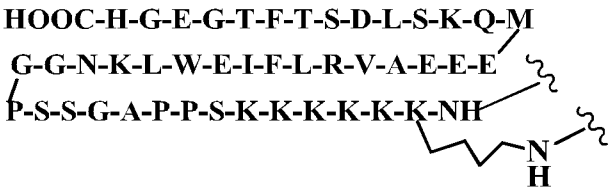
LB47 (Retapamulin analog),



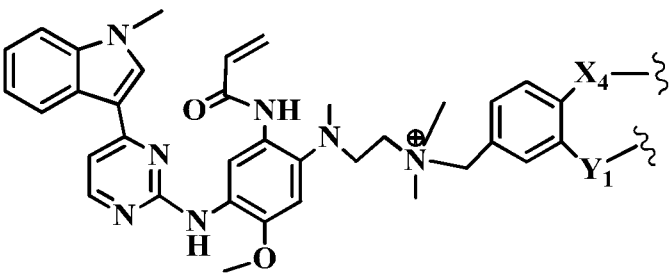
LB48 (Indibulin analog),



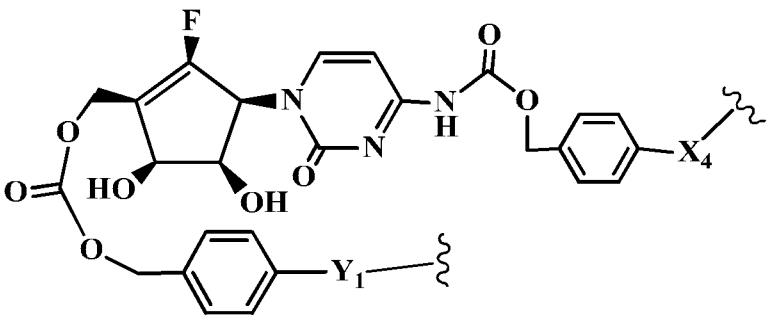
LB49 (Vinblastine analog),



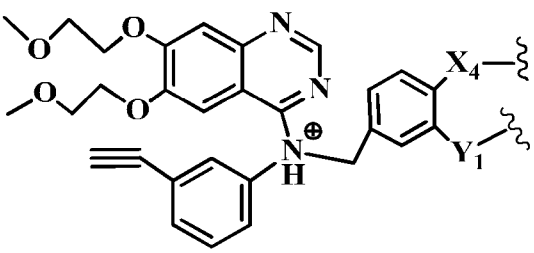
LB50 (Lixisenatide analog),



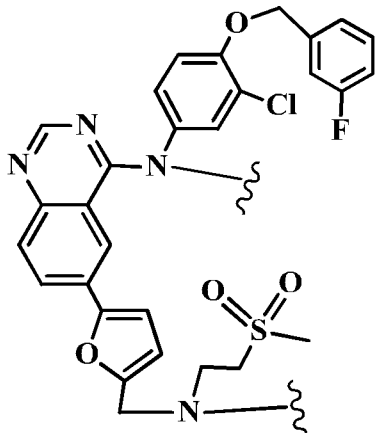
LB51 (Osimertinib analog),



LB52 (a nucleoside analog),



LB53 (Erlotinib analog),



LB54 (Lapatinib analog);

wherein X_4, and Y_1 are independently O, NH, NHNH, NR_1, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH_2, C(O)NHNHC(O) or C(O)NR_1; R_1, and R_2 are defined the same above;

(w) the one, two or more DNA, RNA, mRNA, small interfering RNA (siRNA), micro-RNA (miRNA), and PIWI interacting RNAs (piRNA) have a structure of:



, SI01, or



, SI02;

wherein " ∿∿ " is a site to link the side chain linker;  is single or double strands of DNA, RNA, mRNA, siRNA, miRNA, or piRNA; X_1, and Y are independently O, NH, NHNH, NR_1, S, C(O)O, C(O)NH, OC(O)NH, OC(O)O, NHC(O)NH, NHC(O)S, OC(O)N(R_1), N(R_1)C(O)N(R_1), CH_2, C(O)NHNHC(O) or C(O)NR_1; R_1, and R_2 are defined the same above.

11. The process according to Claim 1, or 3, or 4, wherein the antibody or antibody-like protein is selected from:

(A): the group consisting of probody, nanobody, peptides, a polymeric micelle, a liposome, a lipoprotein-based drug carrier, a nano-particle drug carrier, a dendrimer, and a molecule or a particle said above coating or linking with a cell-binding ligand, or a combination of two or more of the above;

(B): the group consisting of a full-length antibody (polyclonal antibody, monoclonal antibody, antibody dimer, antibody multimer), multispecific antibody (selected from, bispecific antibody, trispecific antibody, or tetraspecific antibody); a single chain antibody, an antibody fragment that binds to the target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that binds the target cell, a chimeric antibody, a chimeric antibody

fragment that binds to the target cell, a domain antibody, a domain antibody fragment that binds to the target cell, a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment that binds to the target cell, a humanized antibody or a resurfaced antibody, a humanized single chain antibody, or a humanized antibody fragment that binds to the target cell, anti-idiotypic (anti-Id) antibodies, CDR's, diabody, triabody, tetrabody, miniantibody, a probody, a probody fragment, small immune antibody-like proteins (SIP), a lymphokine antibody-like protein, a hormone antibody-like protein, a growth factor antibody-like protein, a colony stimulating factor antibody-like protein,, a nutrient-transport antibody-like protein, large molecular weight antibody-like proteins, fusion antibody-like proteins, kinase inhibition antibody-like protein, gene-targeting antibody-like protein, antibody-like protein coated on nanoparticles or polymers modified with antibodies or large molecular weight antibody-like proteins; or

(C): the group consisting of an IgG antibody that is able to bind against tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune disease cells, activated tumor cells, myeloid cells, activated T-cells, an affecting B cells, or melanocytes, or abnormal cells expressing any one of the following antigens or receptors: CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CD12w, CD14, CD15, CD16, CD16a, CD16b, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD32a, CD32b, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD49c, CD49d, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD65s, CD66, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD67, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD76, CD77, CD78, CD79, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, CD85a, CD85b, CD85c, CD85d, CD85e, CD85f, CD85g, CD85g, CD85i, CD85j, CD85k, CD85m, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD120a, CD120b, CD121, CD121a, CD121b, CD122, CD123, CD123a, CD124, CD125, CD126, CD127, CD128, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140, CD140a, CD140b, CD141, CD142, CD143, CD144, CD145, CDw145, CD146, CD147, CD148, CD149, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD156a, CD156b, CD156c, CD156d, CD157, CD158, CD158a, CD158b1, CD158b2, CD158c, CD158d, CD158e1, CD158e2, CD158f2, CD158g, CD158h, CD158i, CD158j, CD158k, CD159, CD159a, CD159b, CD159c, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD167, CD167a,

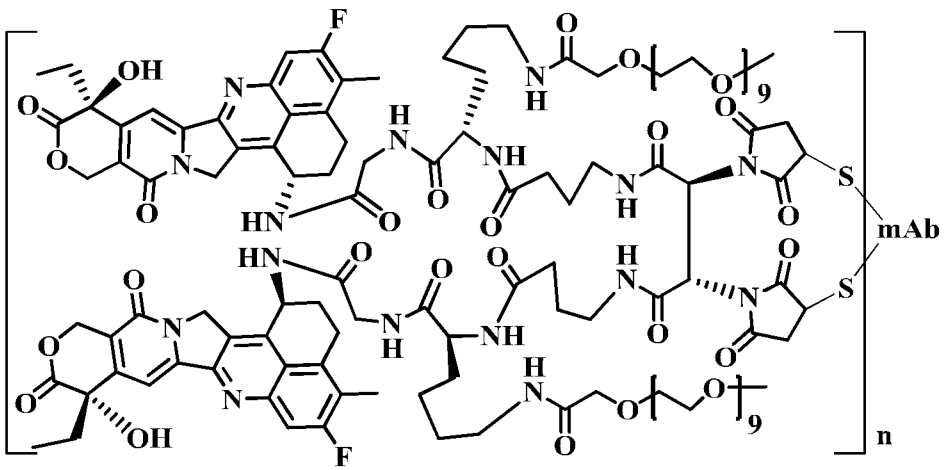
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factor 1 receptor), CSF2 (colony stimulating factor 2, Granulocyte-macrophage colony-stimulating factor (GM-CSF)), CSP4, CTLA4 (cytotoxic T-lymphocyte-associated protein 4), CTAA16.88 tumor antigen, CXCR4, C-X-C chemokine receptor type 4, cyclic ADP ribose hydrolase, Cyclin B1, CYP1B1, Cytomegalovirus, Cytomegalovirus glycoprotein B, Dabigatran, DLL3 (delta-like-ligand 3), DLL4 (delta-like-ligand 4), DPP4 (Dipeptidyl-peptidase 4), DR5 (Death receptor 5), E. coli shiga toxin type-1, E. coli shiga toxin type-2, ED-B, EGFL7 (EGF-like domain-containing protein 7), EGFR, EGFRII, EGFRvIII, Endoglin, Endothelin B receptor, Endotoxin, EpCAM (epithelial cell adhesion molecule), EphA2, Episialin, ERBB2 (Epidermal Growth Factor Receptor 2), ERBB3, ERG (TMPRSS2 ETS fusion gene), Escherichia coli, ETV6-AML, FAP (Fibroblast activation protein alpha), FCGR1, alpha-Fetoprotein, Fibrin II, beta chain, Fibronectin extra domain-B, FOLR (folate receptor), Folate receptor alpha, Folate hydrolase, Fos-related antigen 1F protein of respiratory syncytial virus, Frizzled receptor, Fucosyl GM1, GD2 ganglioside, G-28 (a cell surface antigen glyvolipid), GD3 idiotype, GloboH, Glypican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α-chain, Growth differentiation factor 8, GP100, GPNMB (Trans-membrane glycoprotein NMB), GUCY2C (Guanylate cyclase 2C, guanylyl cyclase C(GC-C), intestinal Guanylate cyclase, Guanylate cyclase-C receptor, Heat-stable enterotoxin receptor (hSTAR)), Heat shock proteins, Hemagglutinin, Hepatitis B surface antigen, Hepatitis B virus, HER1 (human epidermal growth factor receptor 1), HER2, HER2/neu, HER3 (ERBB-3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB , HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGF1R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN-γ, Influenza hemagglutinin, IgE, IgE Fc region, IGHE, interleukins (comprising IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-6R, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17, IL-17A, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-27, or IL-28), IL31RA, ILGF2 (Insulin-like growth factor 2), Integrins (α4, $\alpha_{IIb}\beta_3$, αvβ3, $\alpha_4\beta_7$, α5β1, α6β4, α7β7, αIIβ3, α5β5, αvβ5), Interferon gamma-induced protein, ITGA2, ITGB2, KIR2D, Kappa Ig, LCK, Le, Legumain, Lewis-Y antigen, LFA-1 (Lymphocyte function-associated antigen 1, CD11a), LHRH, LINGO-1, Lipoteichoic acid, LIV1A, LMP2, LTA, MAD-CT-1, MAD-CT-2, MAGE-1, MAGE-2, MAGE-3, MAGE A1, MAGE A3, MAGE 4, MART1, MCP-1, MIF (Macrophage migration inhibitory factor, or glycosylation-inhibiting factor (GIF)), MS4A1 (membrane-spanning 4-domains subfamily A member 1), B7H3, B7H4, MSLN (mesothelin), MUC1(Mucin 1, cell surface associated (MUC1) or polymorphic epithelial mucin (PEM)), MUC1-KLH, MUC16 (CA125), MCP1(monocyte chemotactic protein 1), MelanA/MART1, ML-IAP, MPG, MS4A1 (membrane-spanning 4-domains subfamily A), MYCN, Myelin-associated glycoprotein, Myostatin, NA17, NARP-1, NCA-90 (granu-

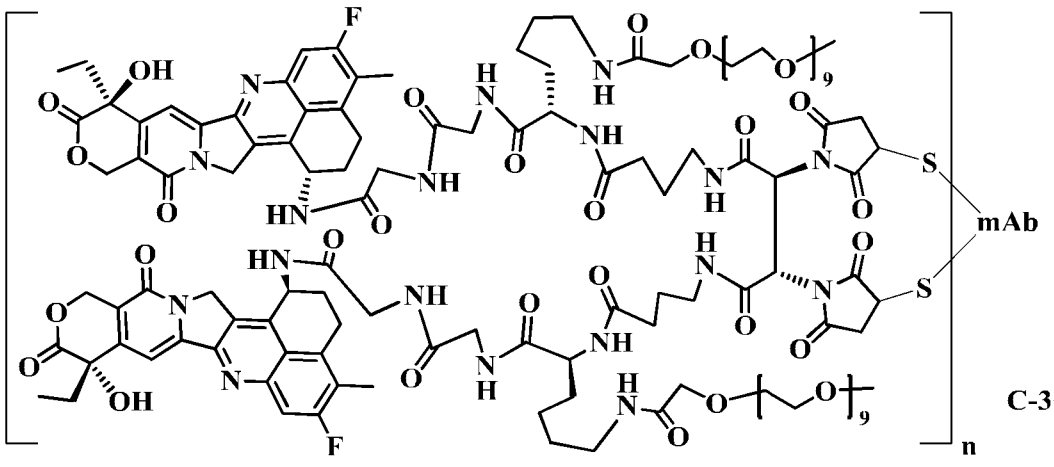
locyte antigen), Nectin-4 (ASG-22ME), NGF, Neural apoptosis-regulated proteinase 1, NOGO-A, Notch receptor, Nucleolin, Neu oncogene product, NY-BR-1, NY-ESO-1, OX-40, OxLDL (Oxidized low-density lipoprotein), OY-TES1, P21, p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolylneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1), PDGF-Rα (Alpha-type platelet-derived growth factor receptor), PDGFR-β, PDL-1, PLAC1, PLAP-like testicular alkaline phosphatase, Platelet-derived growth factor receptor beta, Phosphate-sodium co-transporter, PMEL 17, Polysialic acid, Proteinase3 (PR1), Prostatic carcinoma, PS (Phosphatidyl-serine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhPI)), Rhesus factor, RANKL, RhoC, Ras mutant, RGS5, ROBO4, Respiratory syncytial virus, RON, ROR1, Sarcoma translocation breakpoints, SART3, Sclerostin, SLAMF7 (SLAM family member 7), Selectin P, SDC1 (Syndecan 1), sLe(a), Somatome-din C, SIP (Sphingosine-1-phosphate), Somatostatin, Sperm protein 17, SSX2, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), STEAP2, STn, TAG-72 (tumor associated gly-coprotein 72), Survivin, T-cell receptor, T cell transmembrane protein, TEM1 (Tumor endothelial marker 1), TENB2, Tenascin C (TN-C), TGF-α, TGF-β (Transforming growth factor beta), TGF-β1, TGF-β2 (Transforming growth factor-beta 2), Tie (CD202b), Tie2, TIM-1 (CDX-014), Tn, TNF, TNF-α, TNFRSF8, TNFRSF10B (tumor necrosis factor receptor superfamily member 10B), TNFRSF-13B (tumor necrosis factor receptor superfamily member 13B), TPBG (trophoblast glyco-protein), TRAIL-R1 (Tumor necrosis apoptosis Inducing ligand Receptor 1), TRAILR2 (Death receptor 5 (DR5)), tumor-associated calcium signal transducer 2, tumor specific glycosylation of MUC1, TWEAK receptor, TYRP1(glycoprotein 75), TRP-1 (Trop1), TRP-2 (Trop2), Tyrosinase, VCAM-1, VEGF, VEGF-A, VEGF-2, VEGFR-1, VEGFR2, or vimentin, WT1, XAGE 1, or cells expressing any insulin growth factor receptors, or any epidermal growth factor receptors.

12. The process according to Claim 4, wherein the conjugate, in which the drugs are mainly conjugated to sites of disulfide bond between heavy-light chains (Fab) of an antibody has one of the structures of C-25, C-30, C-36, C-45, C-51, C-58a, C-68a, C-68b, C-68c, C-72a, C-72b, C-73, C-83, C-88, C-91, C-96, C-102, C-115, C-120, C-121, C-126, C-130, C-137, C-140, C-152 C-157, C-168, C-178, C-181a, C-181b, C-181c, C-181d, C-190, C-192 C-195, C-202, C-209, C-215, C-221, C-221b, C-227, C-233, C-241, C-255, C-258, C-267, C-277, C-283, C-284, C-290, C-295, C-300, C-306, C-309, C-310, C-312, C319, C-322, C-326, C-328, C-330, C-334, C-335, C-336, C-337, C-338, C-361, C-362, C-372, C-373, C-374, C-375, C-384, C-385, C-390, C-396, C-399, C-400, C-402, C-406, C-409, C-416, C-417, C-427, C-432, C-437, C-441, C-450, C-455, C-462, C-464, C-470, C-478, C-481, C-484, C-487, C-488, C-494, C-498, C-499, C-500, C-501, C-504, C-508, C-511, C-514, C-518, C-519, C-521, C-527, C-531, C-532, C-537, C-542, C-543, C-544, C-546, C-550, C-553, C-560, C-561,

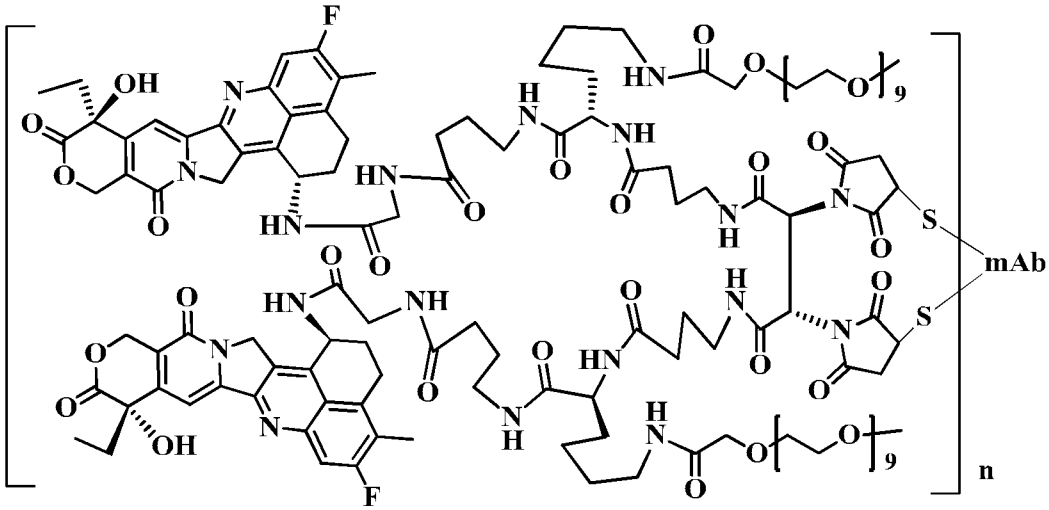
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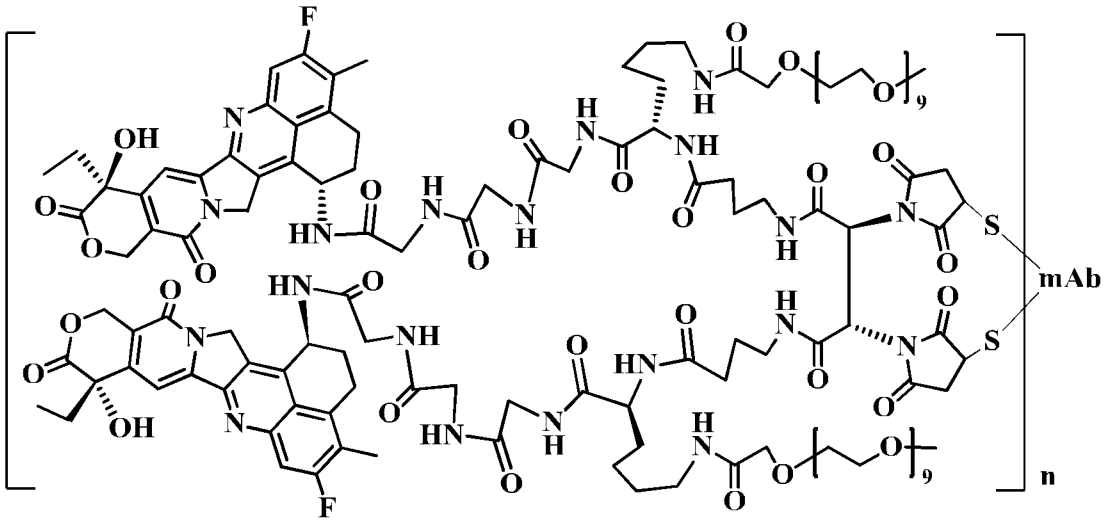


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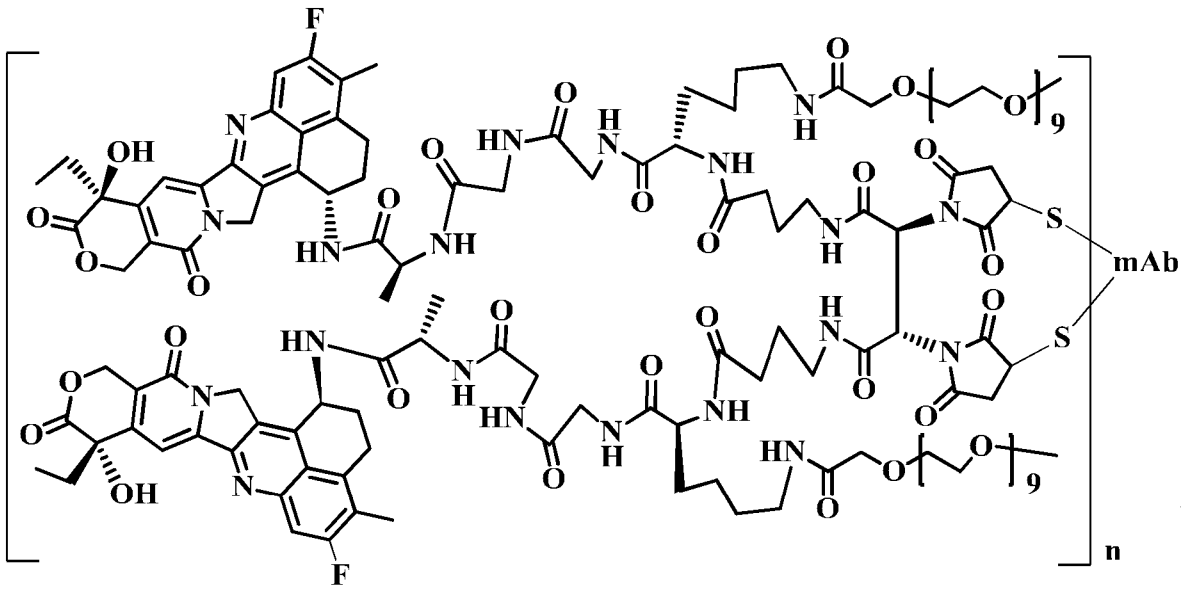
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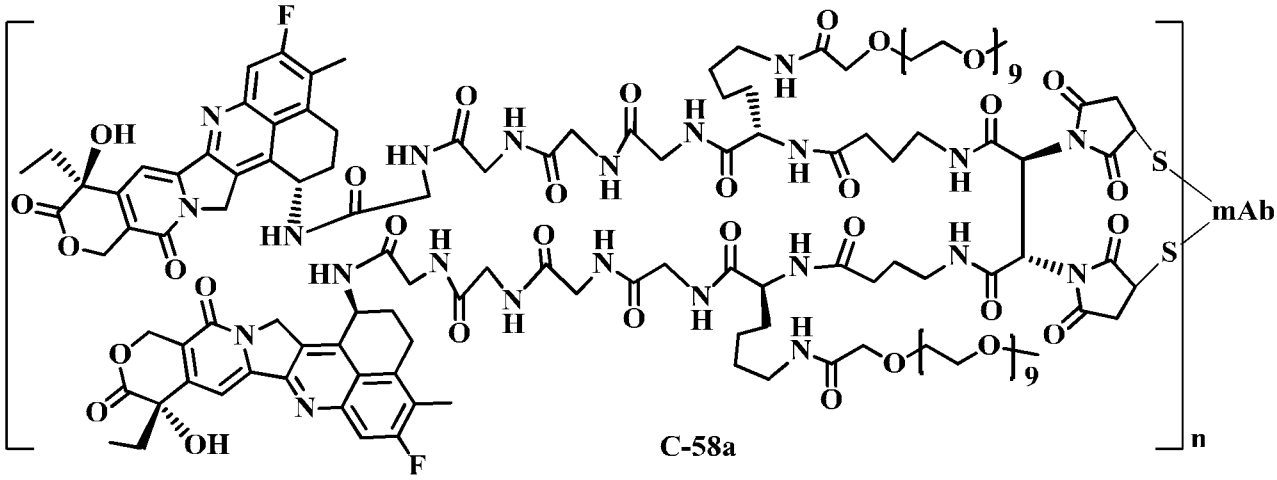
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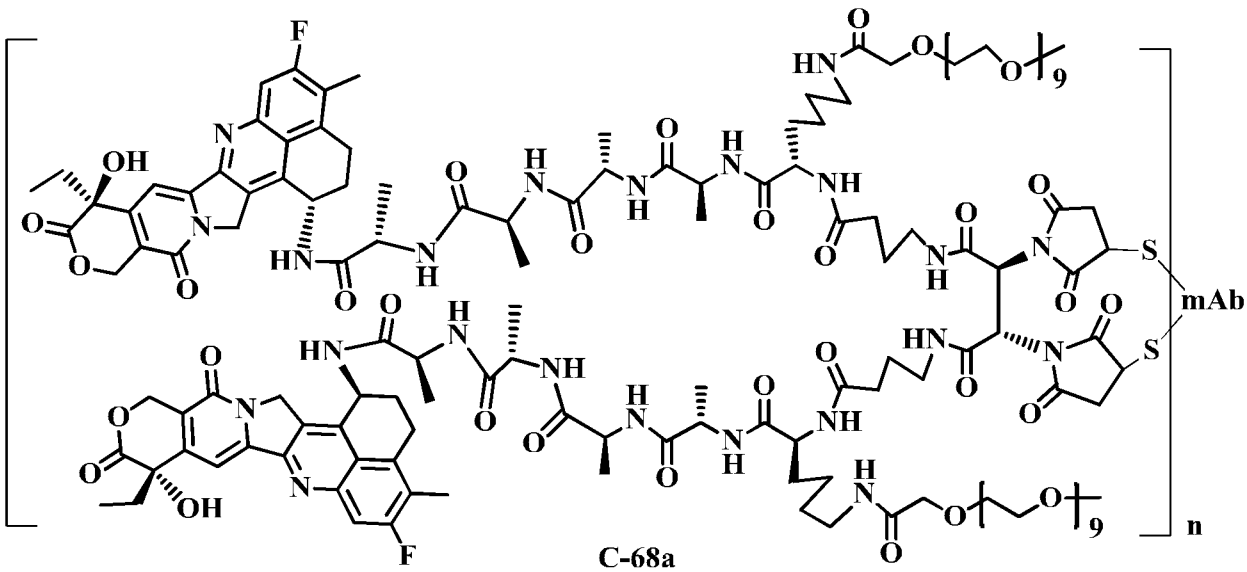


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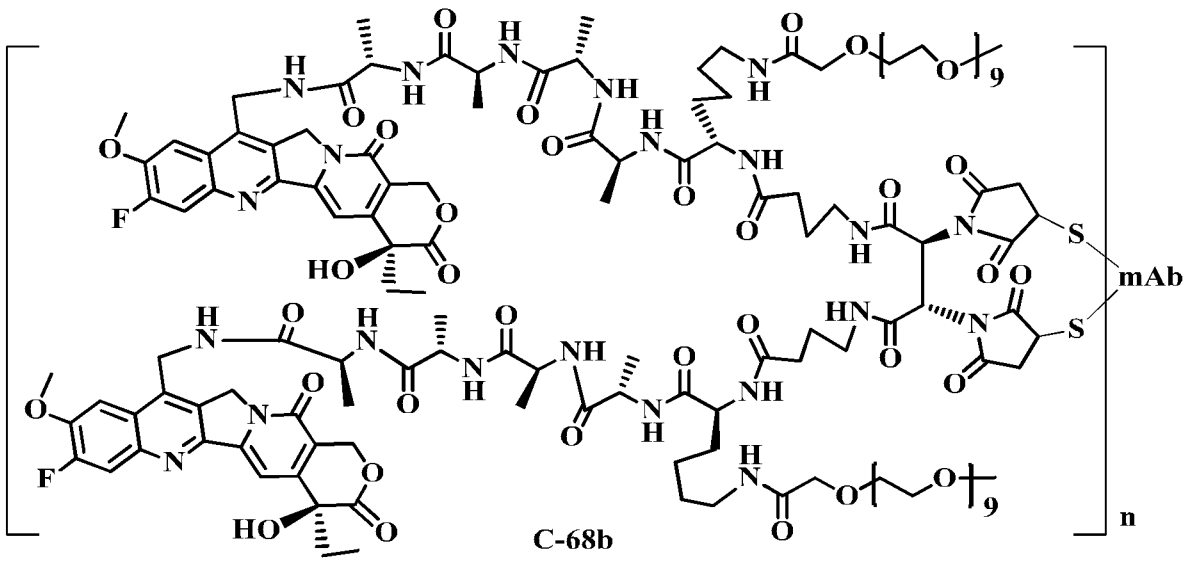
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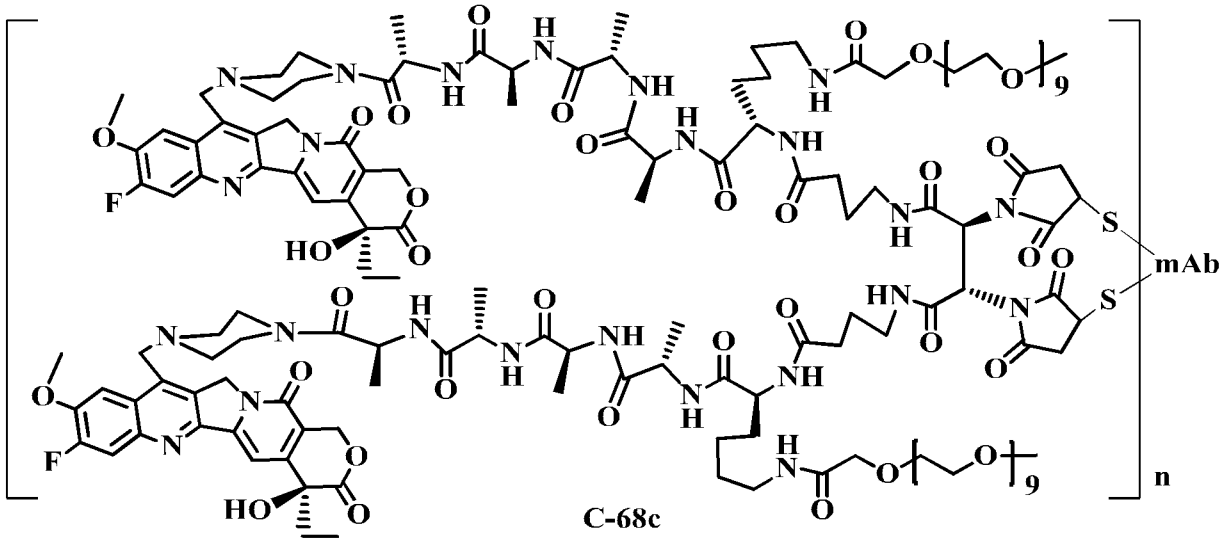
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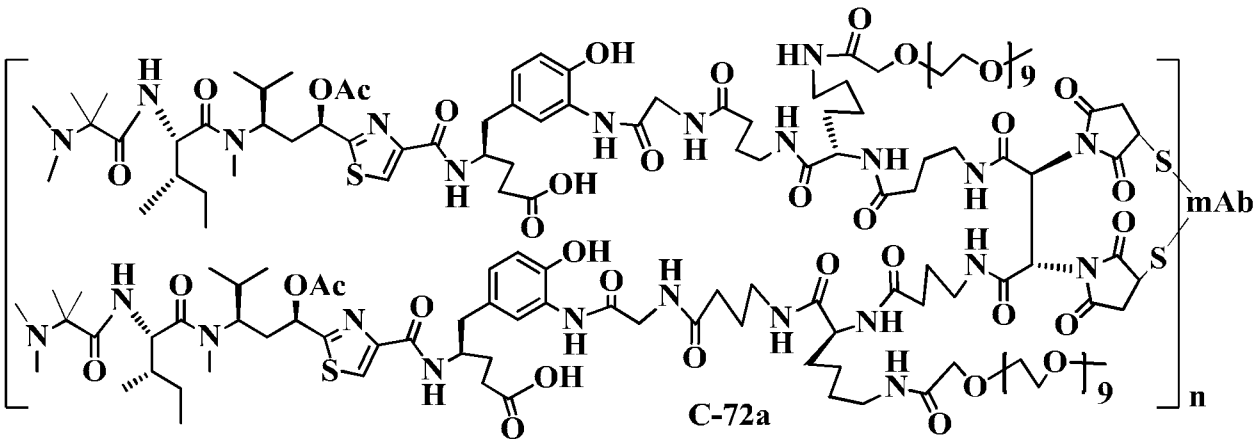
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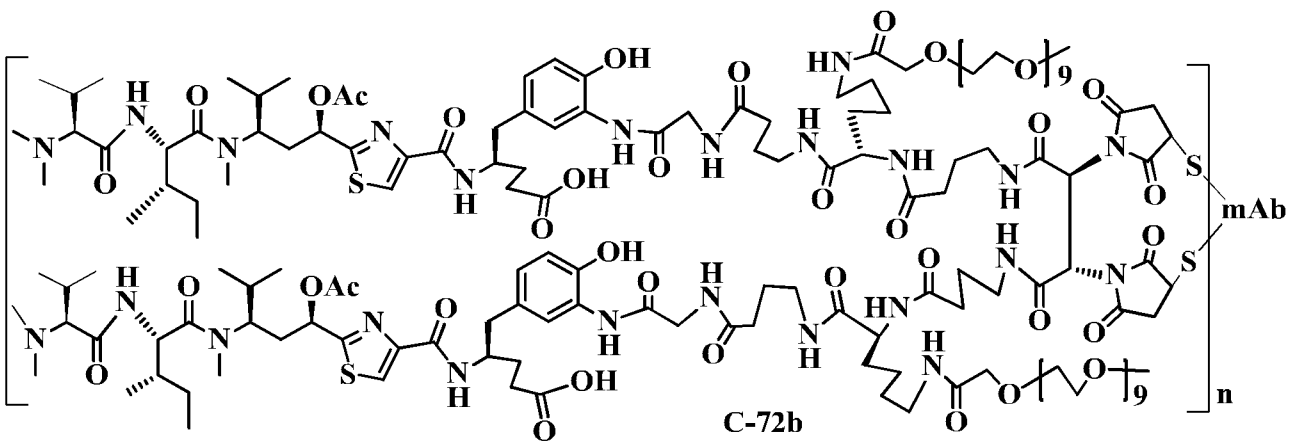
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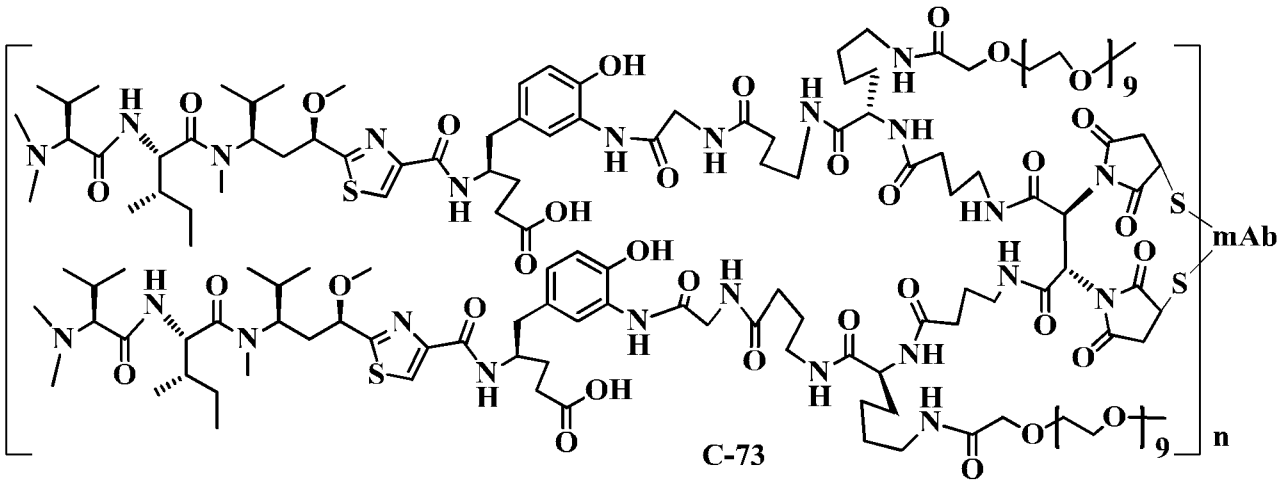
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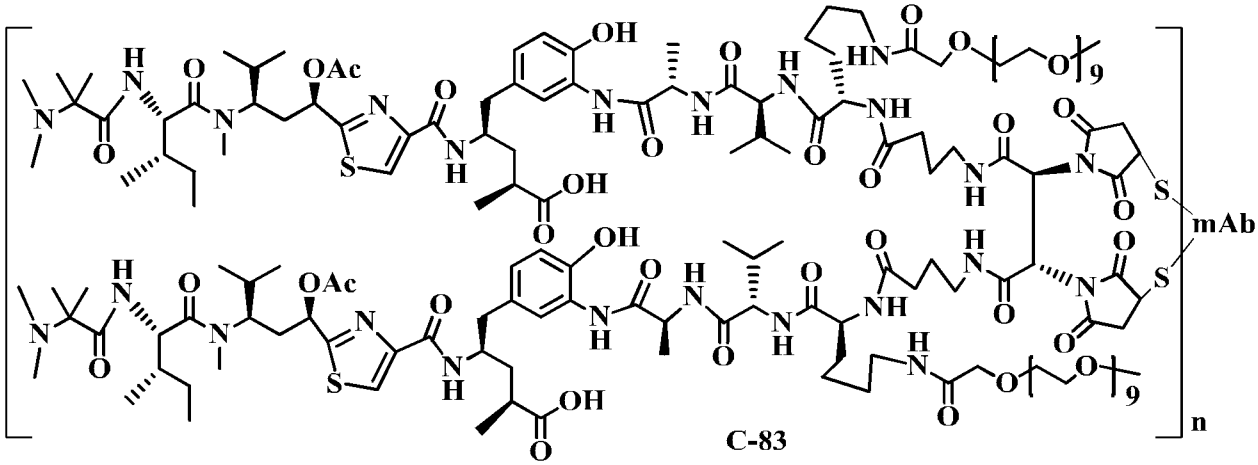
C-72a ,



C-72b ,

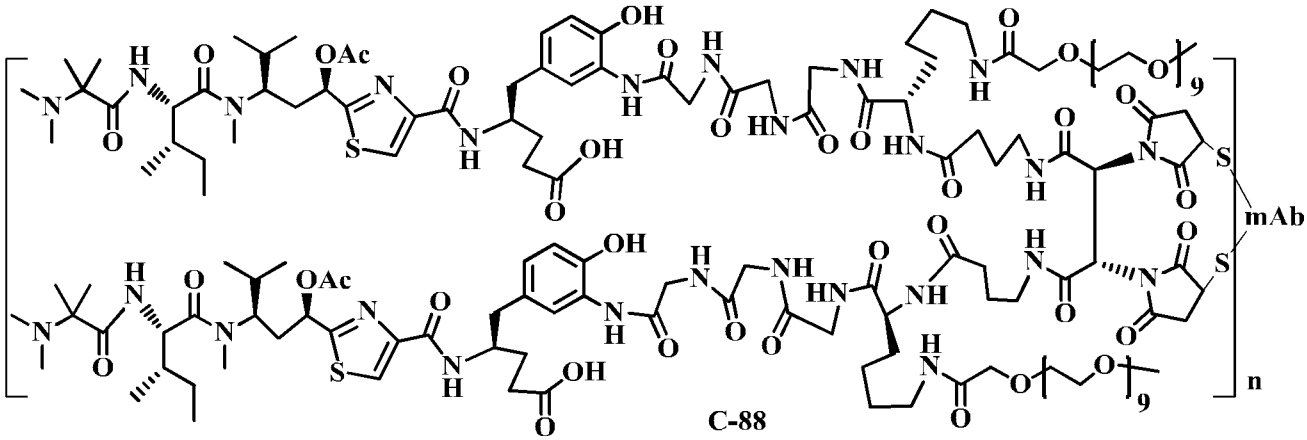


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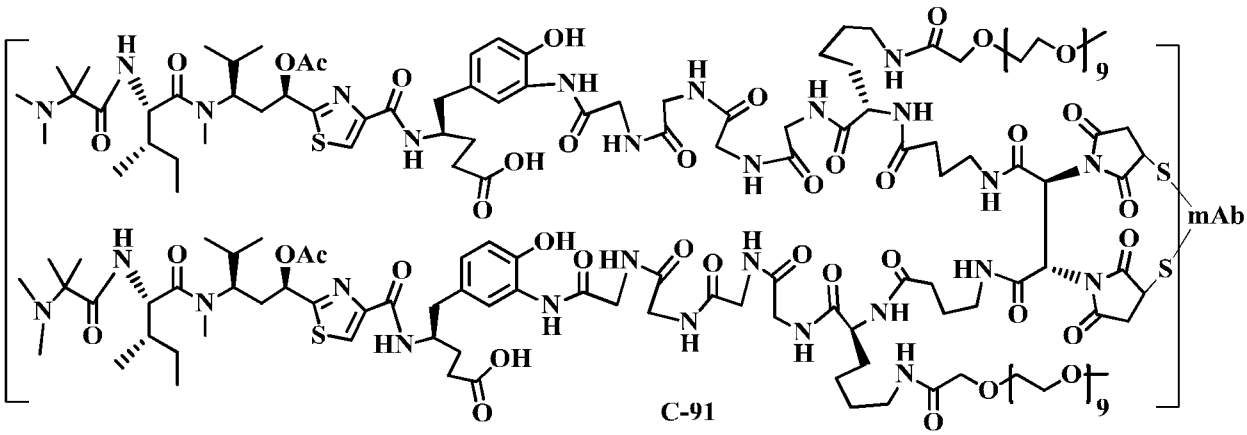
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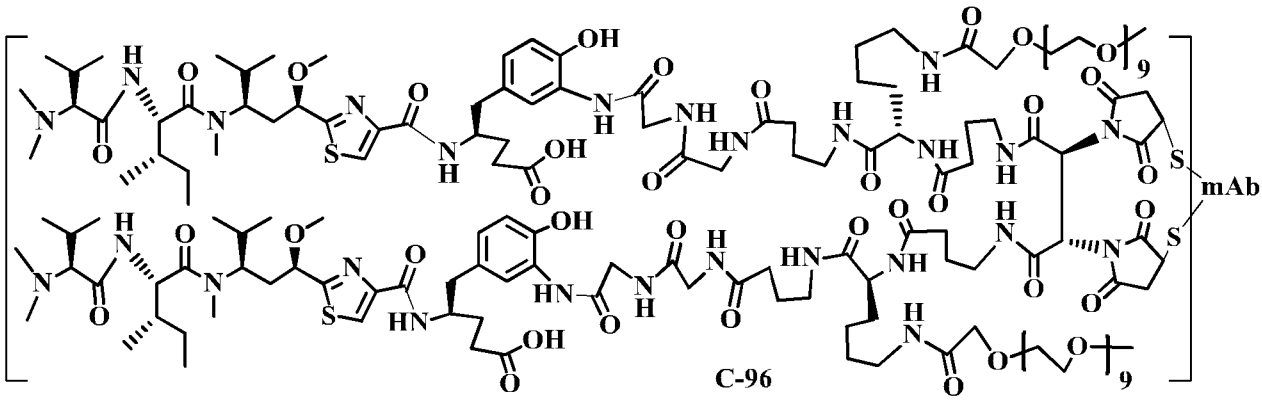
C-88

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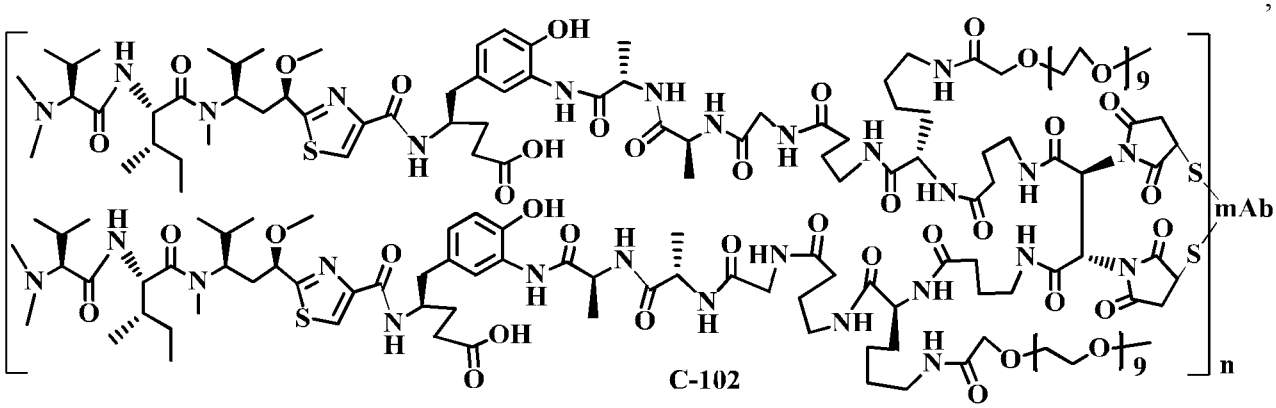


C-91

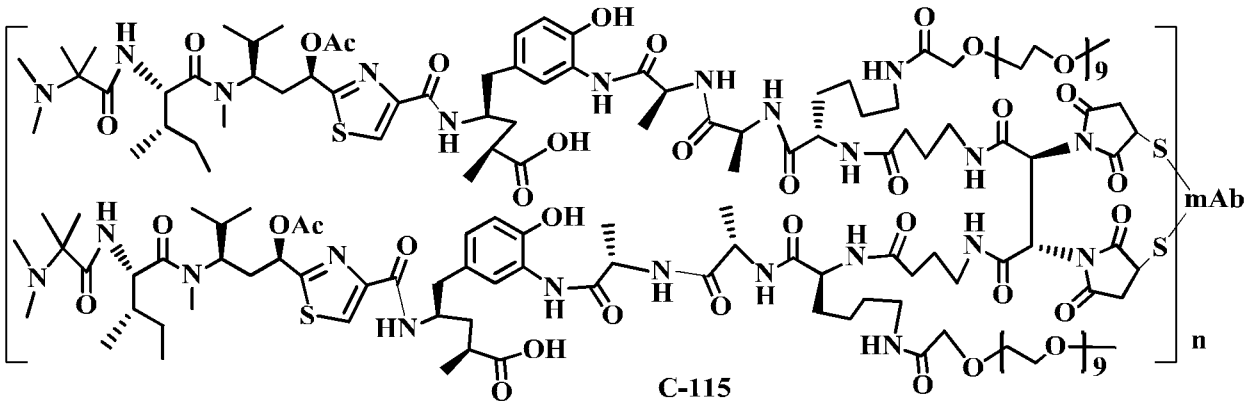
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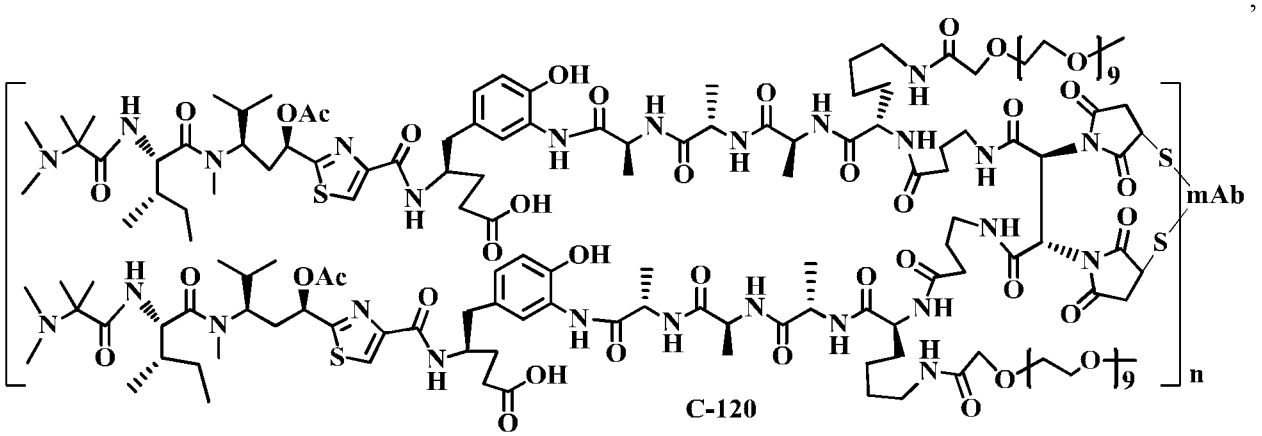
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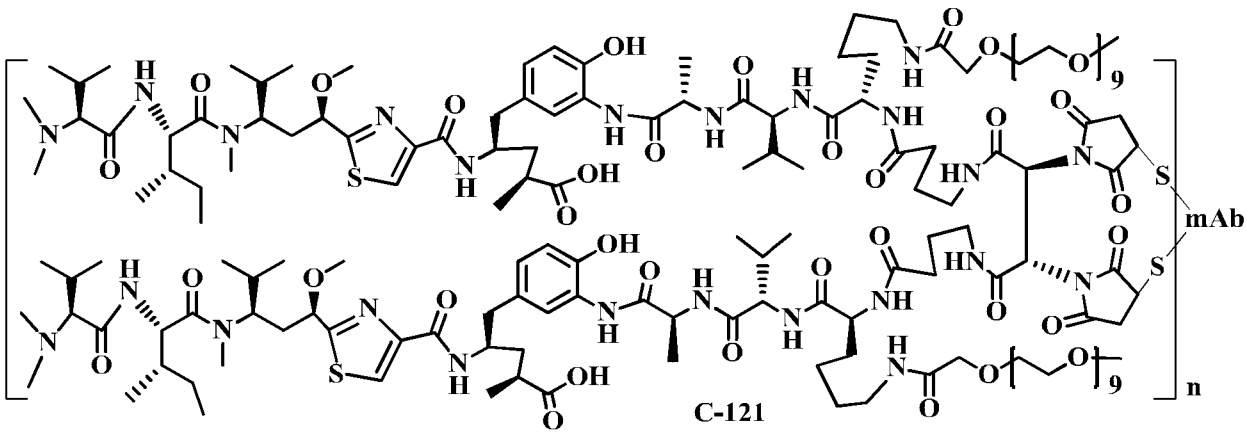
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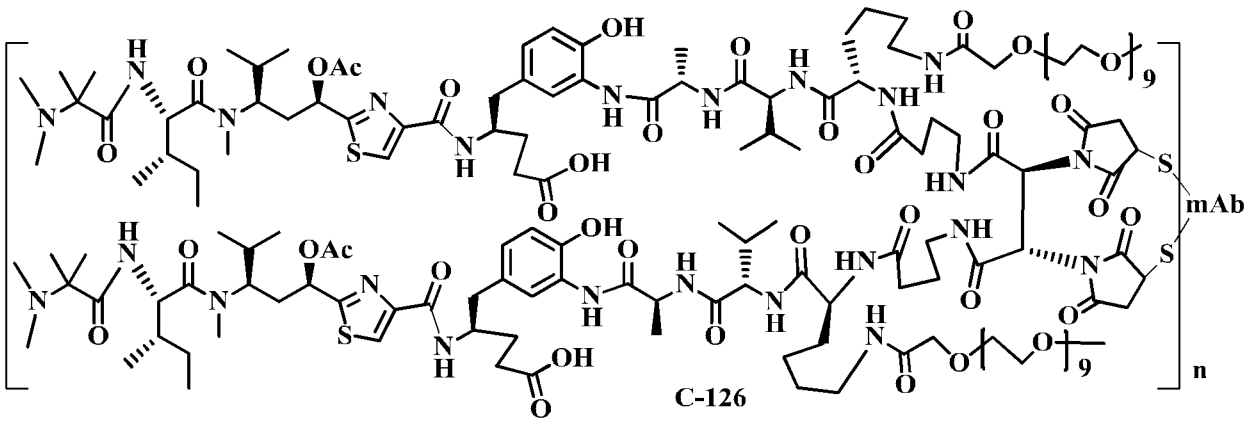
C-115 ,



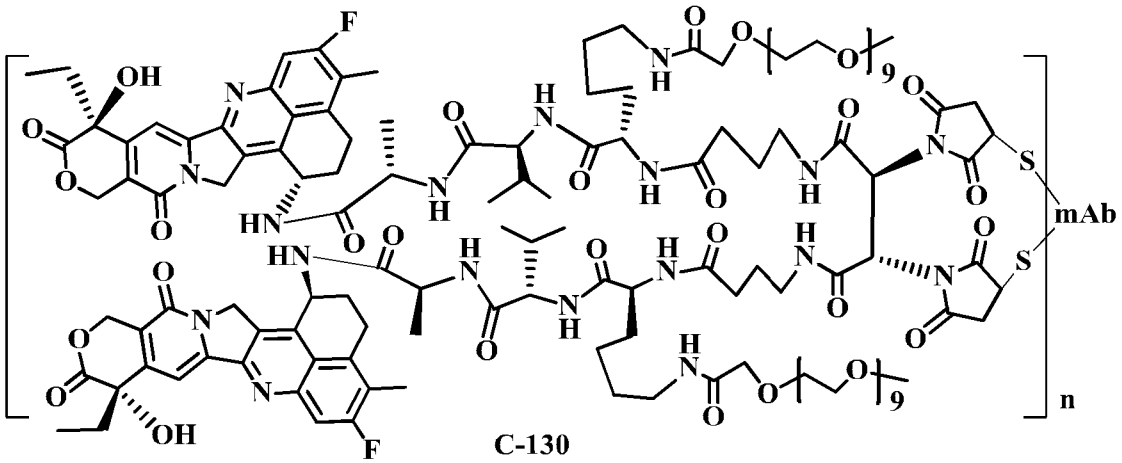
C-120 ,



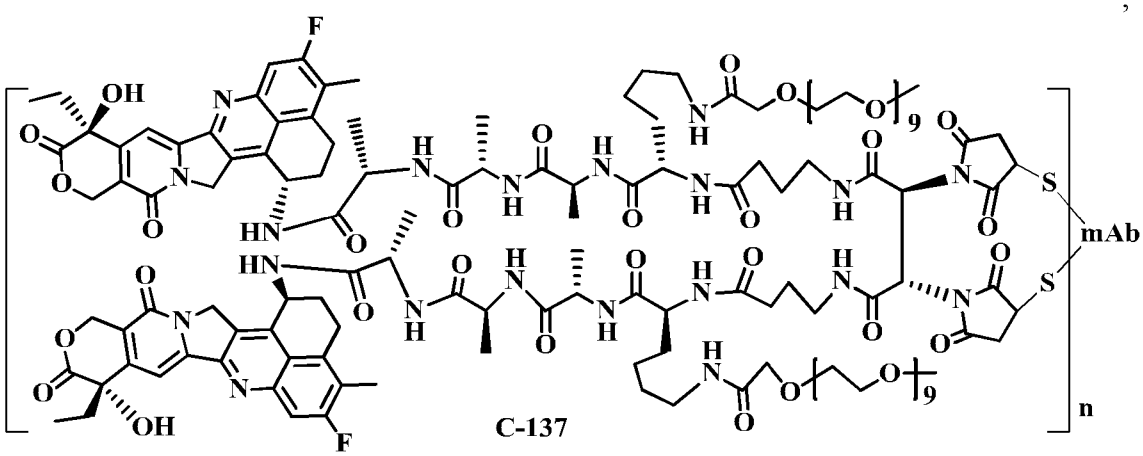
C-121 ,



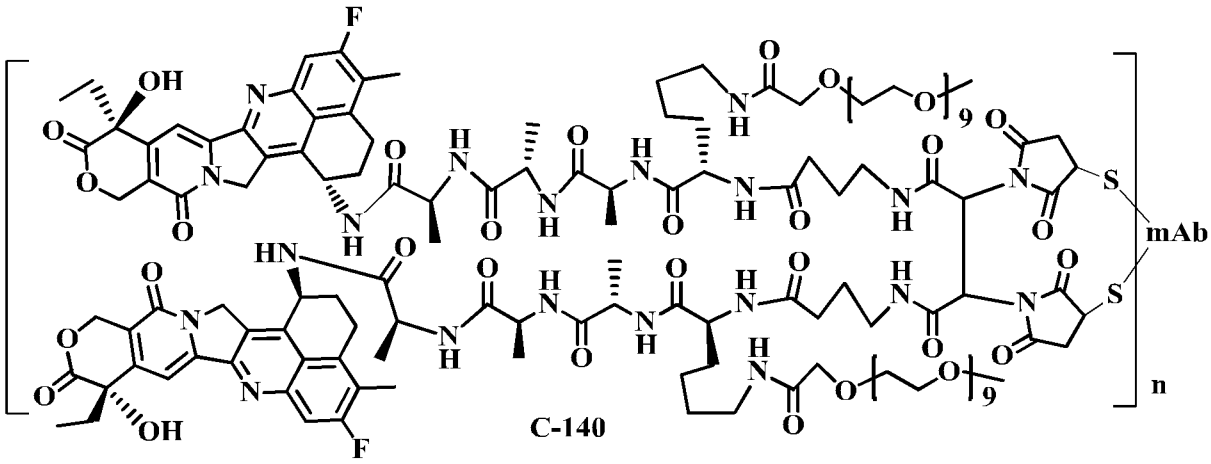
C-126 ,



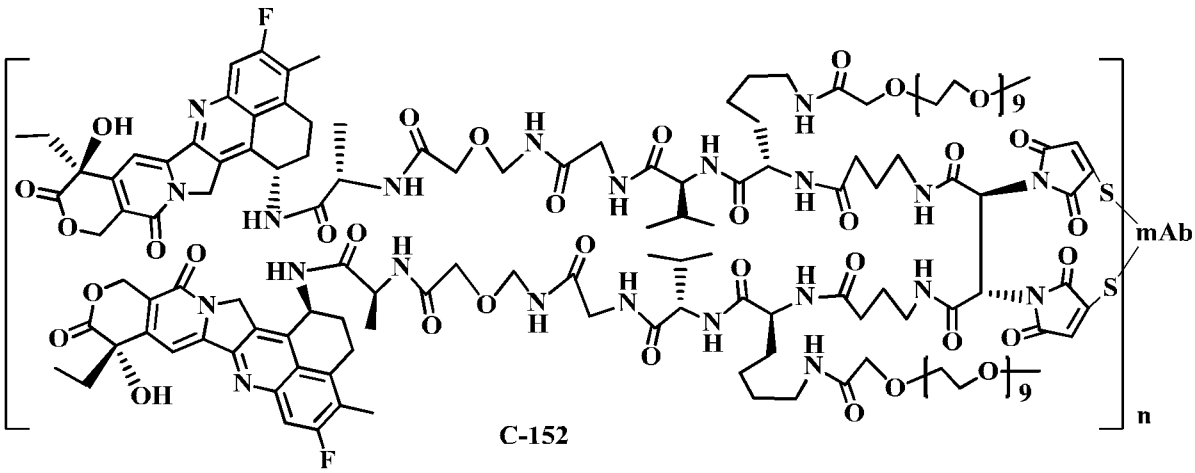
C-130 ,



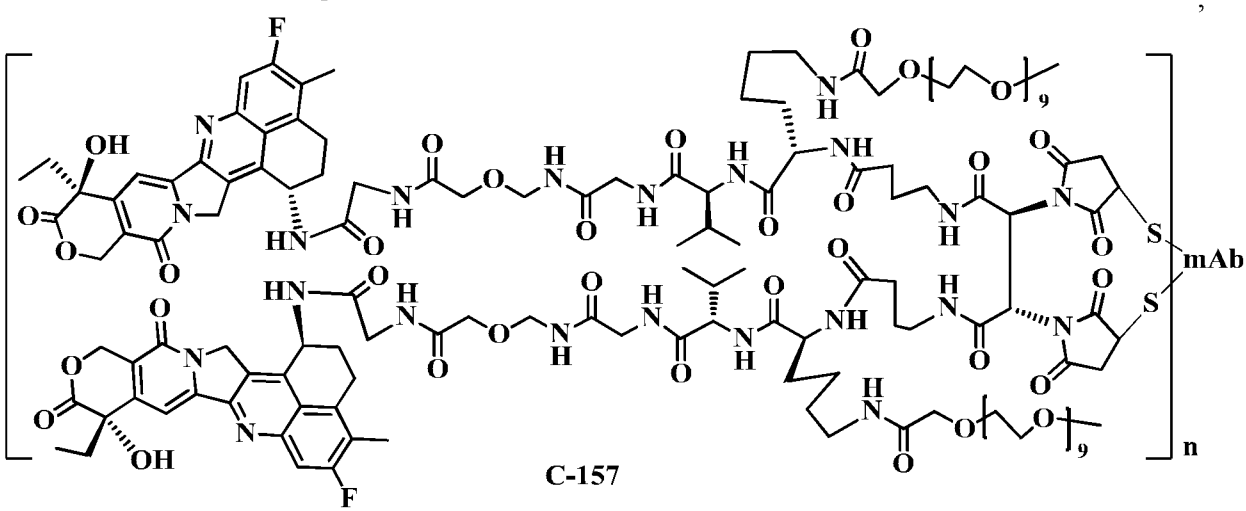
C-137 ,



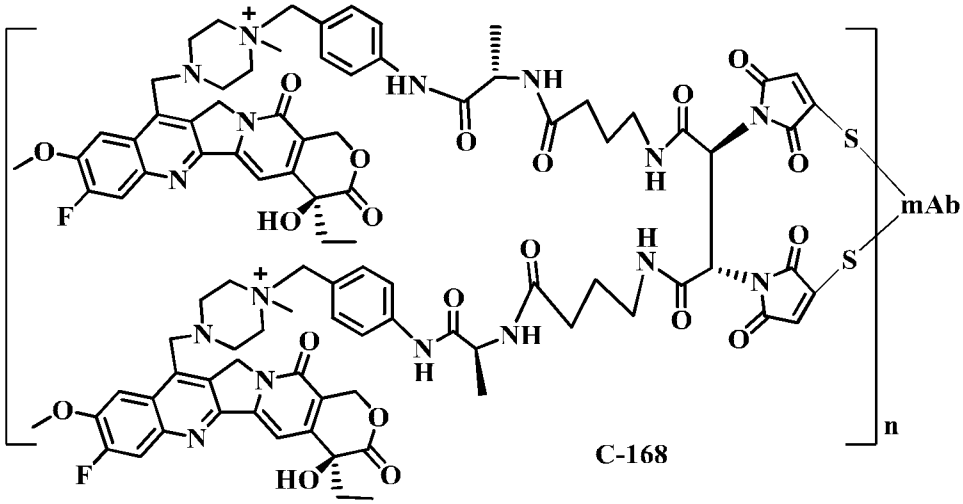
C-140 ,



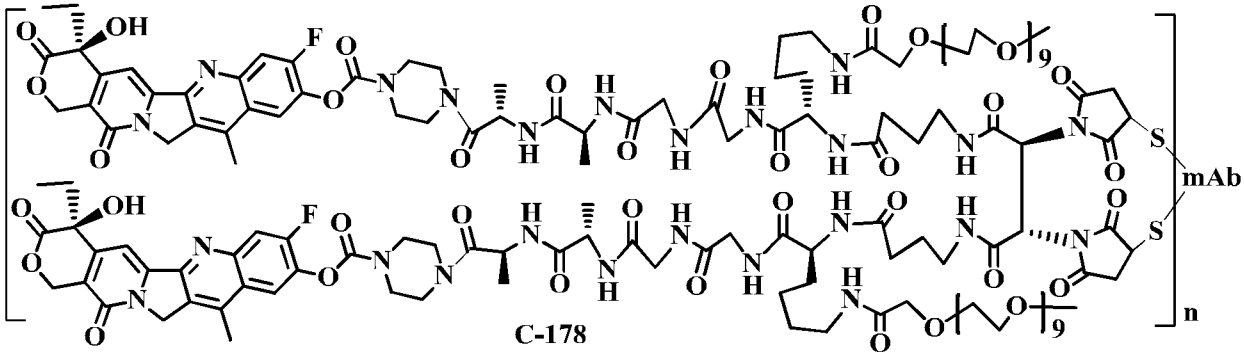
C-152 ,



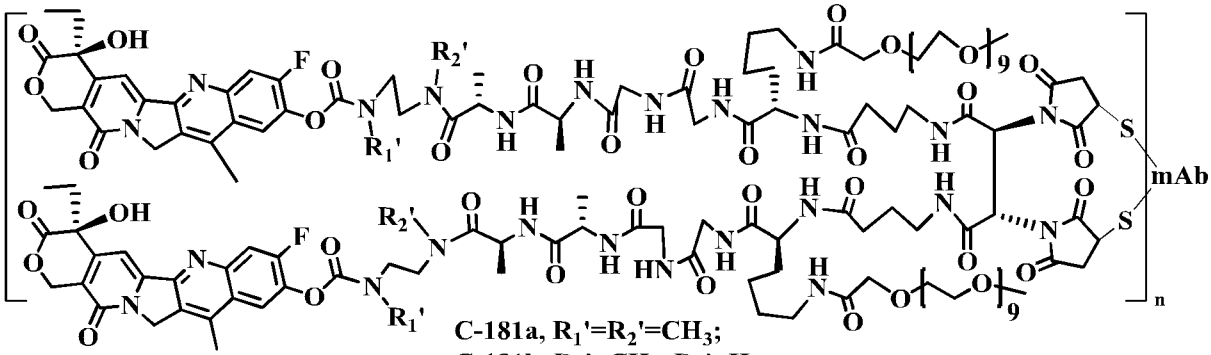
C-157 ,



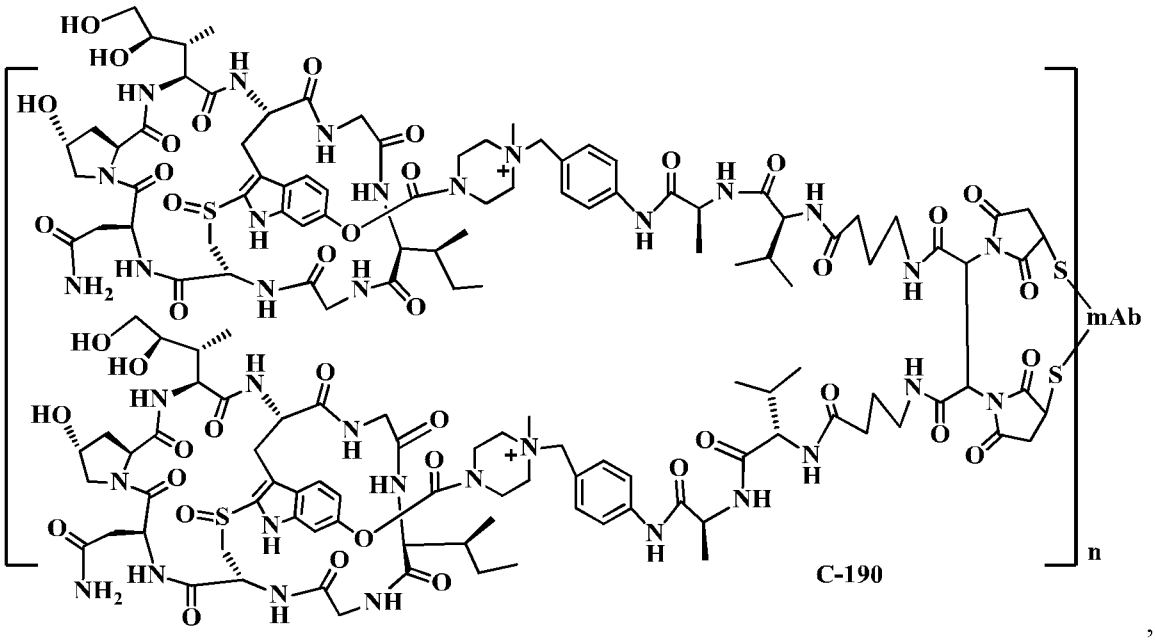
C-168



C-178

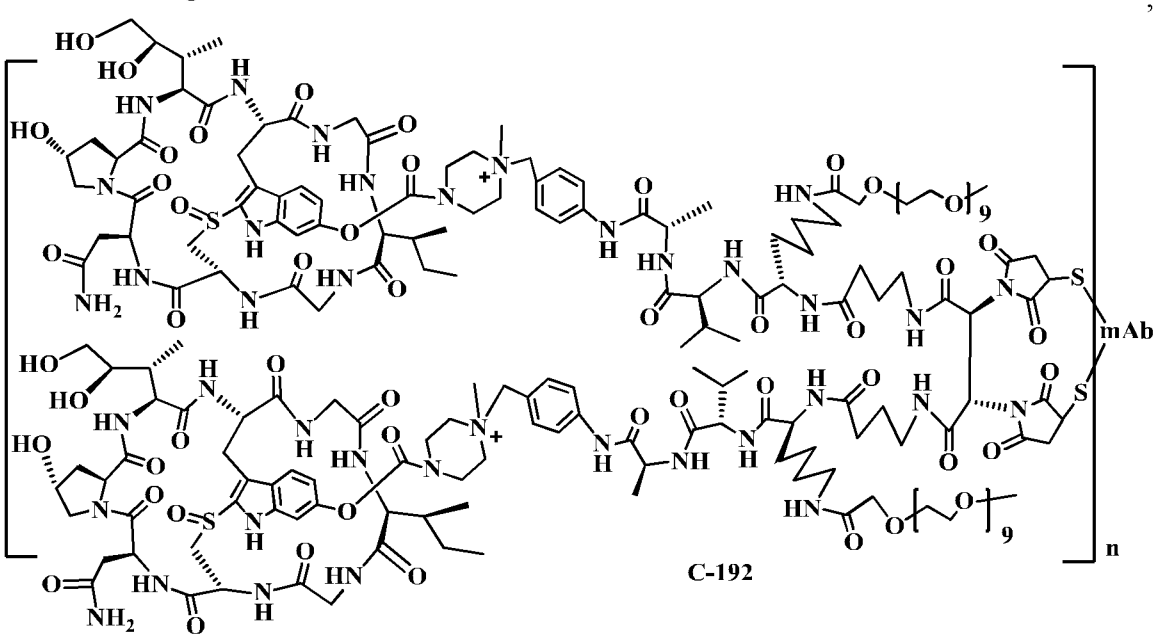


C-181a, $R_1'=R_2'=CH_3$;
C-181b, $R_1'=CH_3$, $R_2'=H$;
C-181c, $R_1'=H$, $R_2'=CH_3$;
C-181d, $R_1'=R_2'=H$;



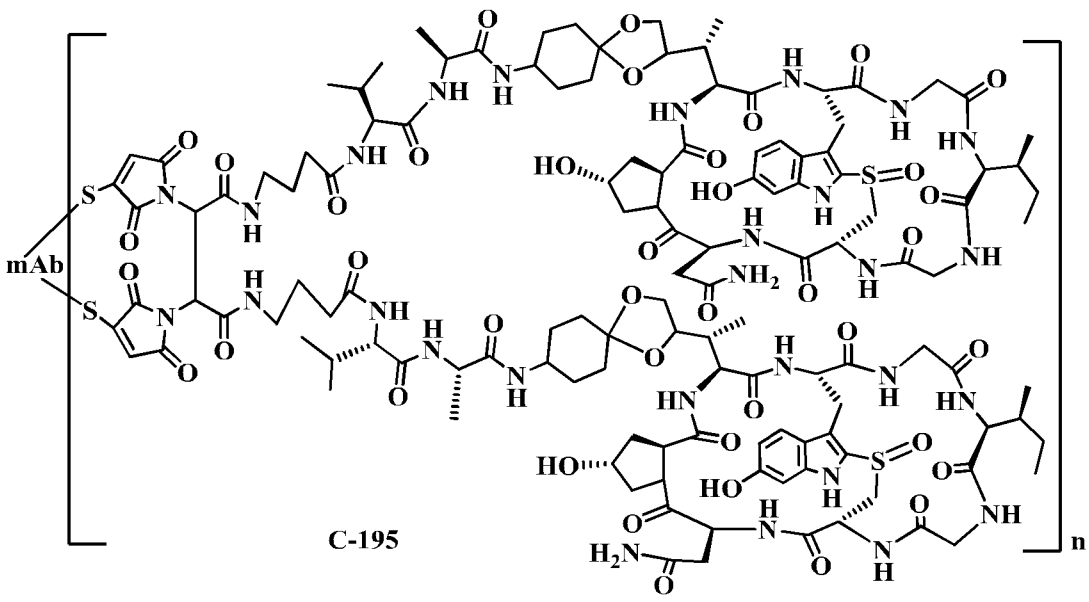
C-190

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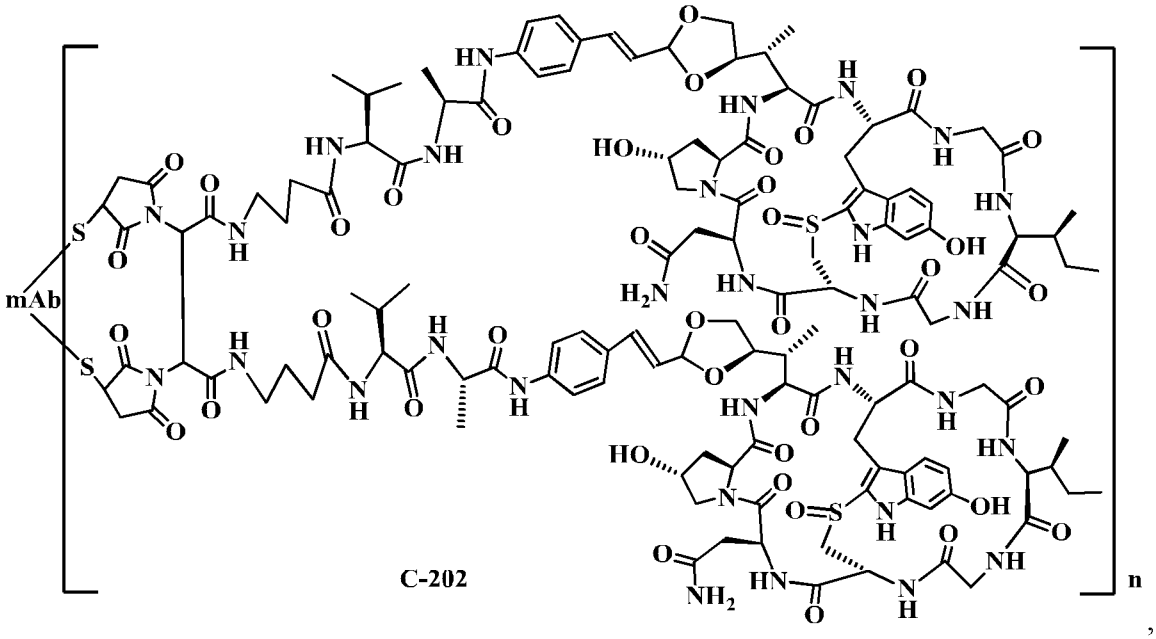
C-192

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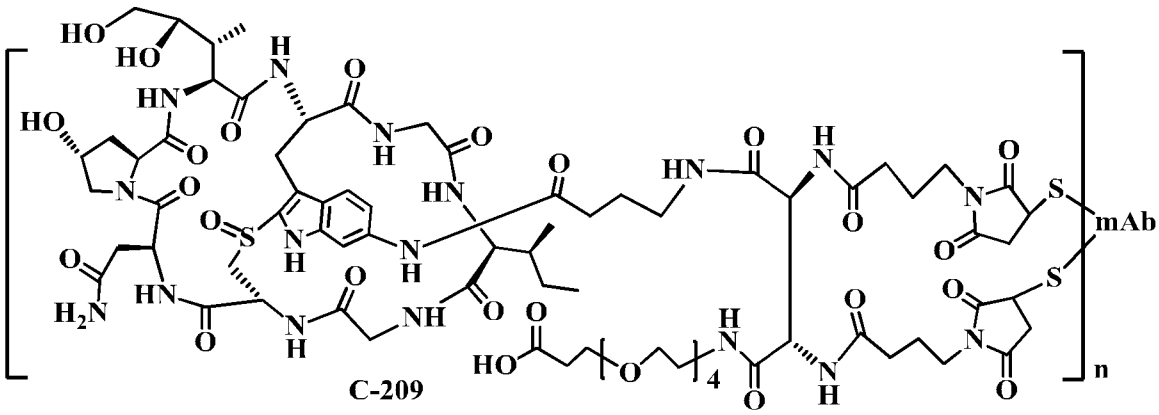


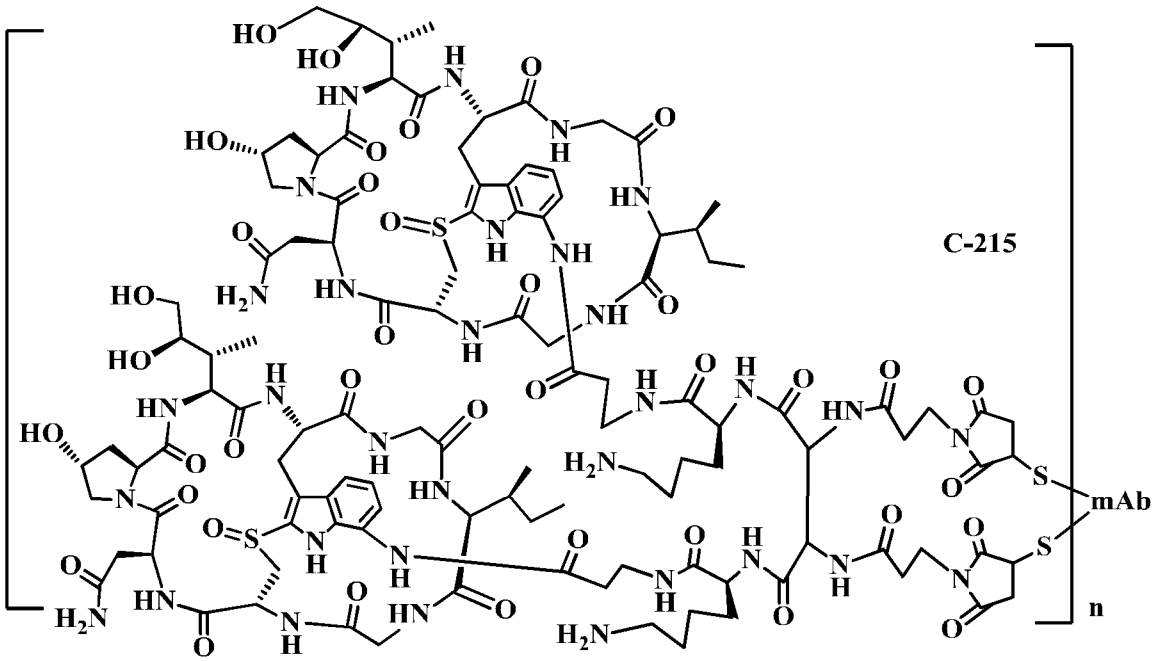
C-195

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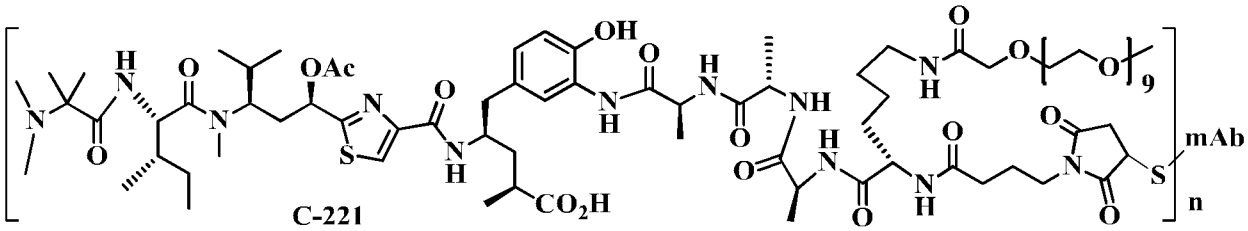

mAb
C-202
n

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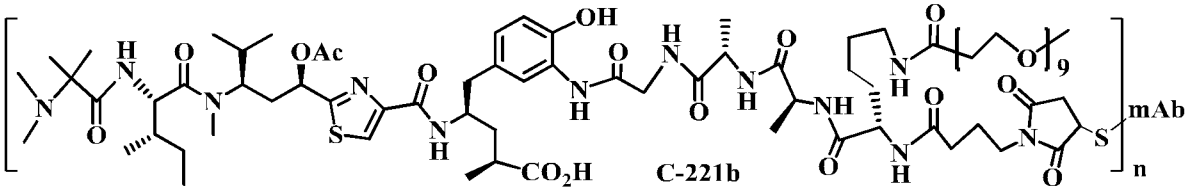

mAb
C-209
n


C-215
mAb
n

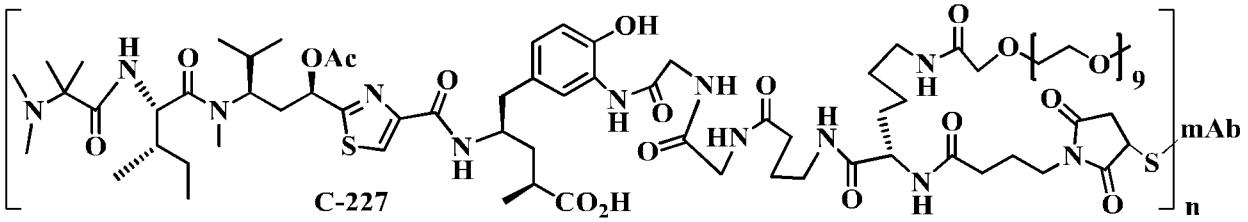
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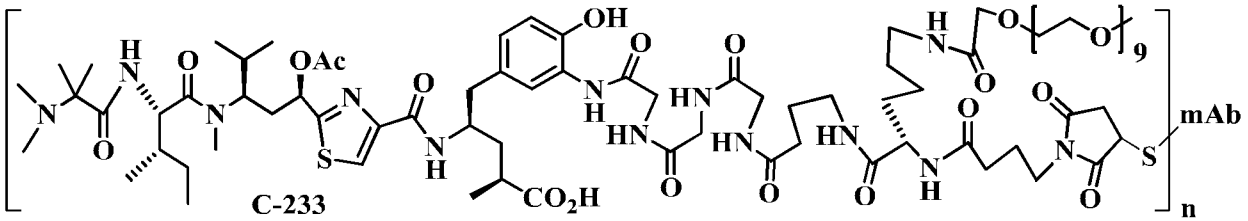
C-221,



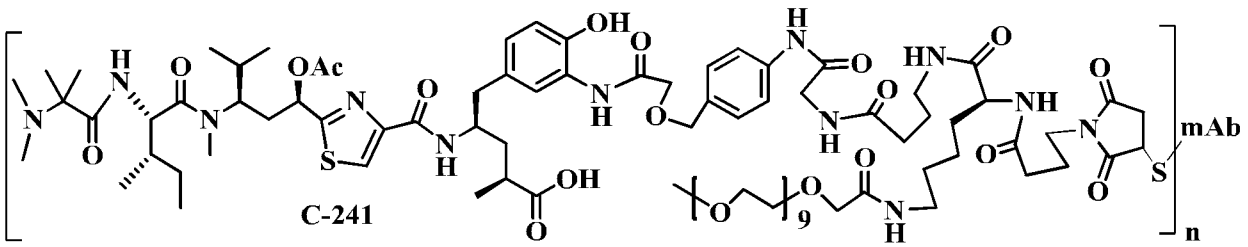
C-221b,



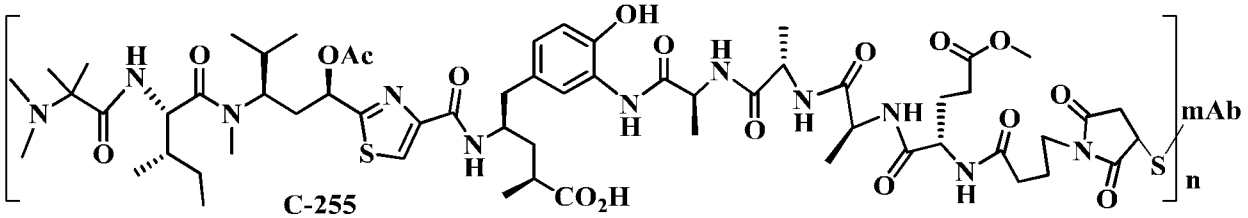
C-227,



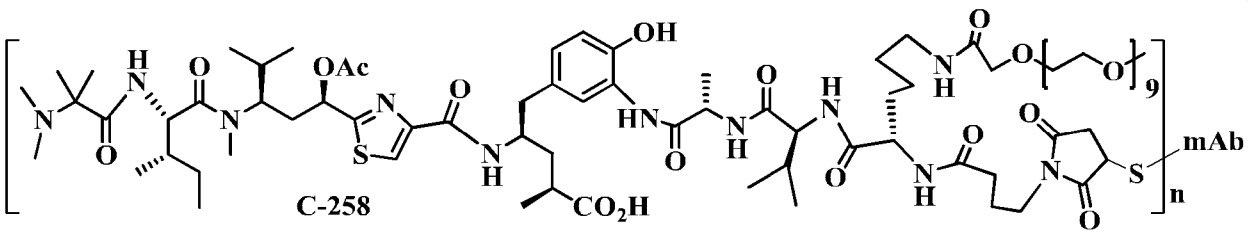
C-233,



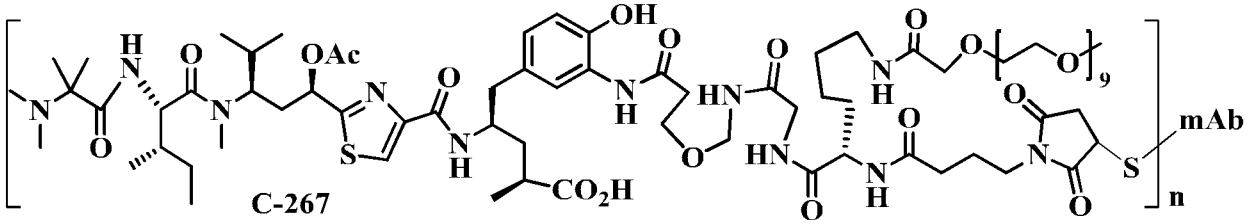
C-241,



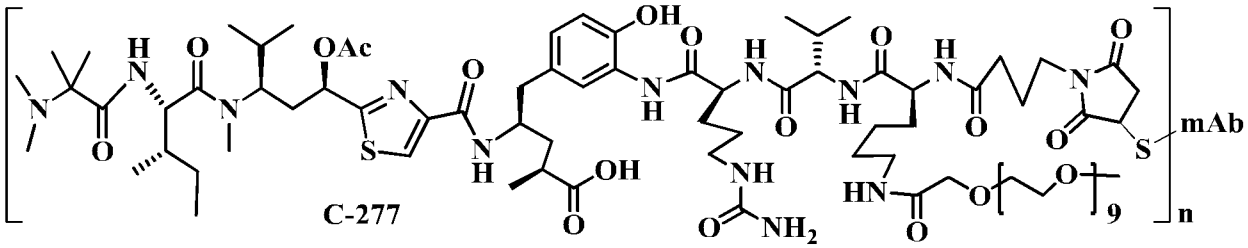
C-255,



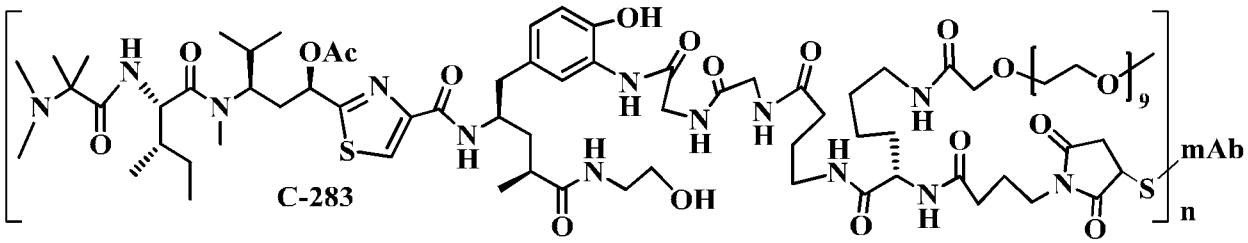
C-258,



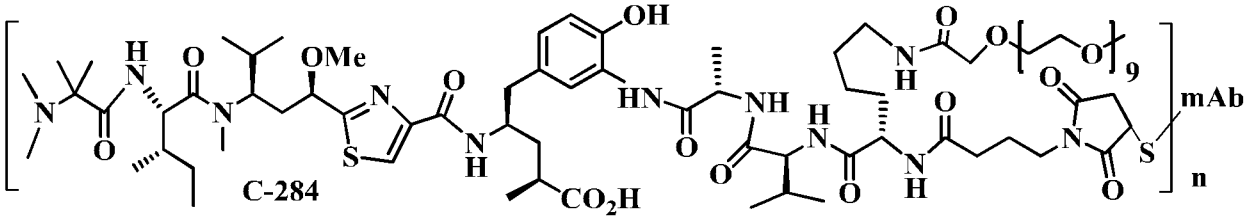
C-267,



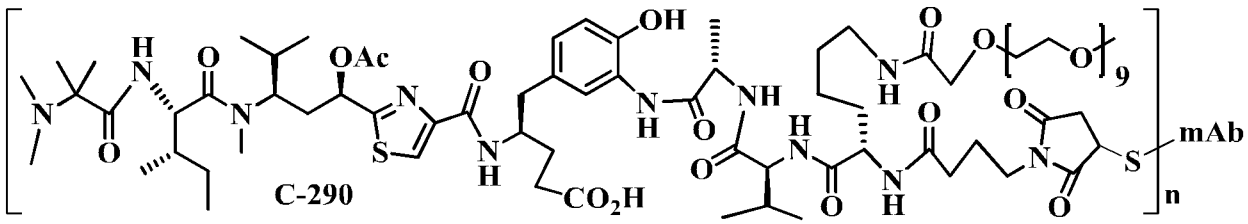
C-277 ,



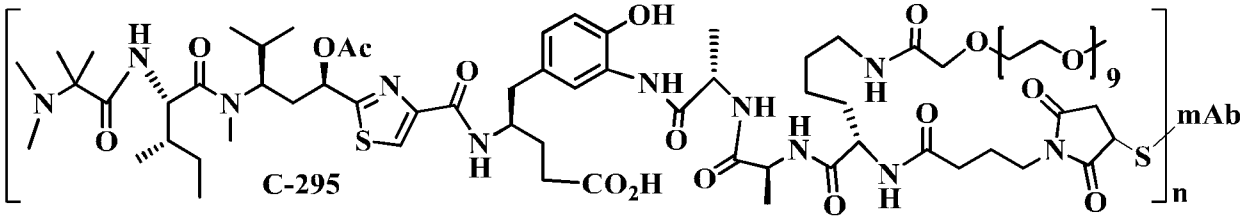
C-283 ,



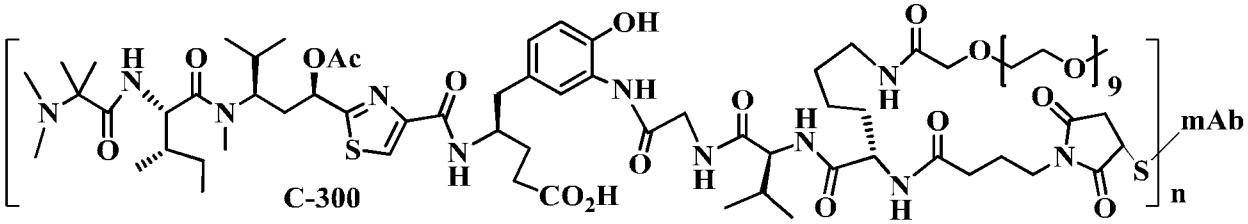
C-284 ,



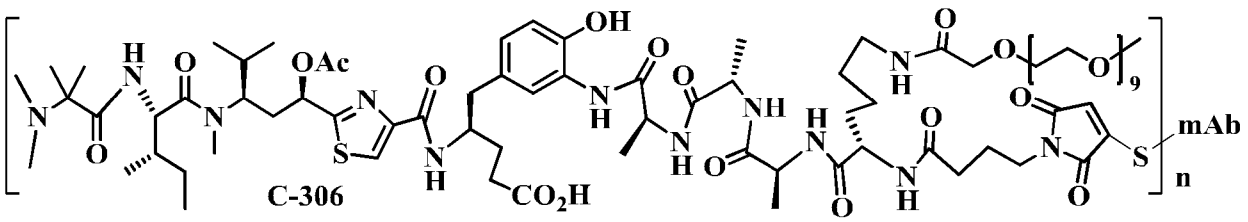
C-290 ,



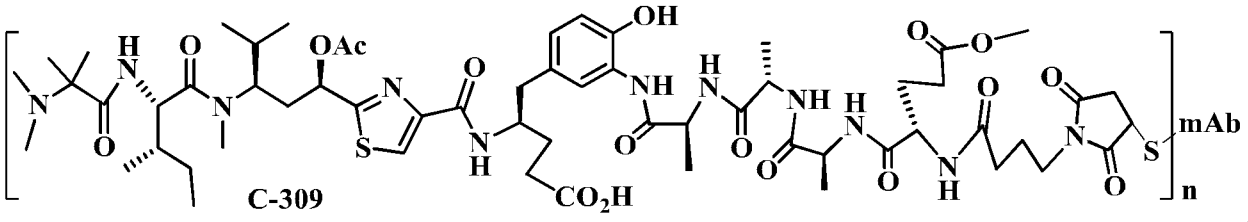
C-295 ,



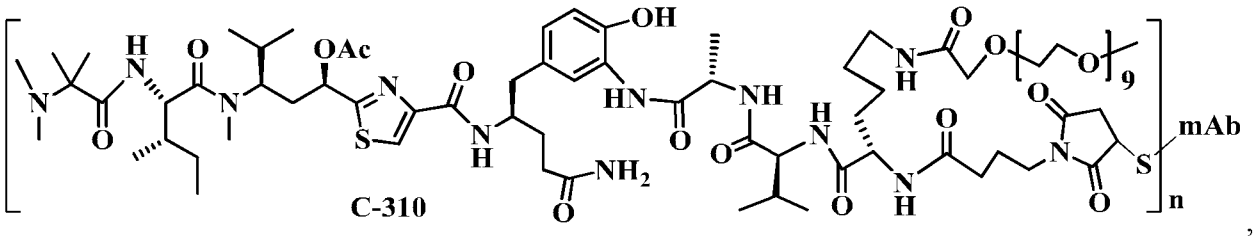
C-300 ,



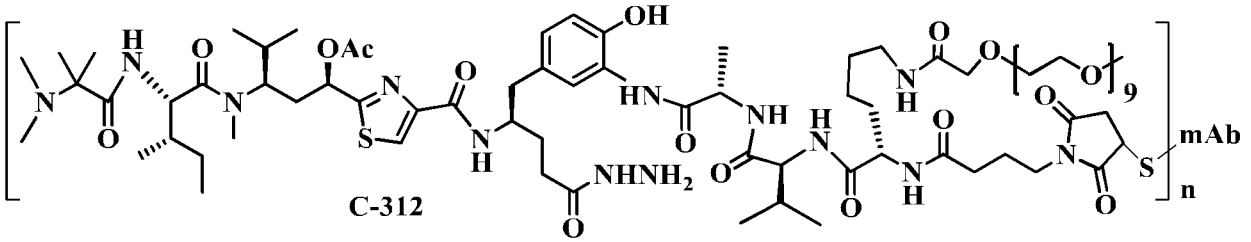
C-306 ,



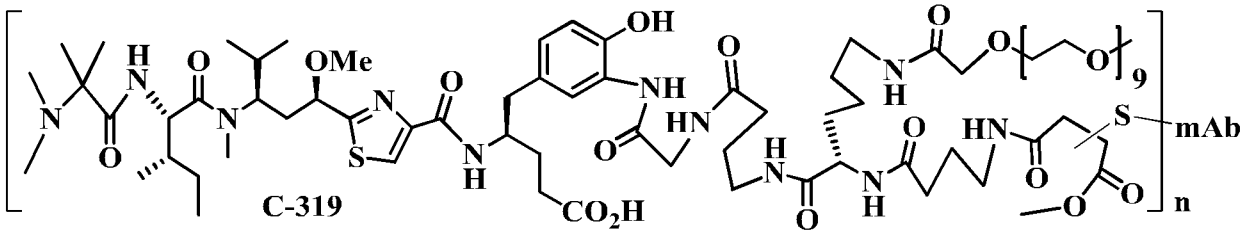
C-309 ,



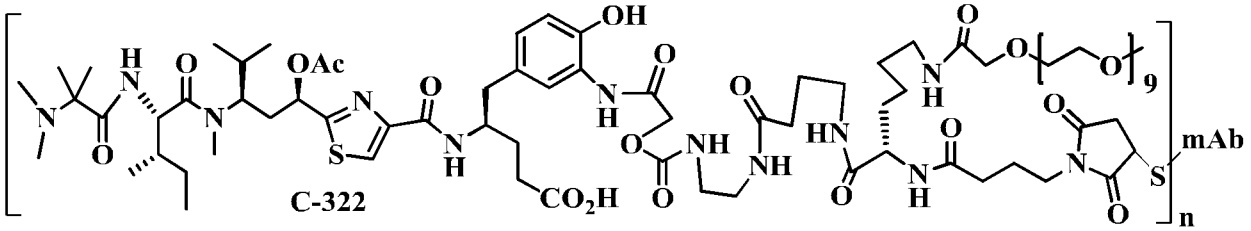
C-310 ,



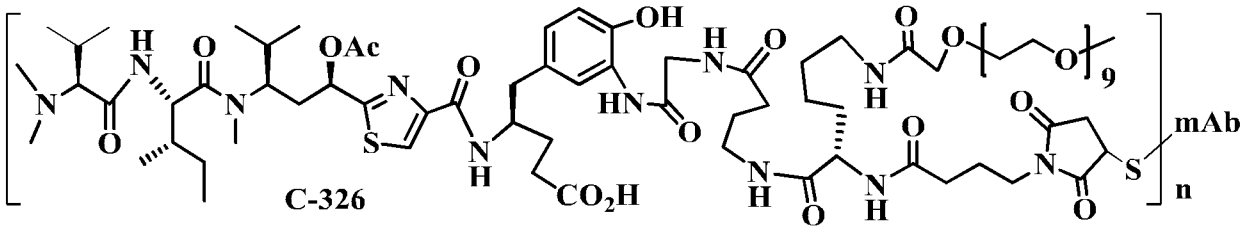
C-312 ,



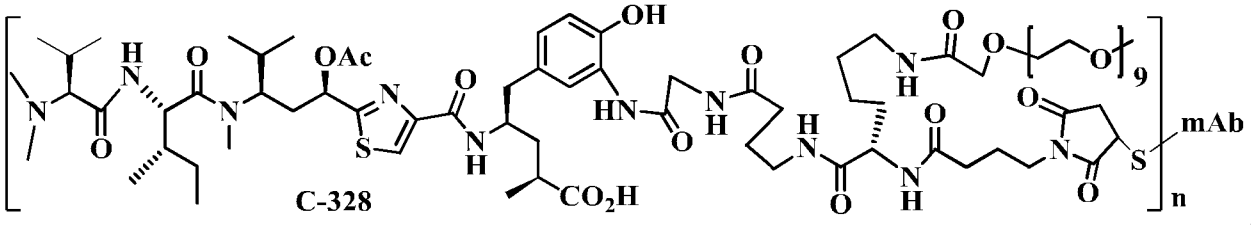
C-319 ,



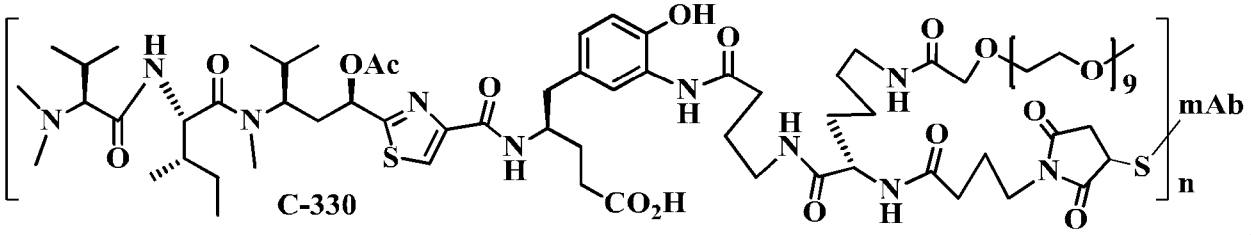
C-322 ,



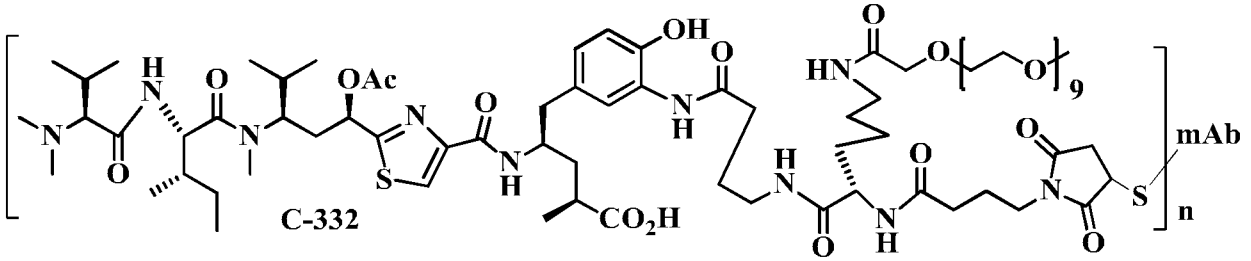
C-326 ,



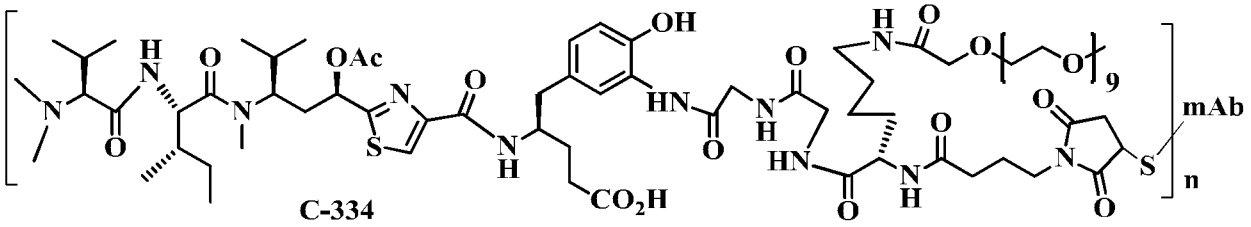
C-328 ,



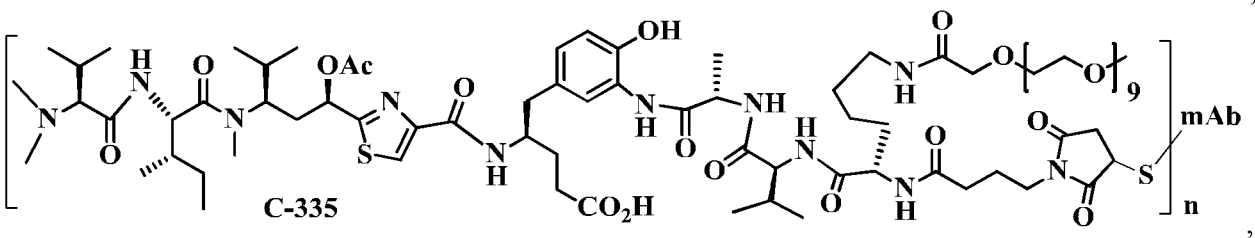
C-330 ,


C-332
mAb
n

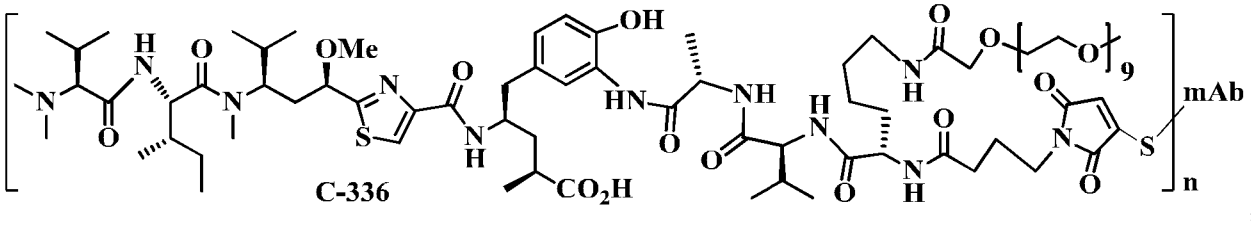
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C-334
mAb
n

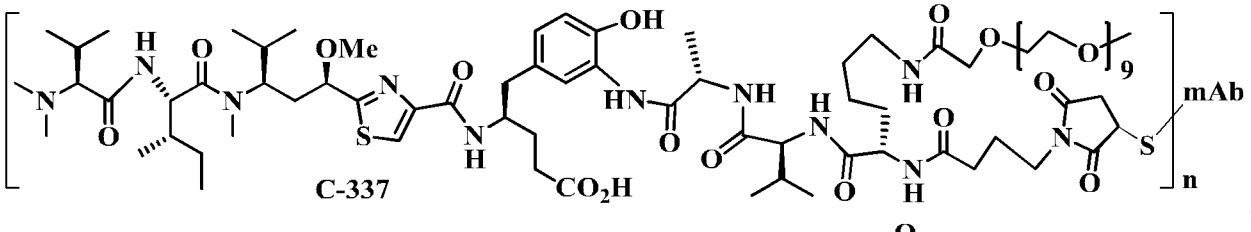
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C-335
mAb
n

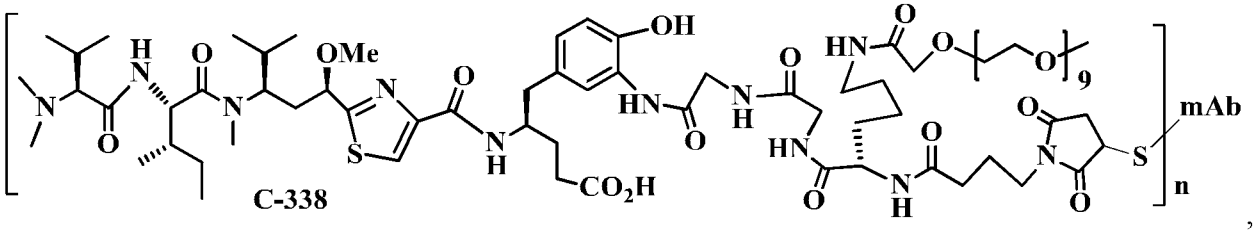
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C-336
mAb
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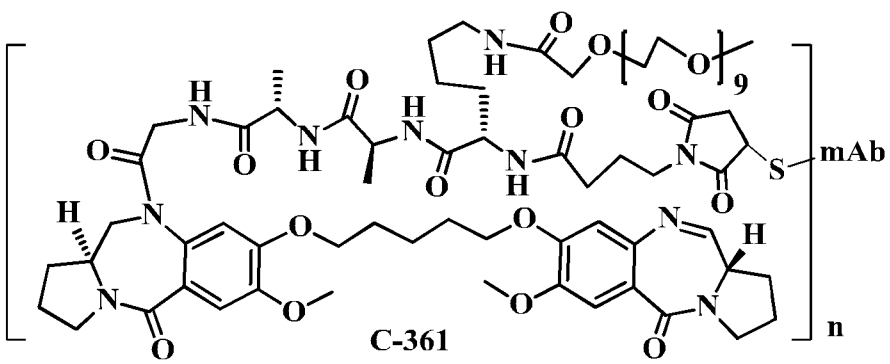
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C-337
mAb
n

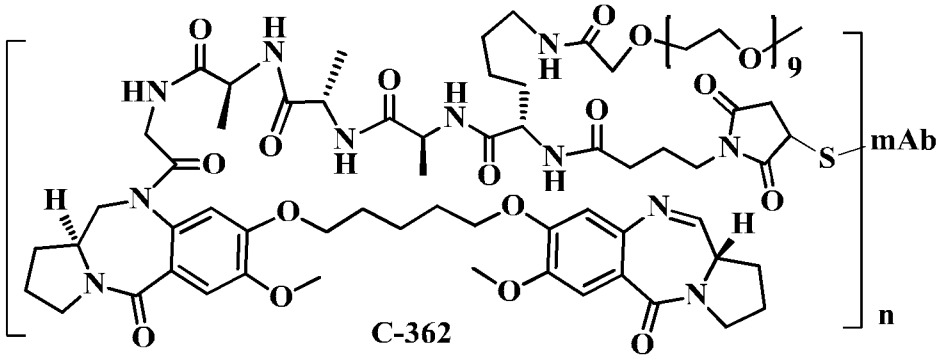
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C-338
mAb
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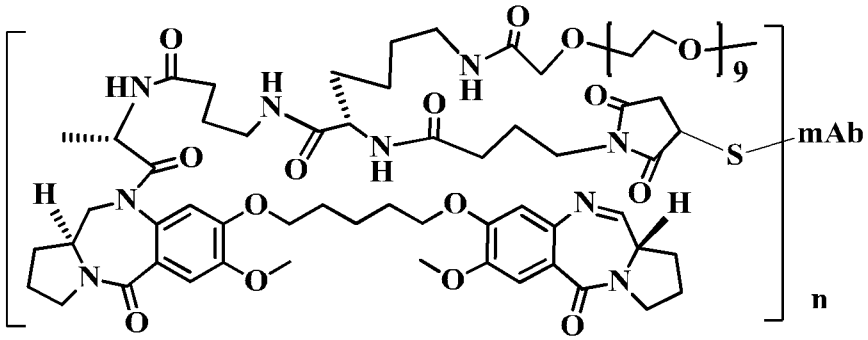
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C-361
mAb
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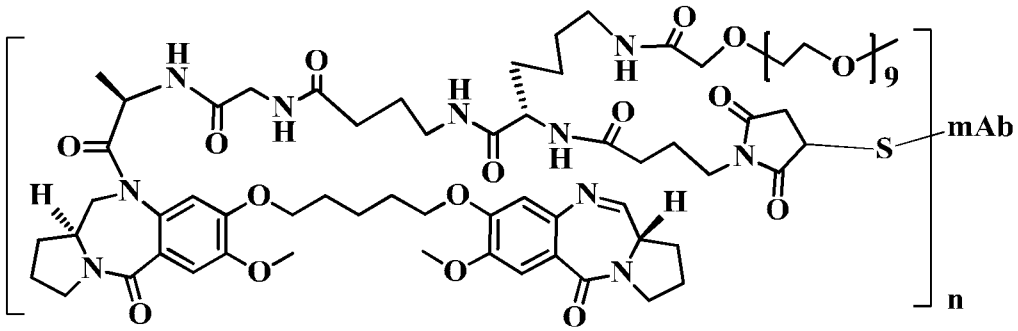
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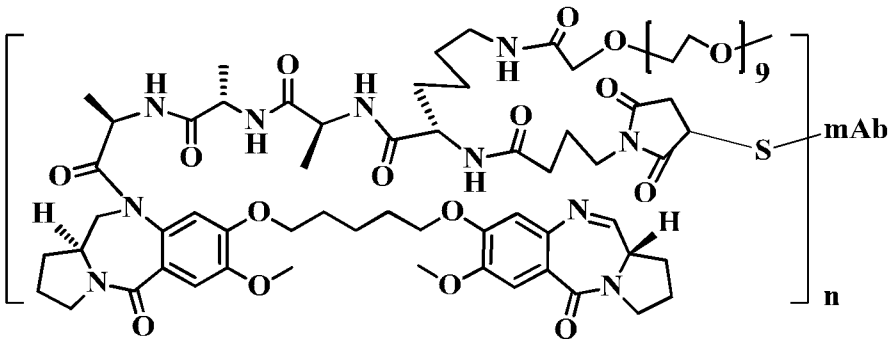
C-362 ,



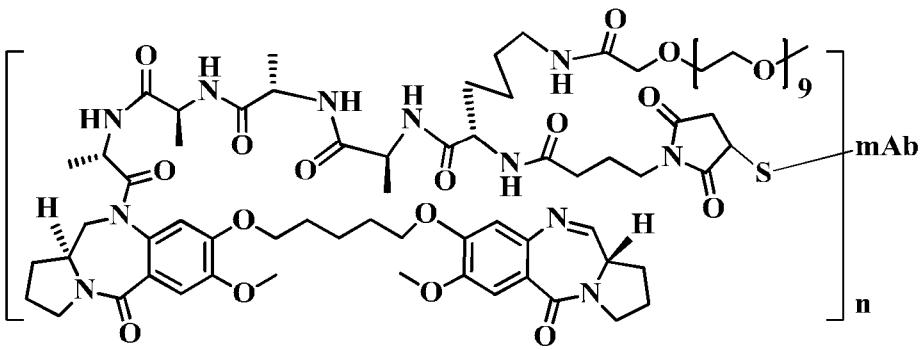
C-372 ,



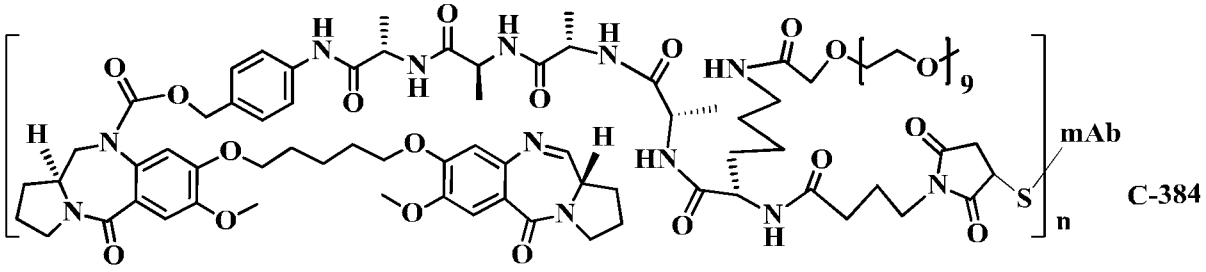
C-373 ,



C-374 ,

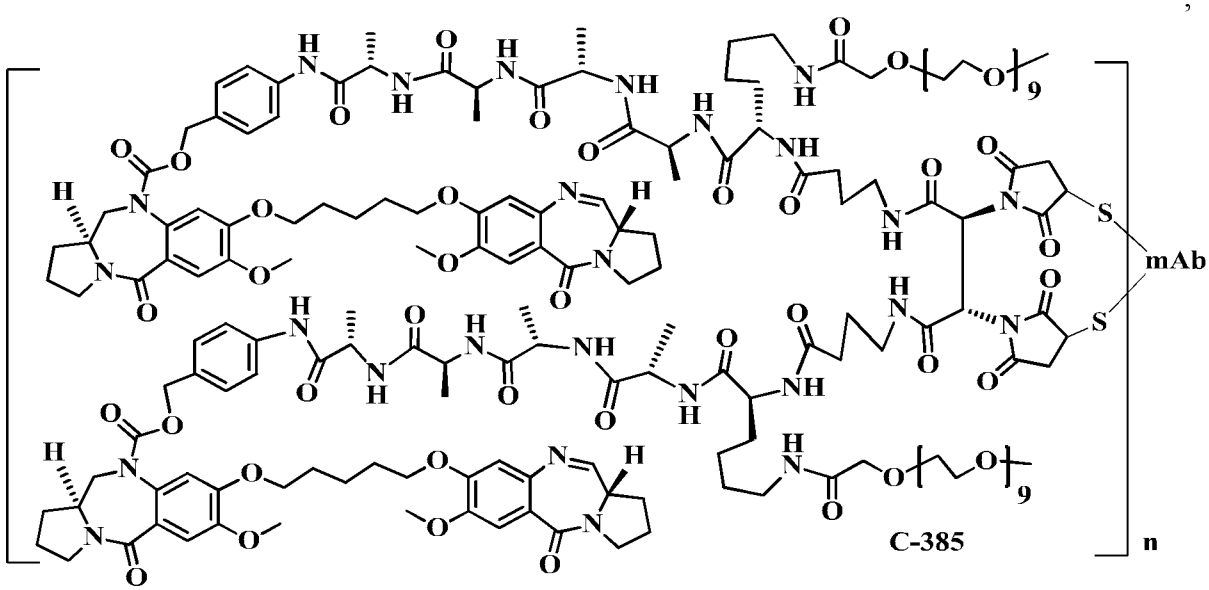


C-375 ,



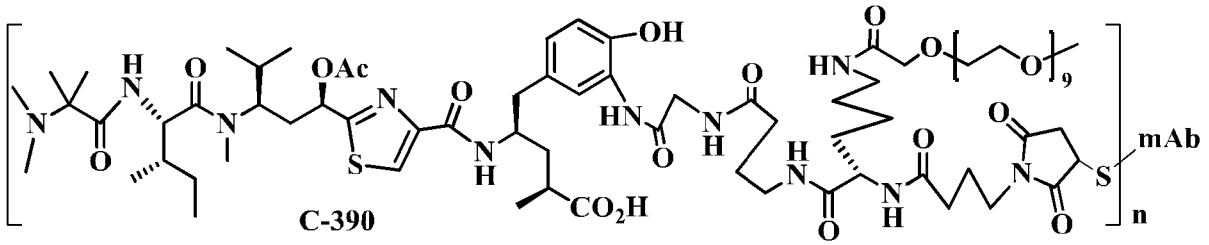
C-384

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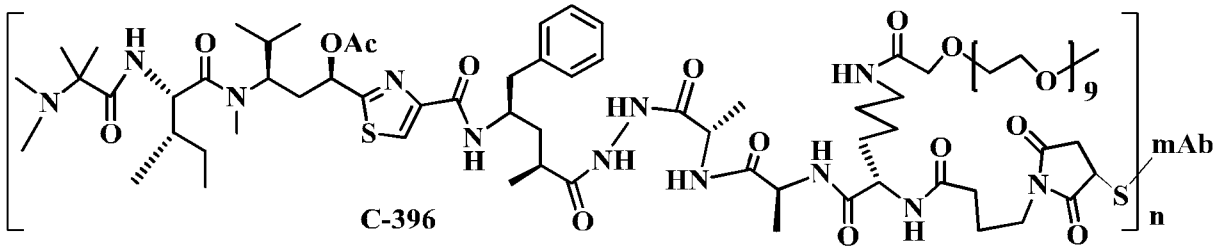
C-385

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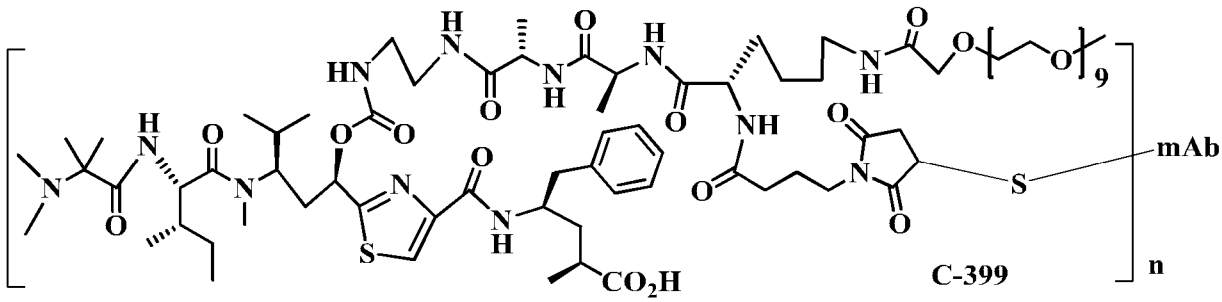
C-390

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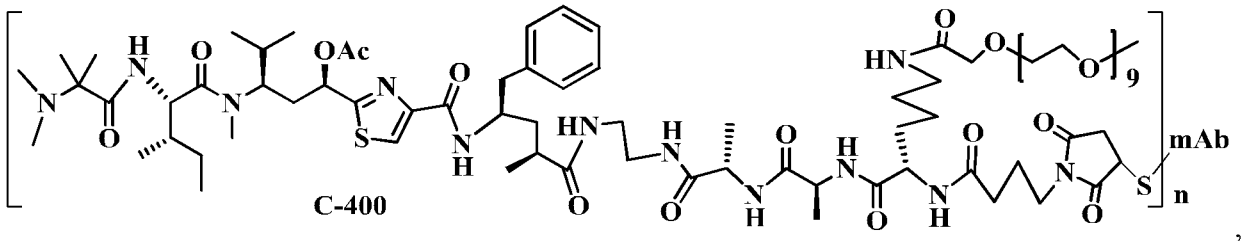
C-396

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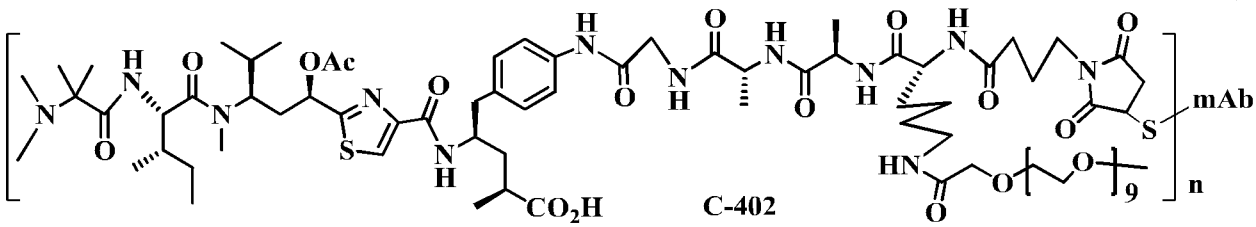


C-399

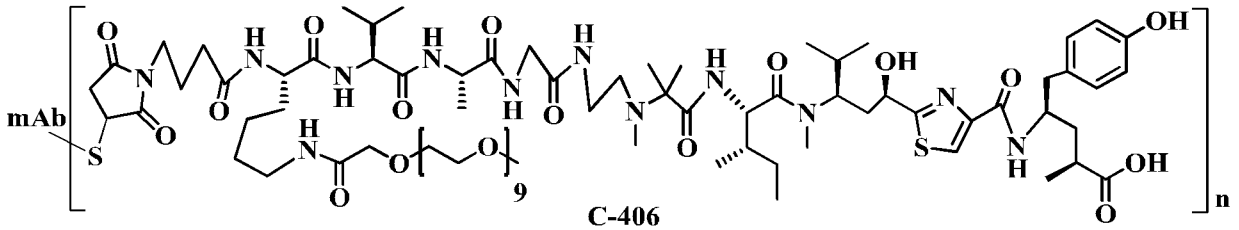
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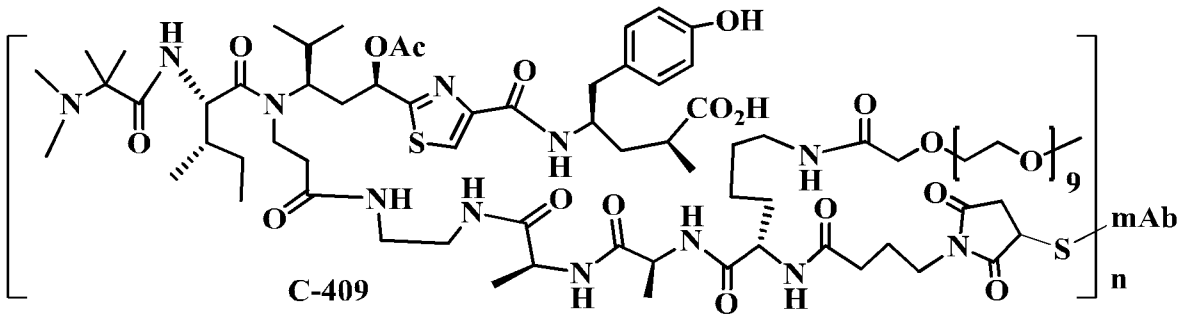
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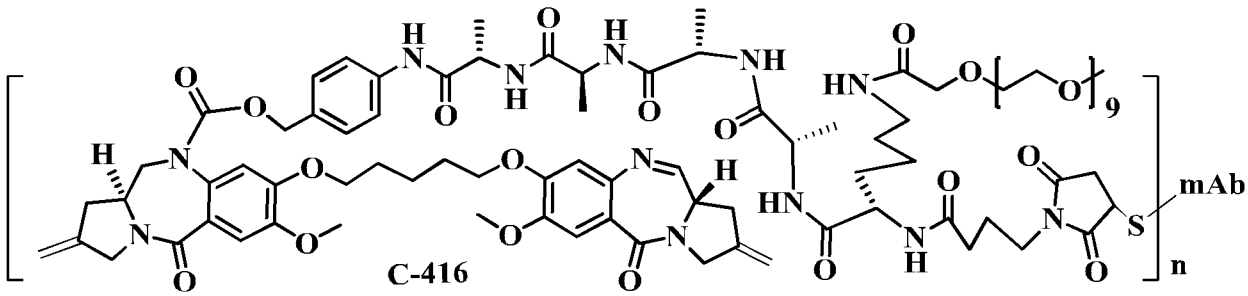
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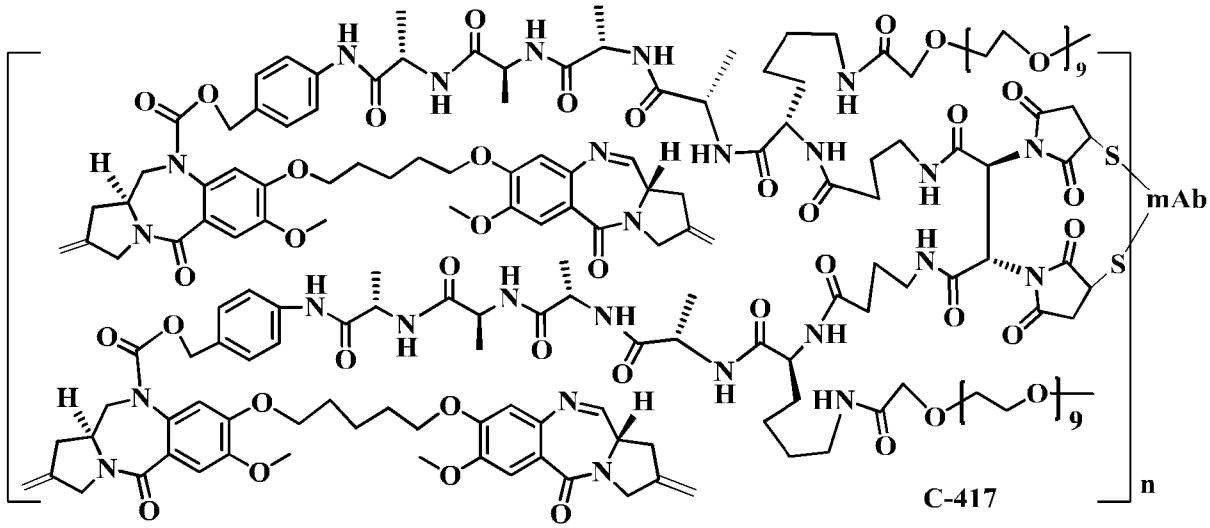
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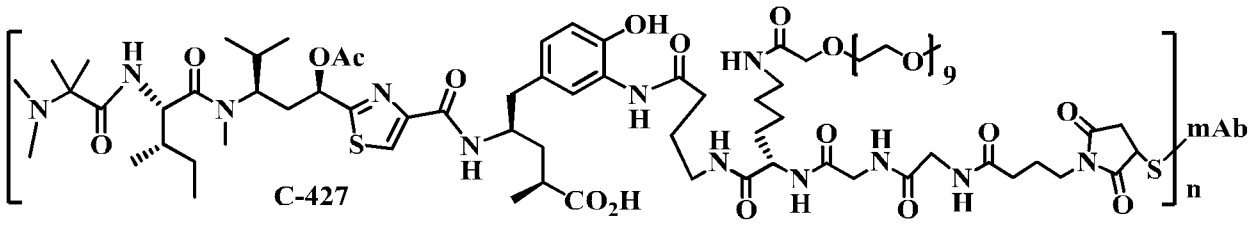
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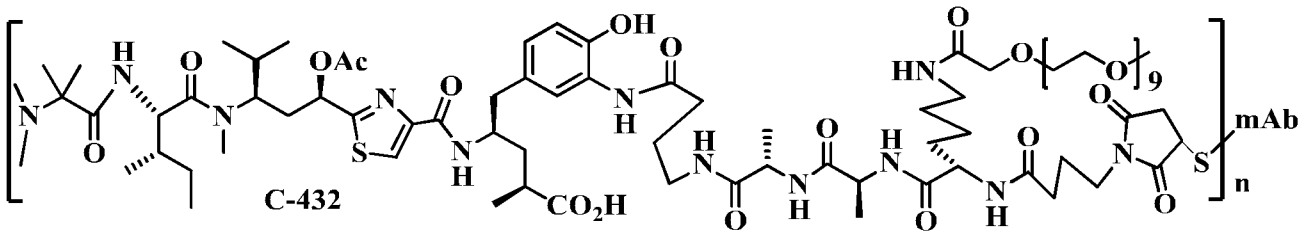
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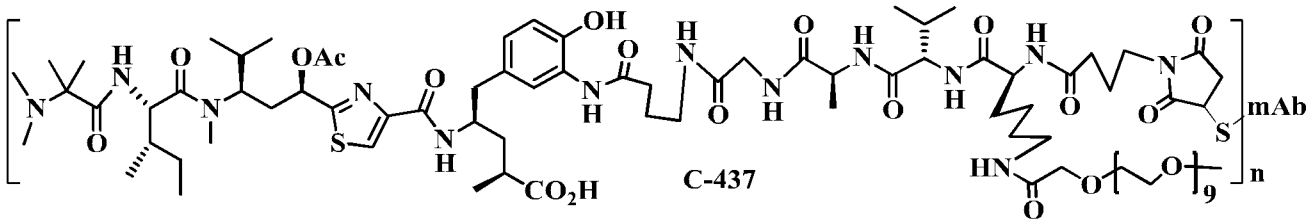


C-417 ,

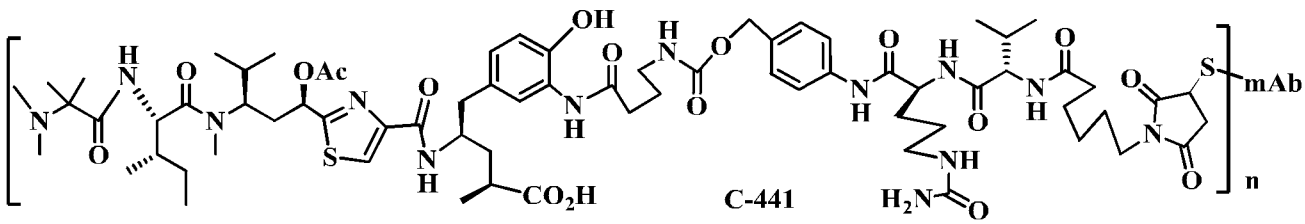

C-427
mAb
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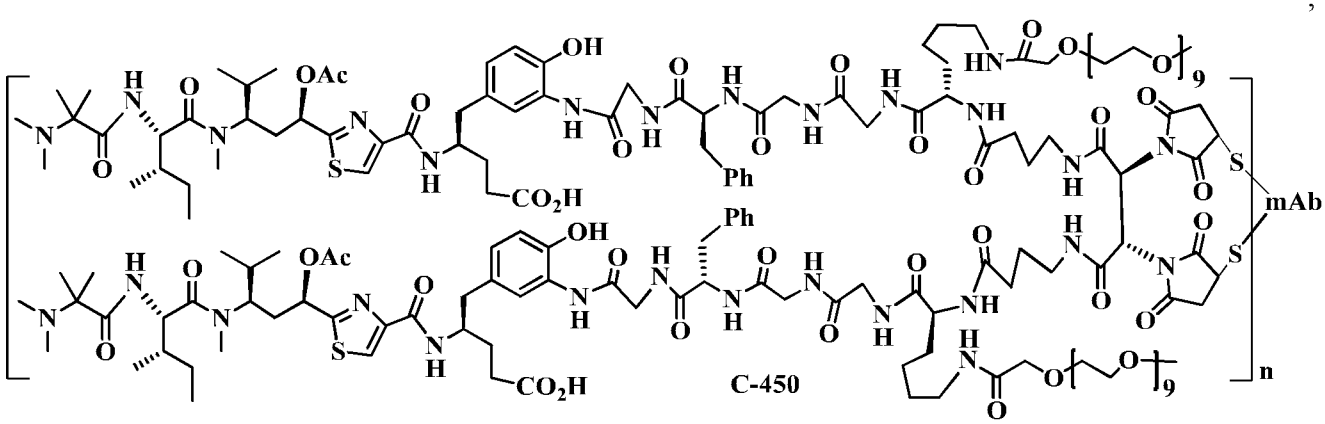

C-432
mAb
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C-437
mAb
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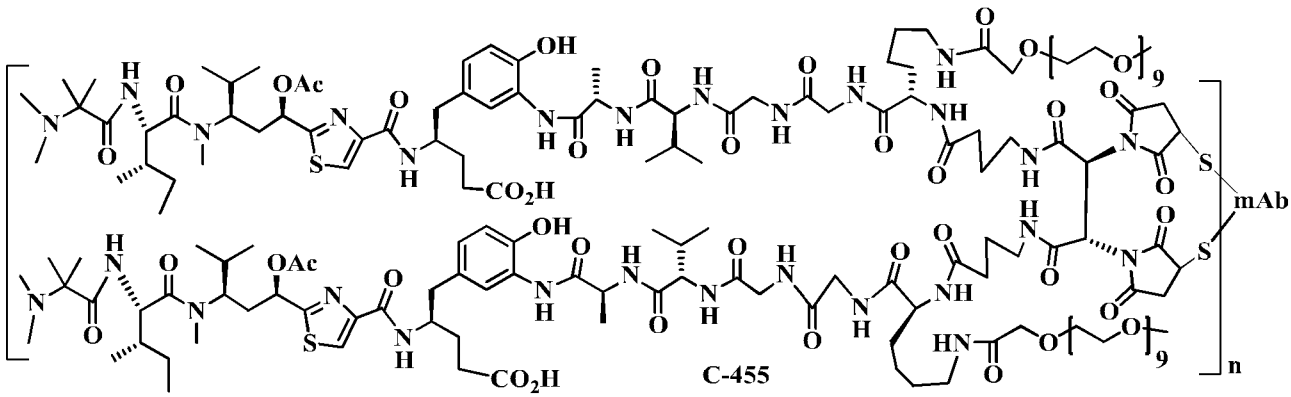
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C-441
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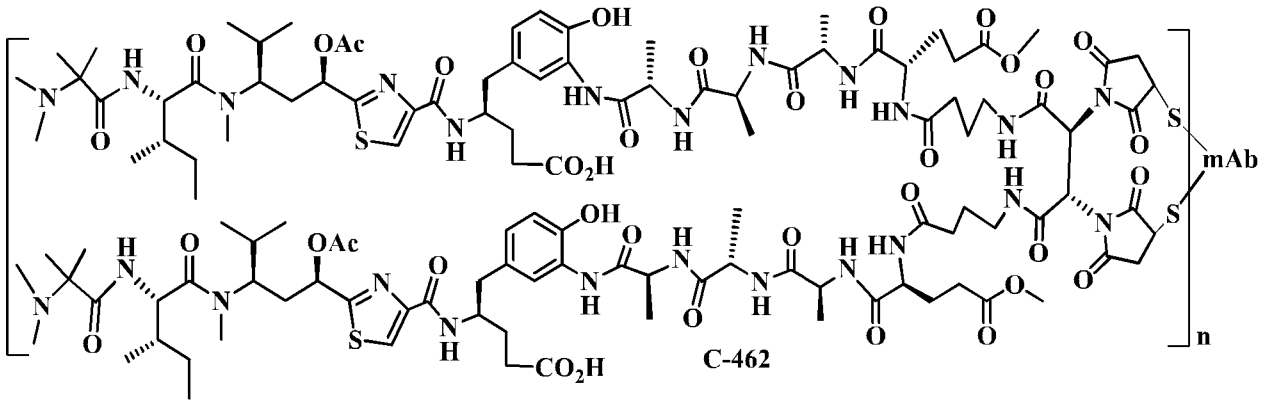
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C-450
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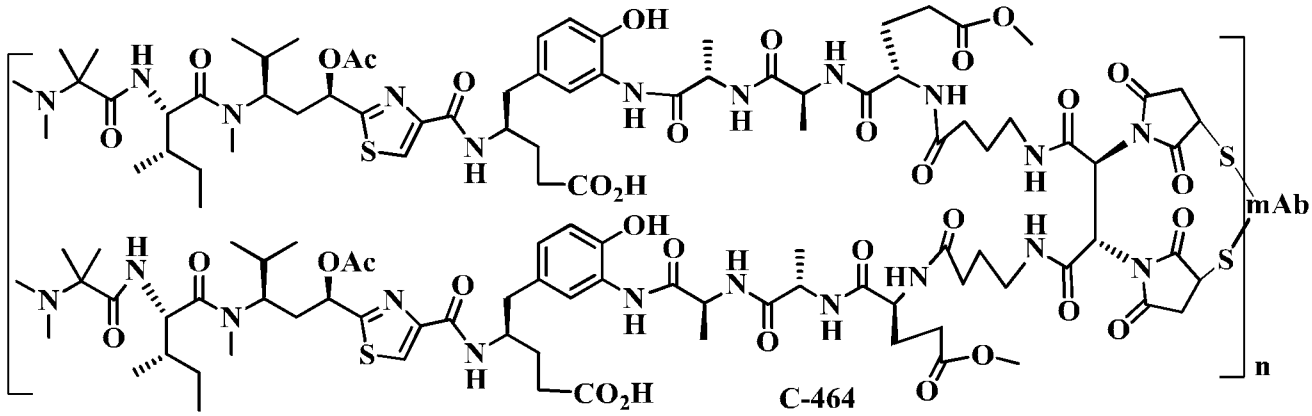
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C-455
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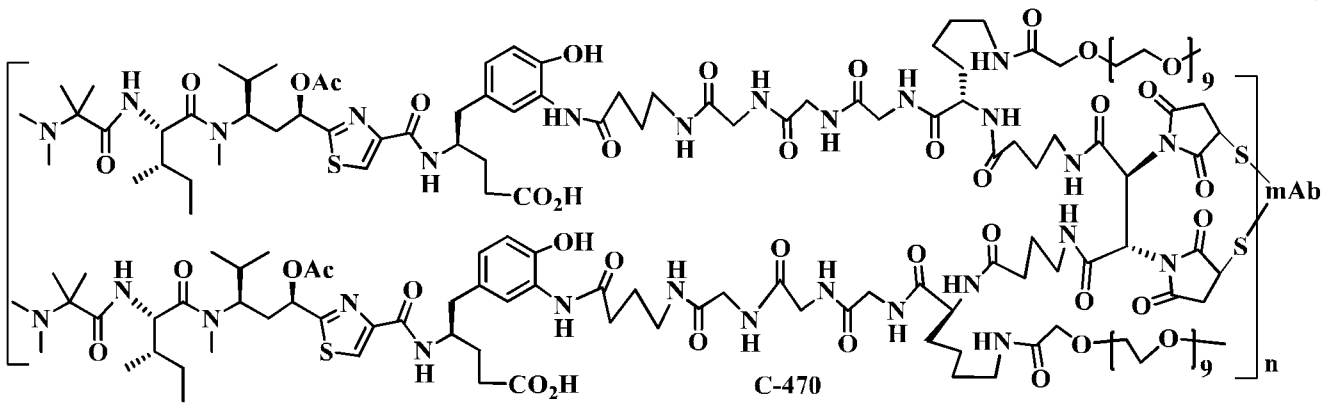
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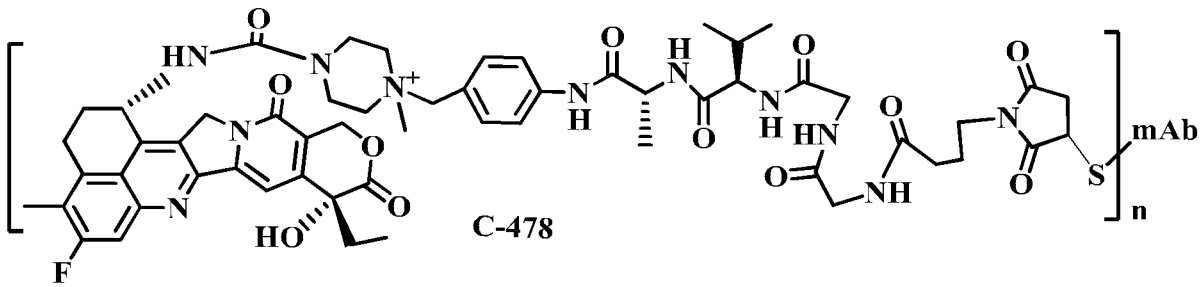
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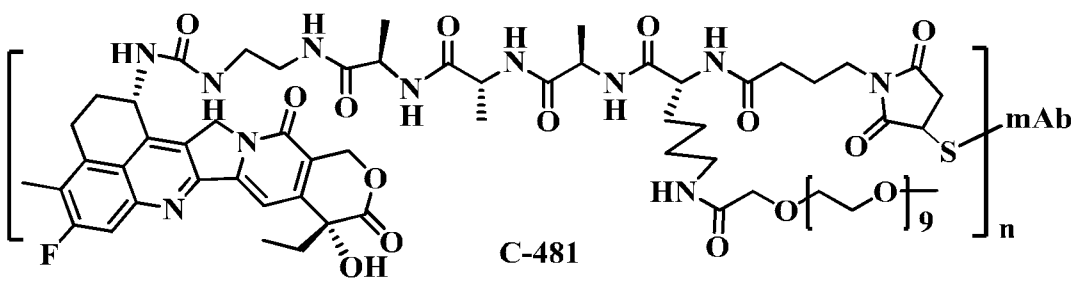
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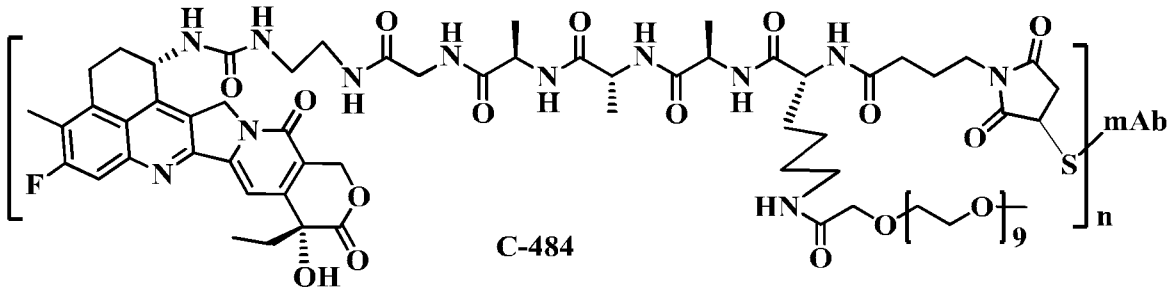
C-470 ,



C-478 ,

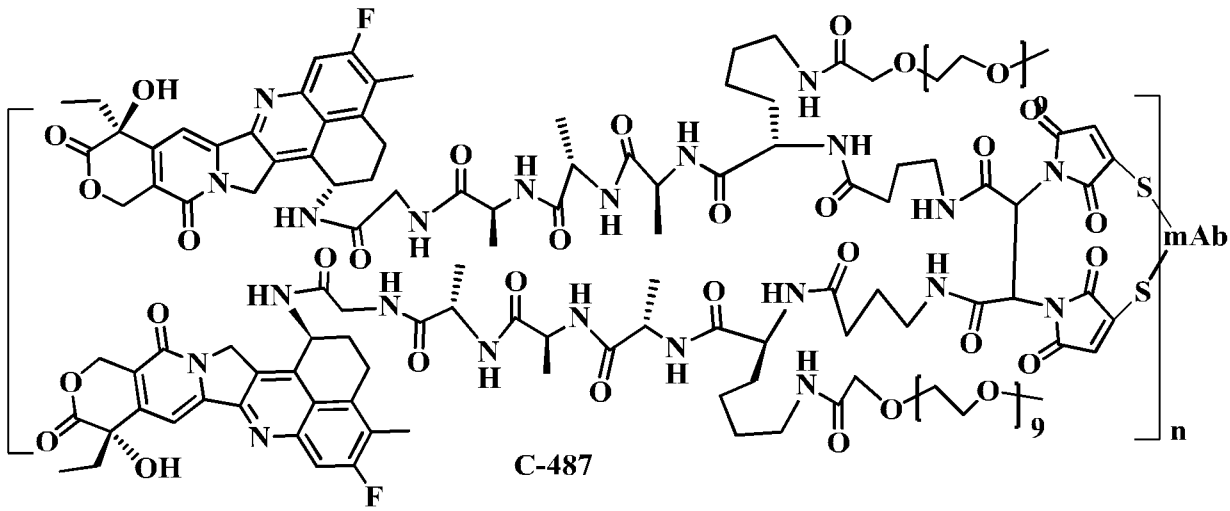


C-481 ,

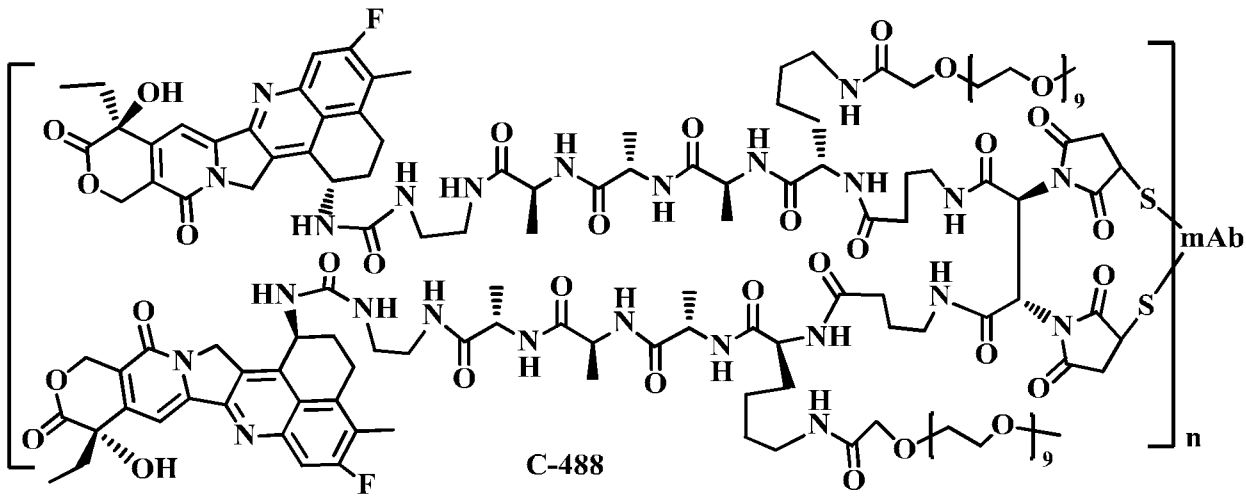


C-484

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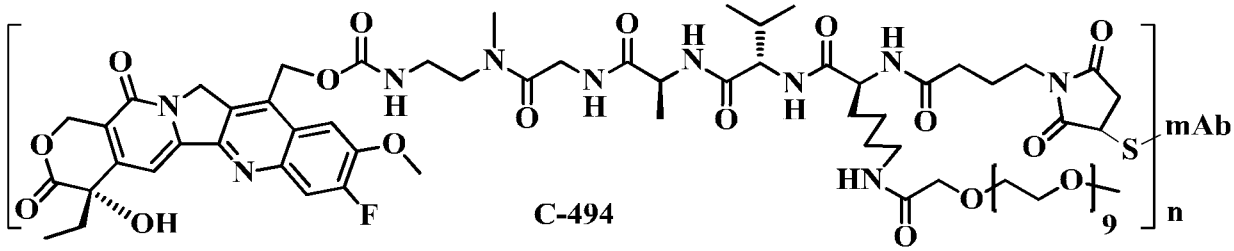


C-487



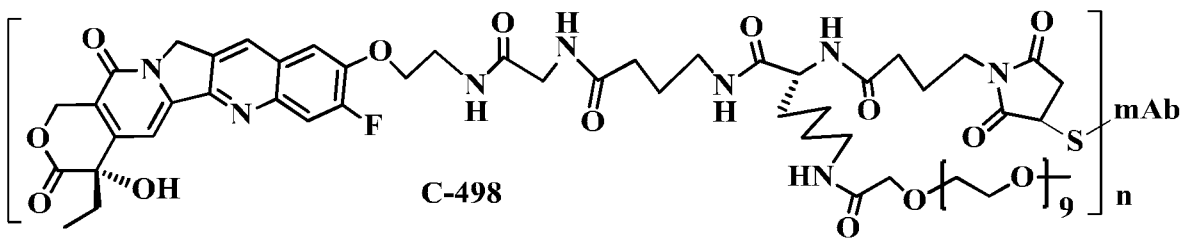
C-488

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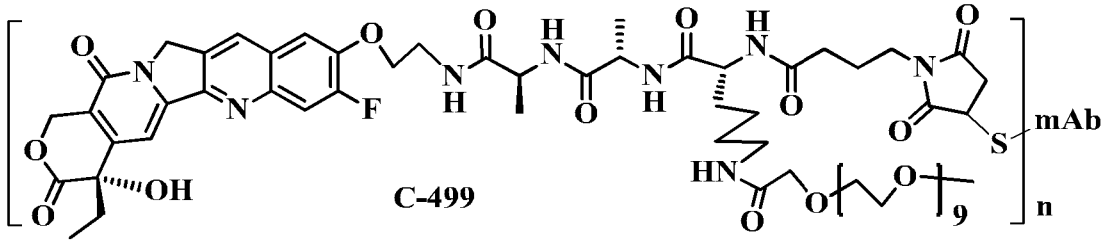
C-494

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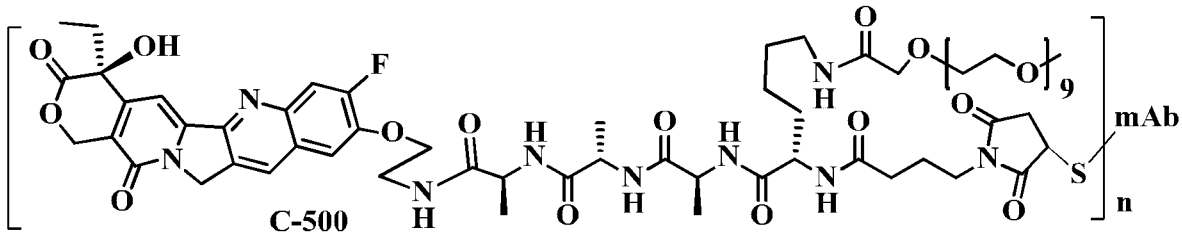


C-498

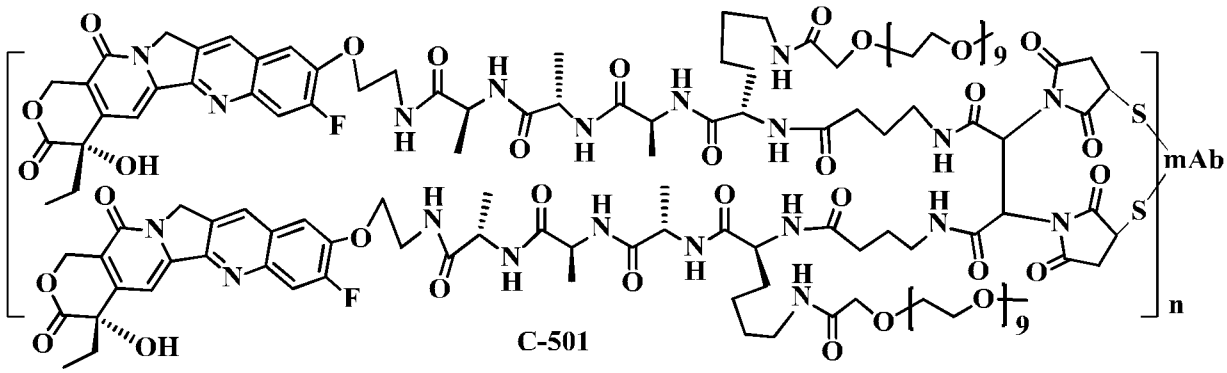
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C-499
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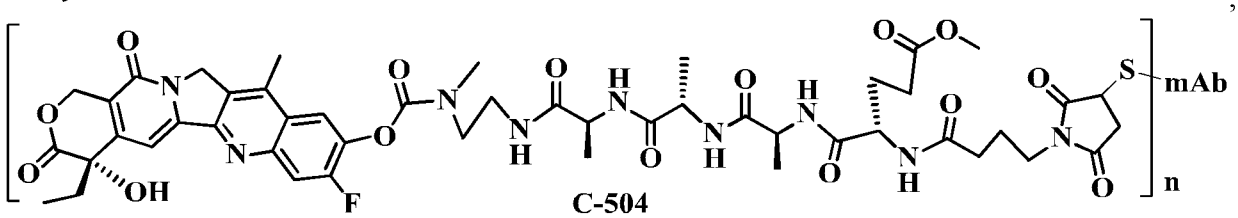
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C-500
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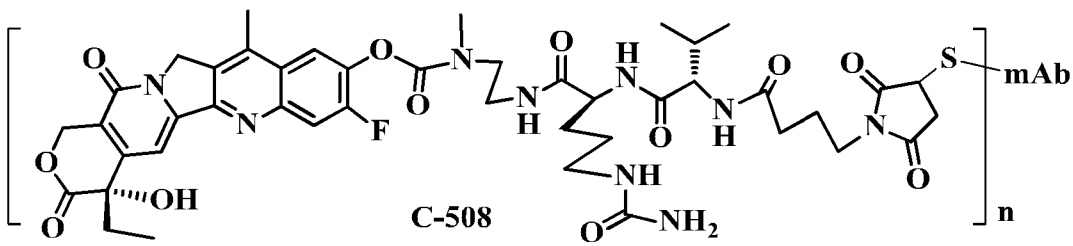
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C-501
mAb
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C-504
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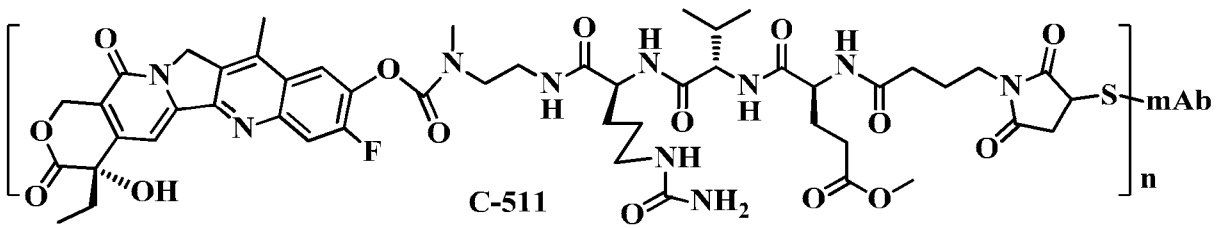
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C-508
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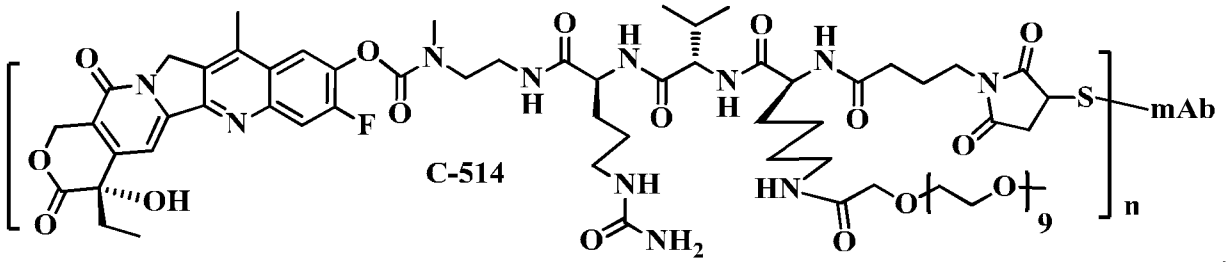
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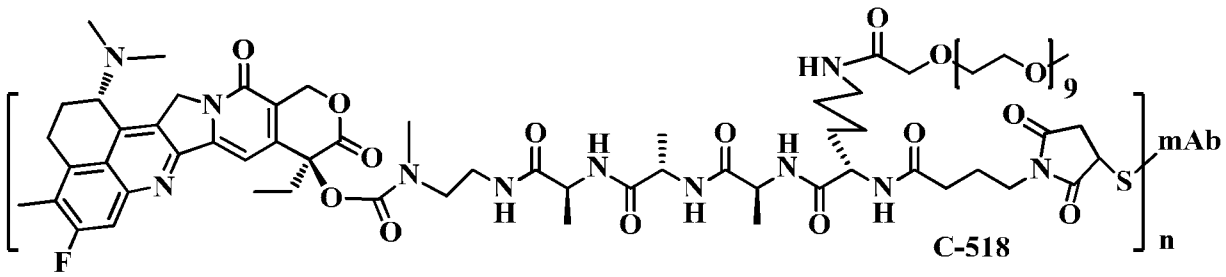
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C-511
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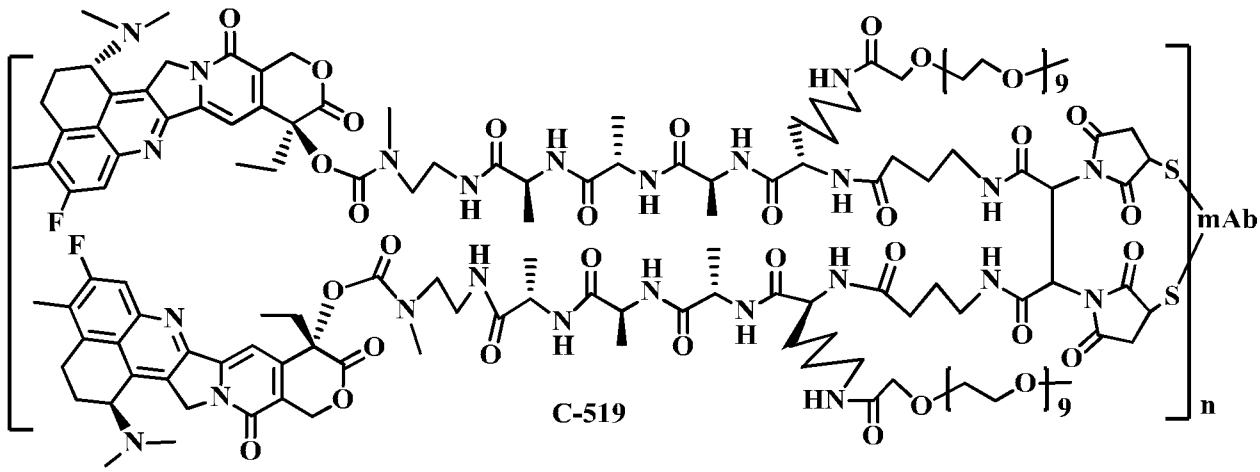
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C-514
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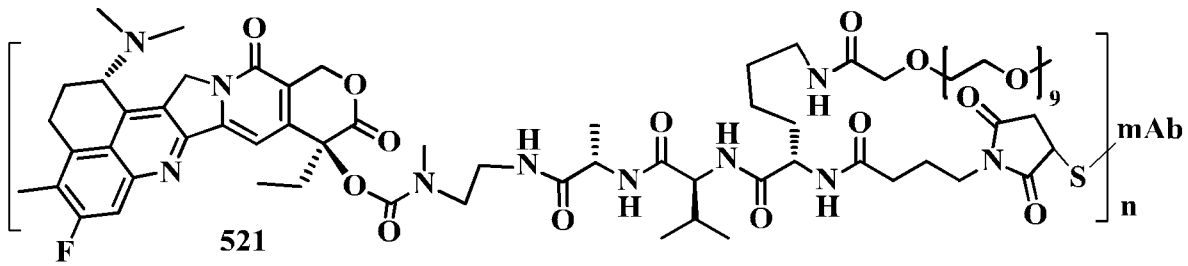
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C-518
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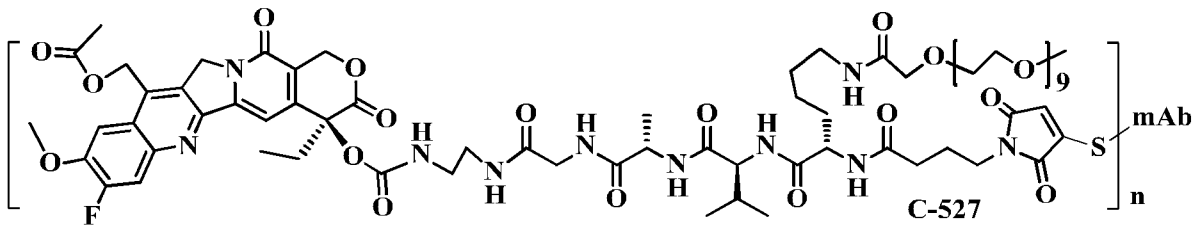
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C-519
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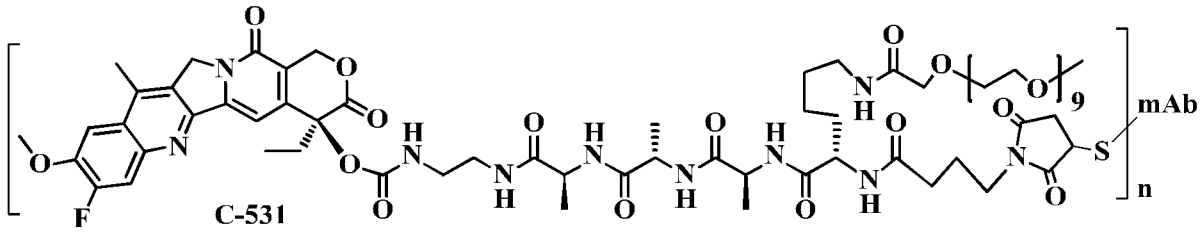
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521
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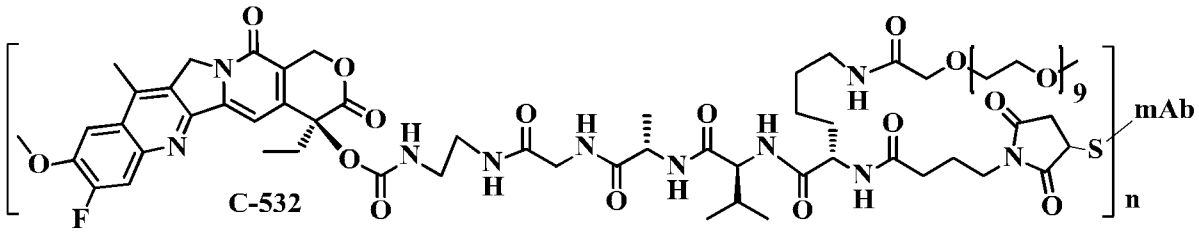
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C-527
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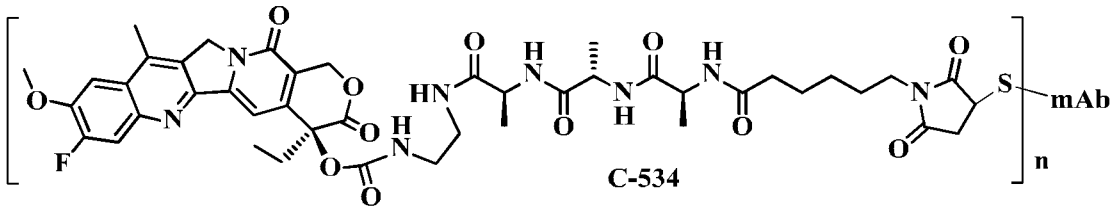
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C-531
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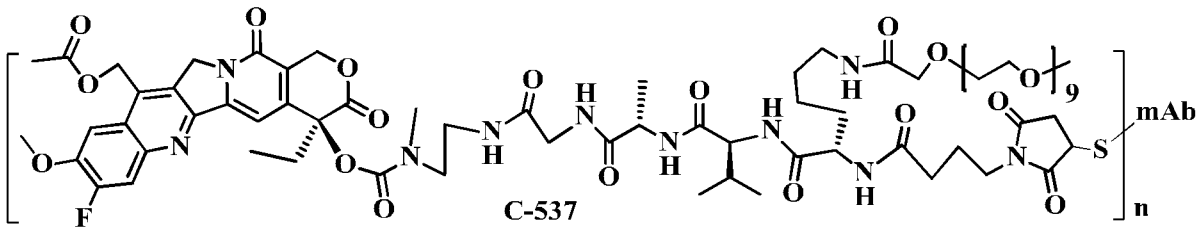
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C-532
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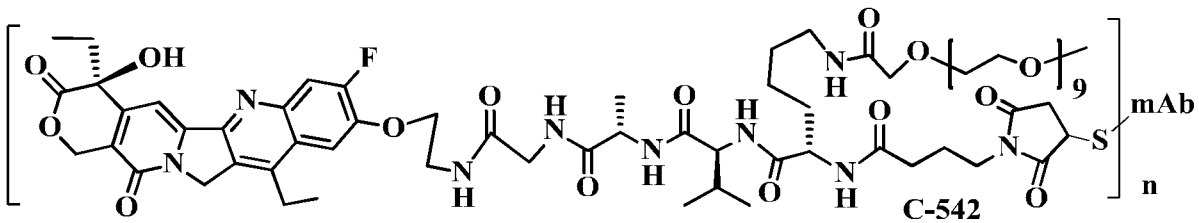
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C-534
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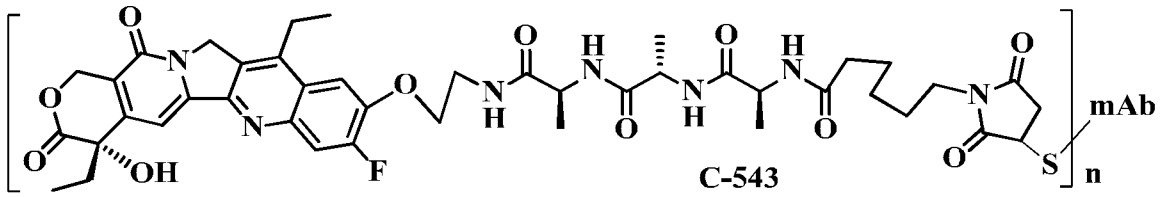
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C-537
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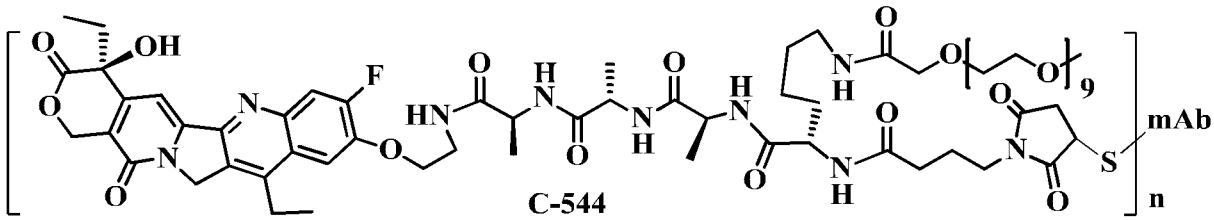
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C-542
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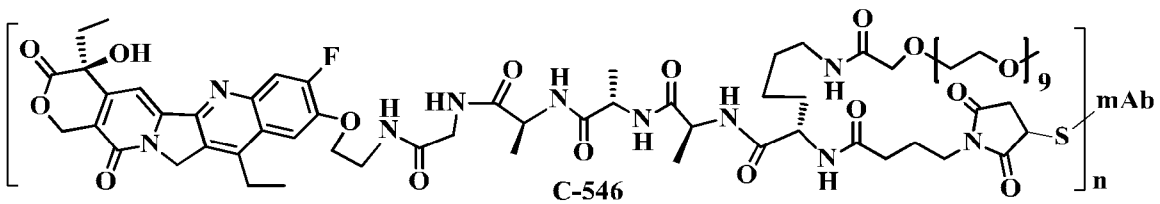
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C-543
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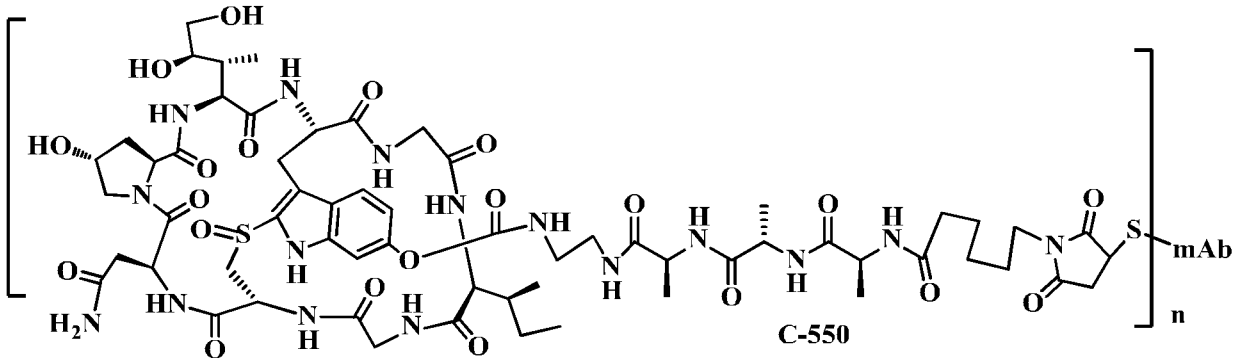
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C-544
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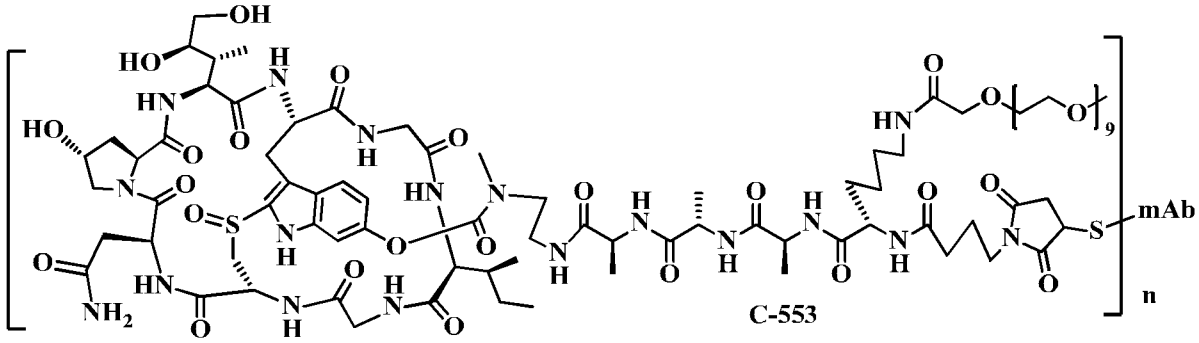
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C-546
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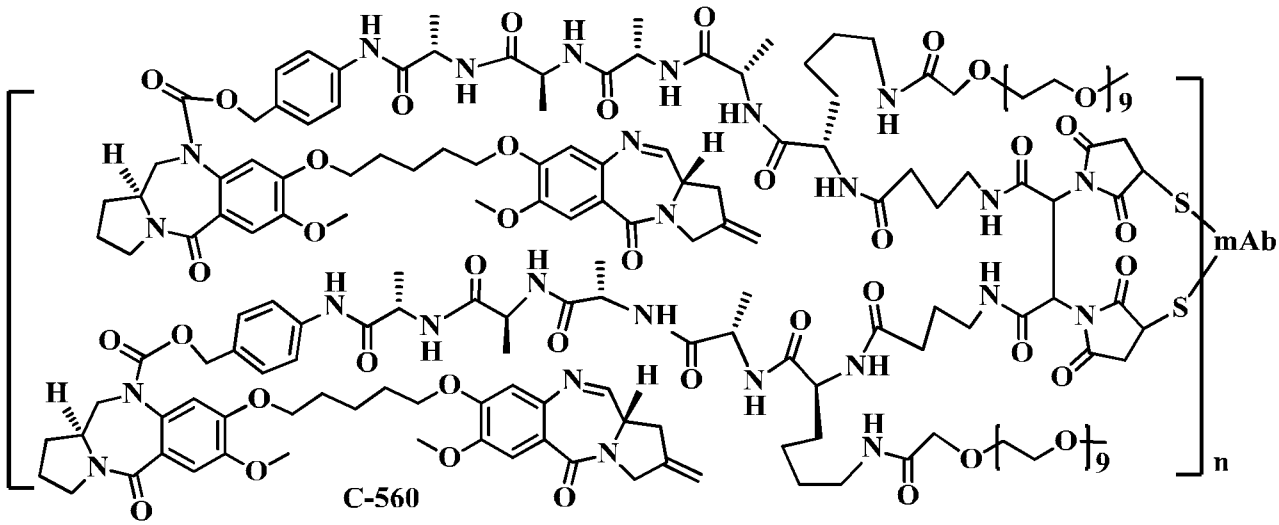
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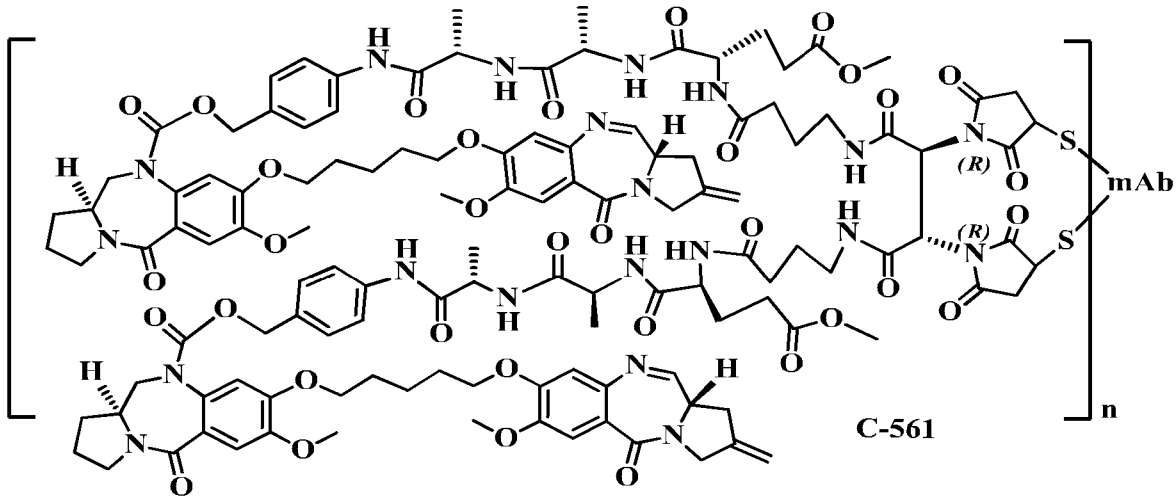
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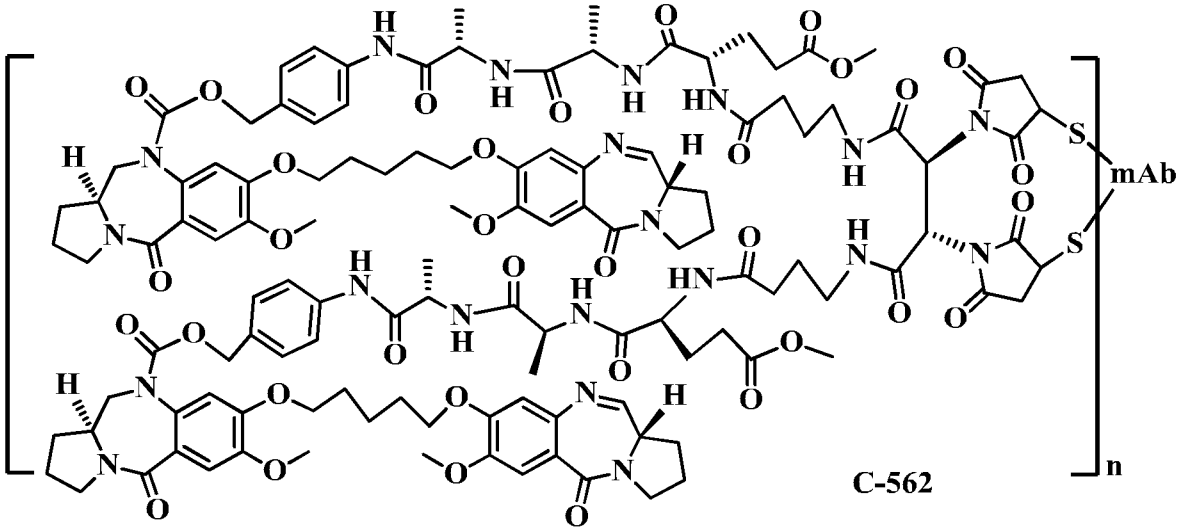
C-553 ,



C-560 ,

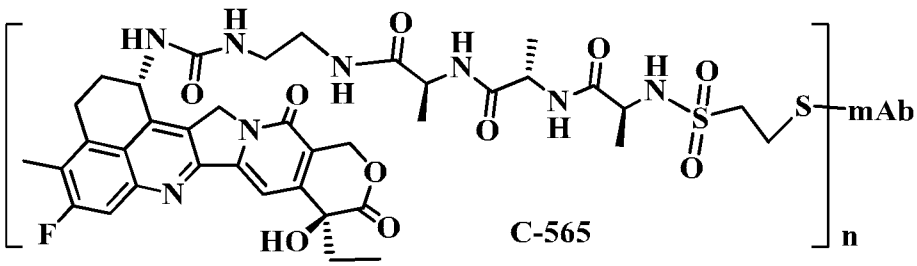


C-561 ,

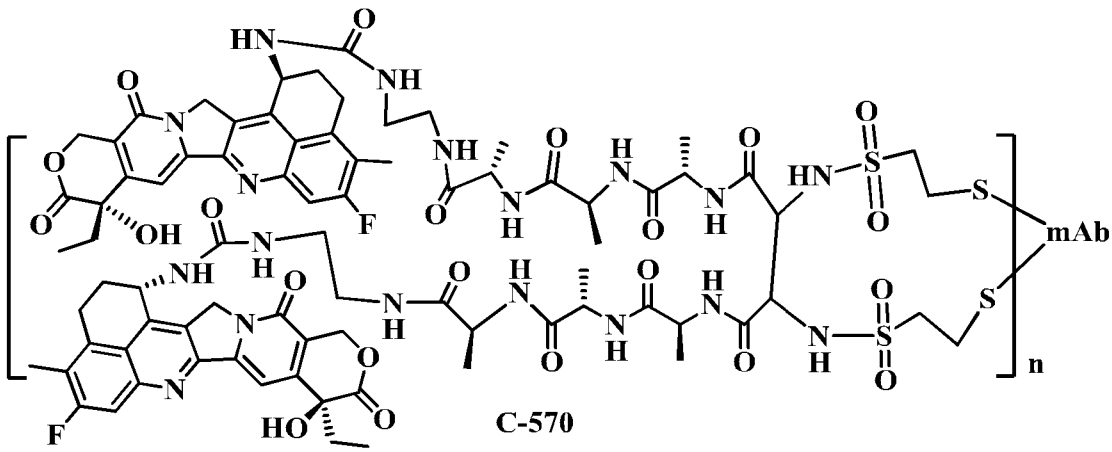


C-562

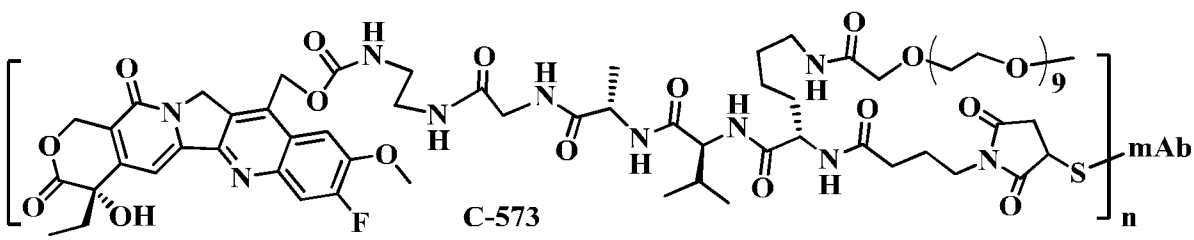
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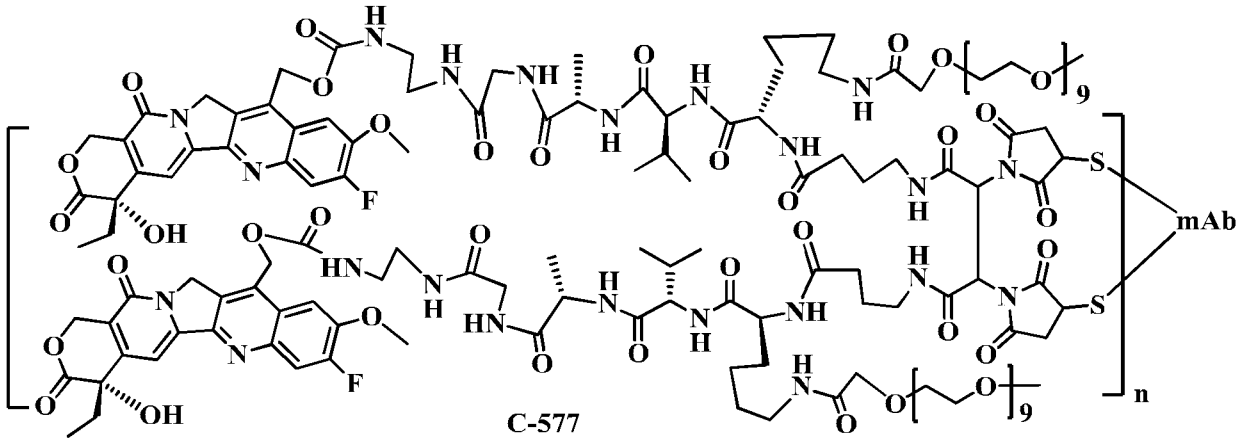
C-565



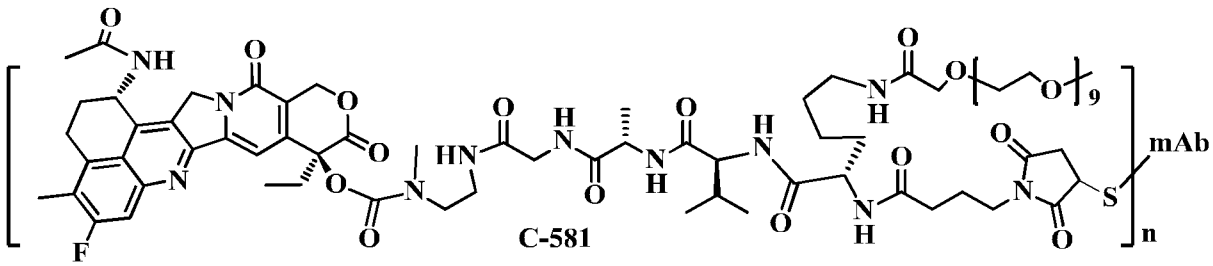
C-570



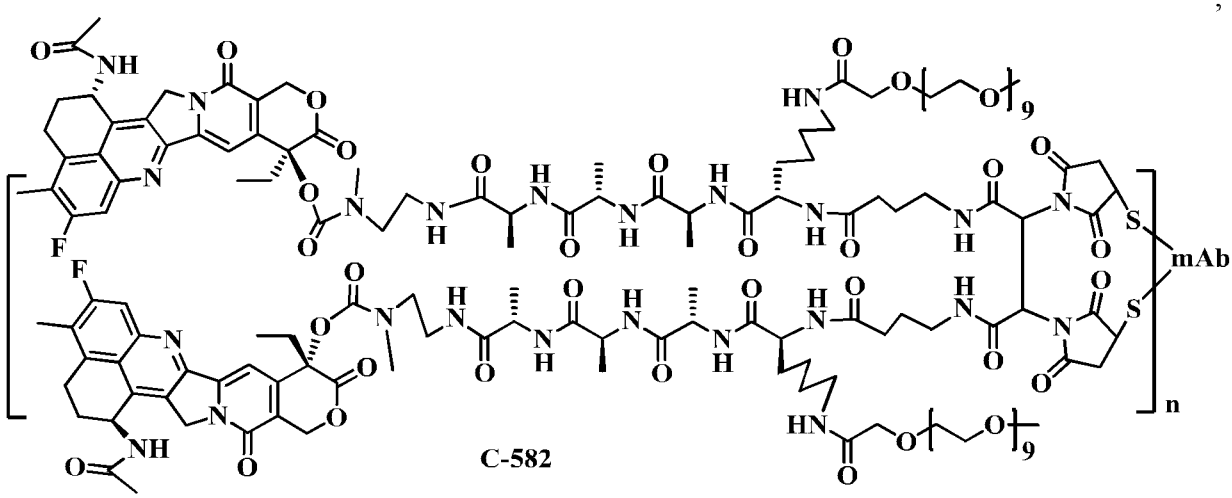
C-573



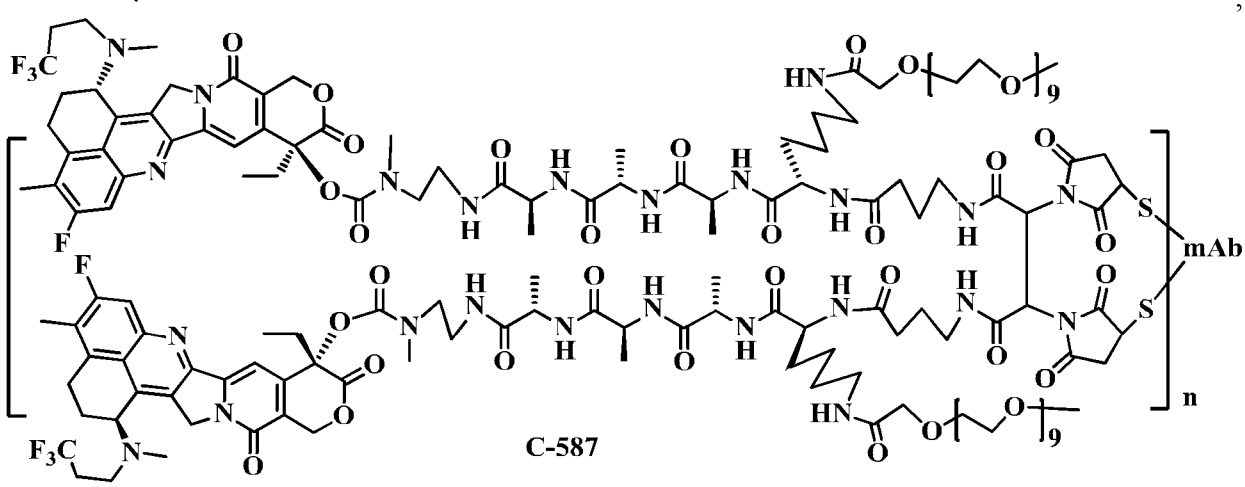
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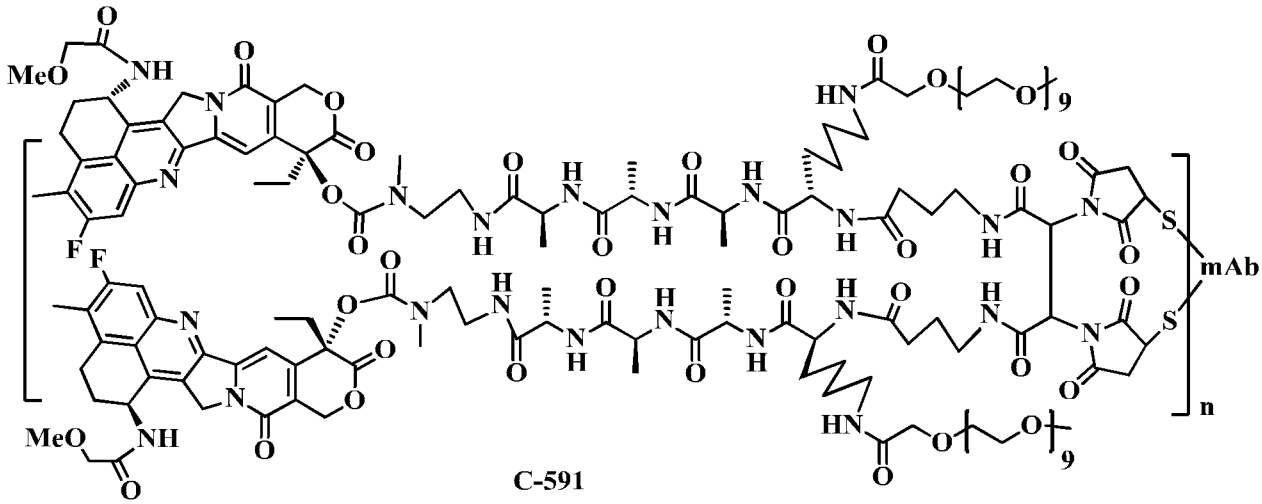
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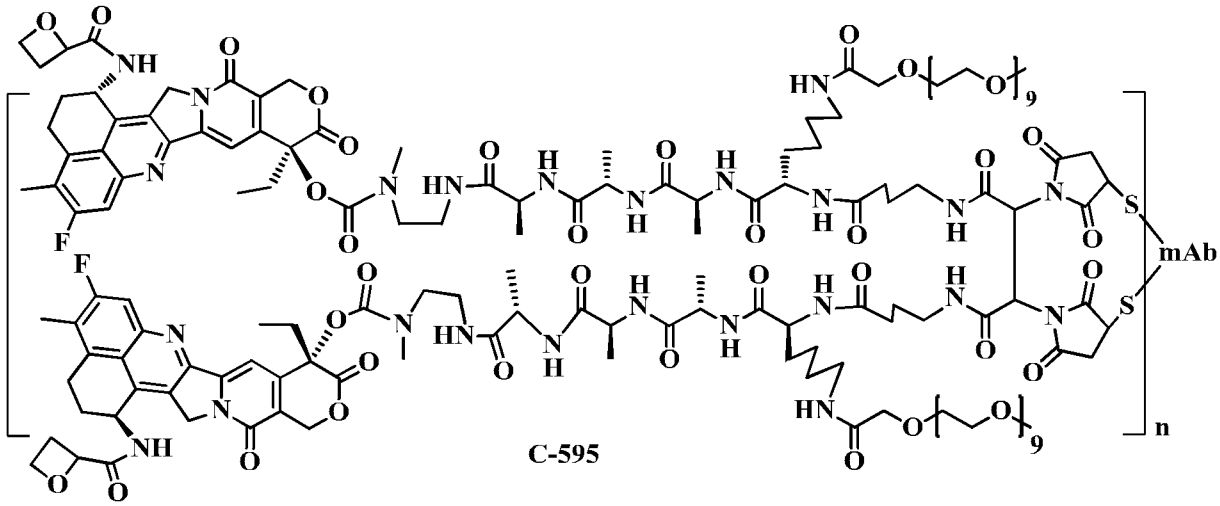


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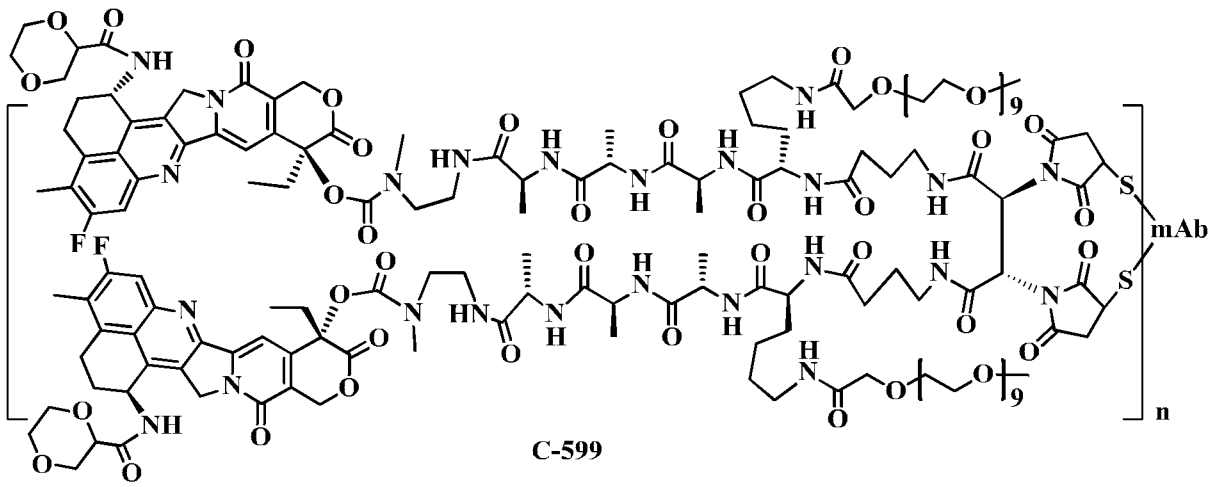
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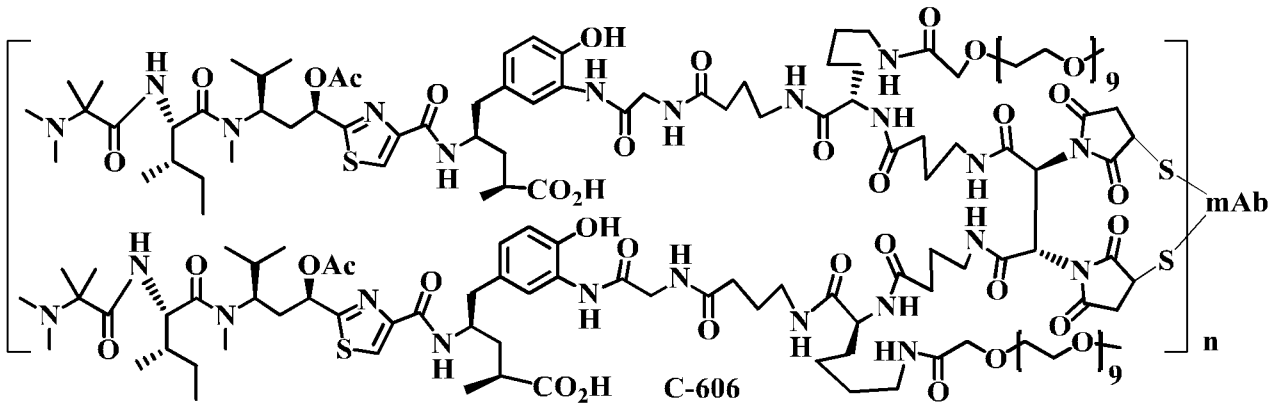
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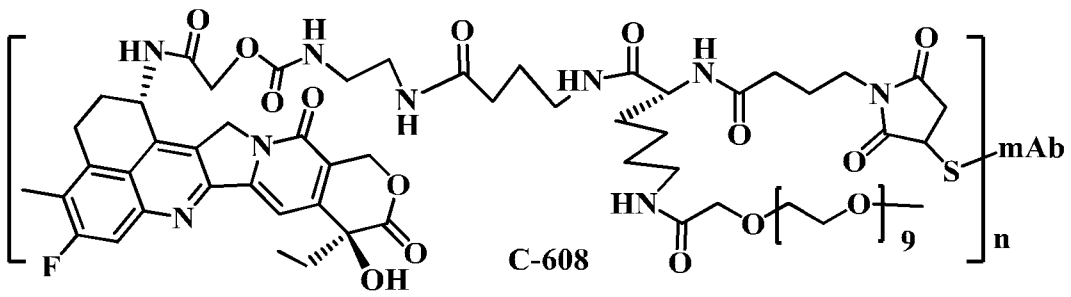


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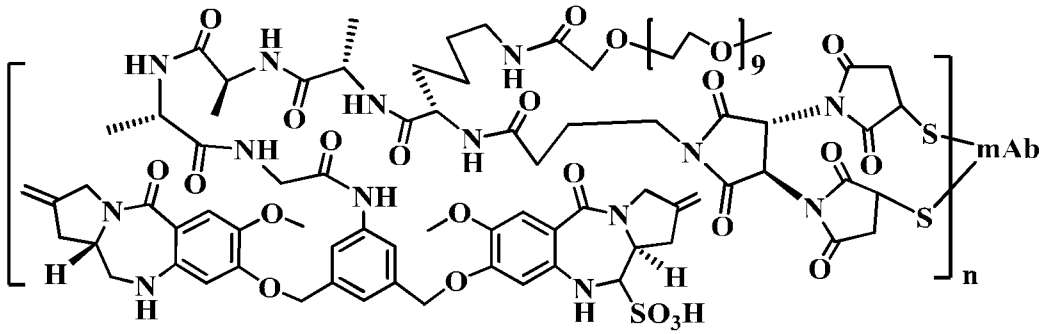
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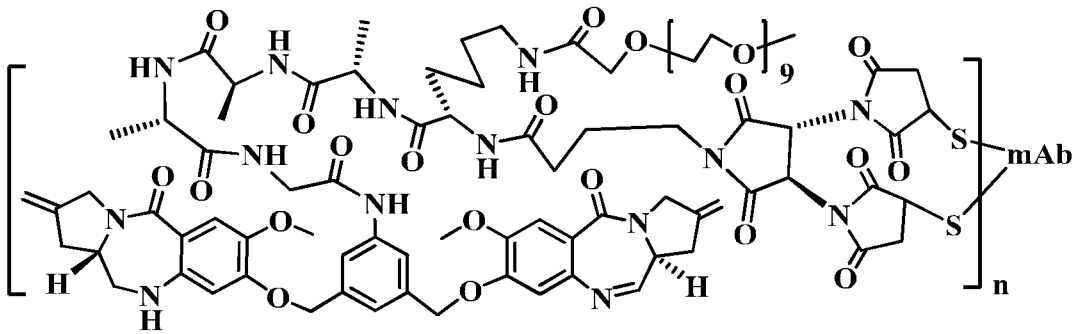
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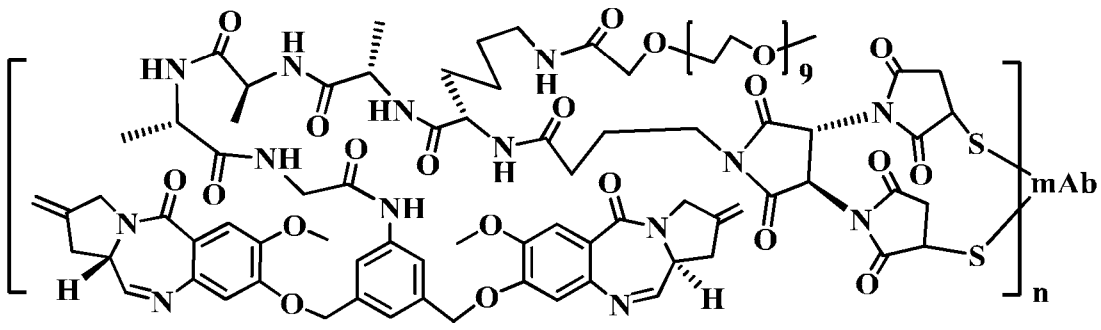
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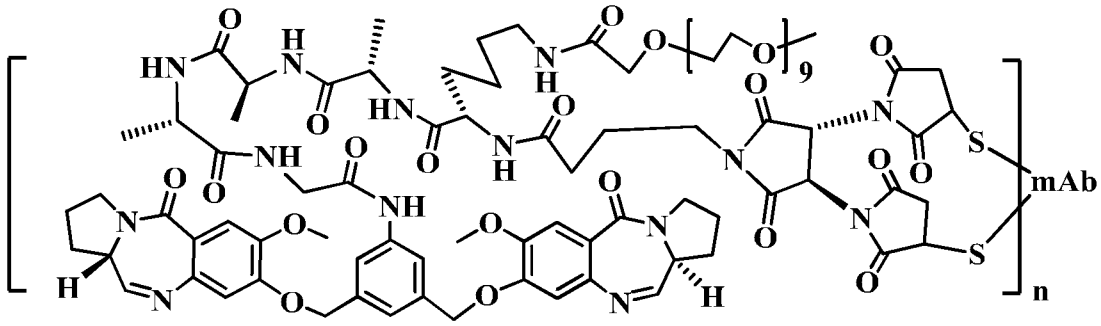
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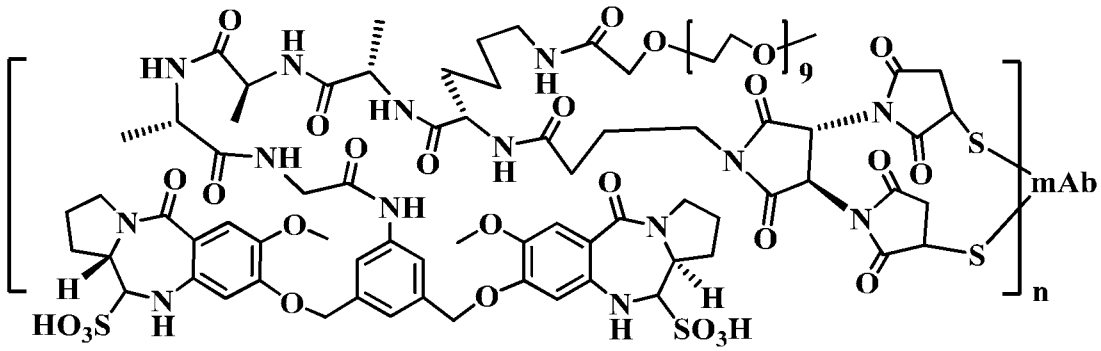
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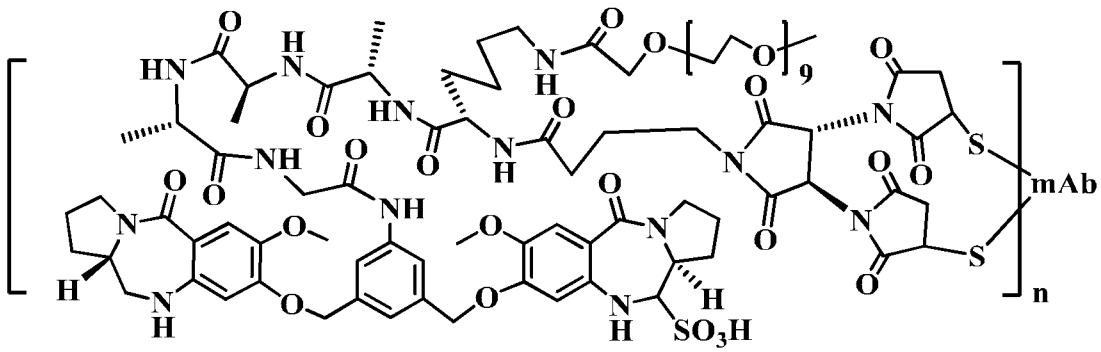
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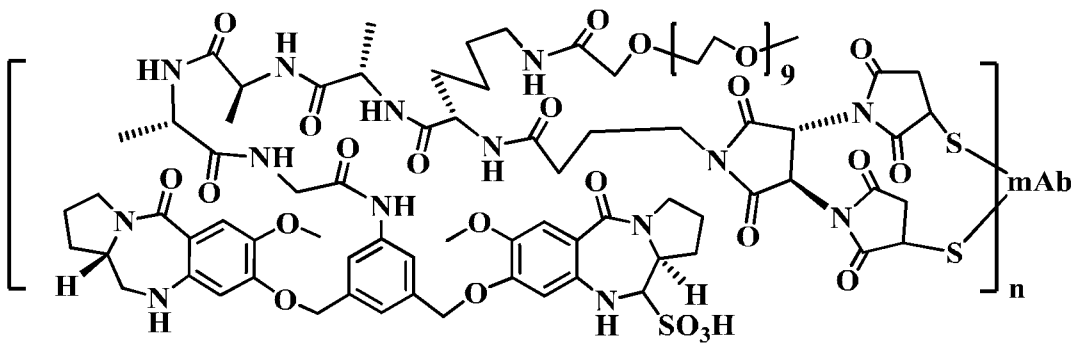
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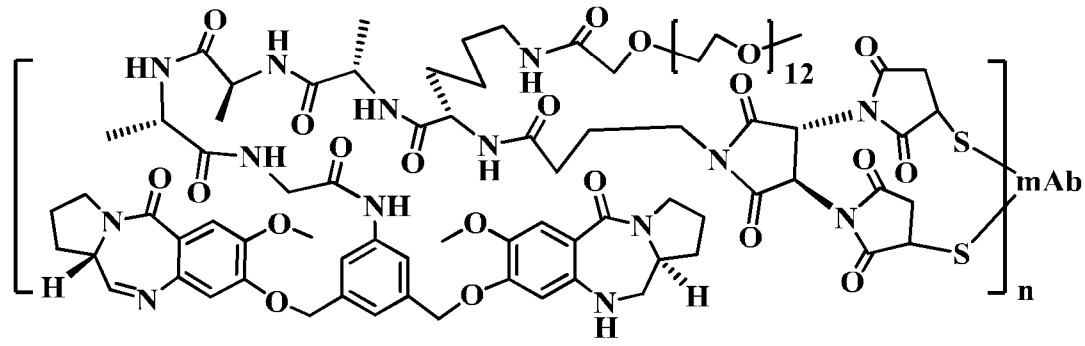
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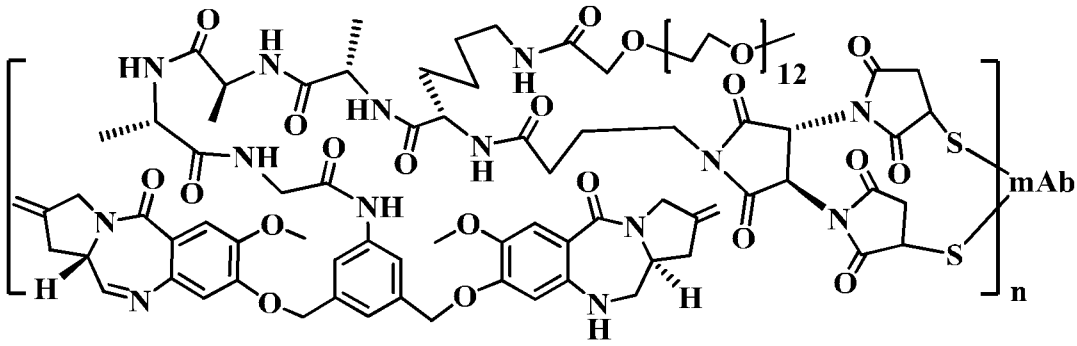
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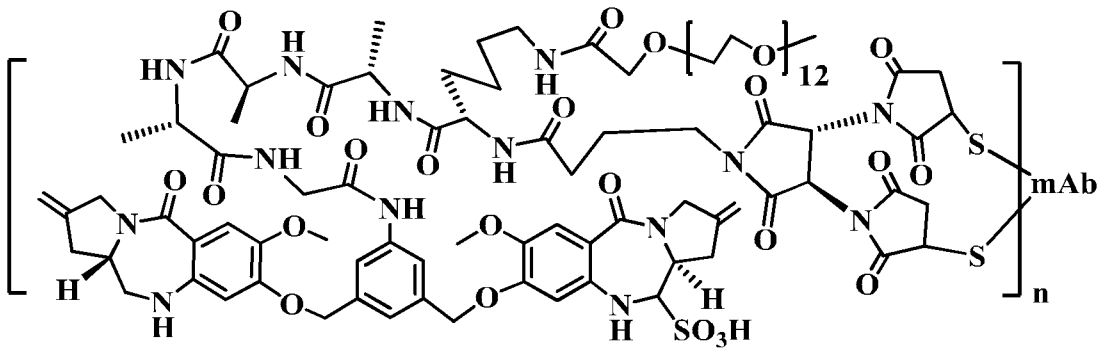
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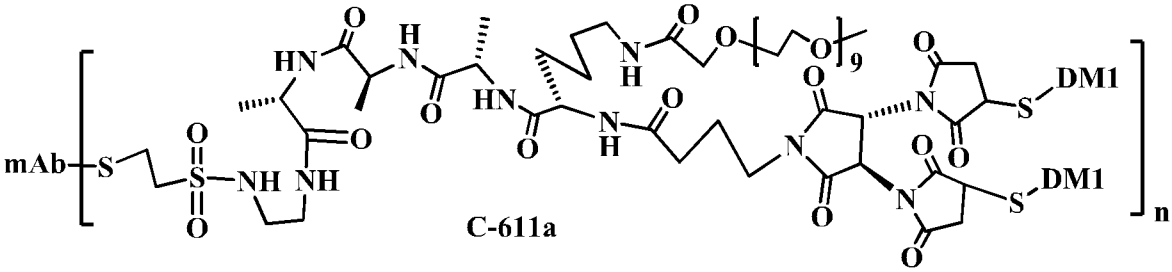
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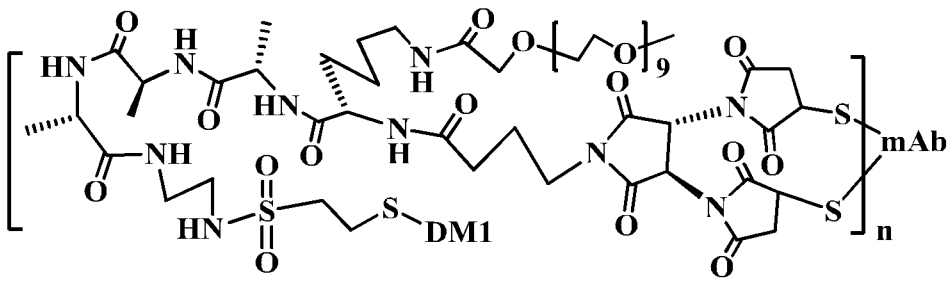


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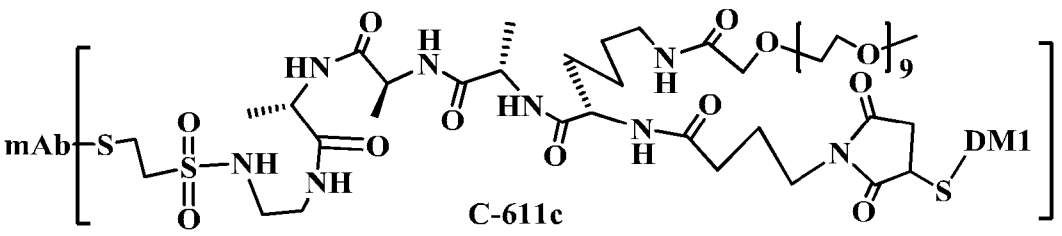
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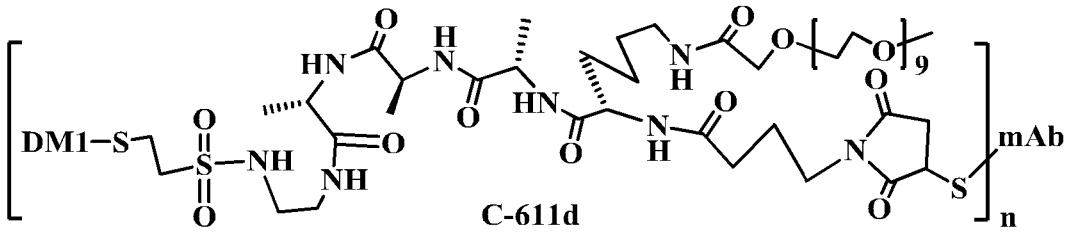
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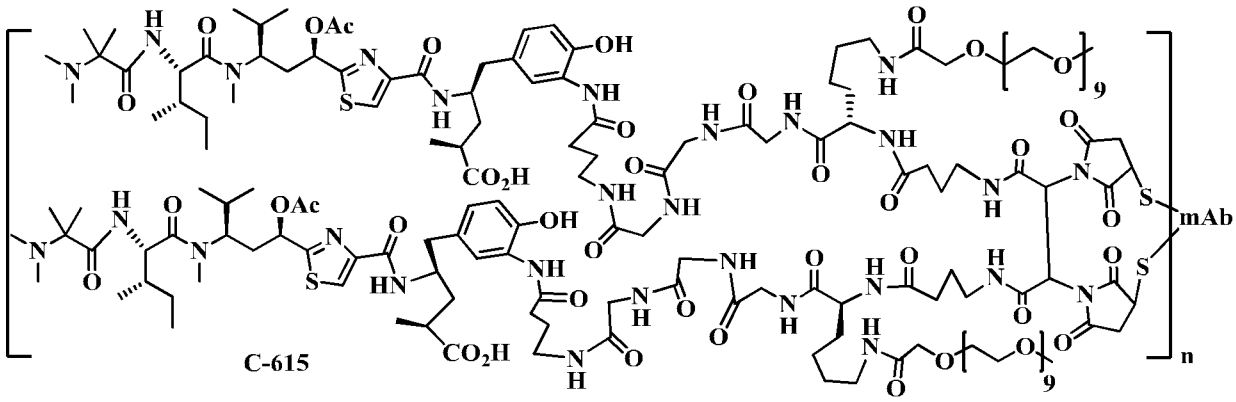
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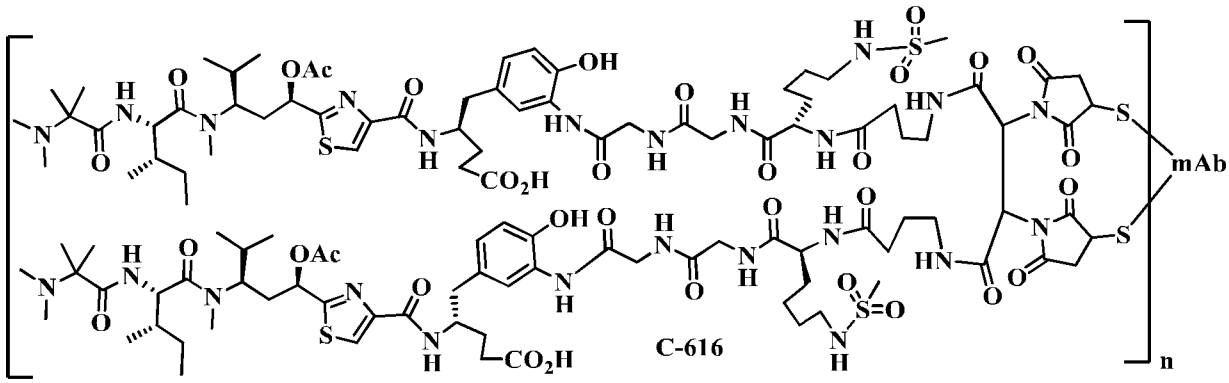
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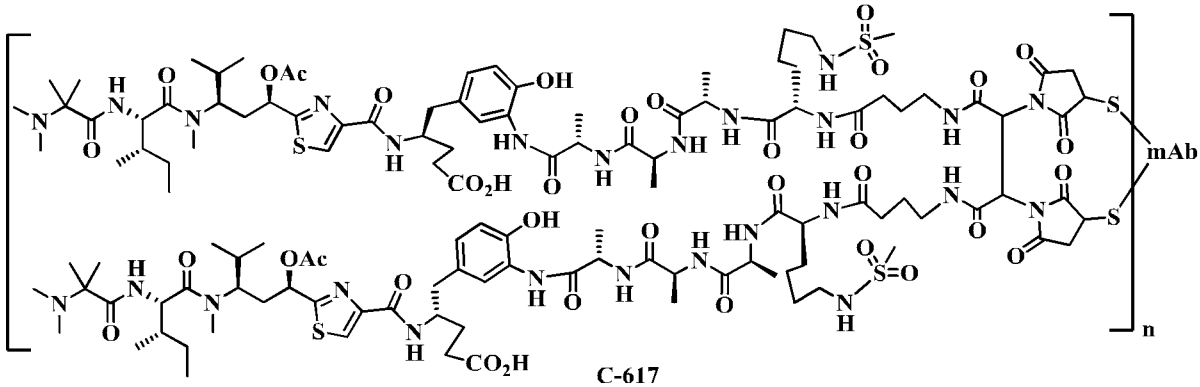

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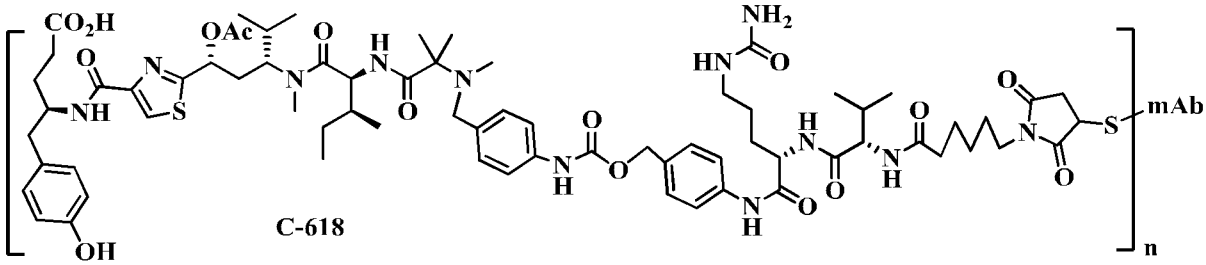
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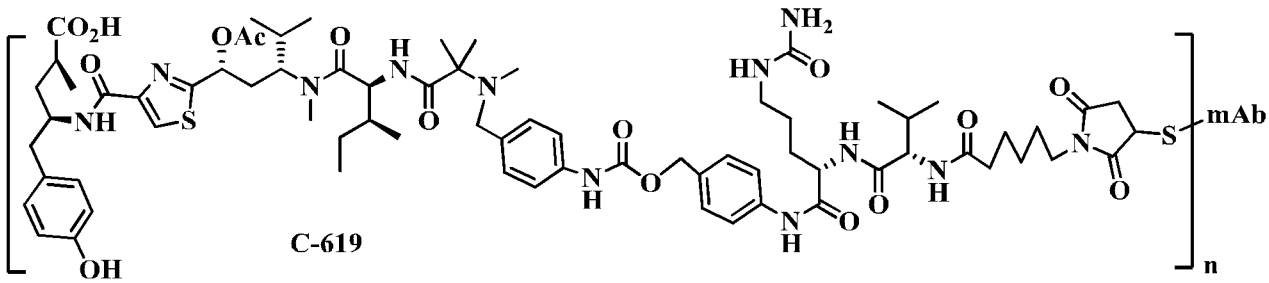
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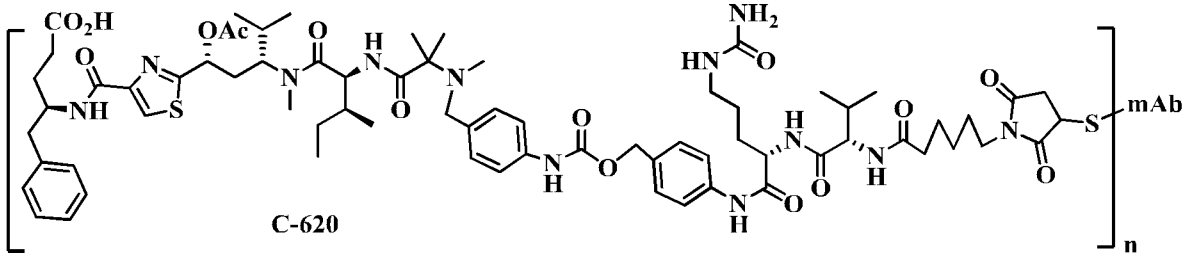
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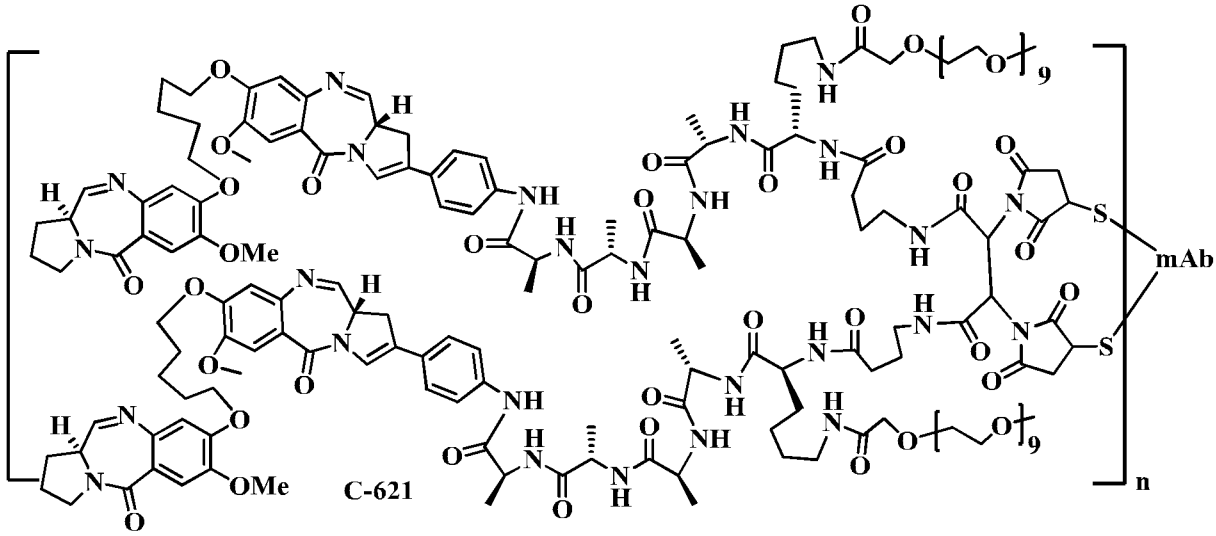
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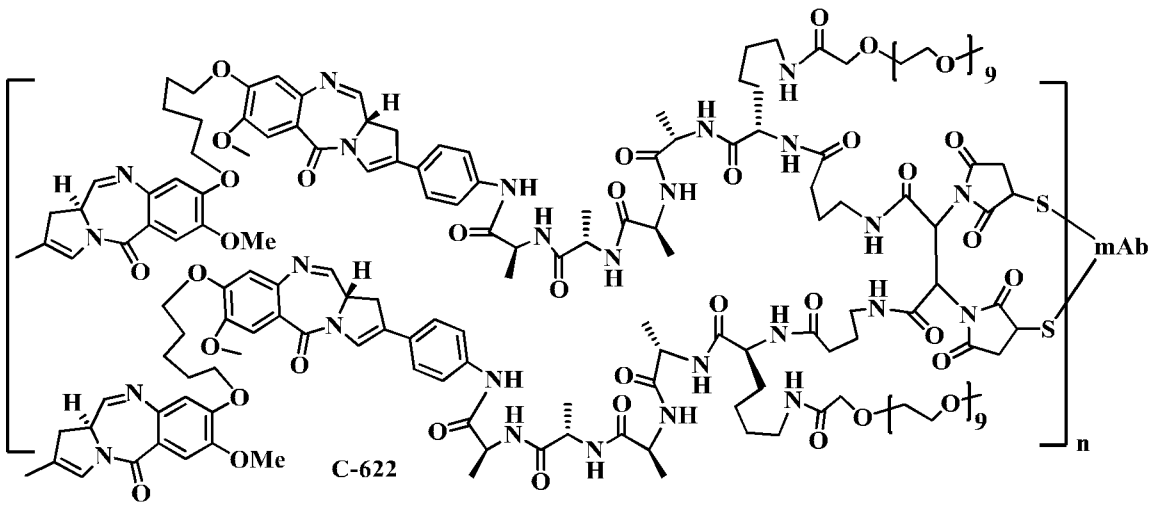
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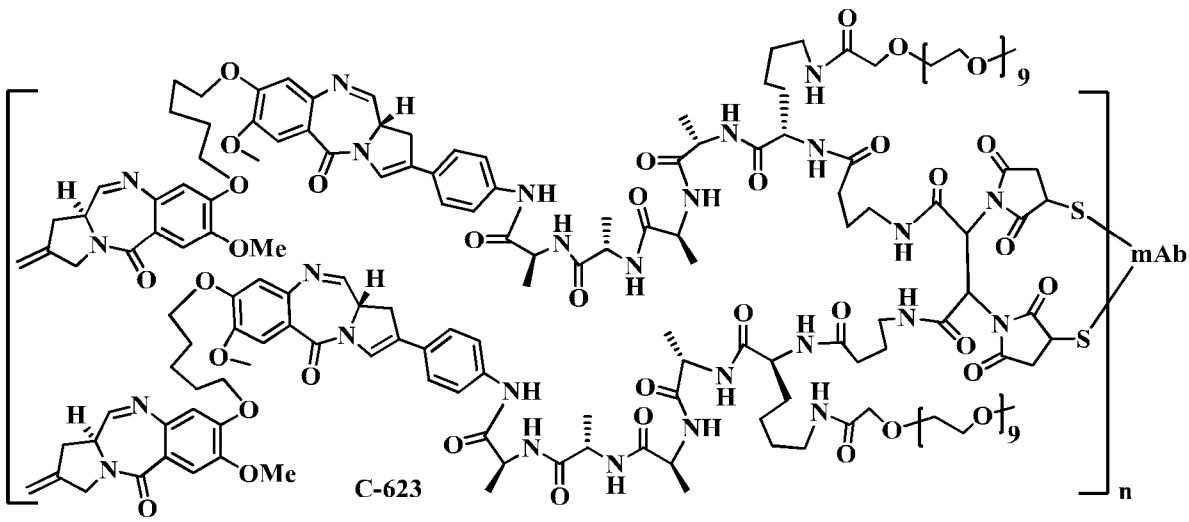
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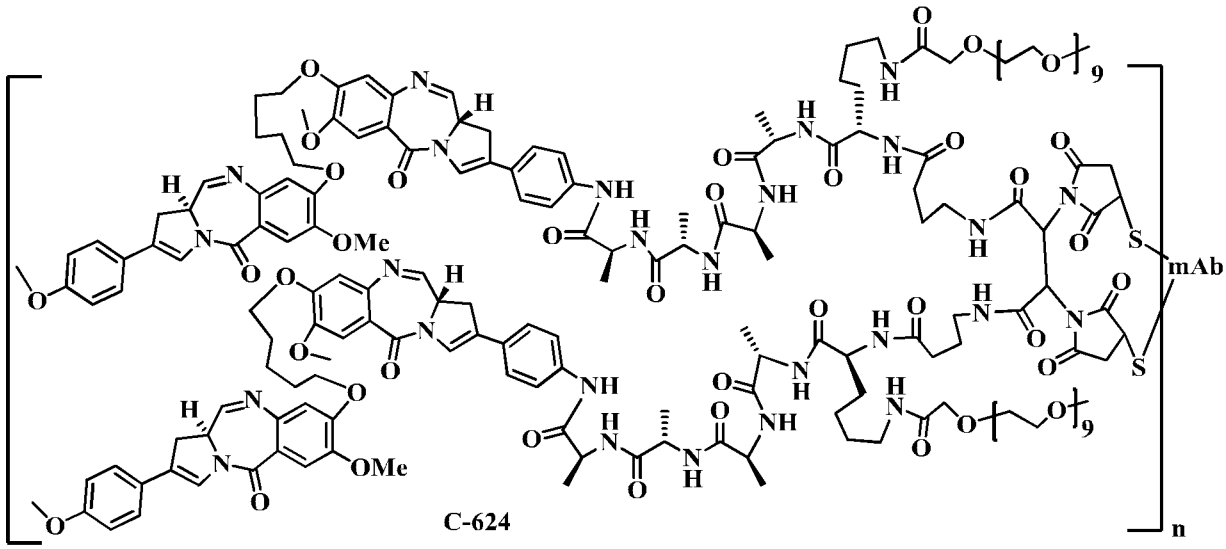
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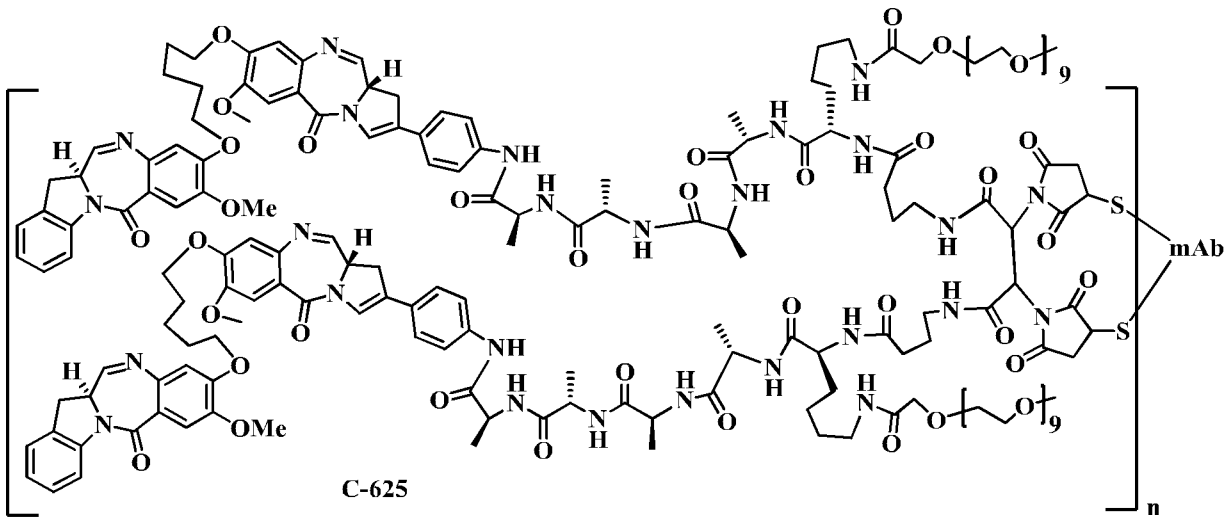
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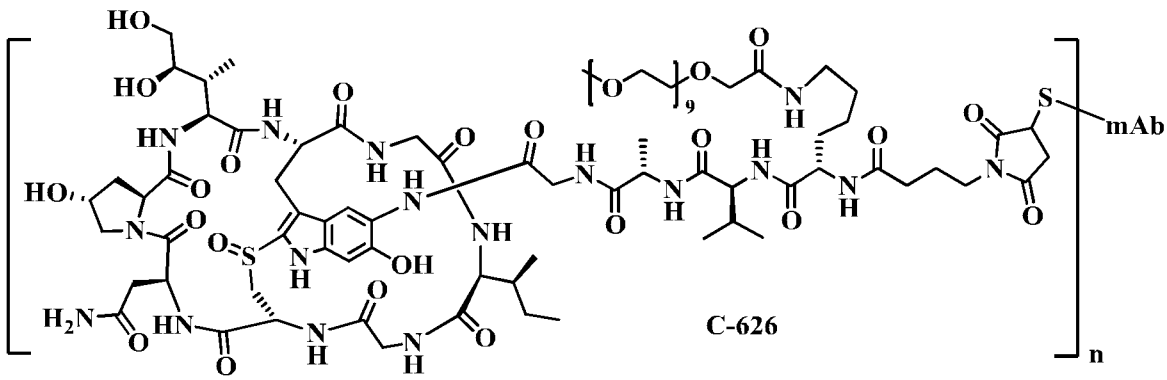
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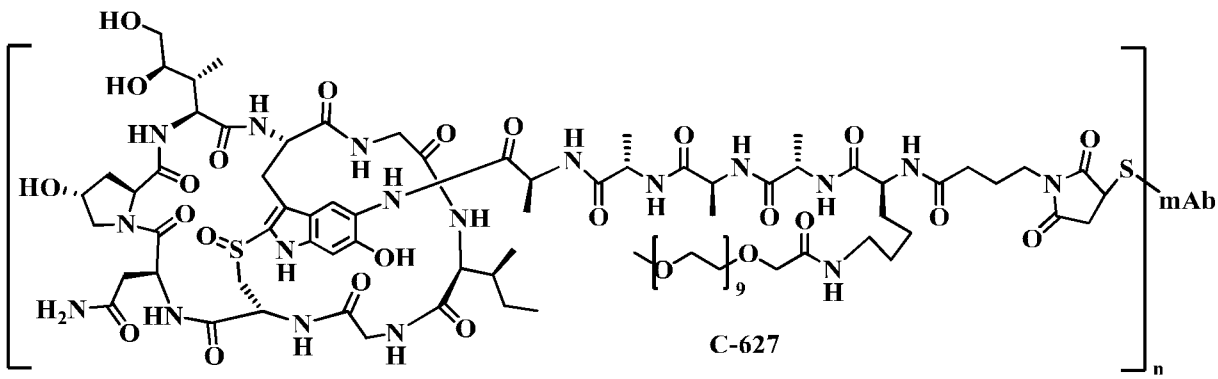
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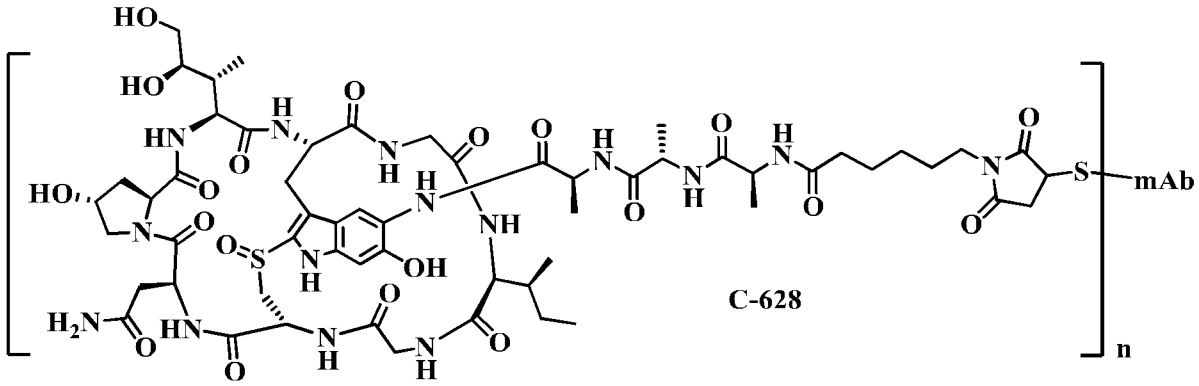
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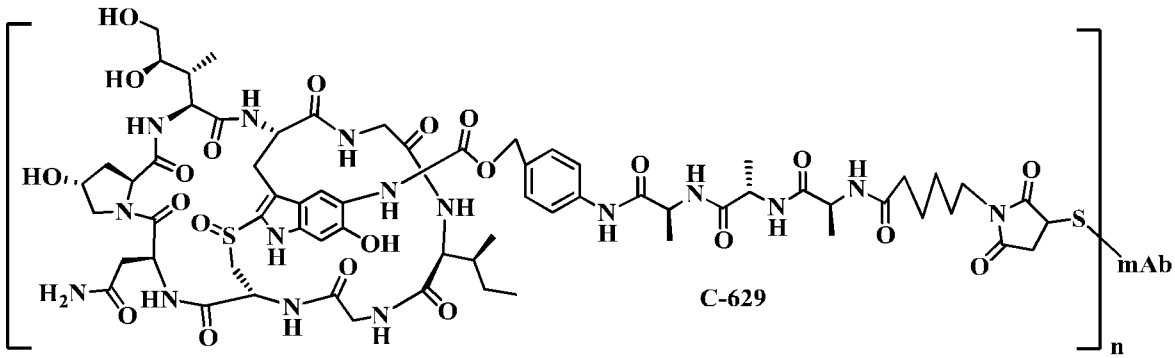
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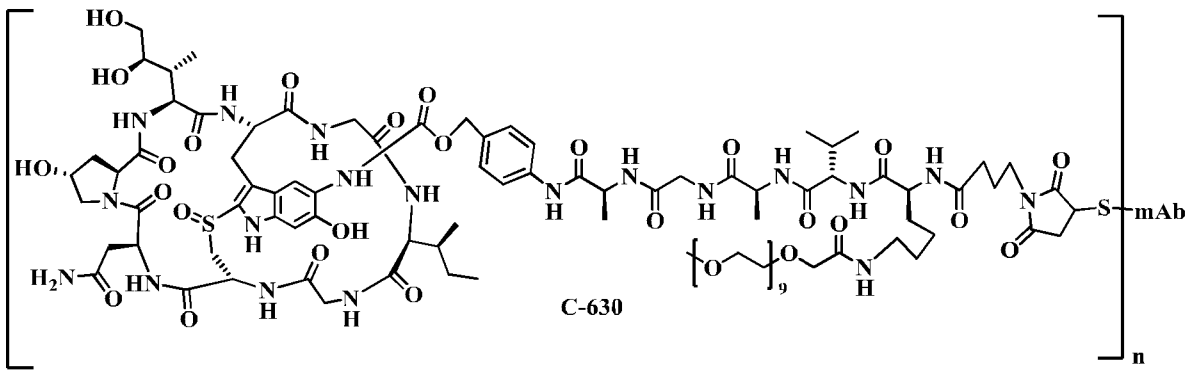
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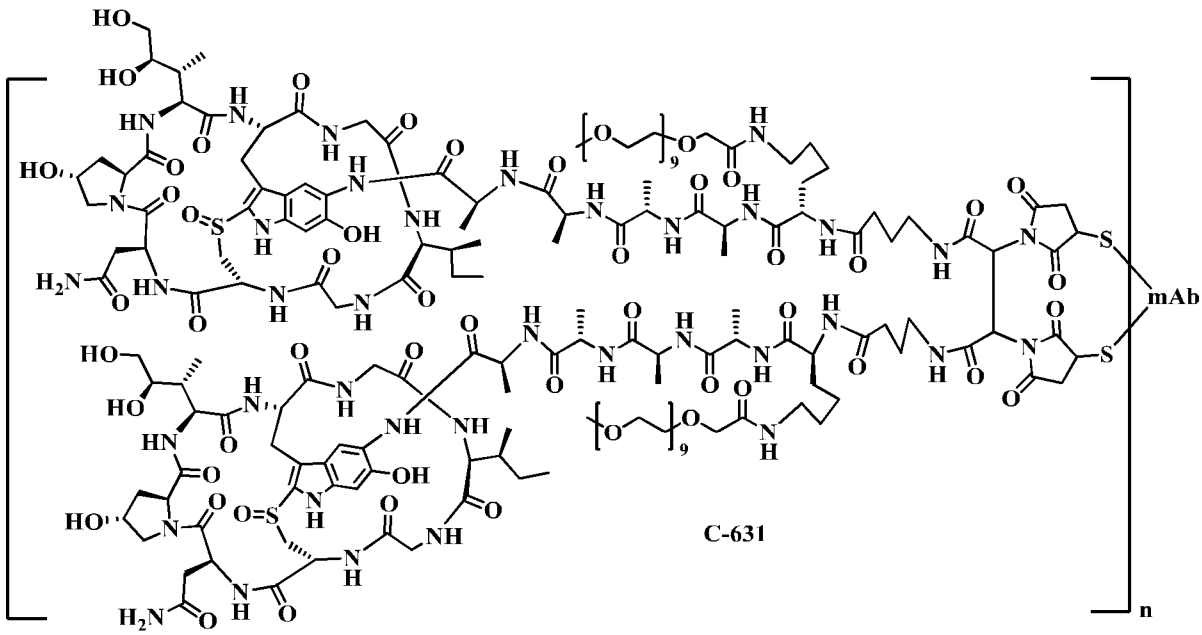
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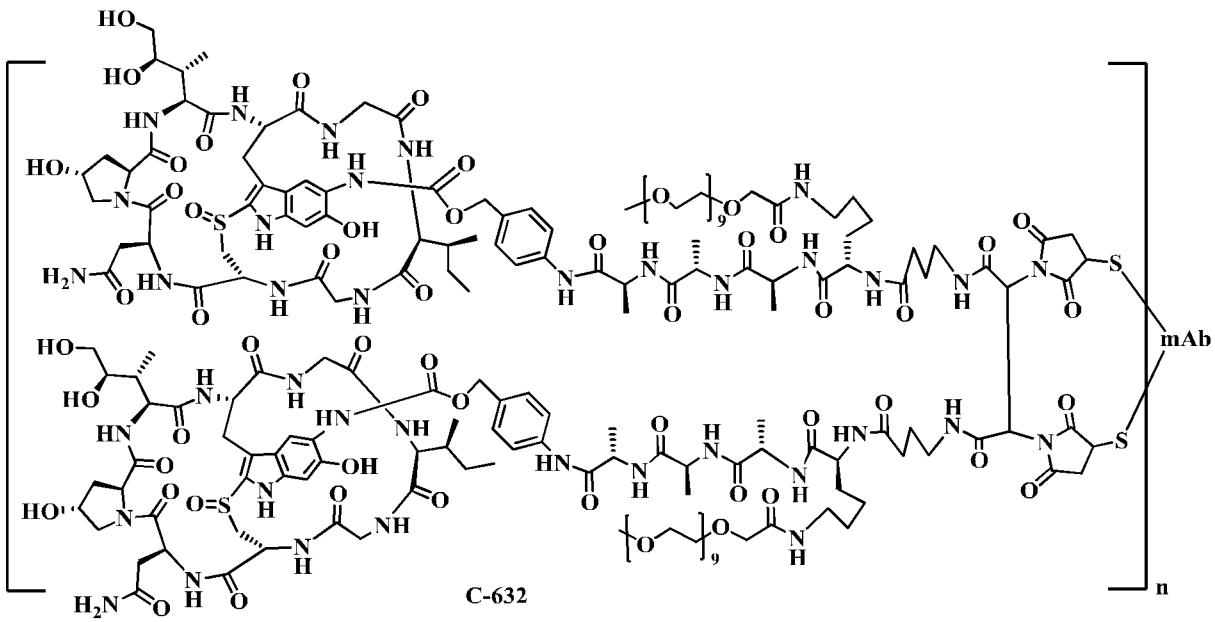
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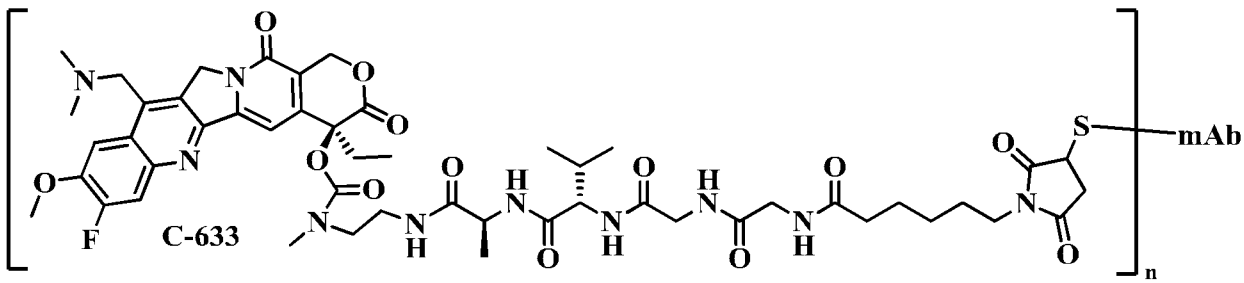
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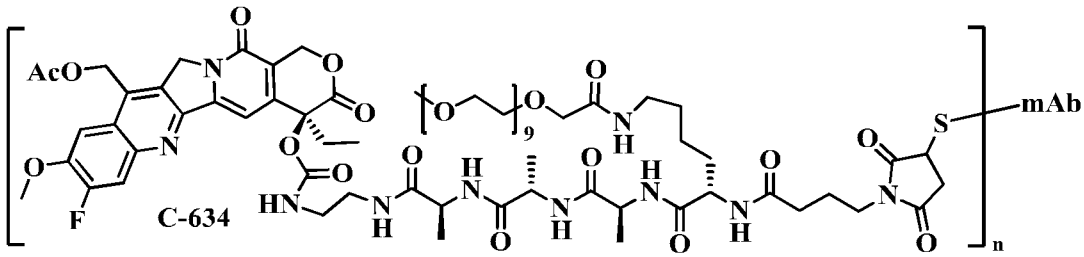
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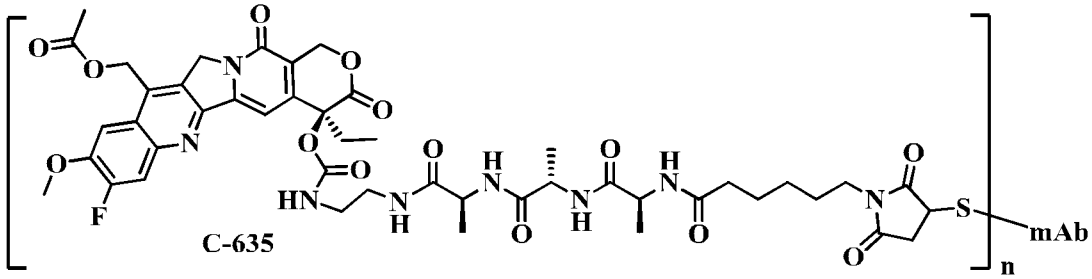
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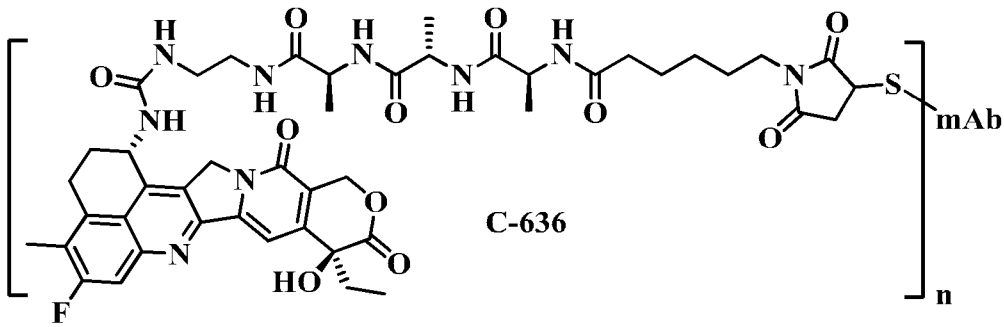
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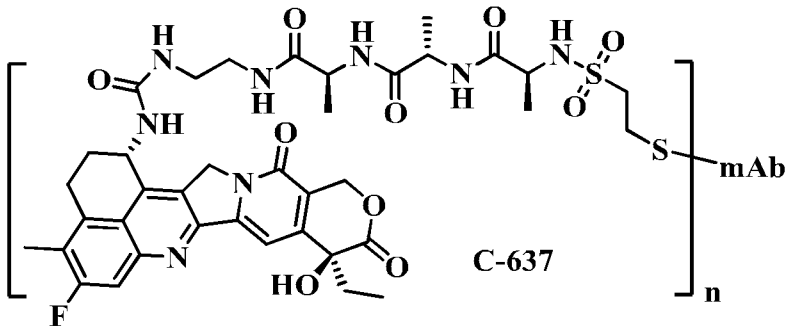
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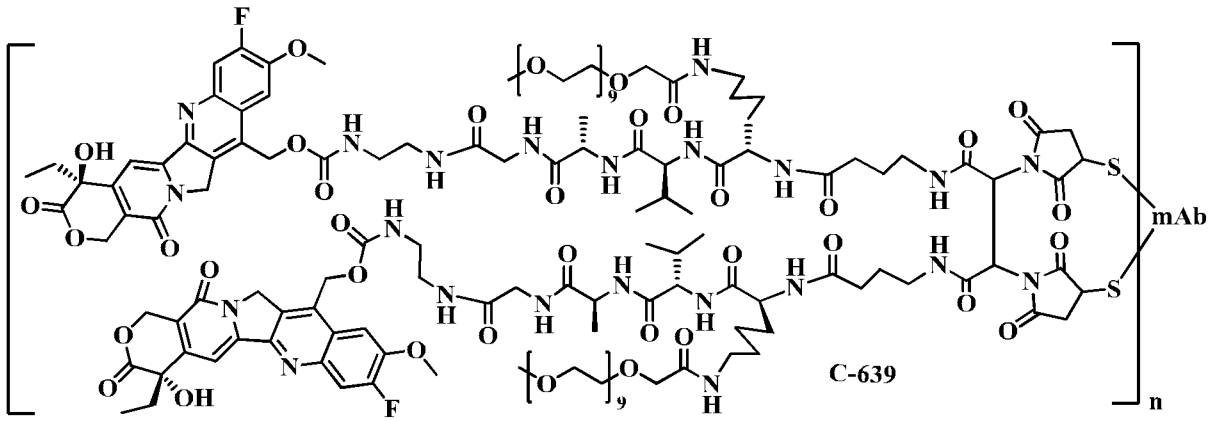


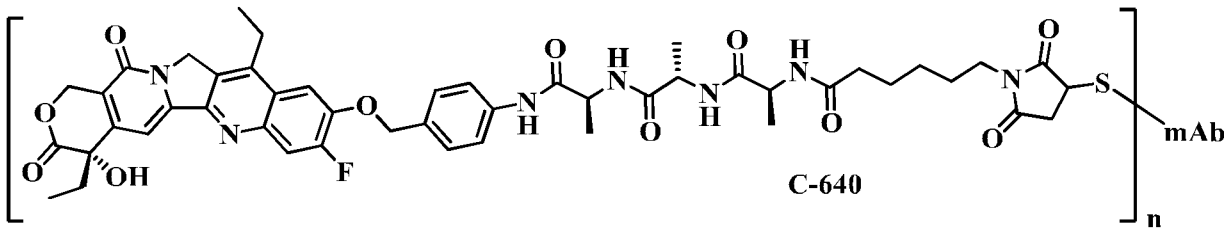
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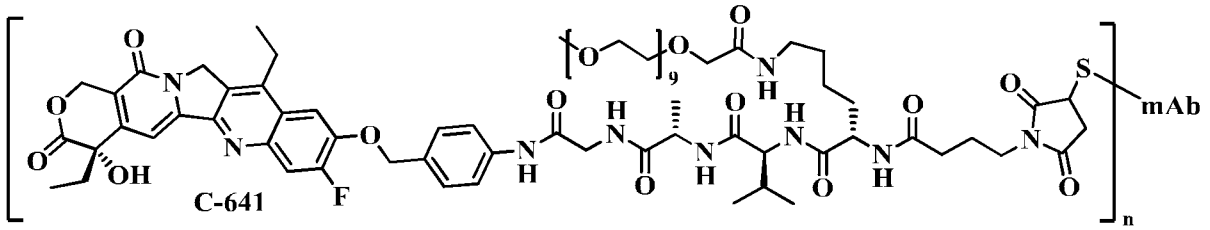


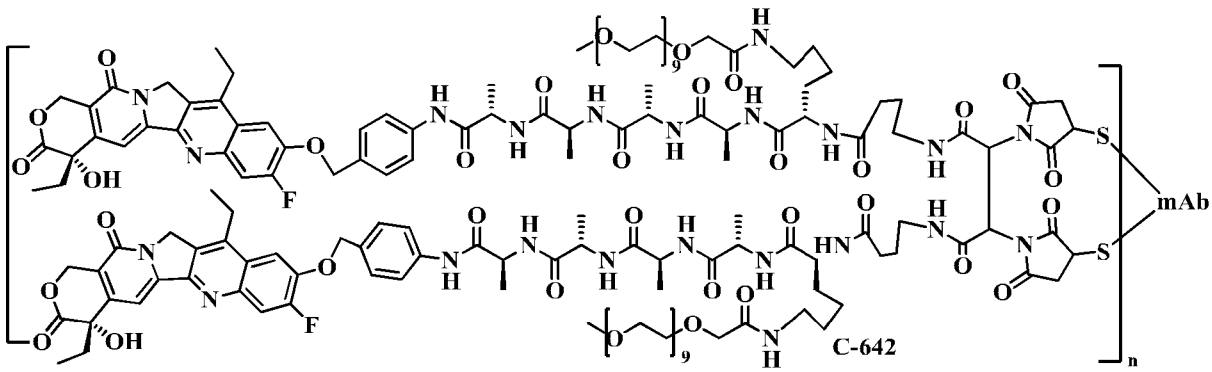
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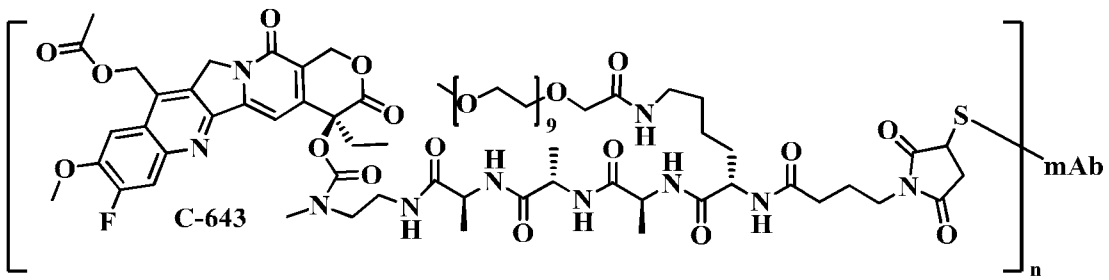

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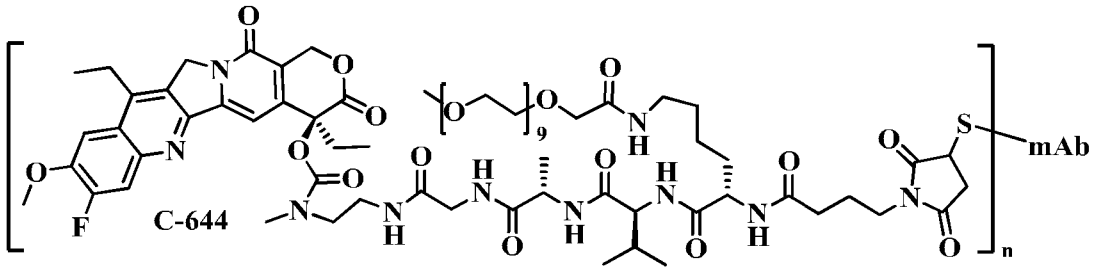

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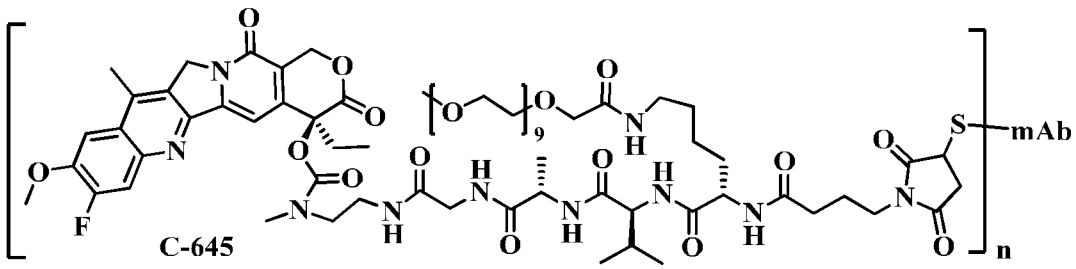

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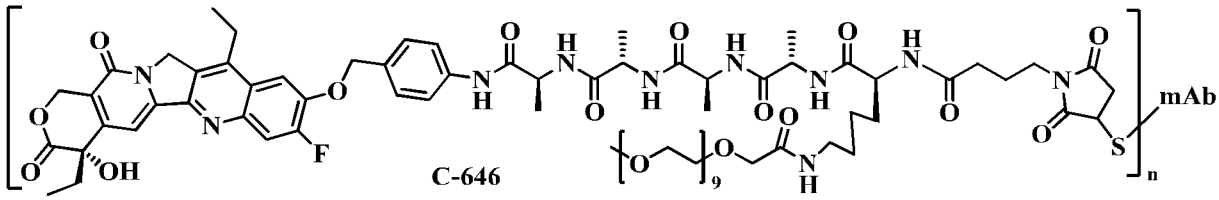

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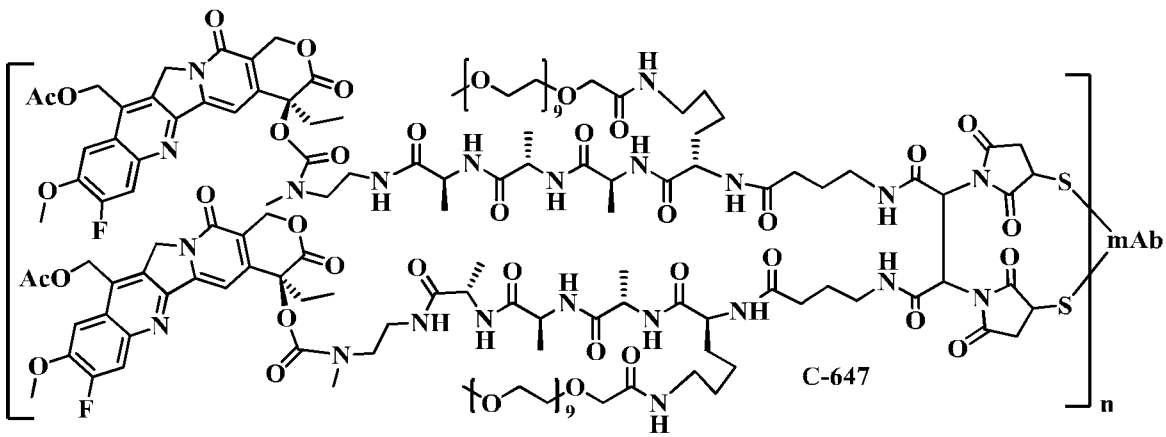

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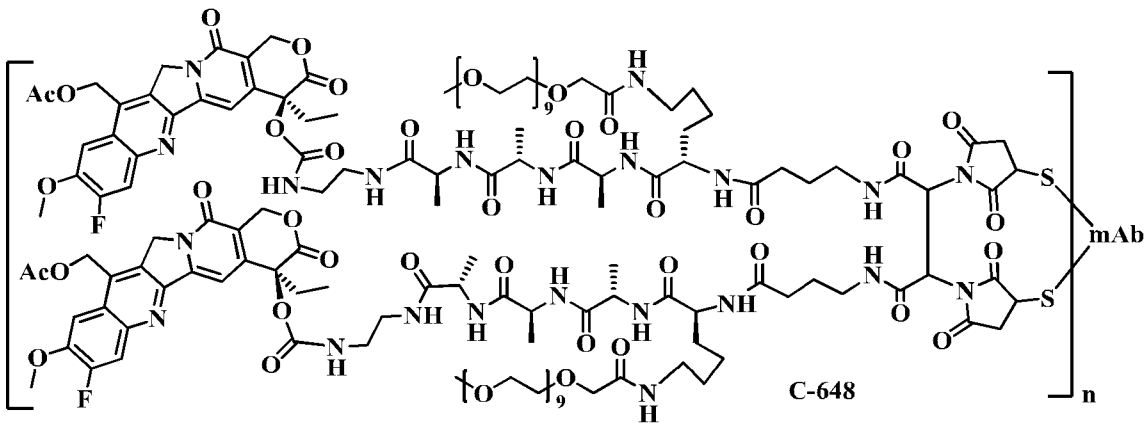

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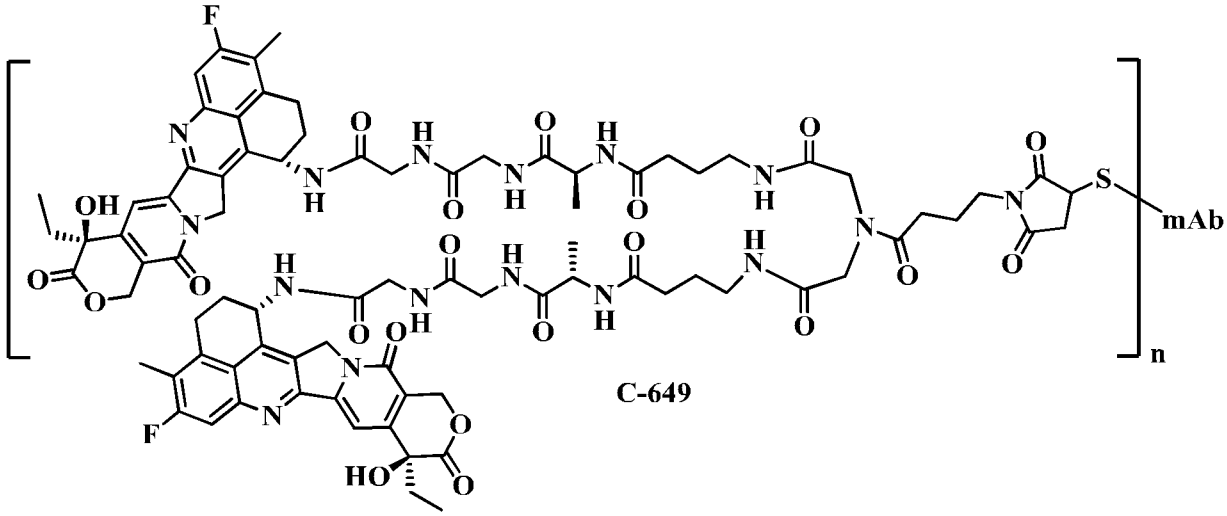

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C-645
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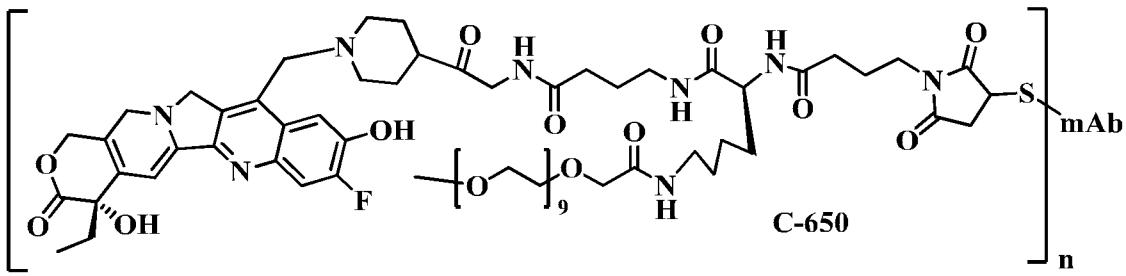

C-646
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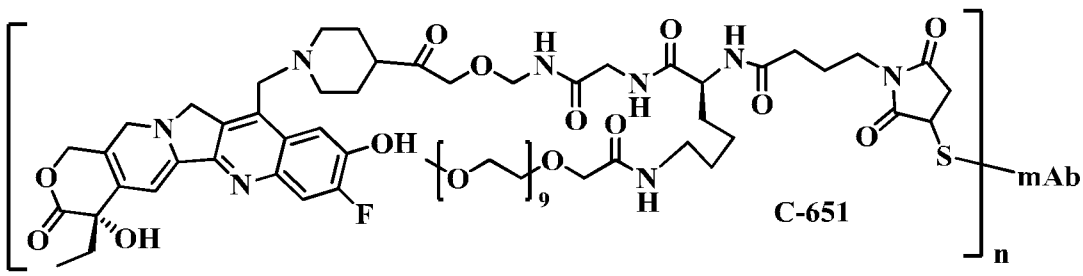

C-648
mAb
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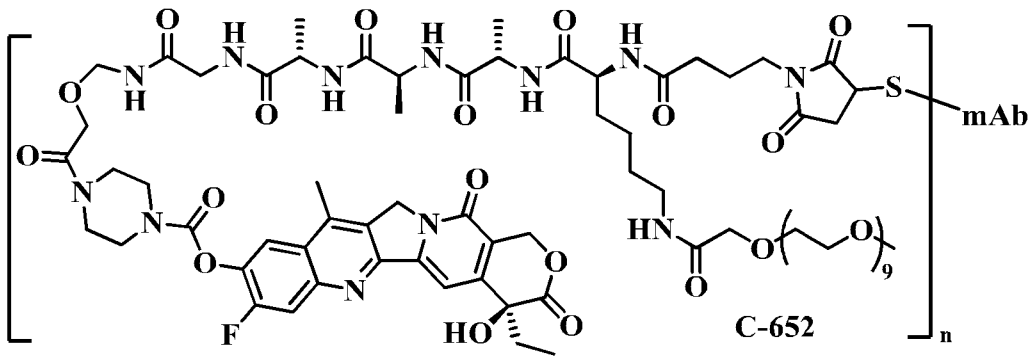
C-649



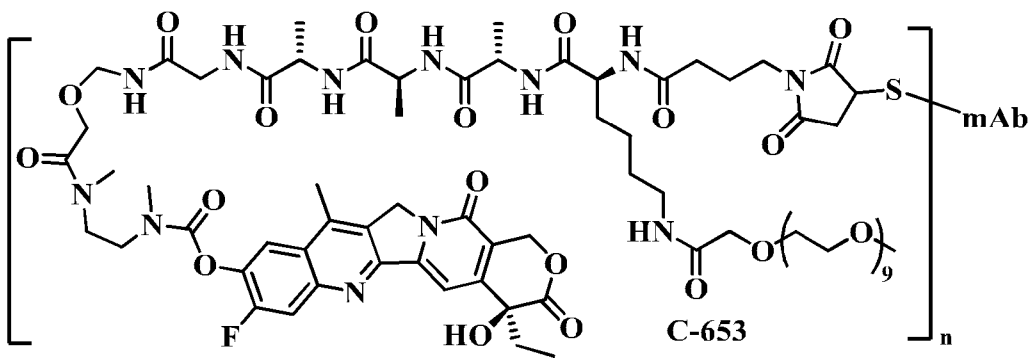
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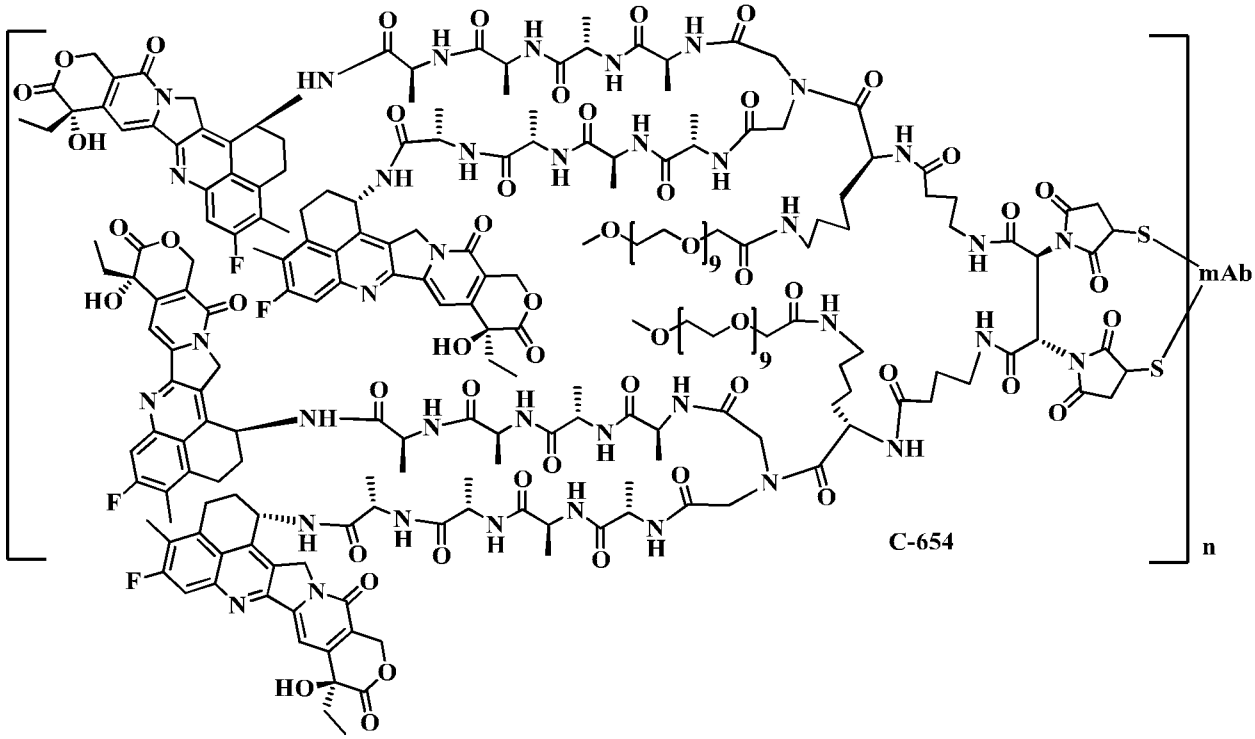
C-651



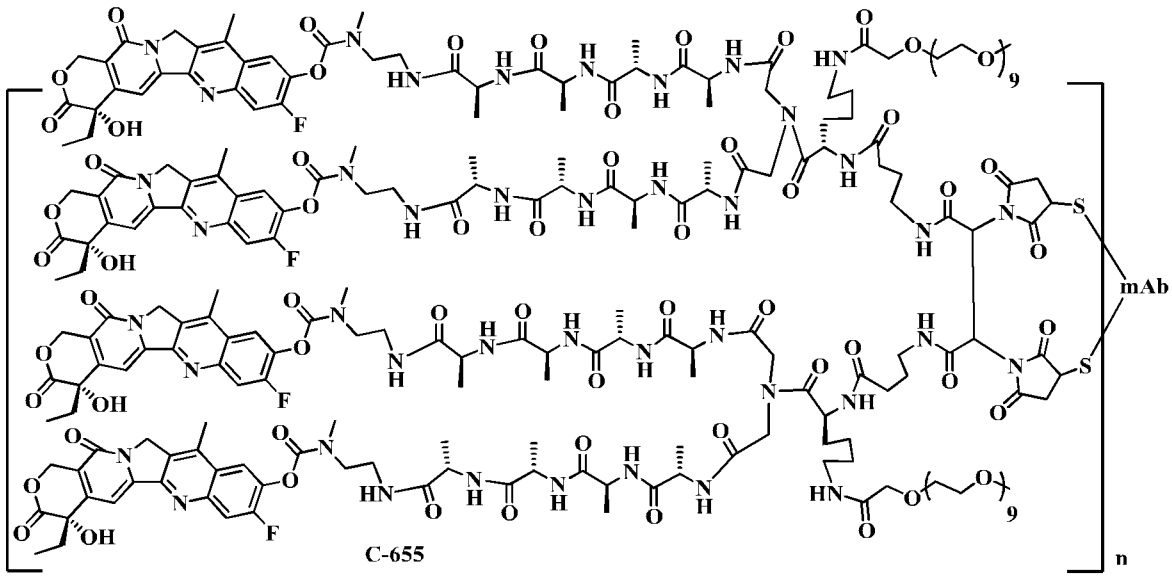
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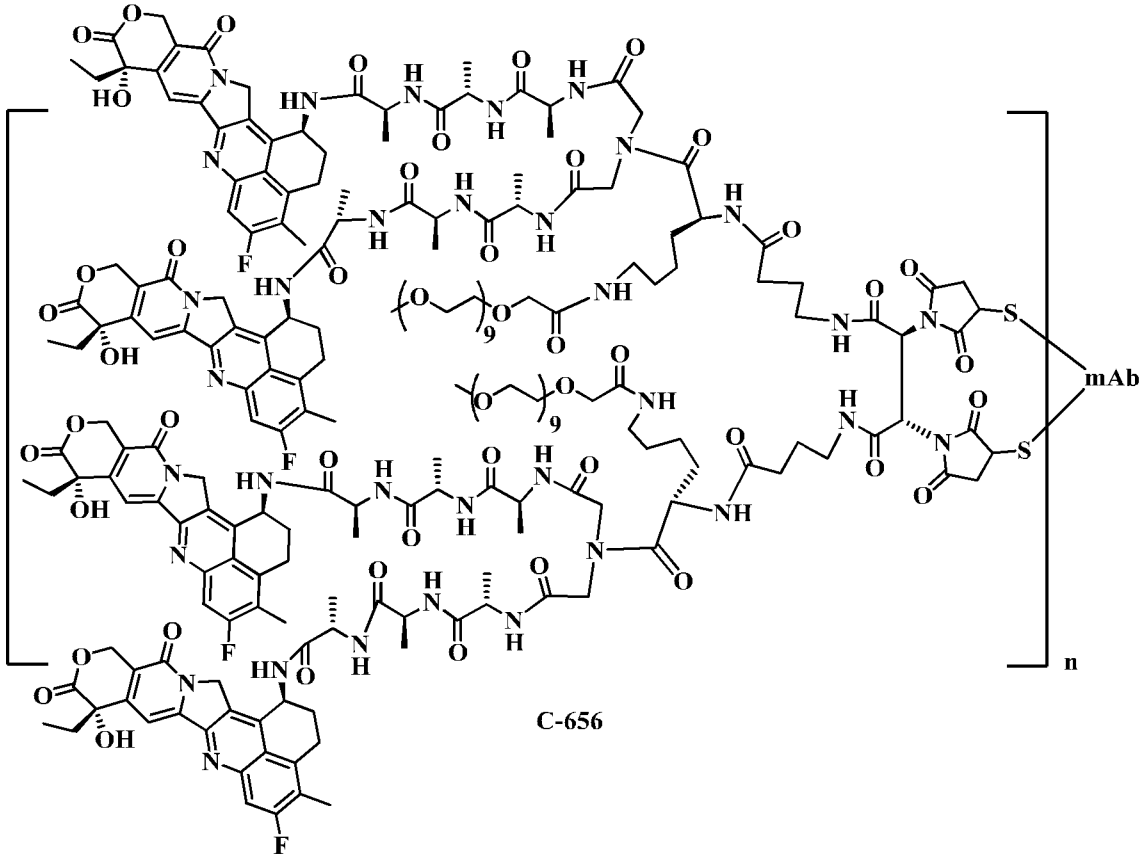
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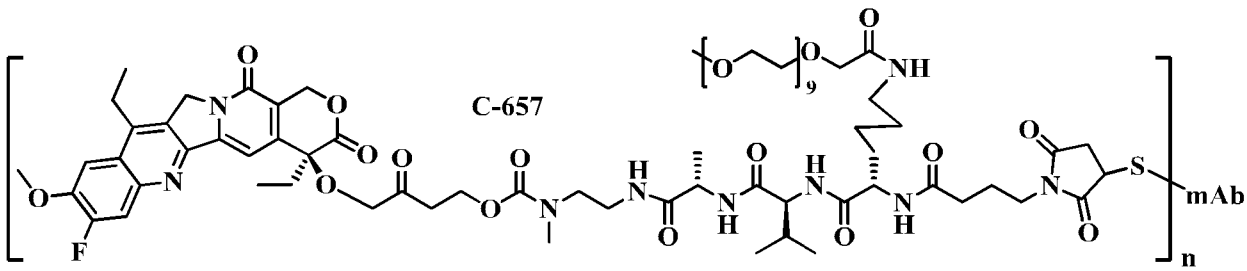
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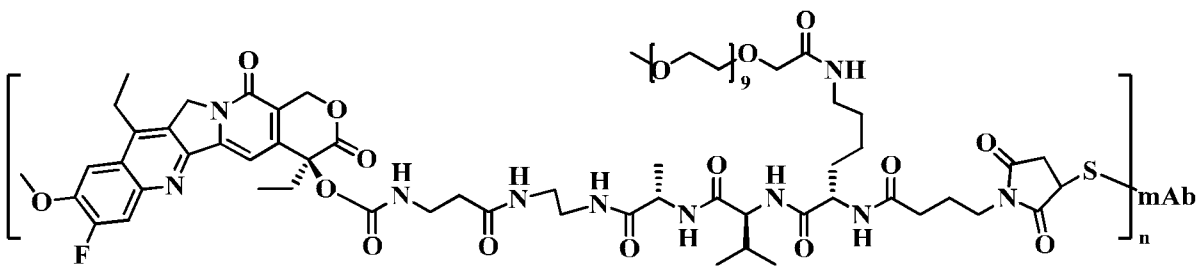
C-655



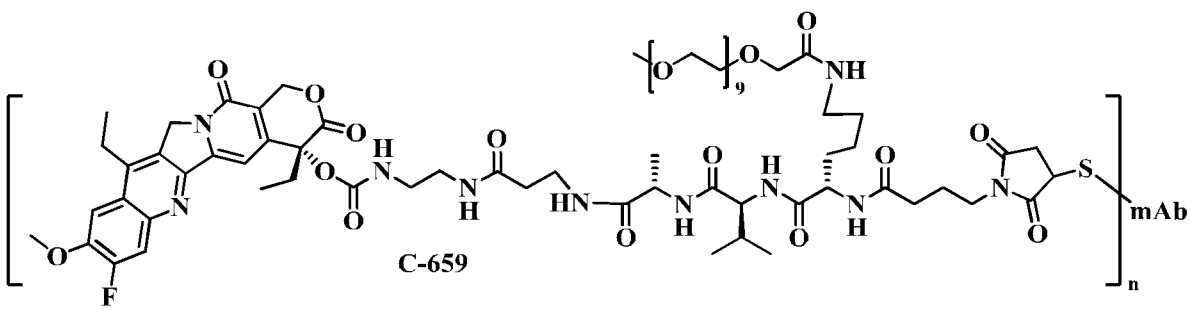
C-656



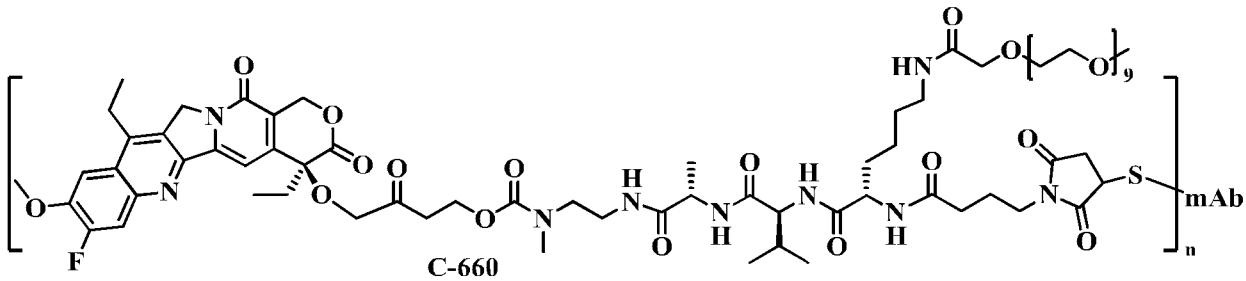
C-657



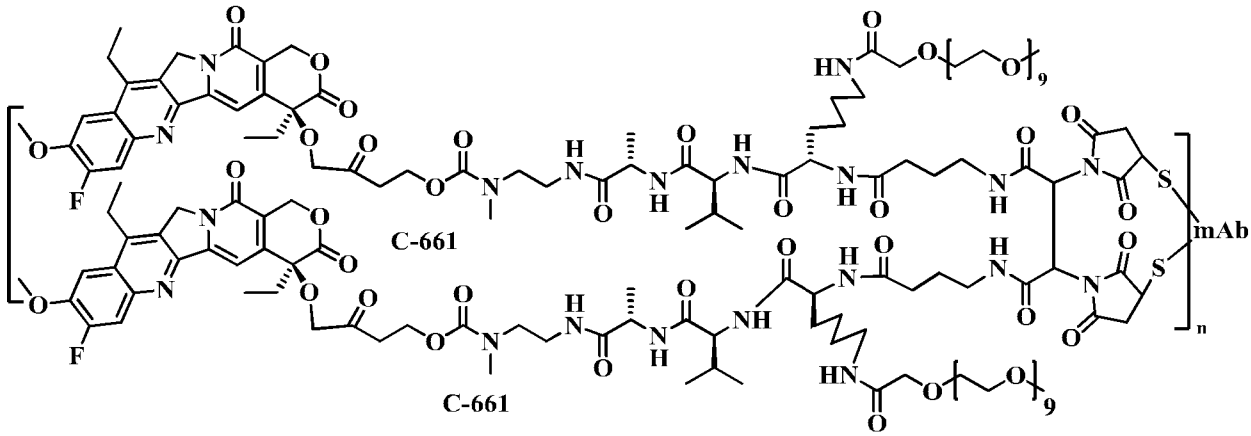
C-658



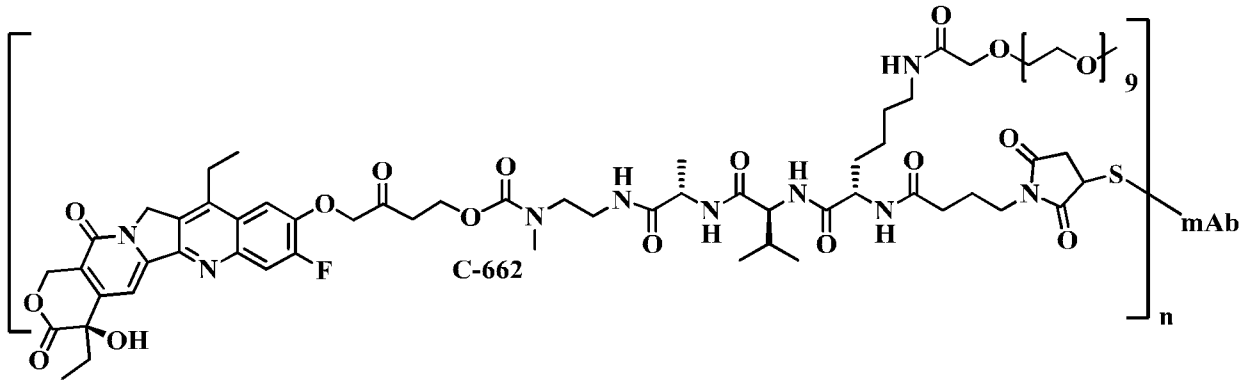
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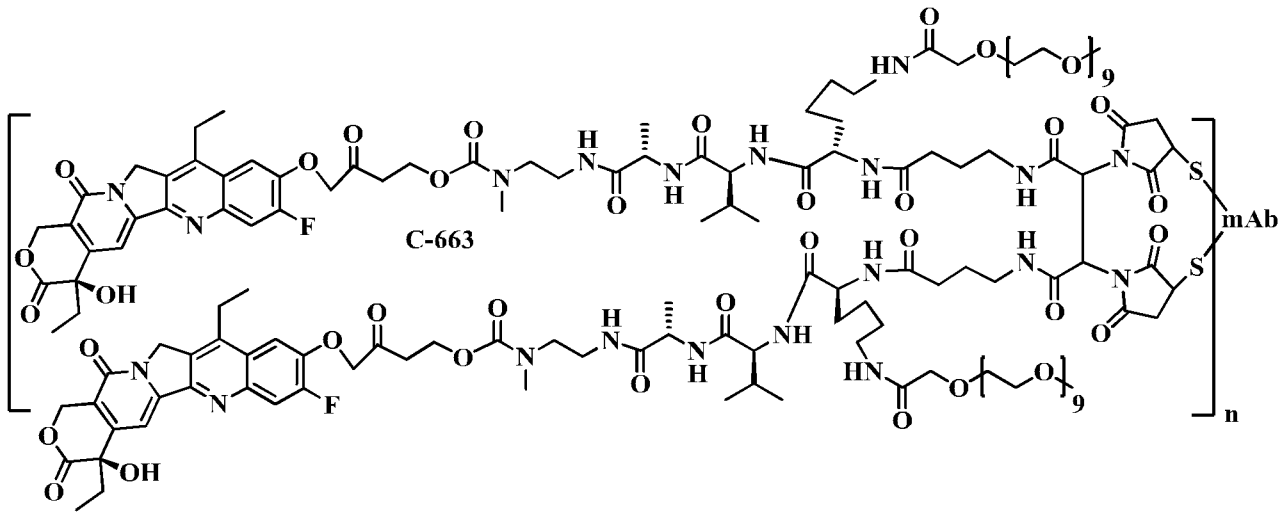
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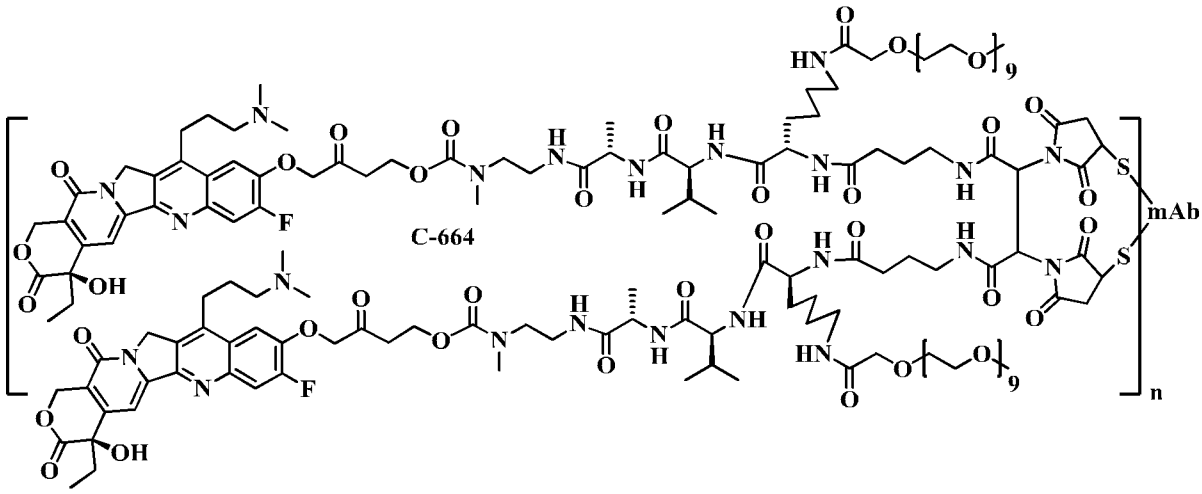
C-661



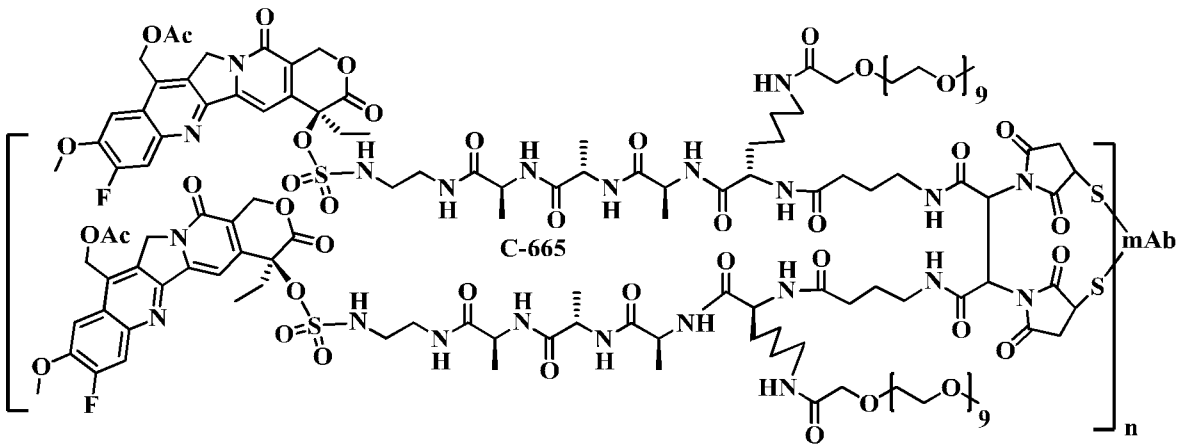
C-662



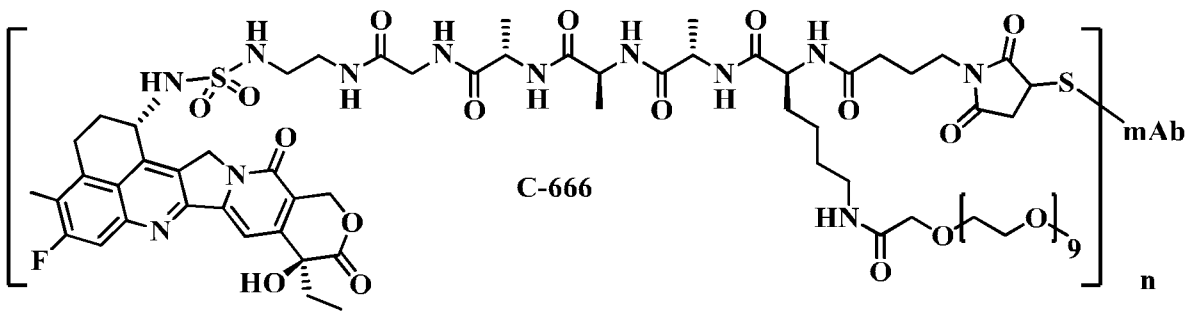
C-663



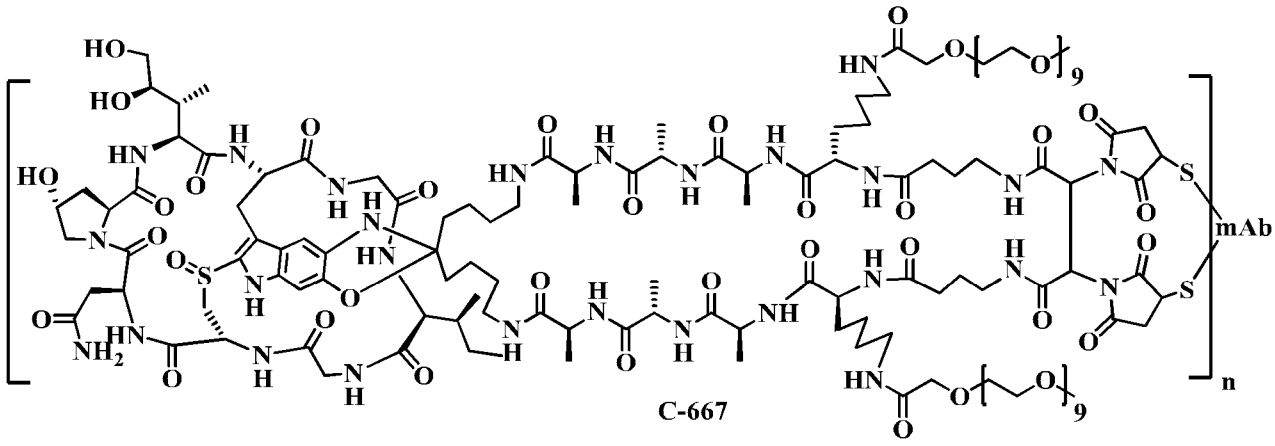
C-664



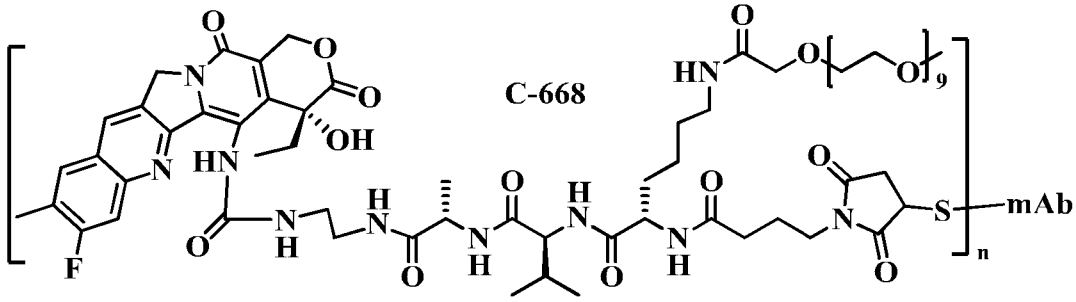
C-665



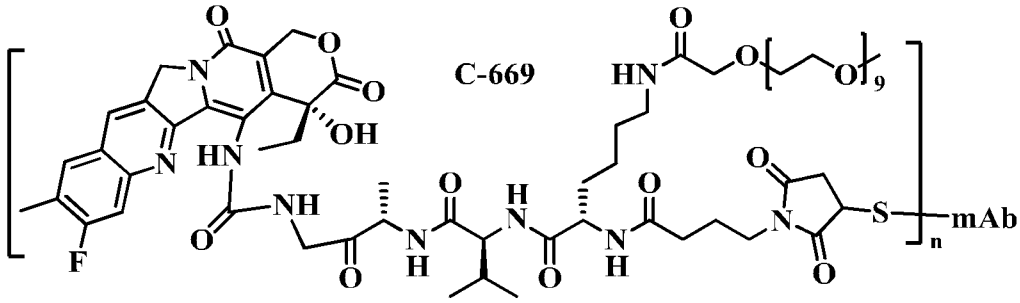
C-666



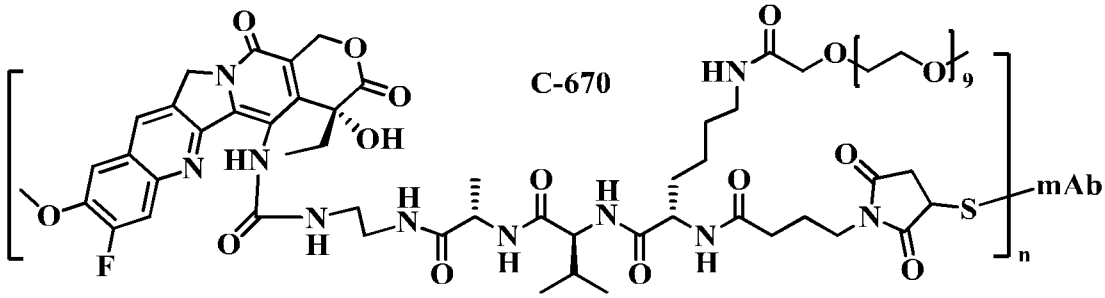
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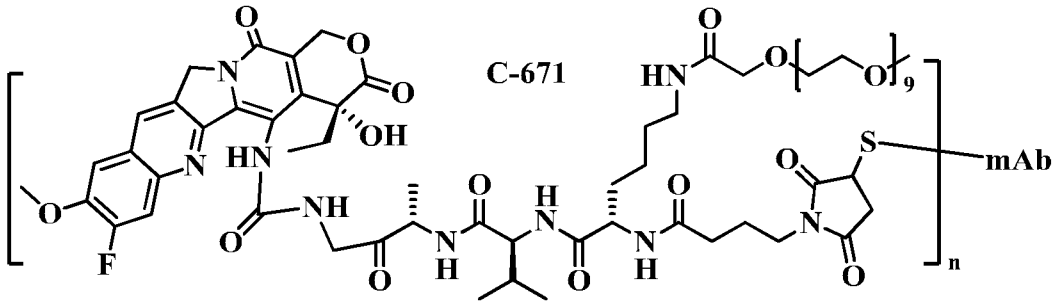
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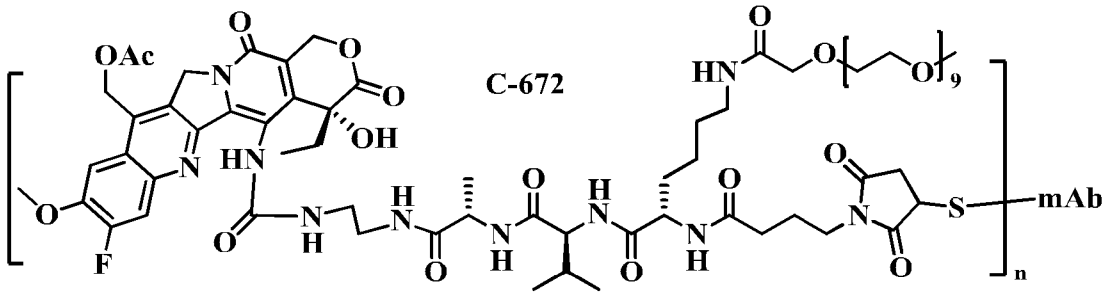
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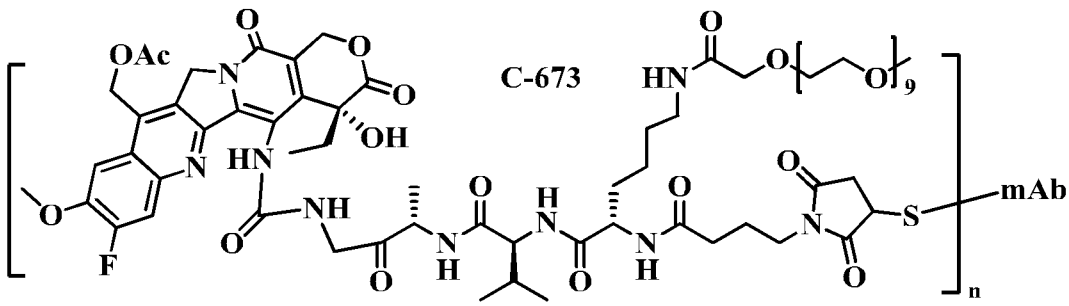
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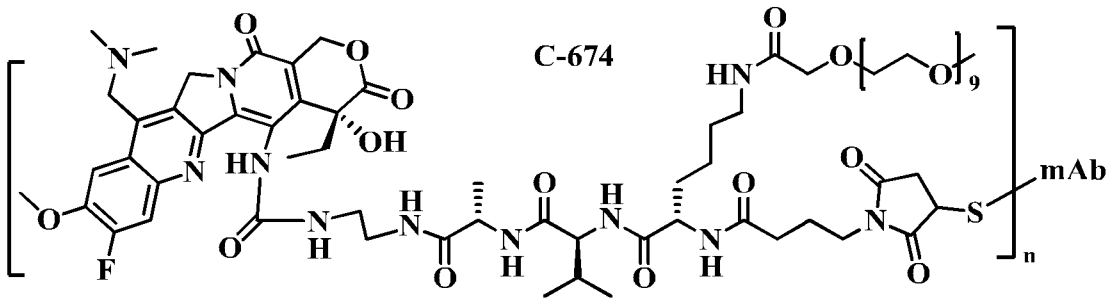
C-671



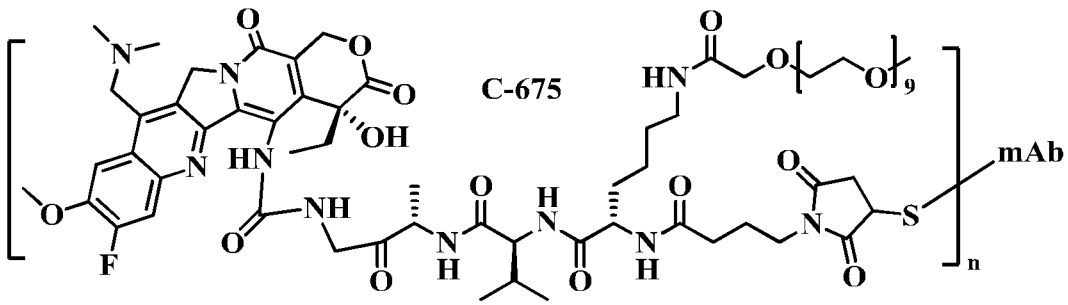
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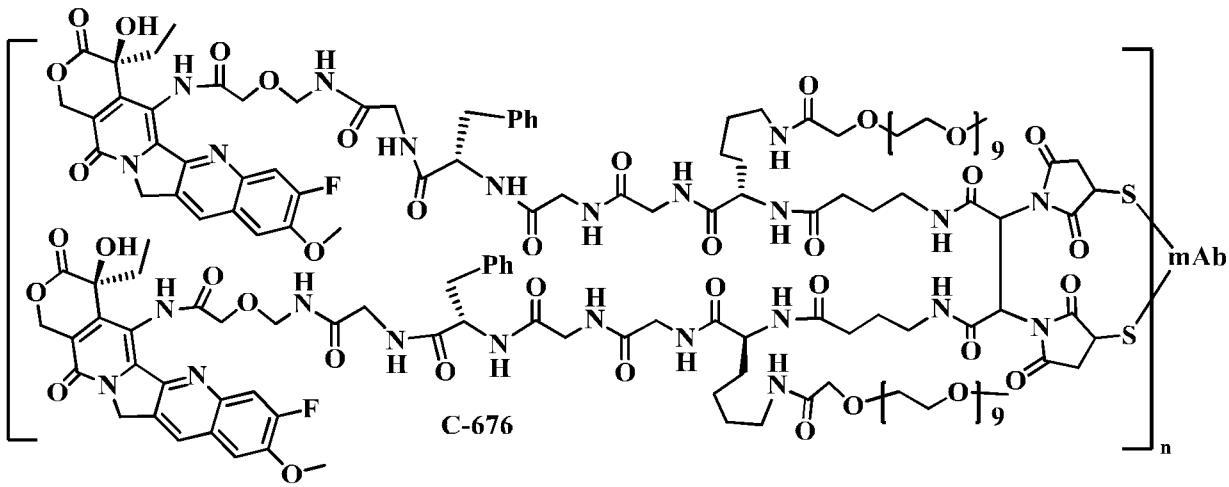
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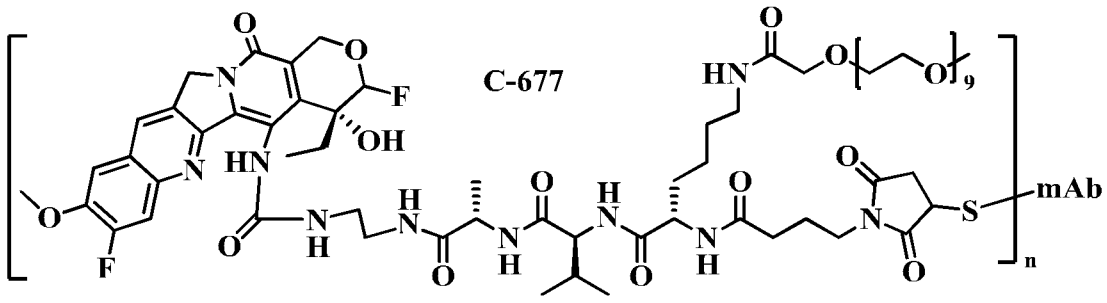
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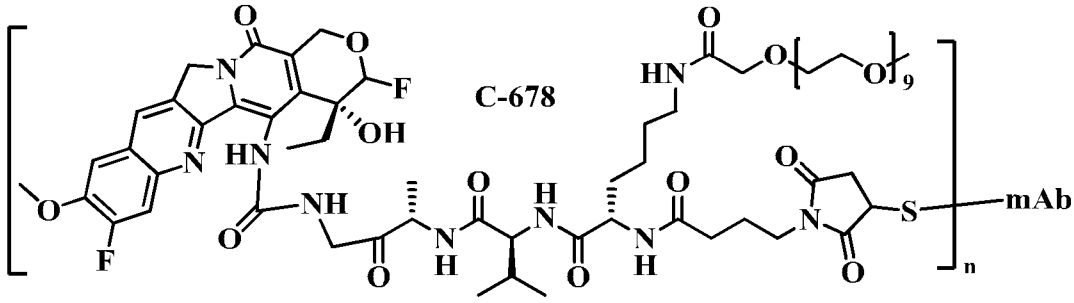
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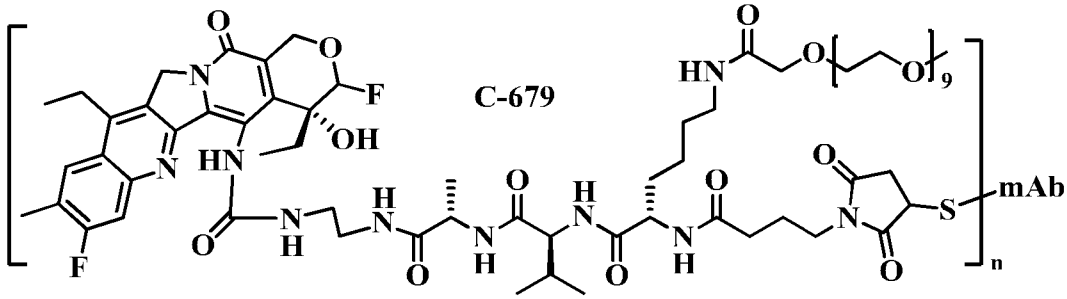
C-676



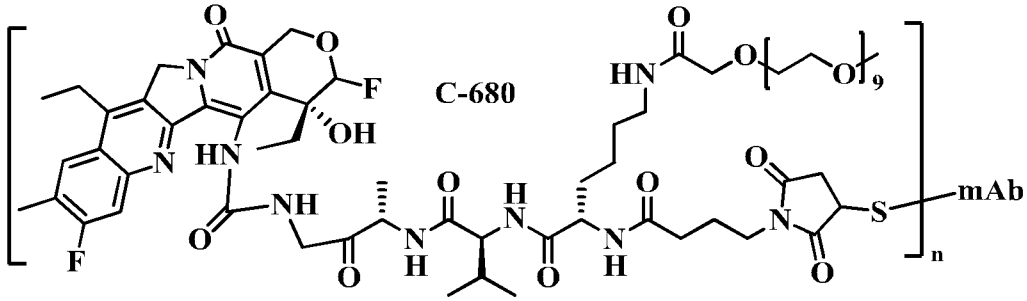
C-677



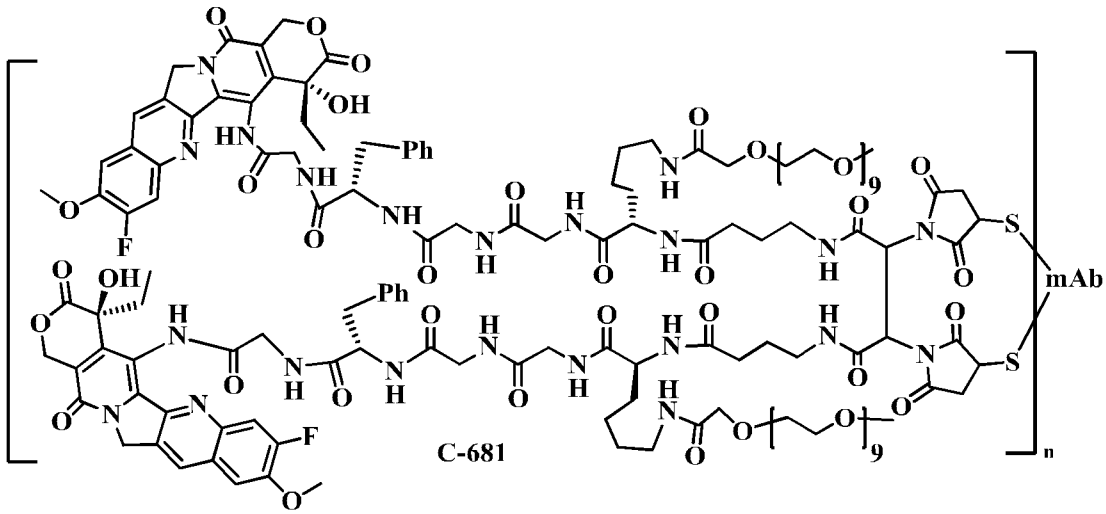
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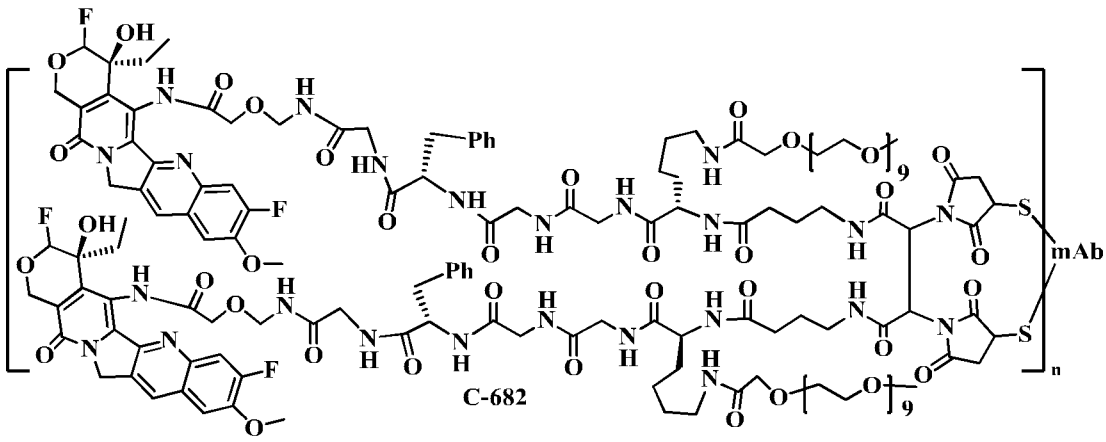
C-679



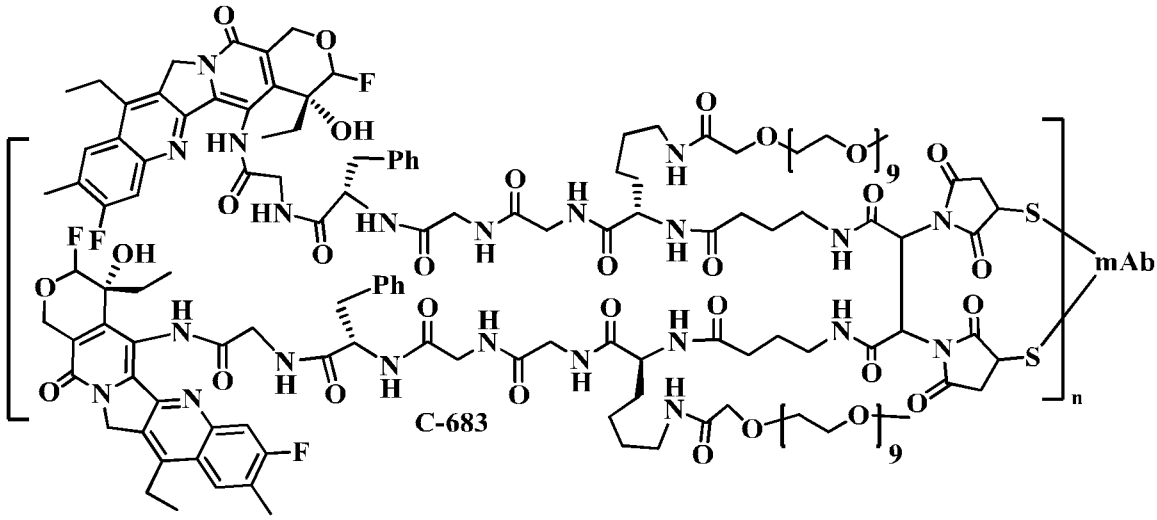
C-680

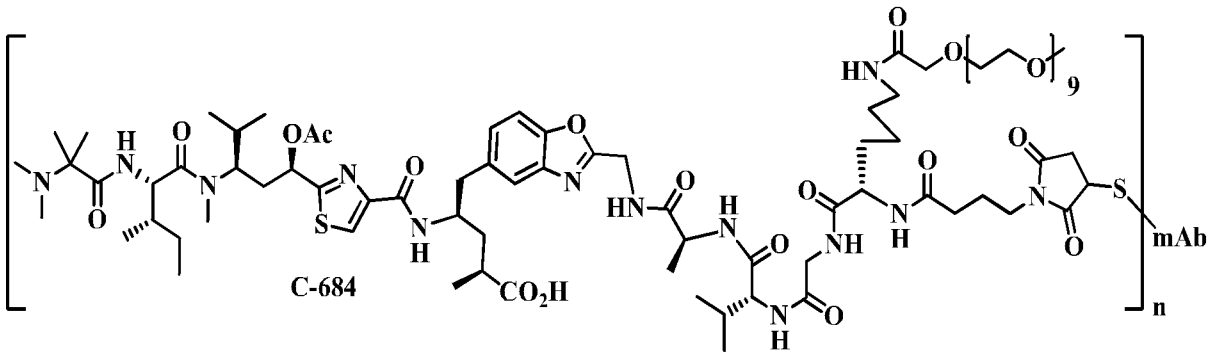


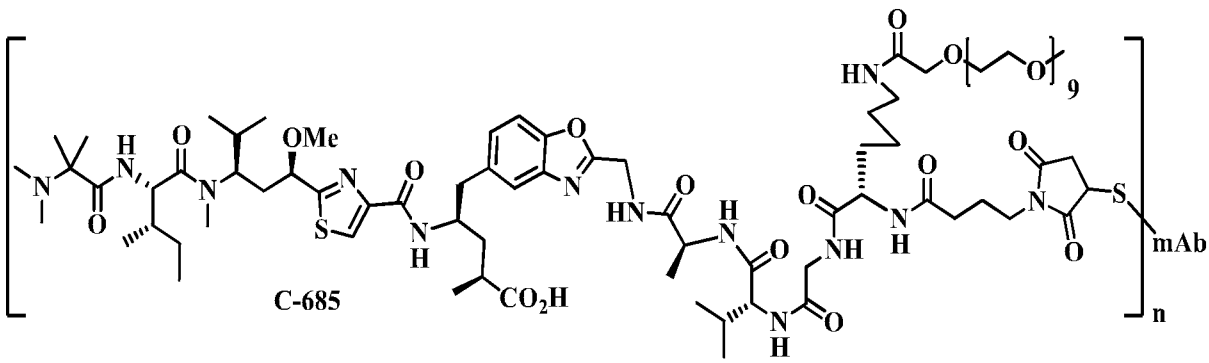
C-681

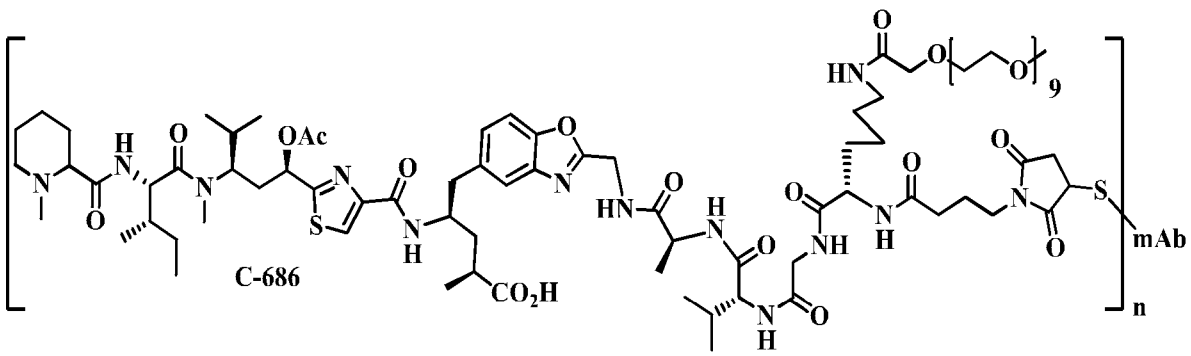


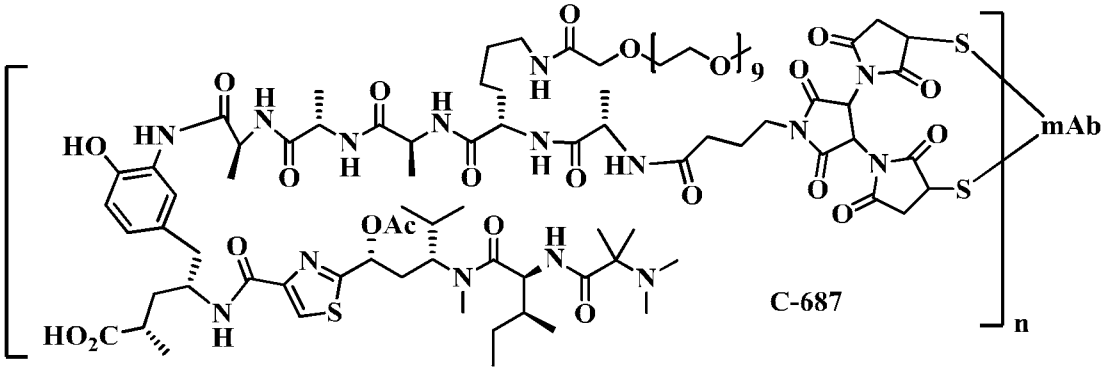
C-682


C-683
mAb
n

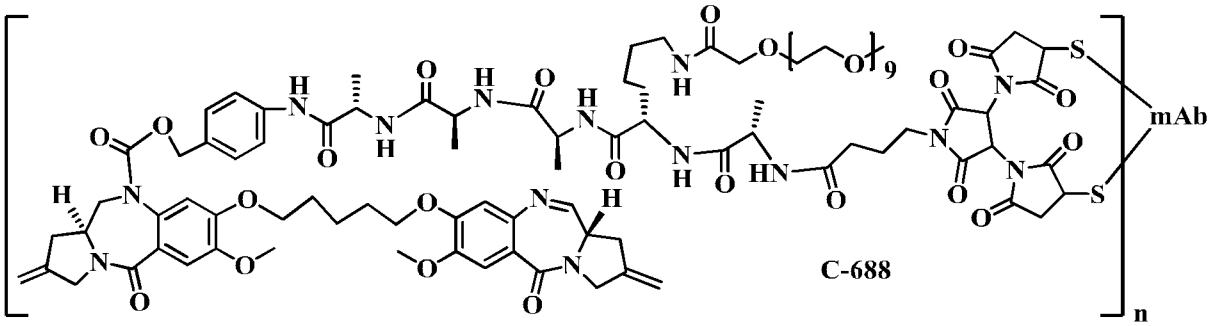

C-684
OAc
CO2H
mAb
n


C-685
OMe
CO2H
mAb
n

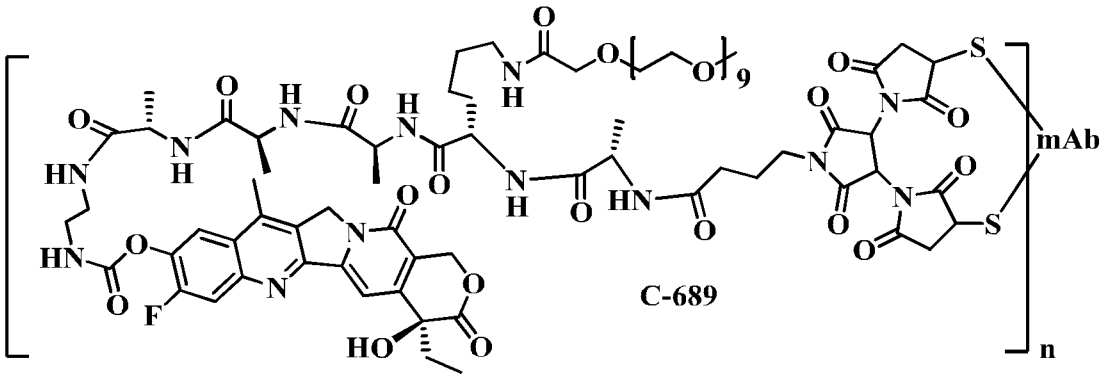

C-686
OAc
CO2H
mAb
n



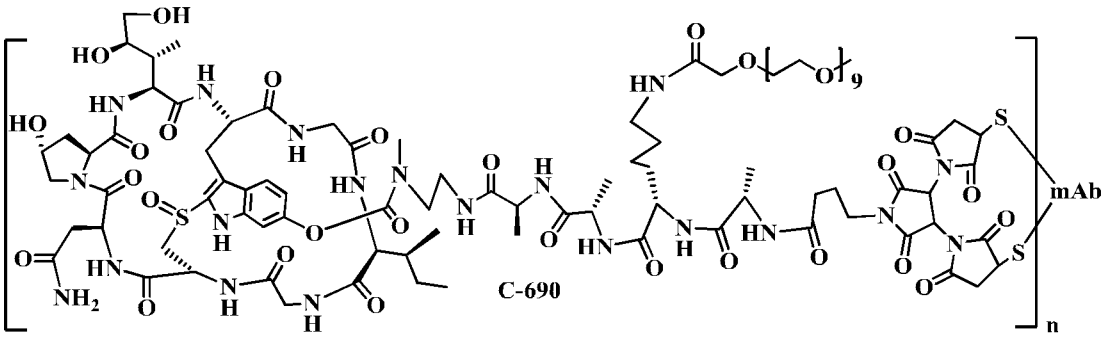
C-687



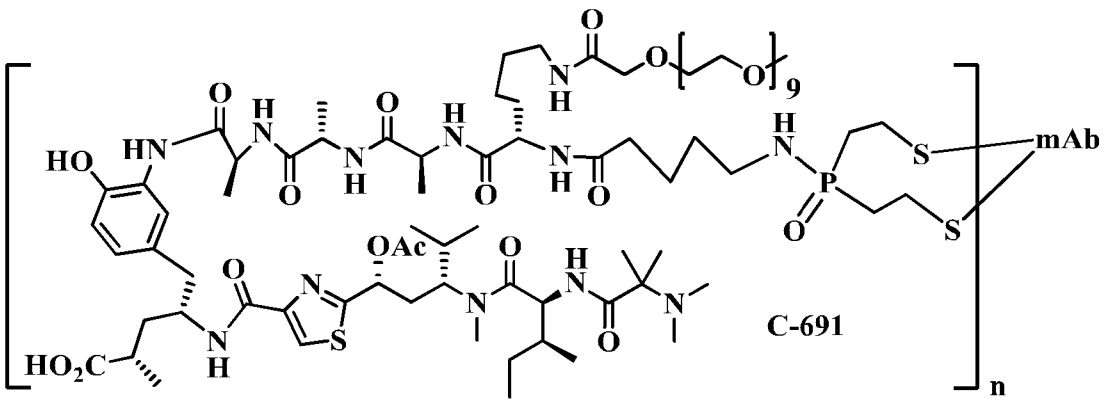
C-688



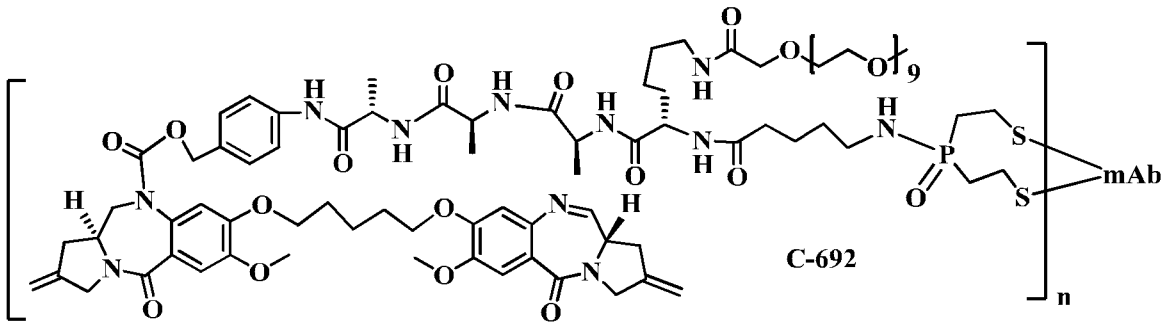
C-689



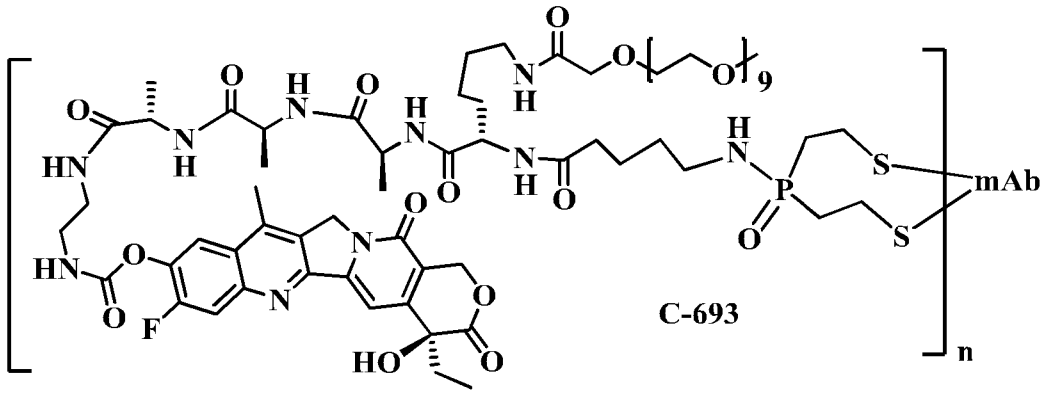
C-690



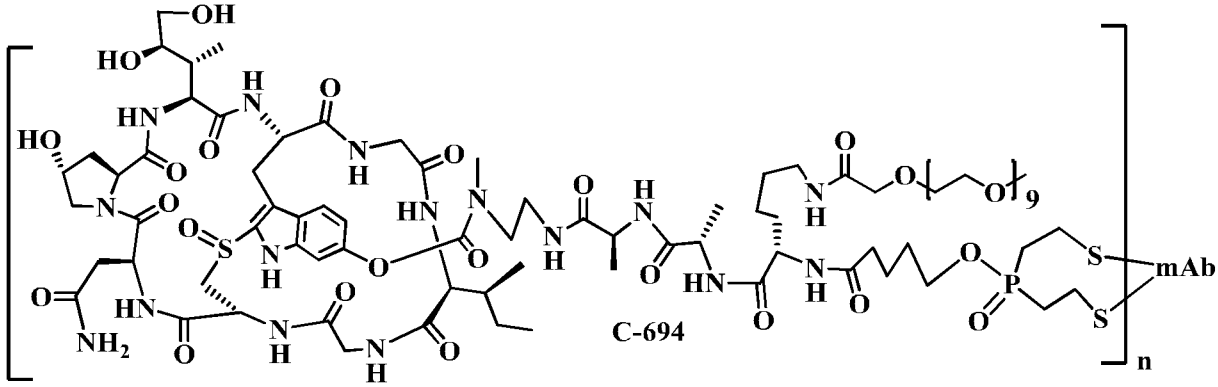
C-691



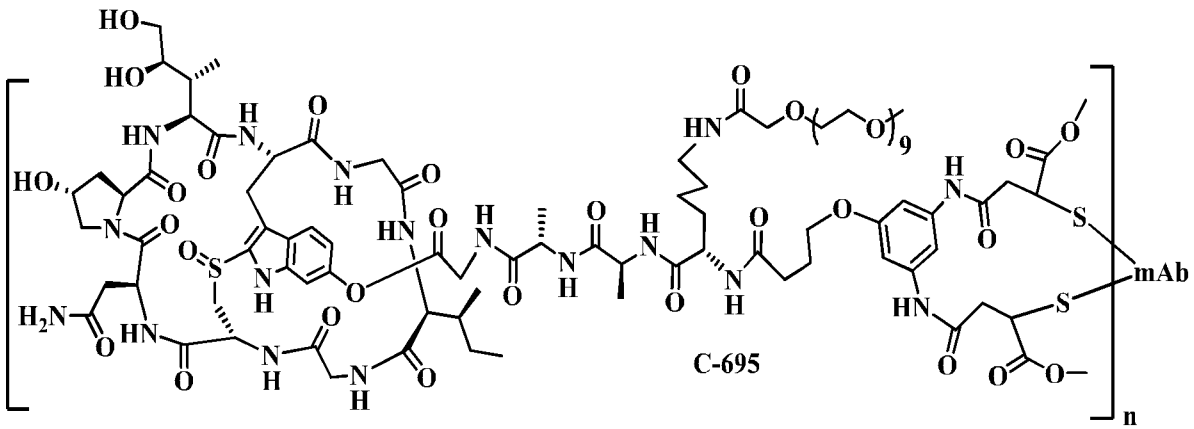
C-692



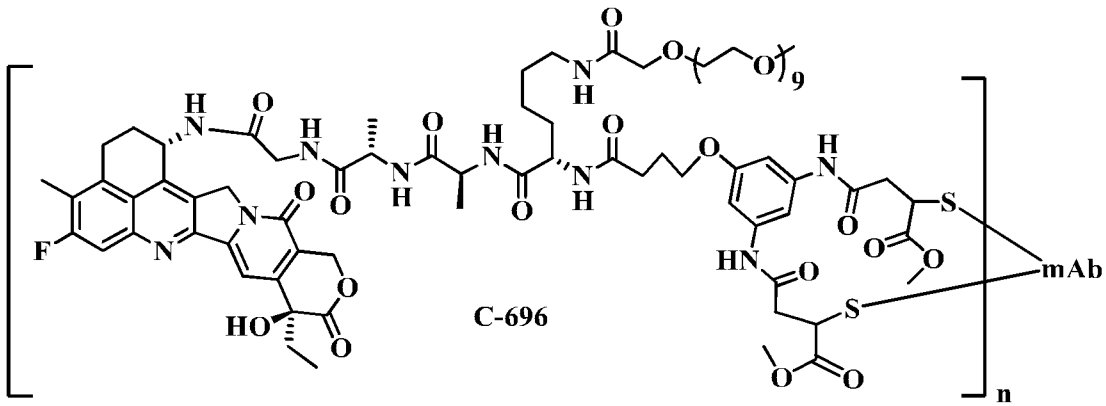
C-693



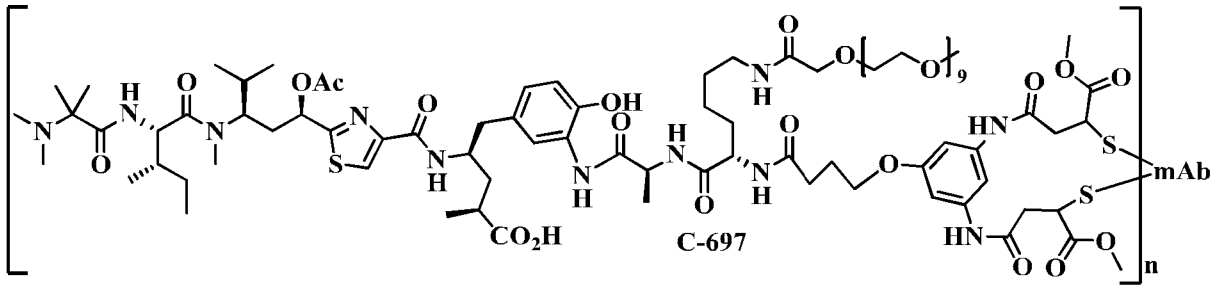
C-694



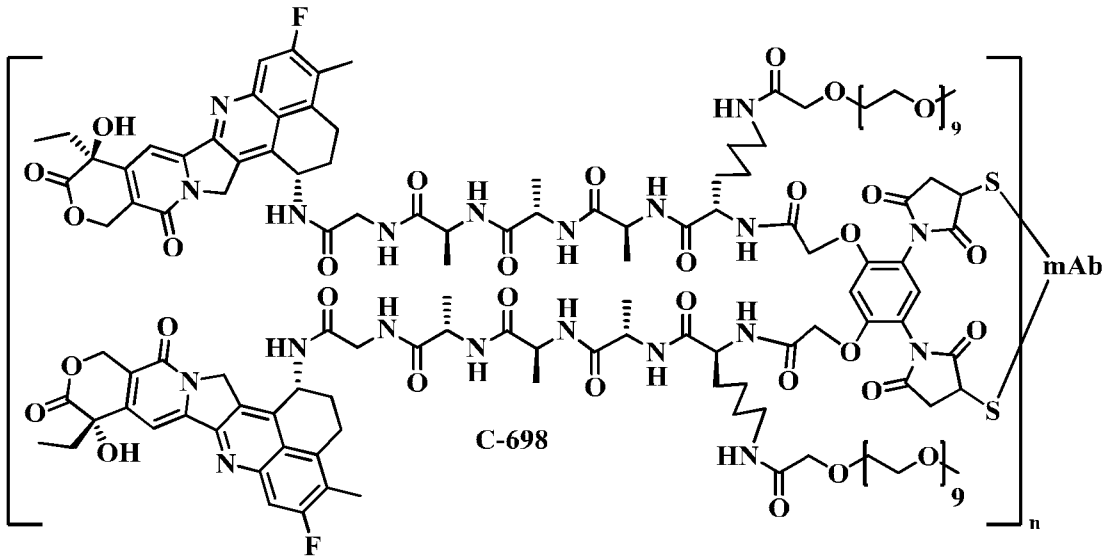
C-695



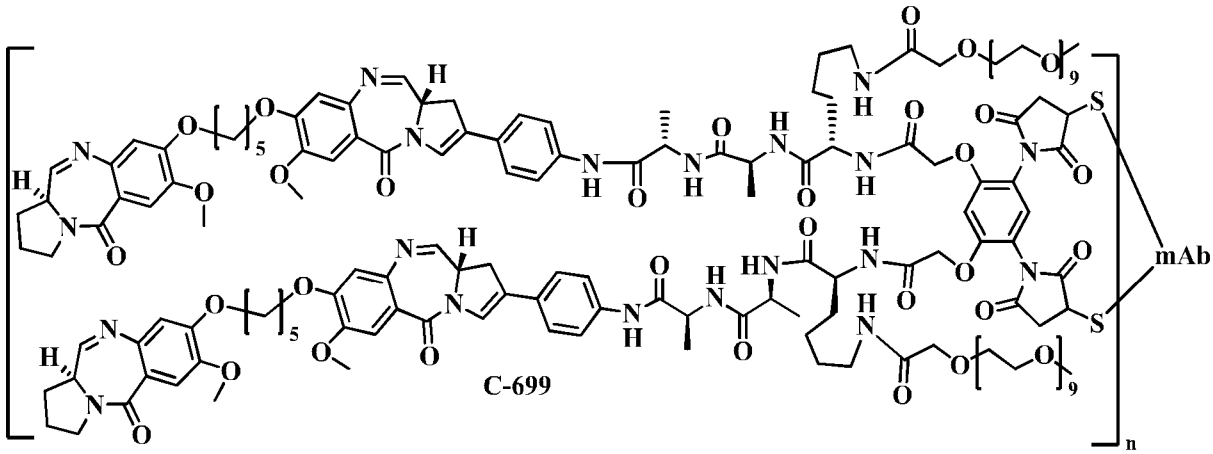
C-696



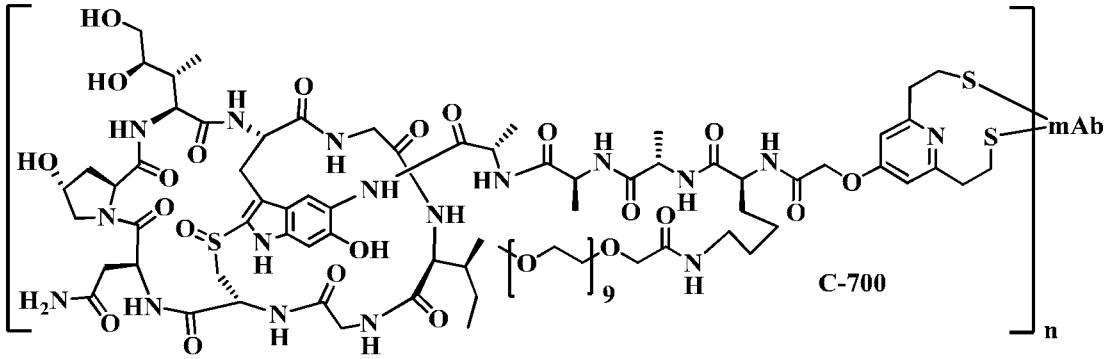
C-697



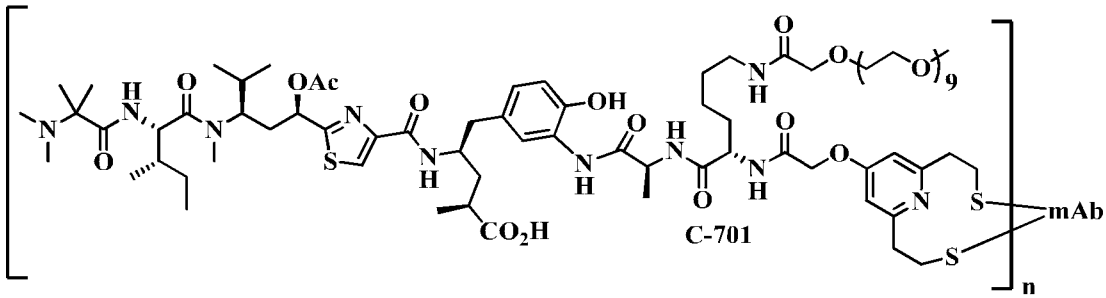
C-698



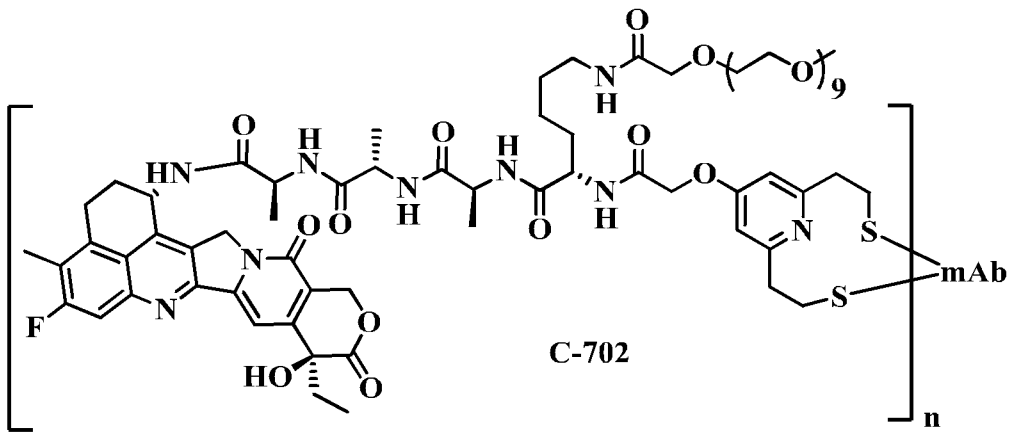
C-699



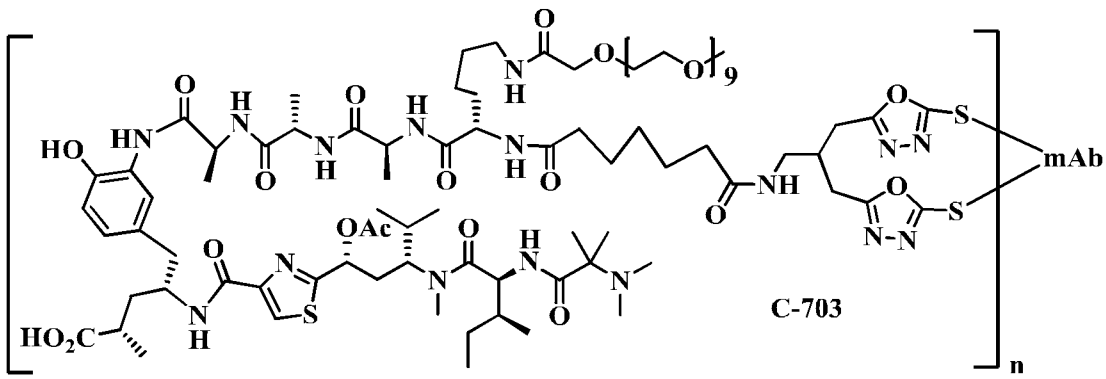
C-700



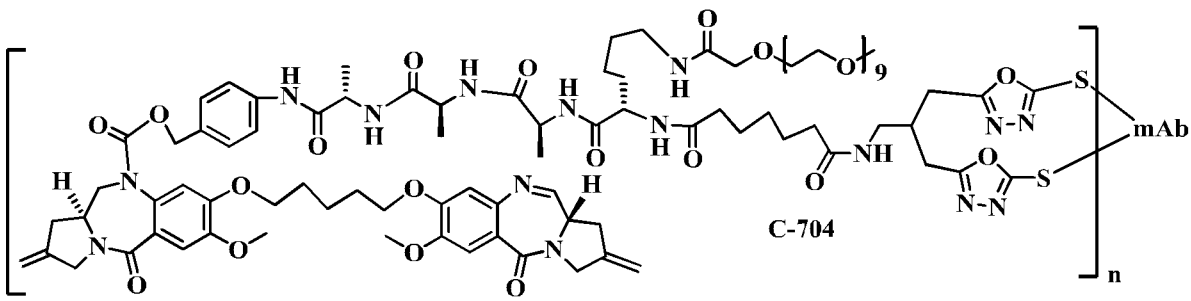
C-701



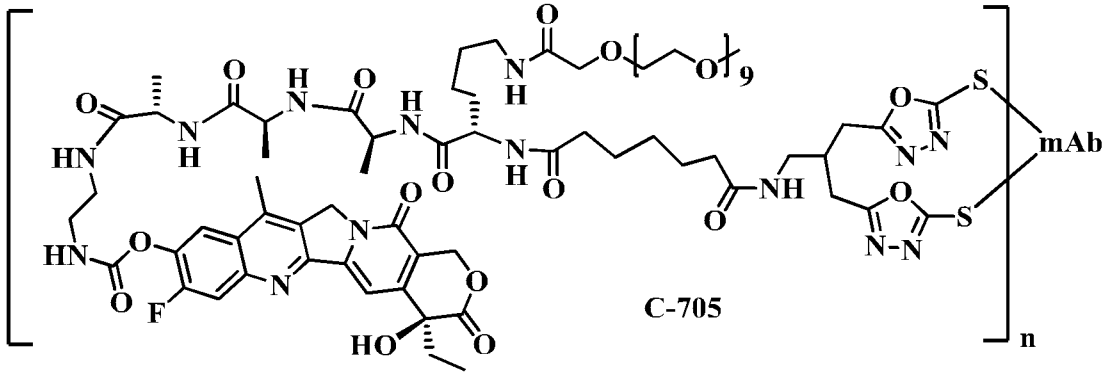
C-702



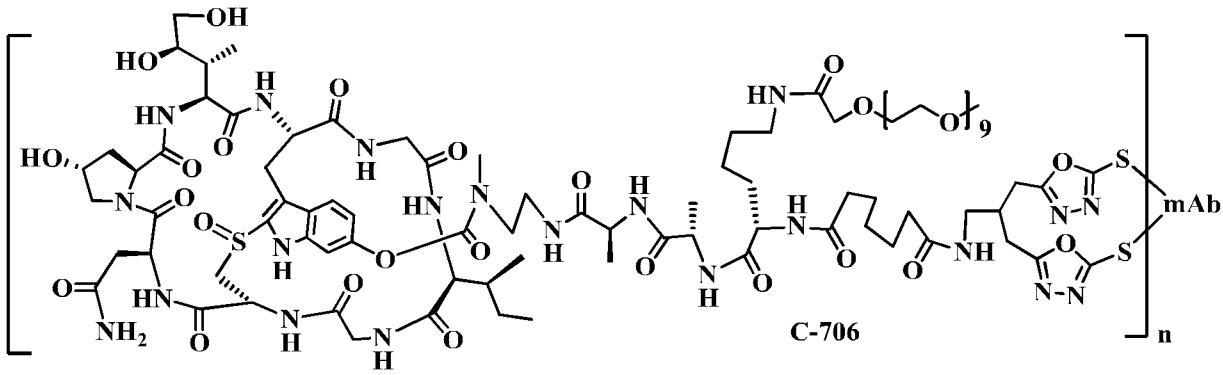
C-703



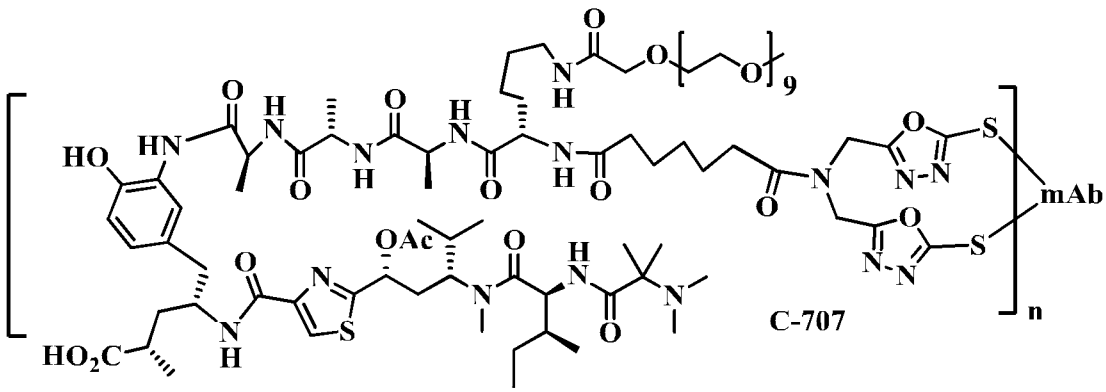
C-704



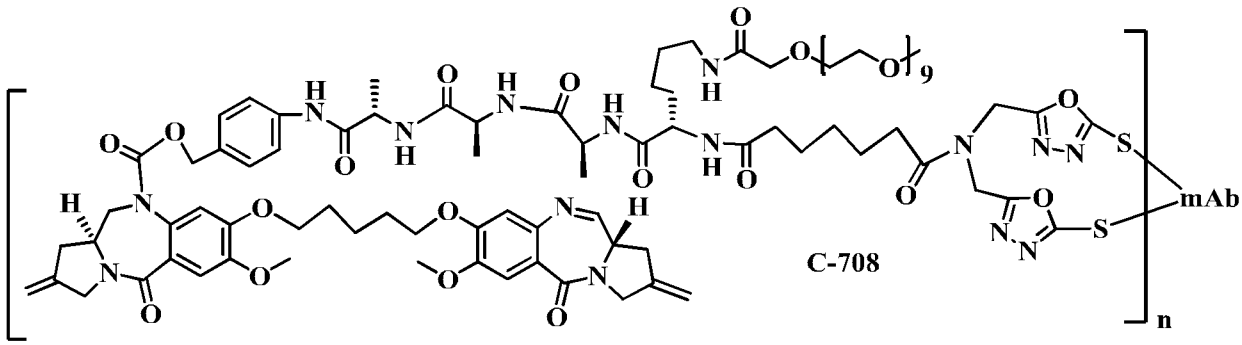
C-705



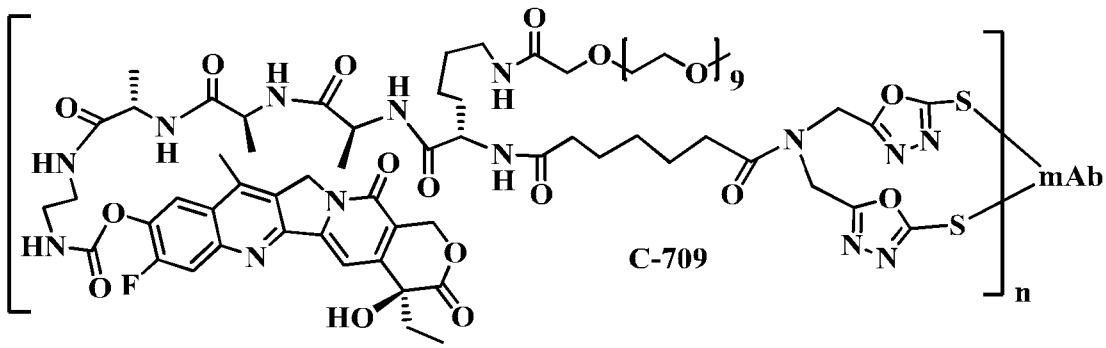
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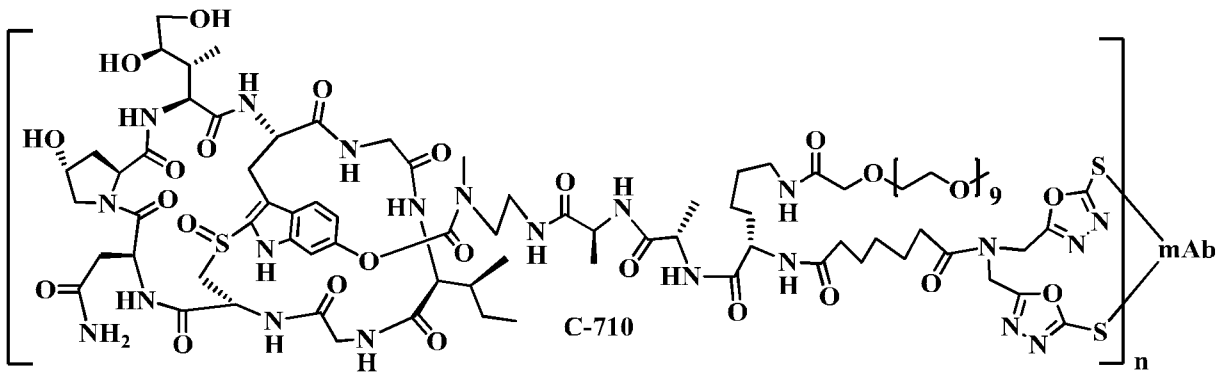
C-707



C-708

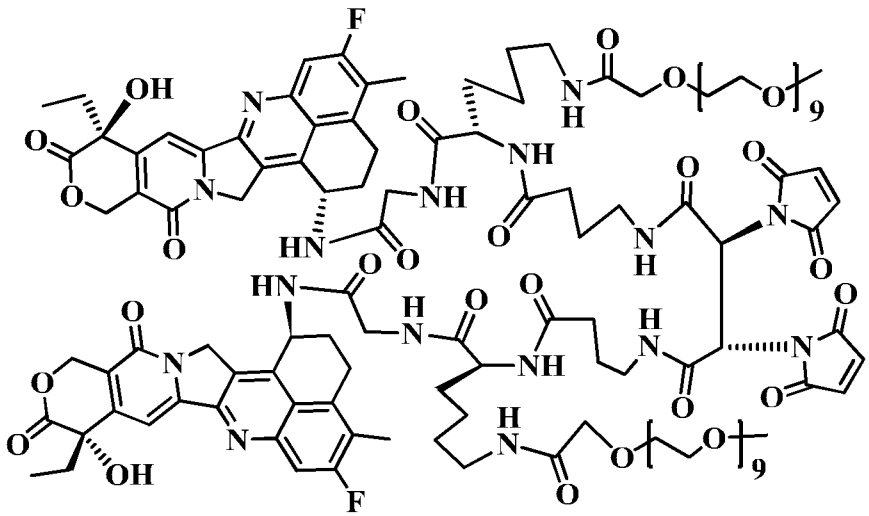


C-709



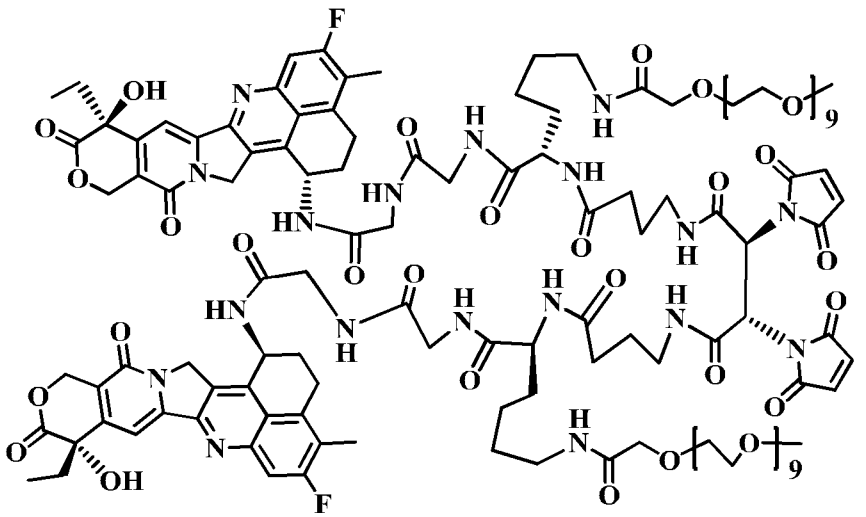
or one or more isotope of chemical elements, pharmaceutically acceptable salts, hydrates, or hydrated salts; or the polymorphic crystalline structures of these compounds; or the optical isomers, racemates, diastereomers or enantiomers; wherein n is 1 -10, mAb is an antibody or antibody-like protein.

13. The process according to claim 3, wherein the drug/linker complex , has one of the structures of below:



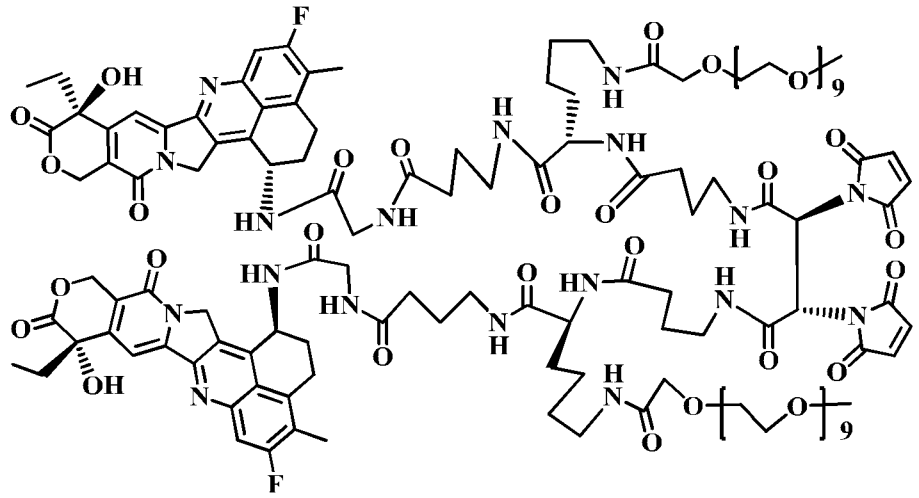
25

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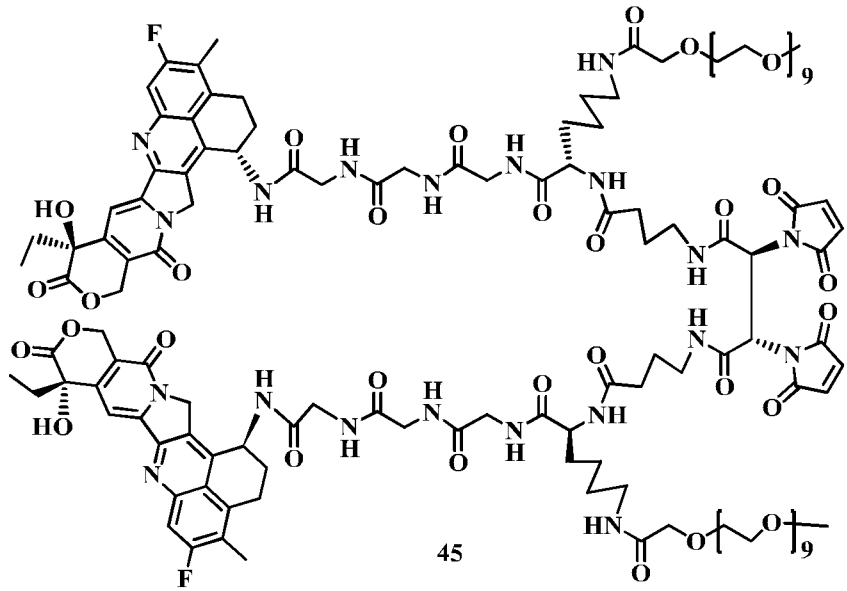
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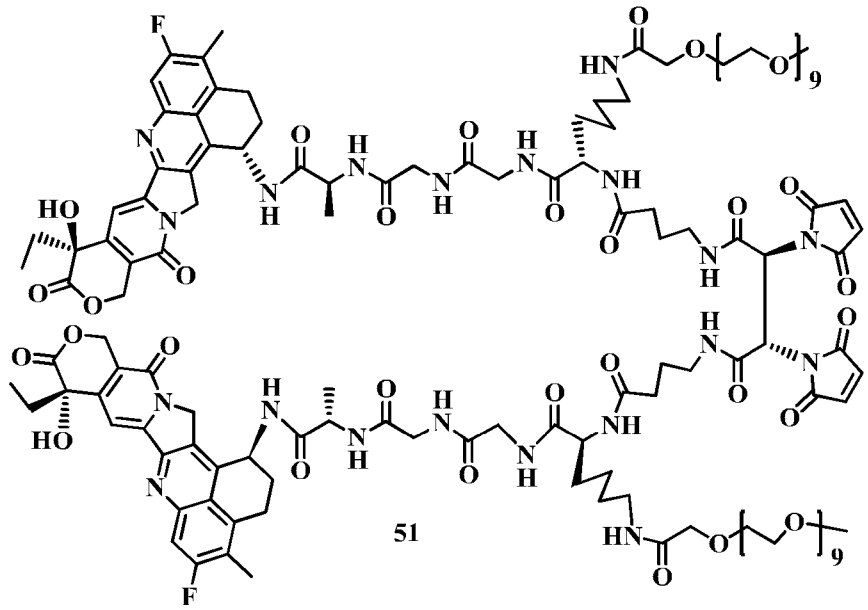
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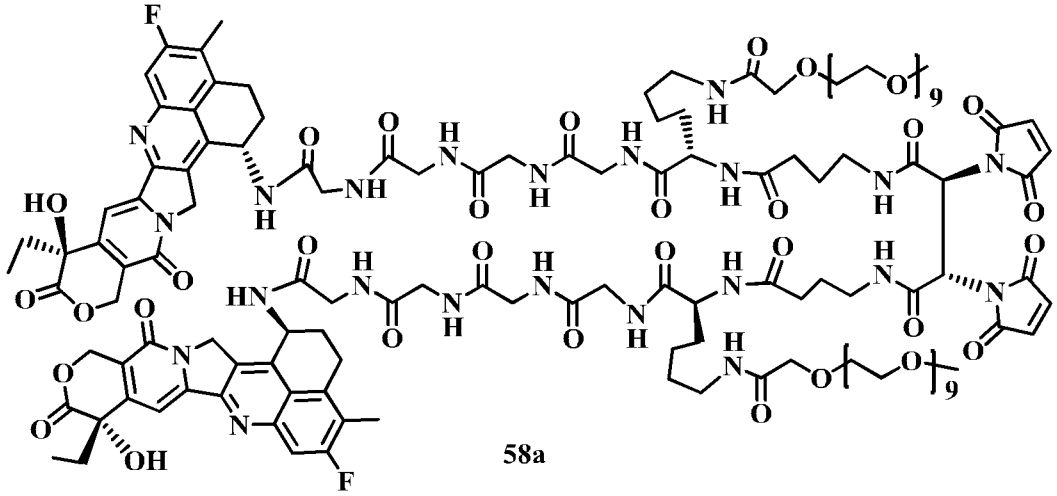
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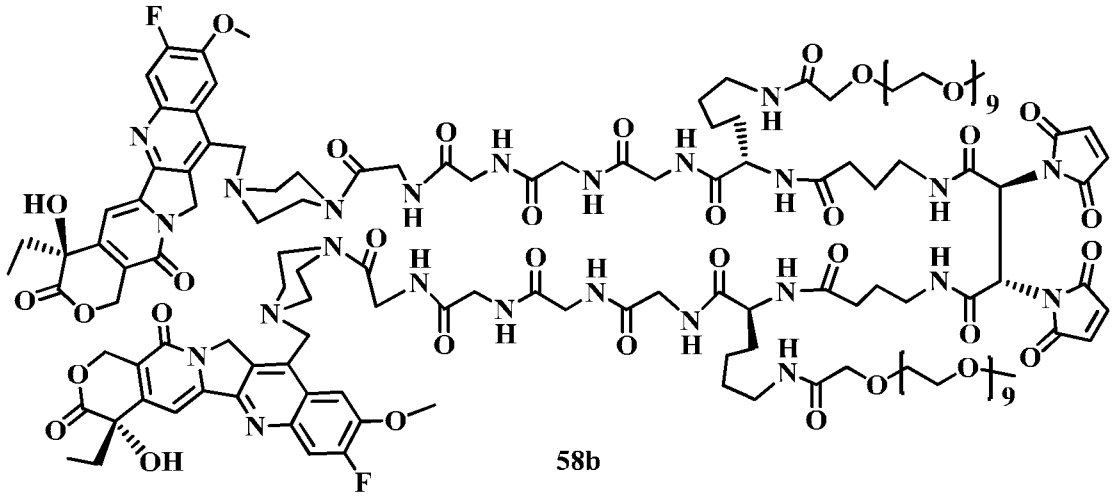


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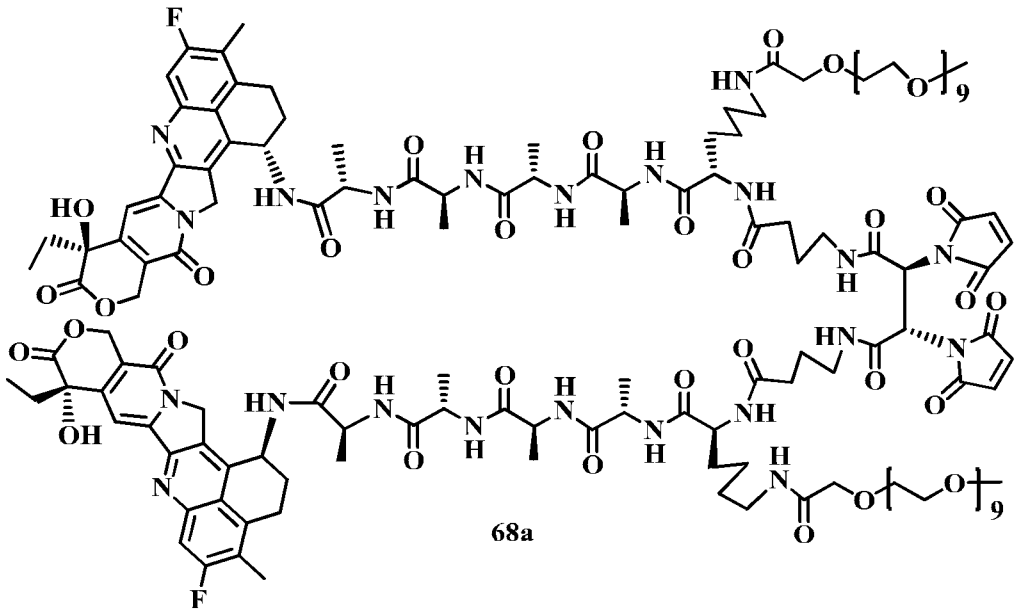
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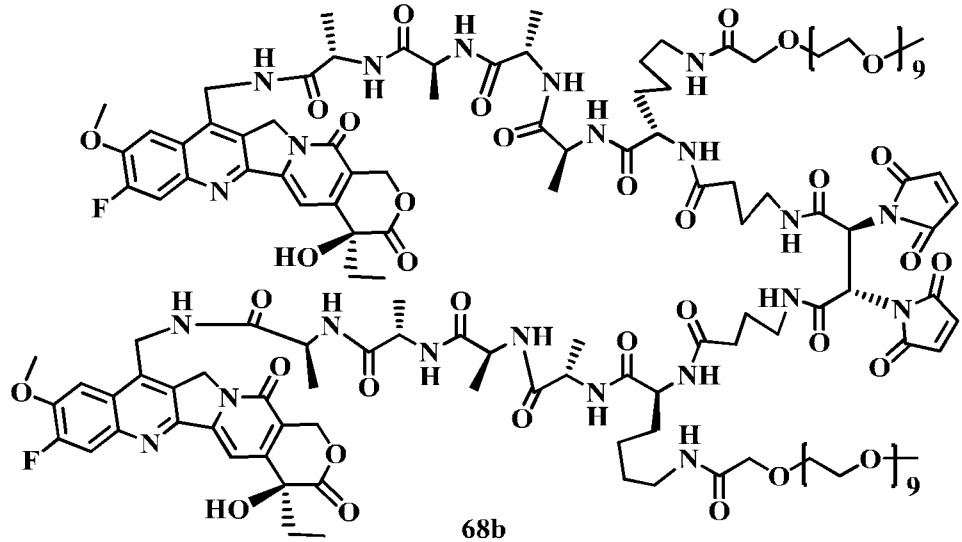
58a ,



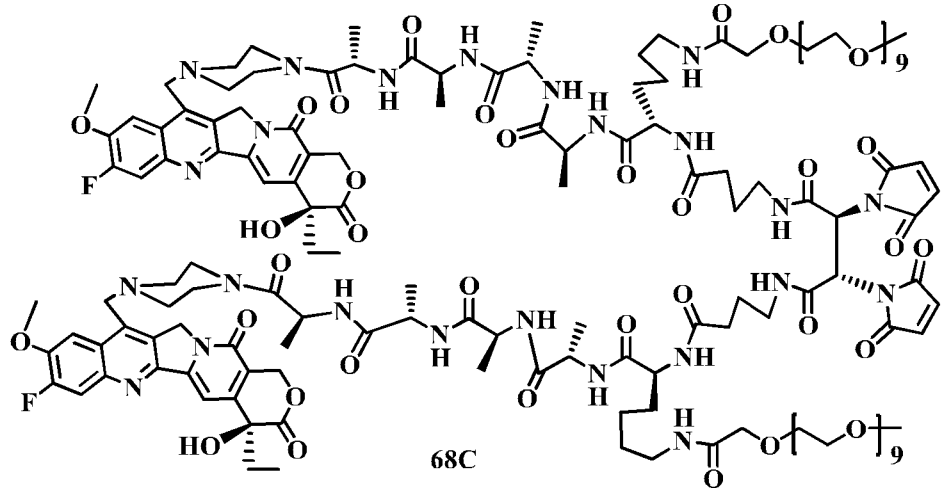
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68a ,

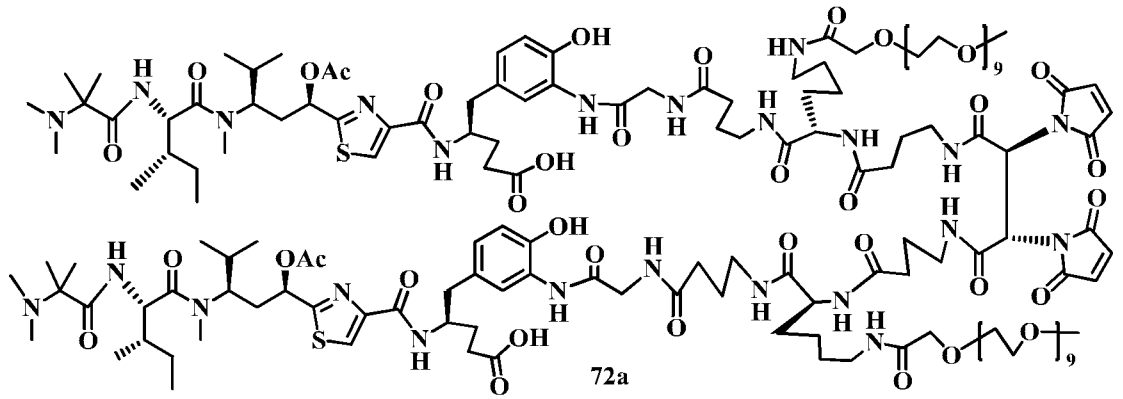


68b



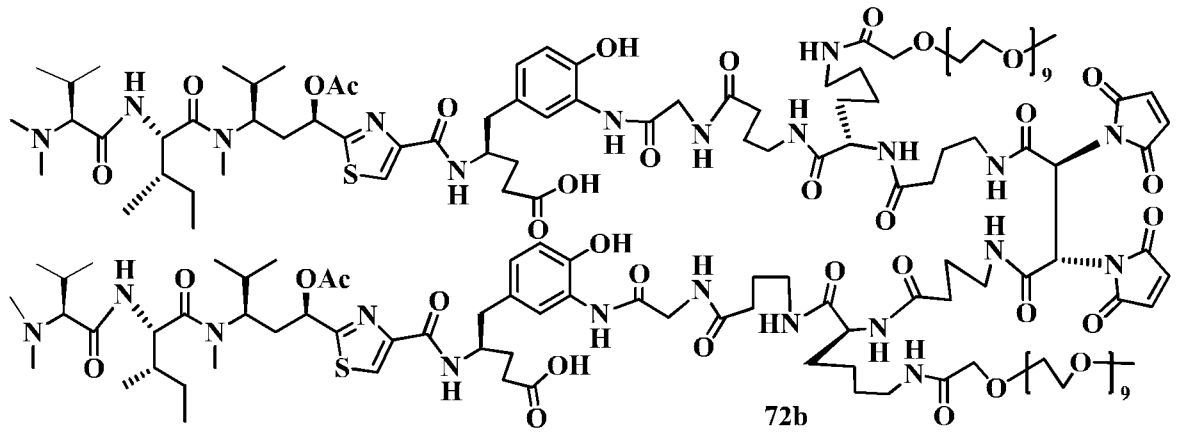
68C

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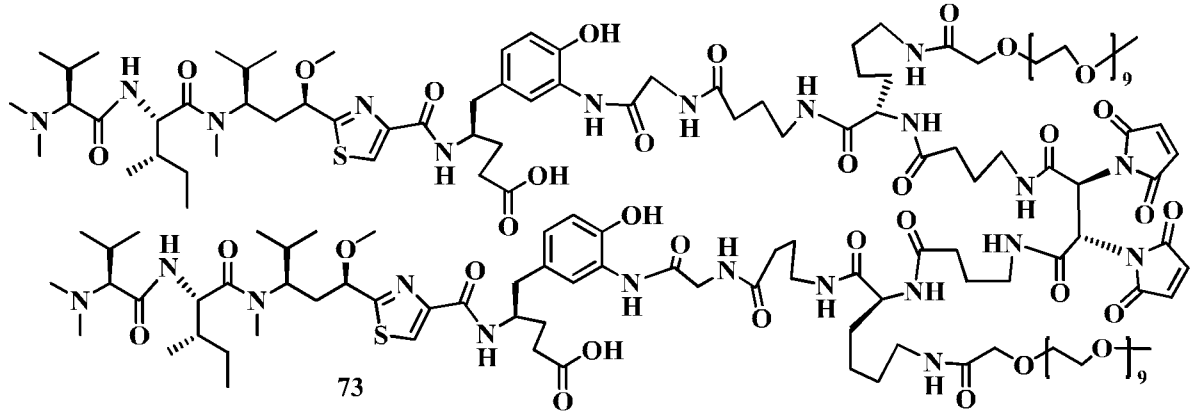


72a

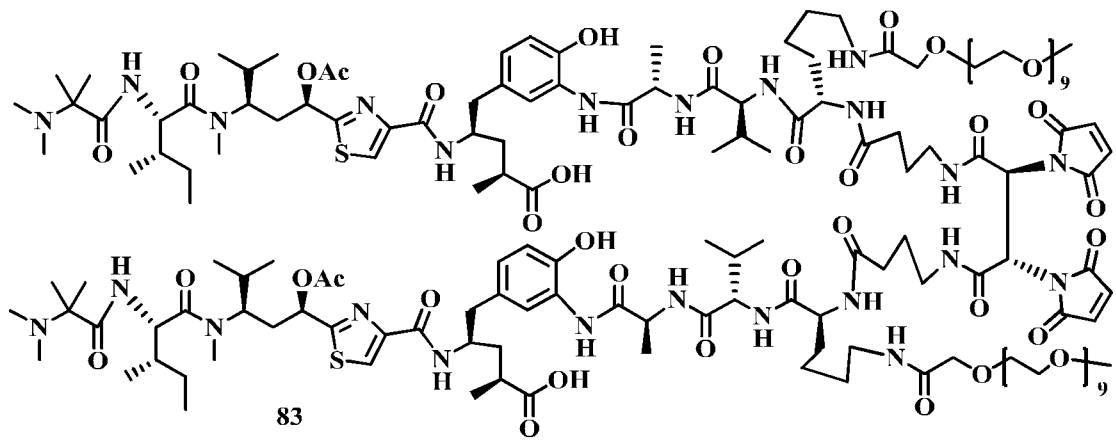
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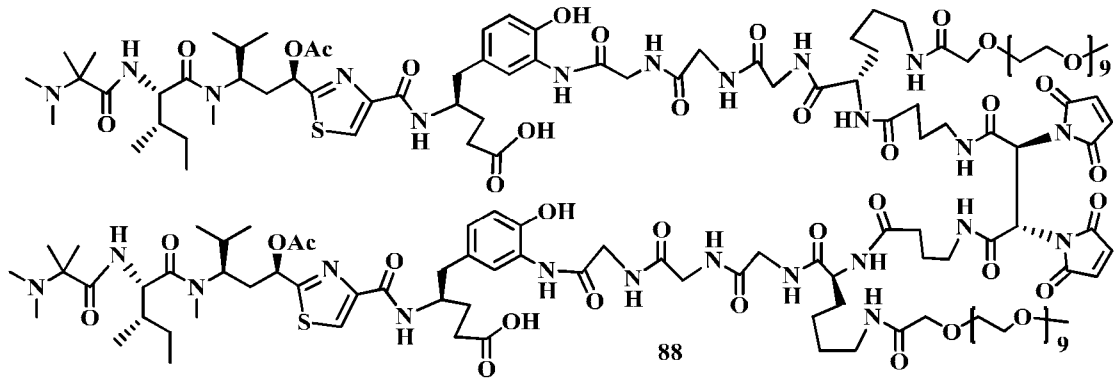
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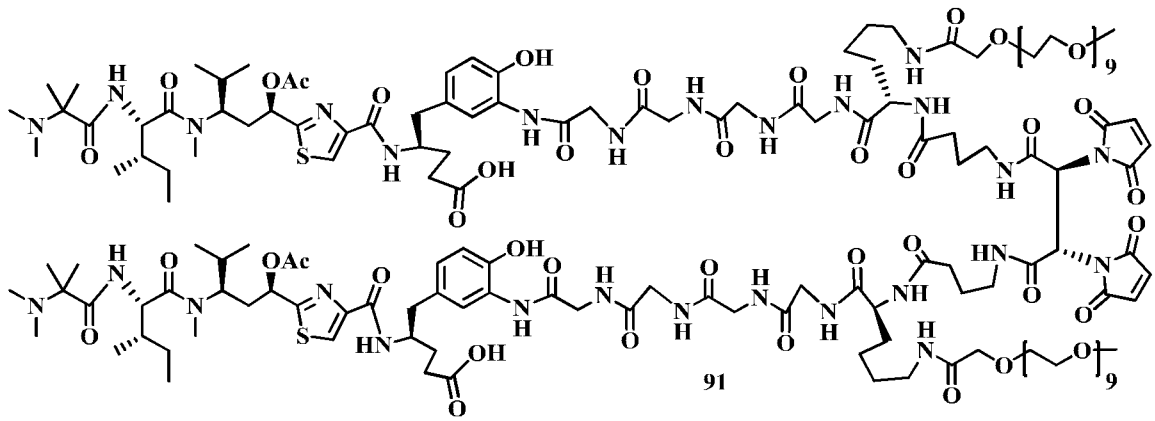
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83 ,

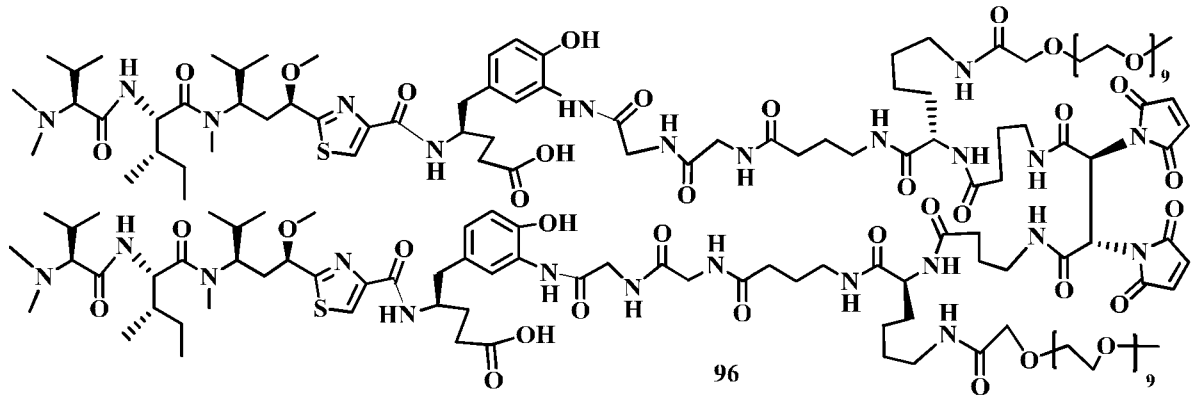


88 ,



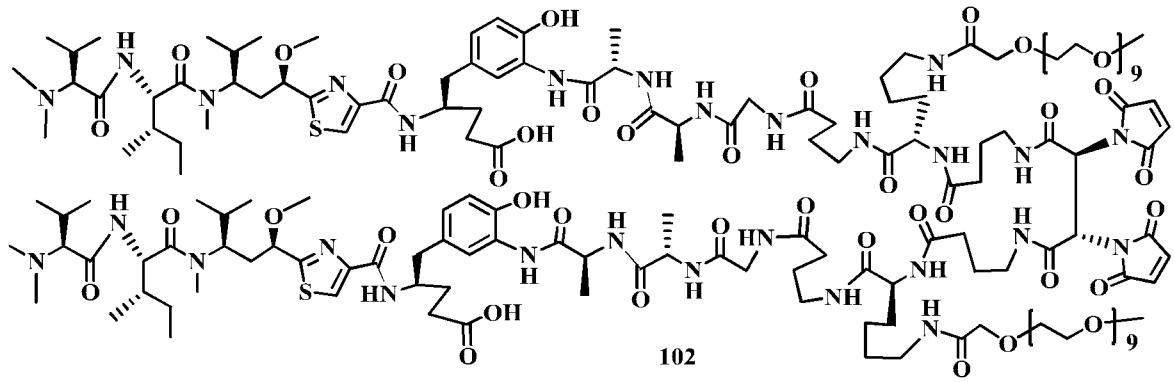
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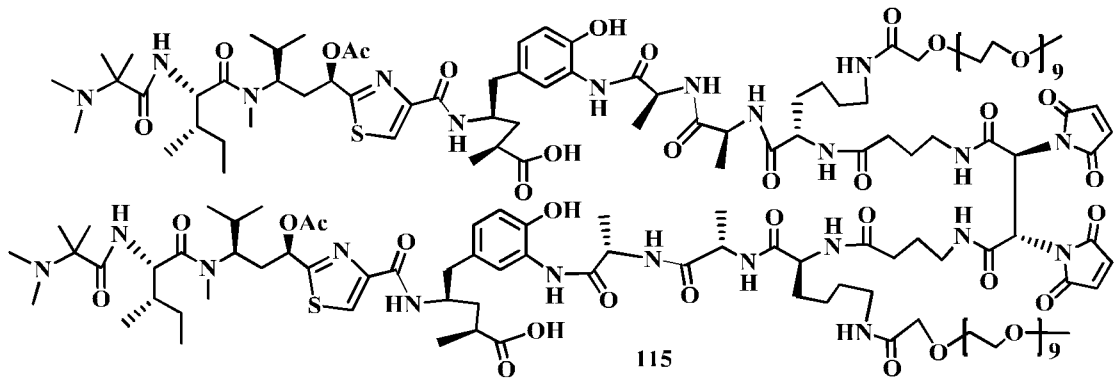
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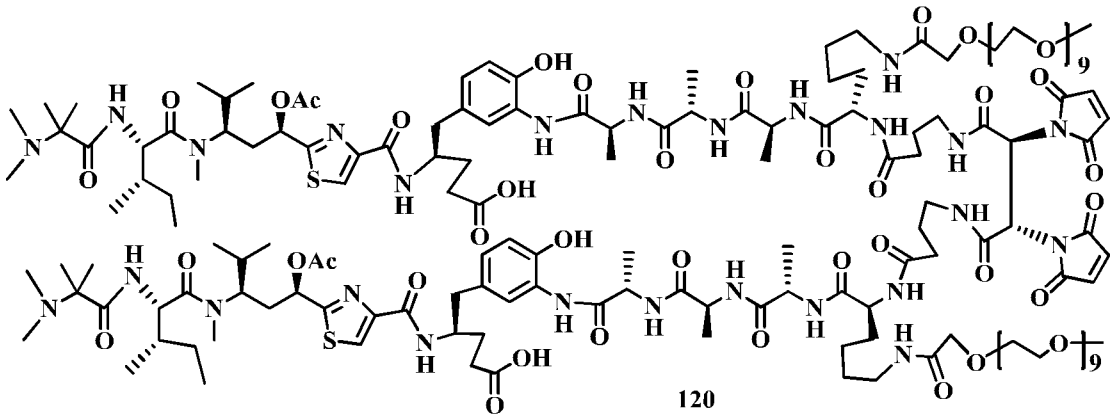
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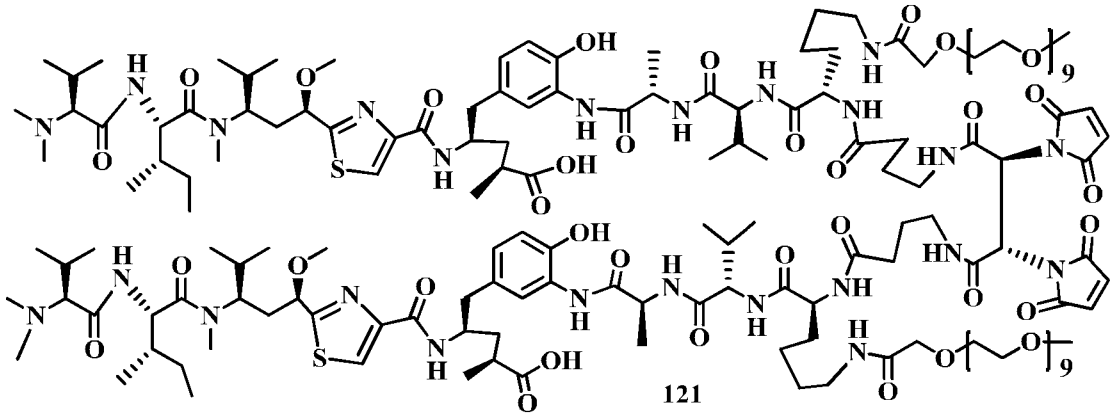


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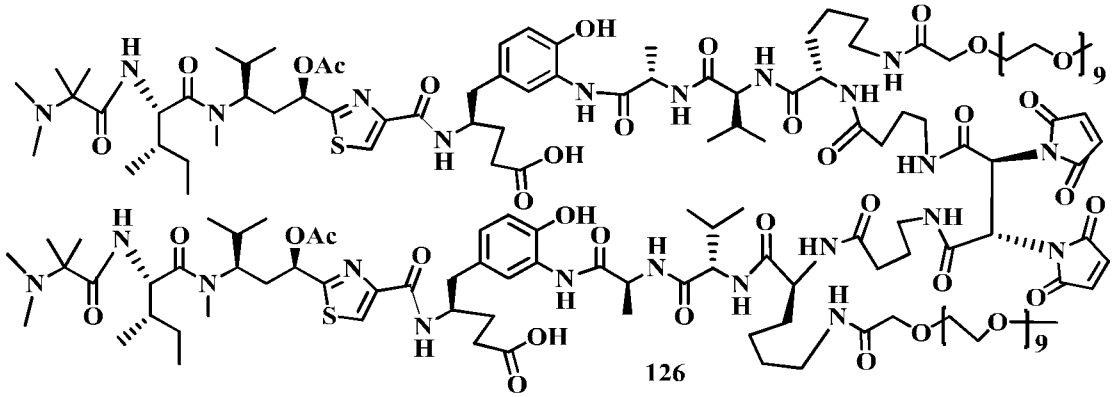
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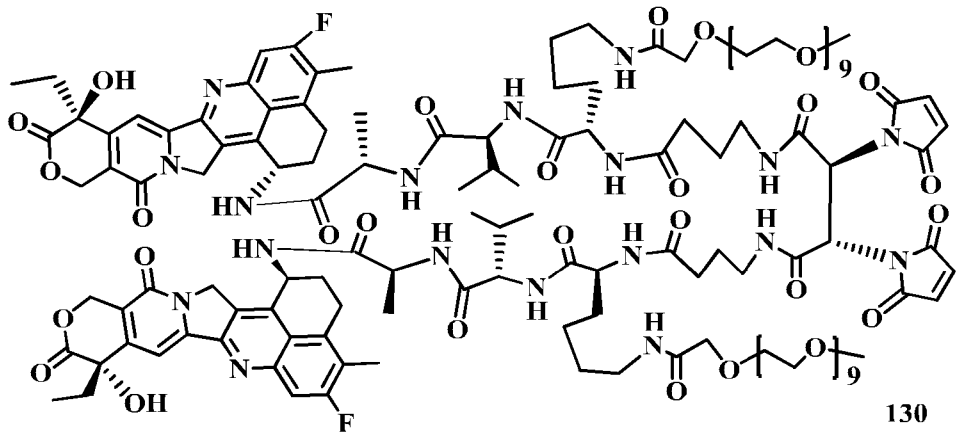
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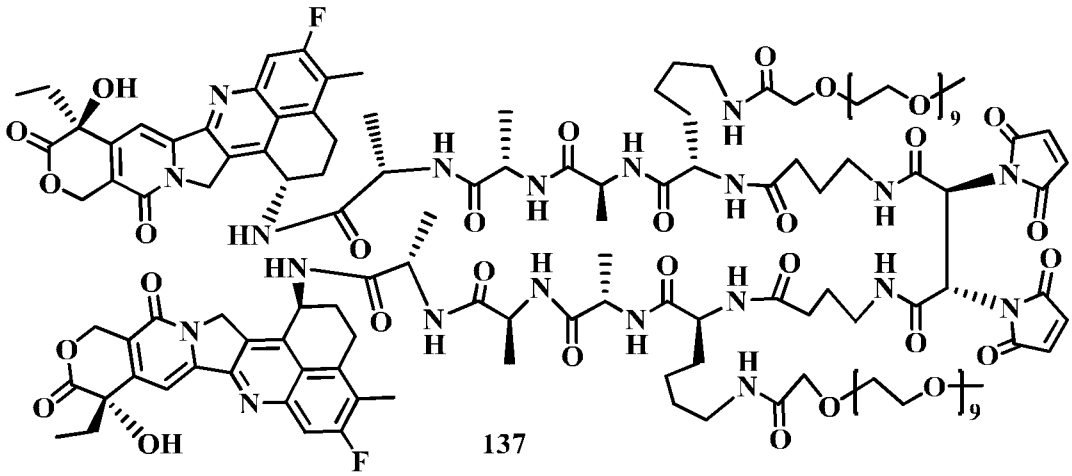
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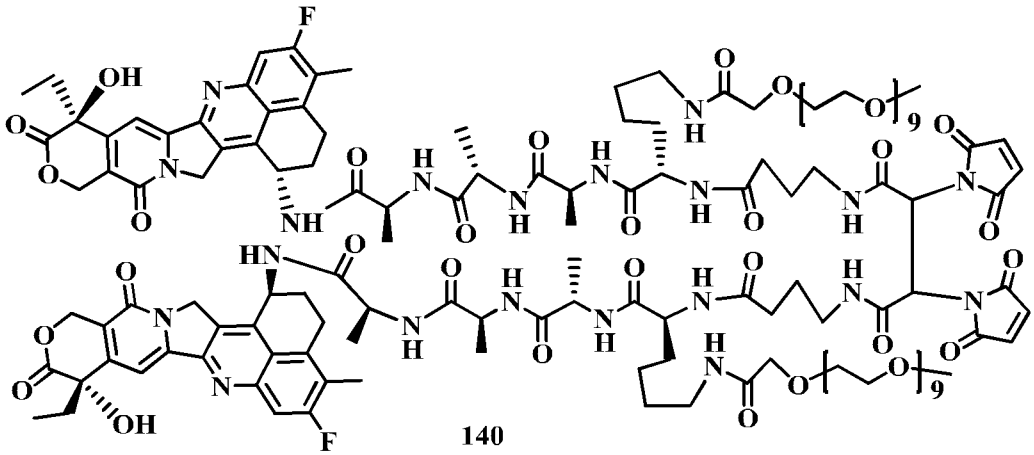
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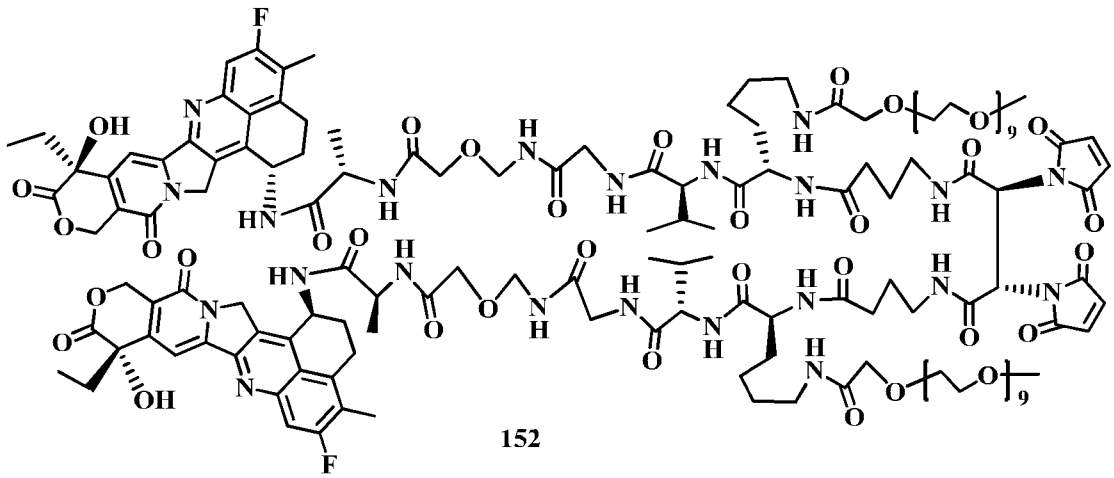
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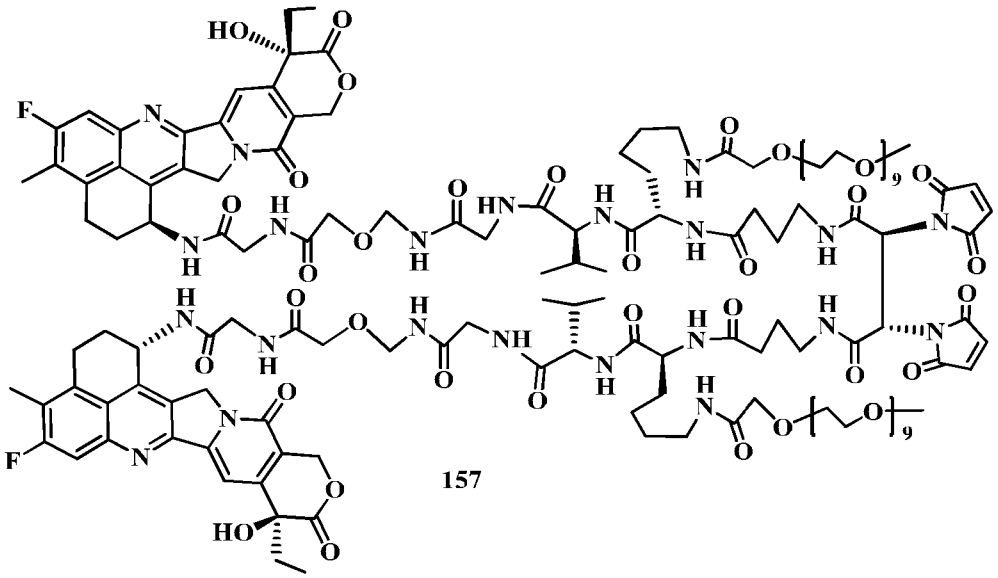
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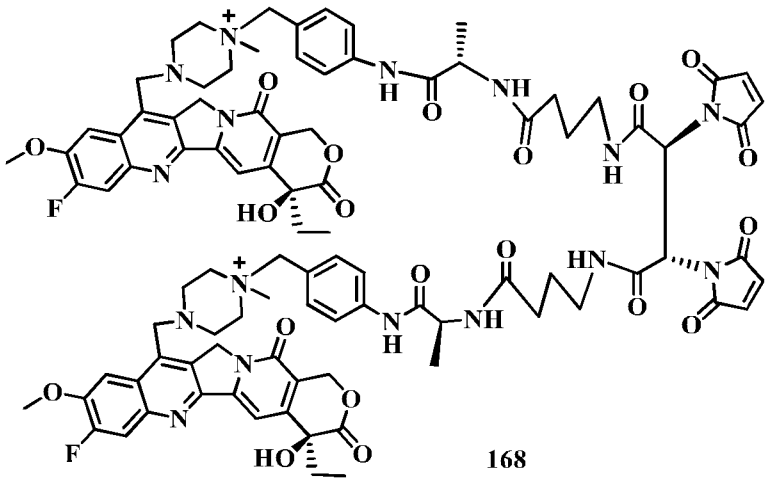
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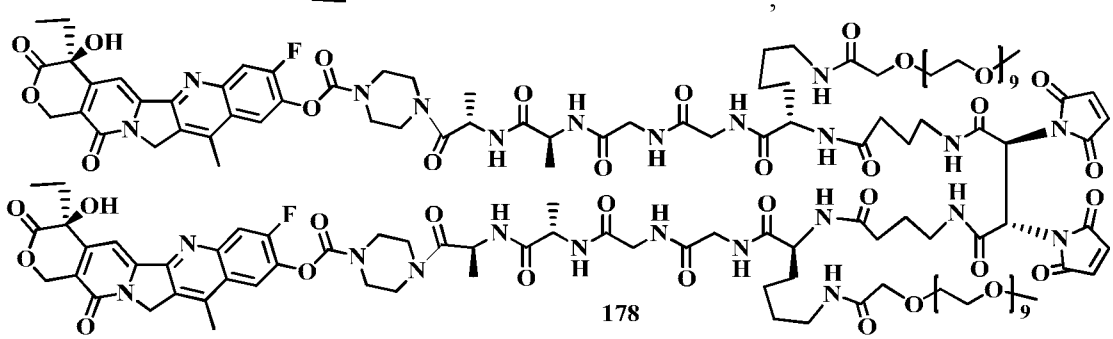
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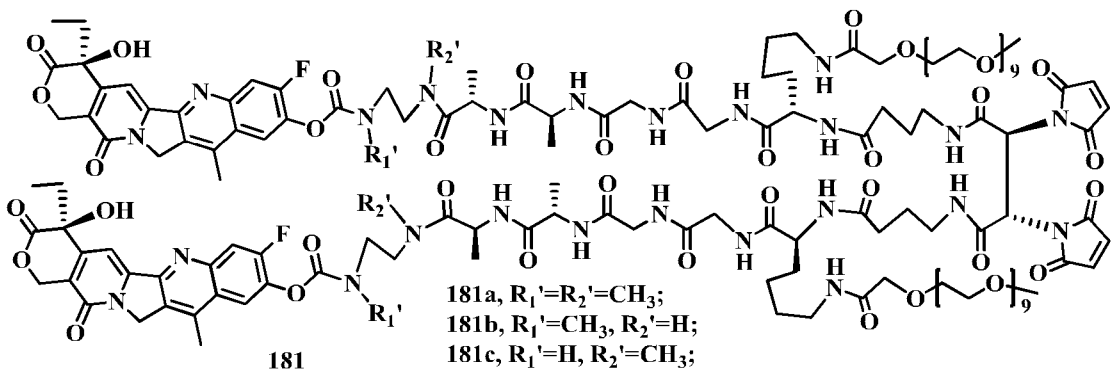
157



168

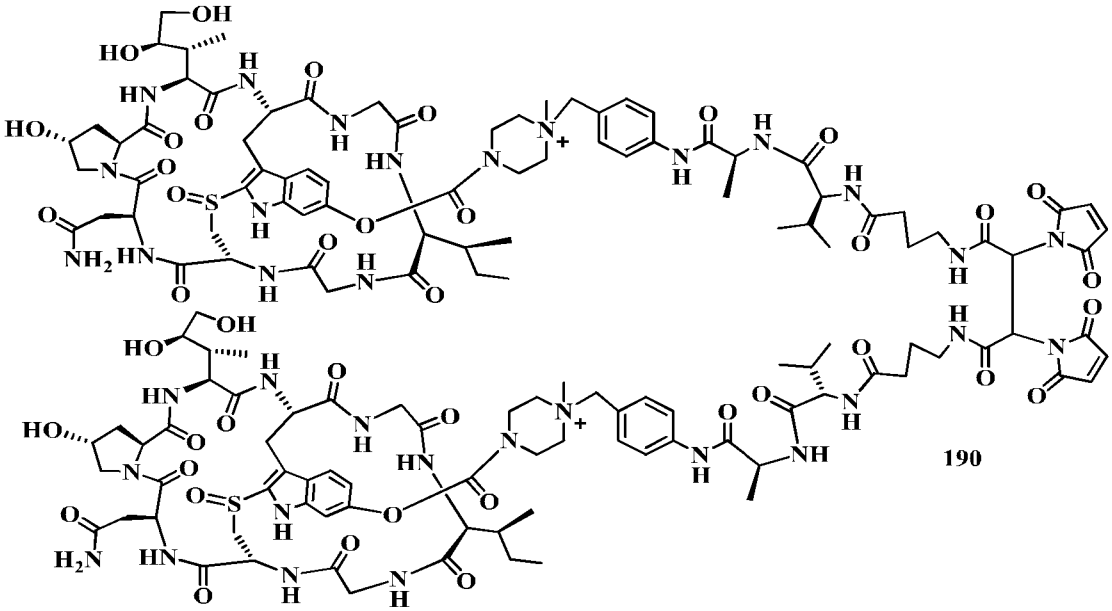


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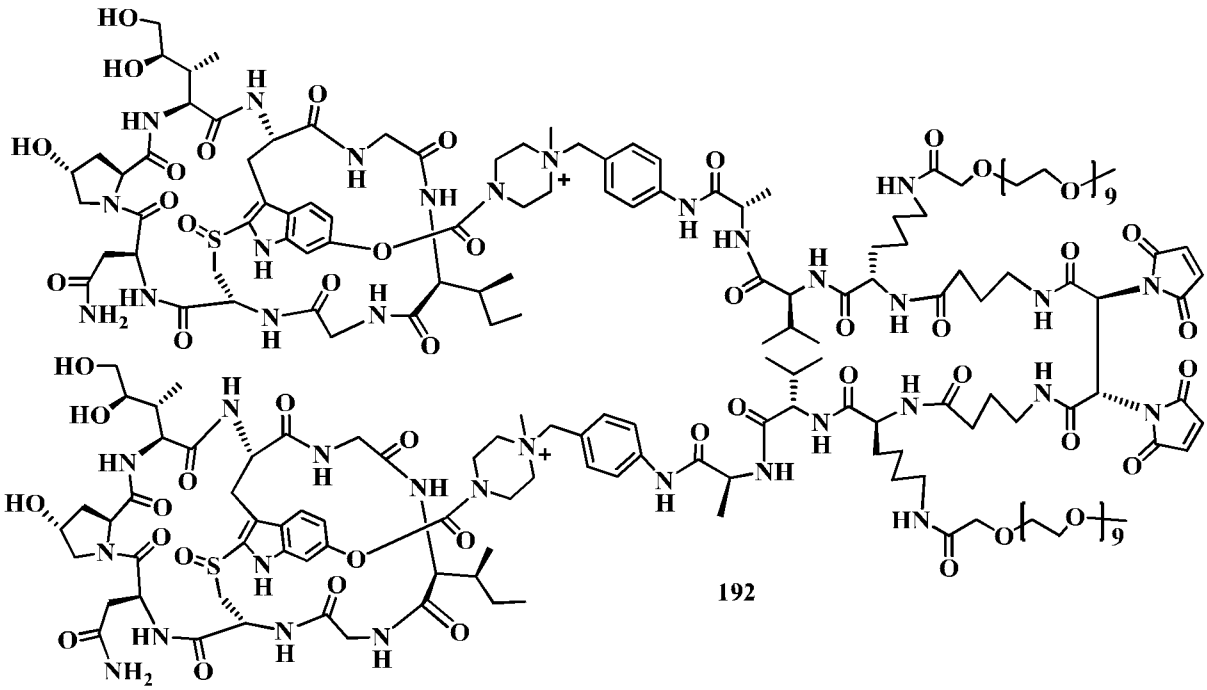
181

181a, $R_1'=R_2'=CH_3$;
181b, $R_1'=CH_3$, $R_2'=H$;
181c, $R_1'=H$, $R_2'=CH_3$;
181d, $R_1'=R_2'=H$;



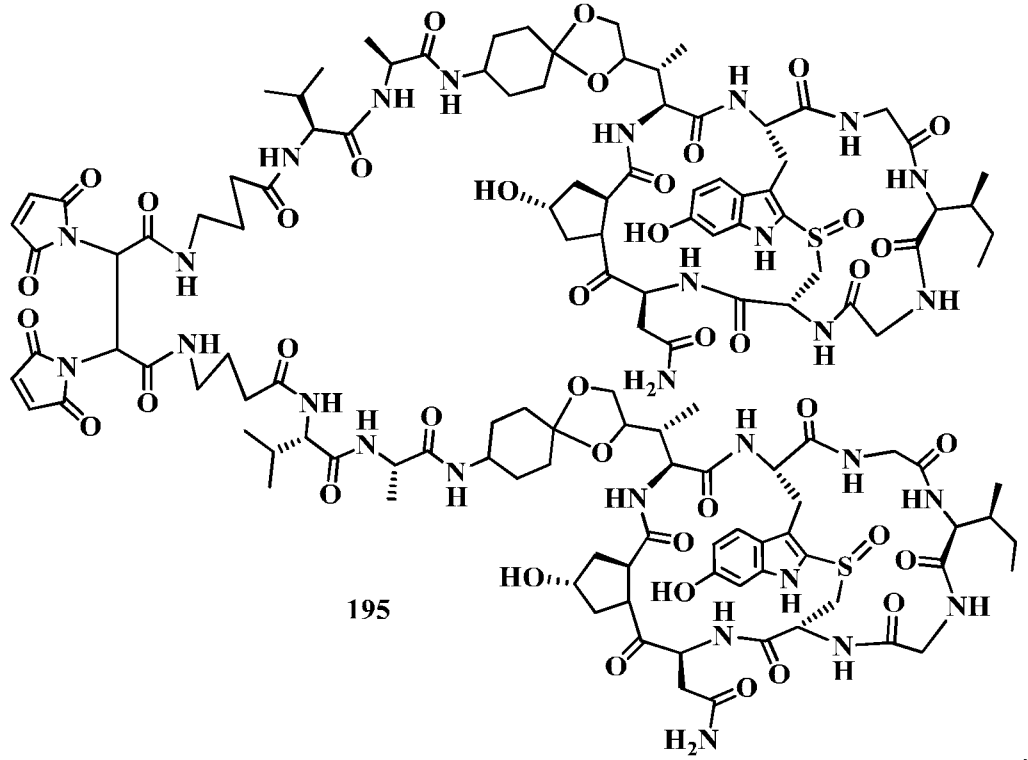
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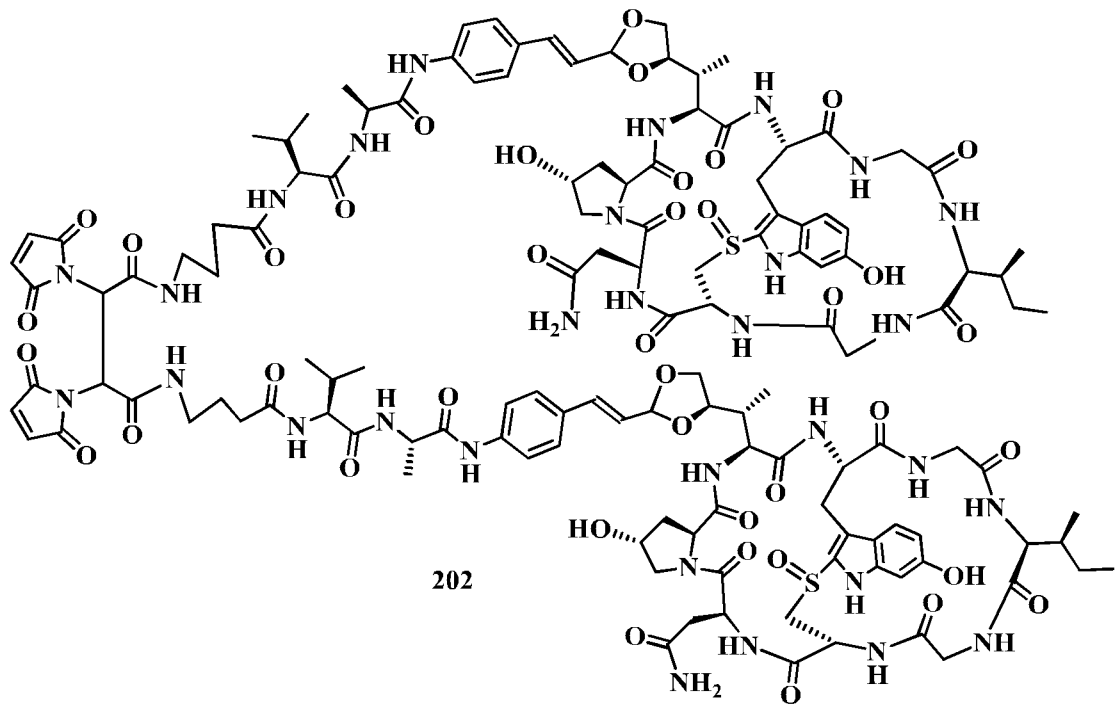
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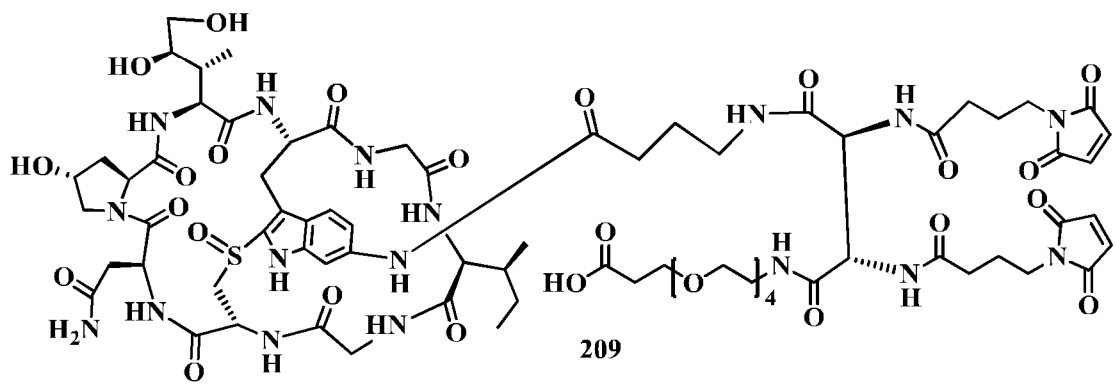
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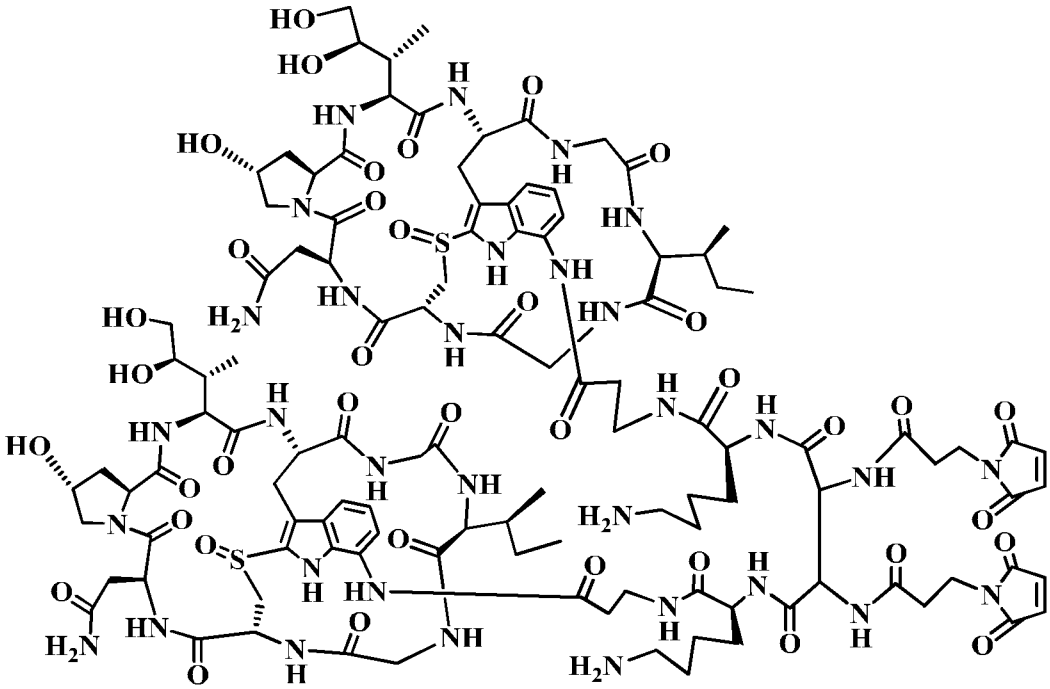
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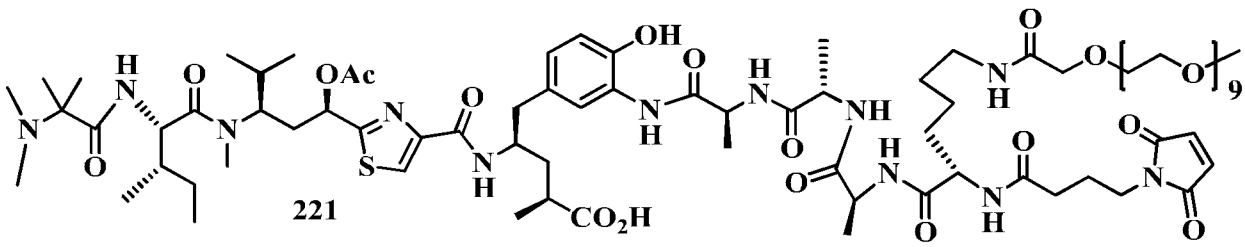


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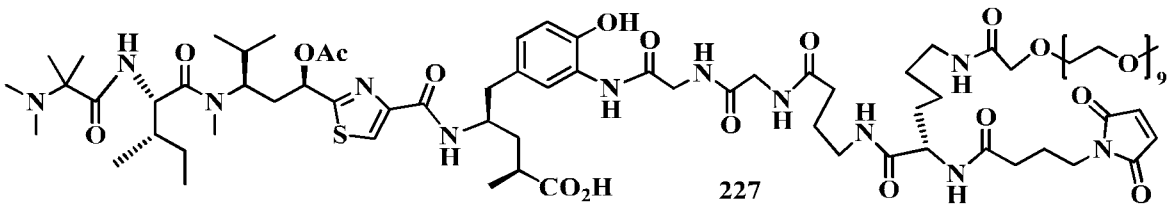
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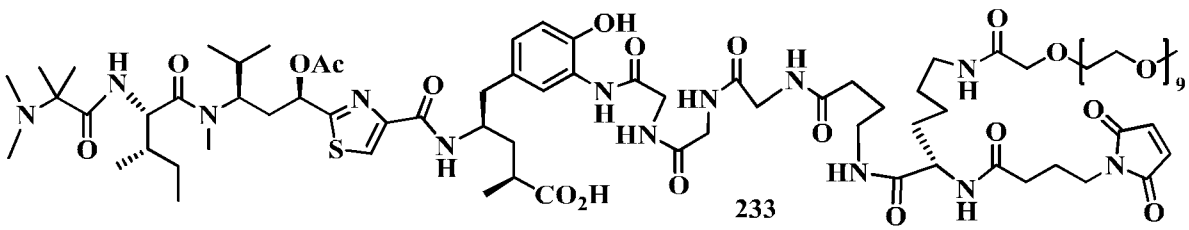
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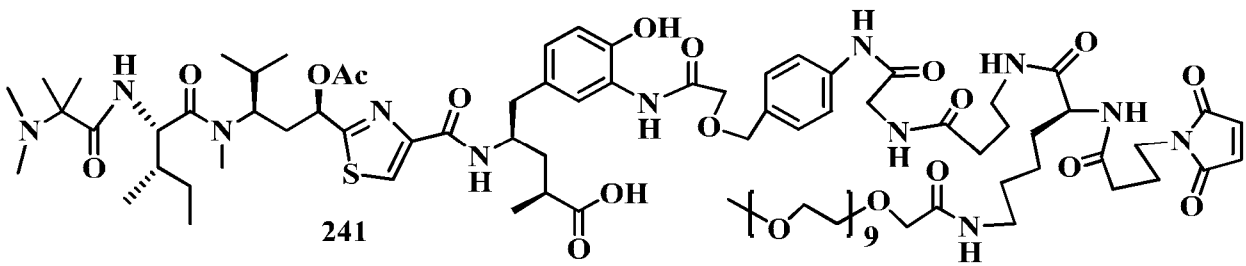
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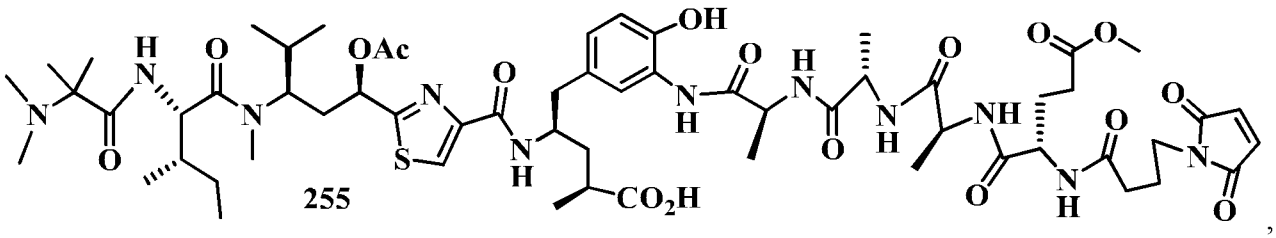
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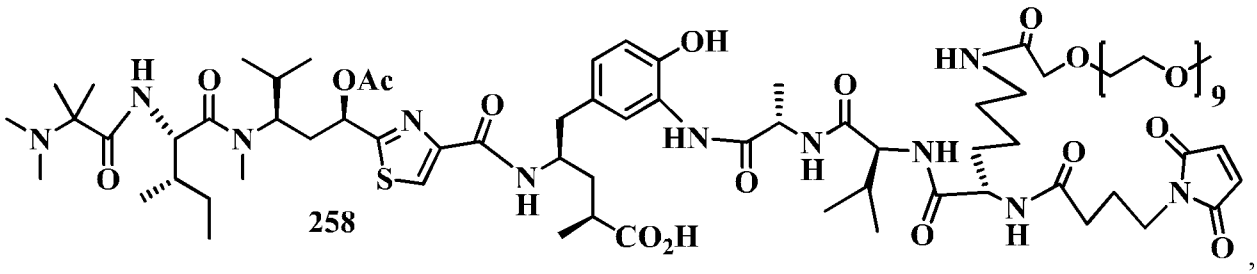
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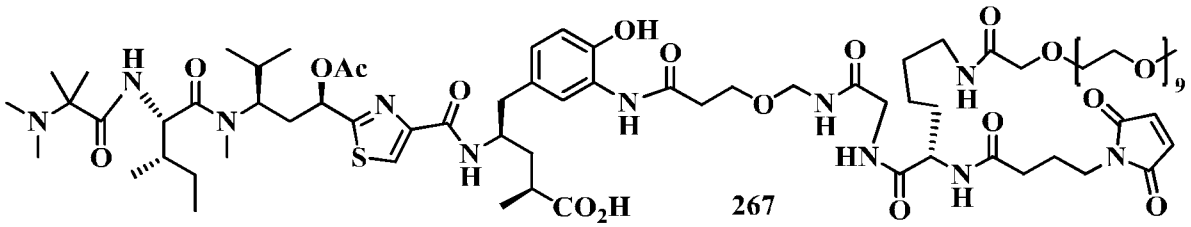
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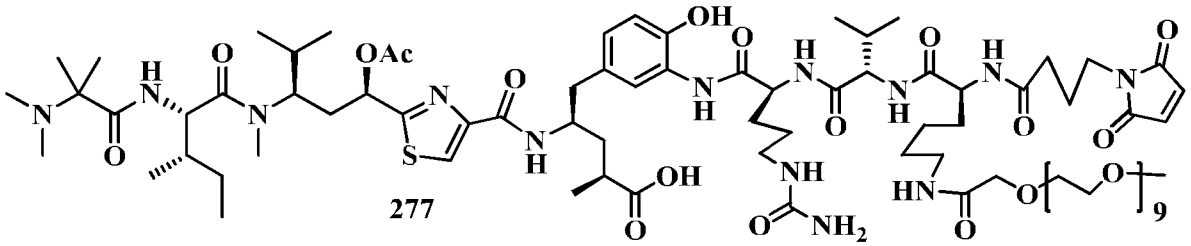
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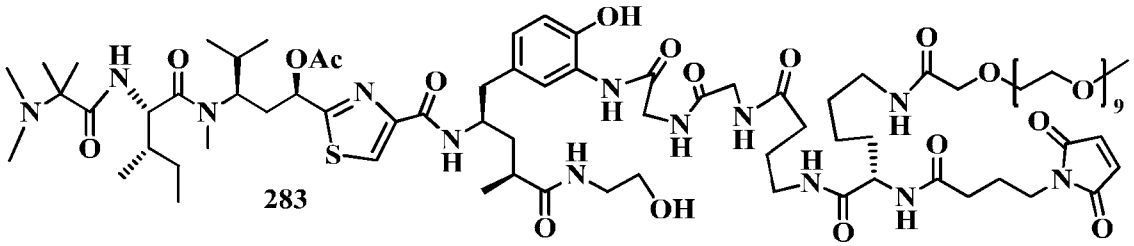
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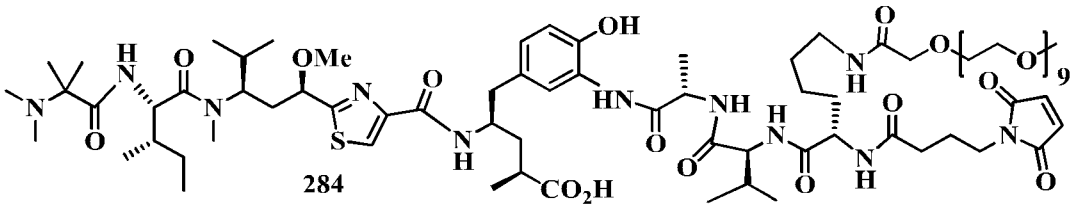
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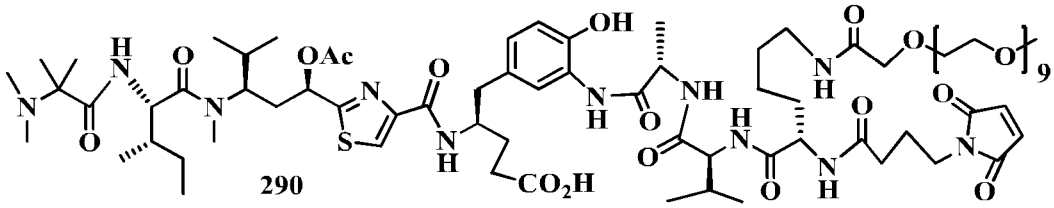
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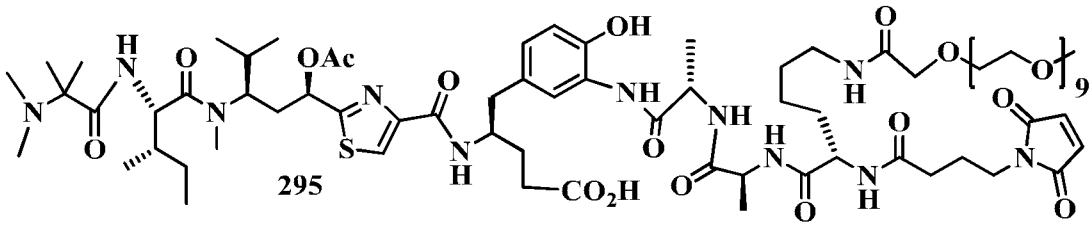
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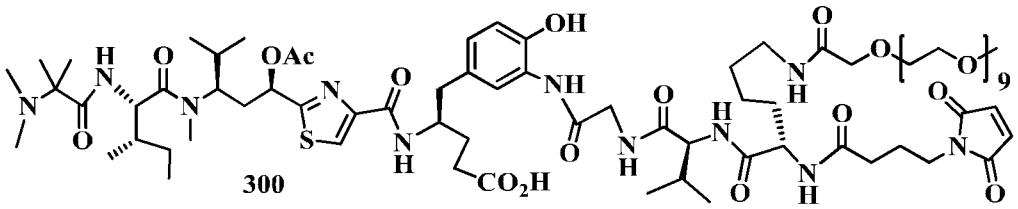
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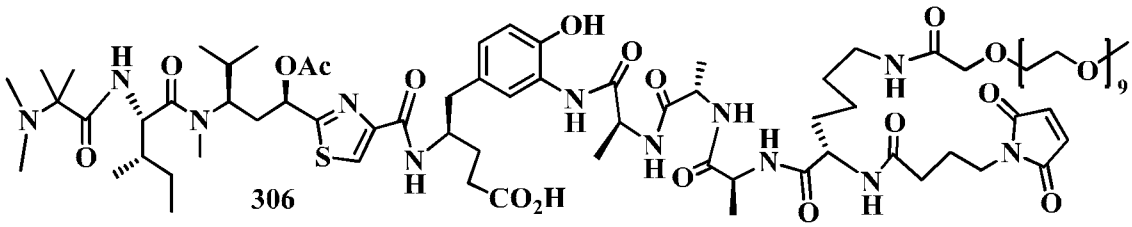
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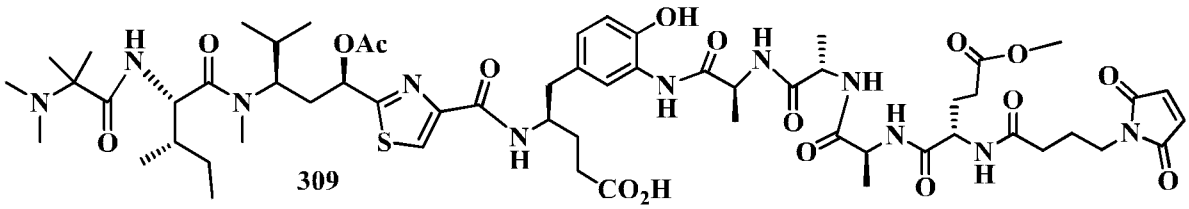
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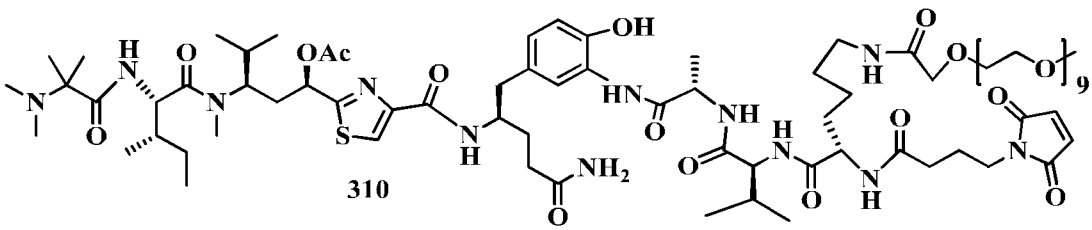
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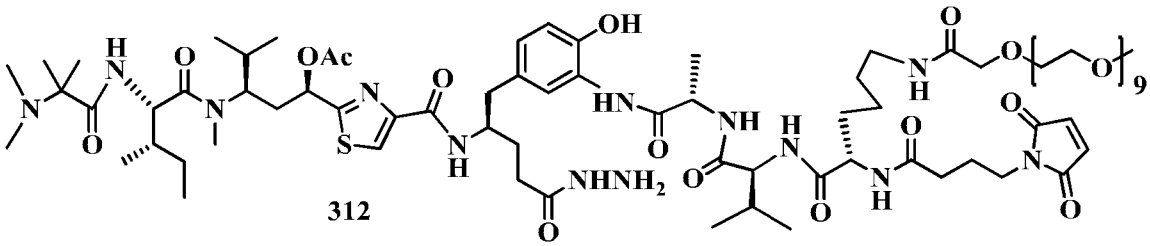
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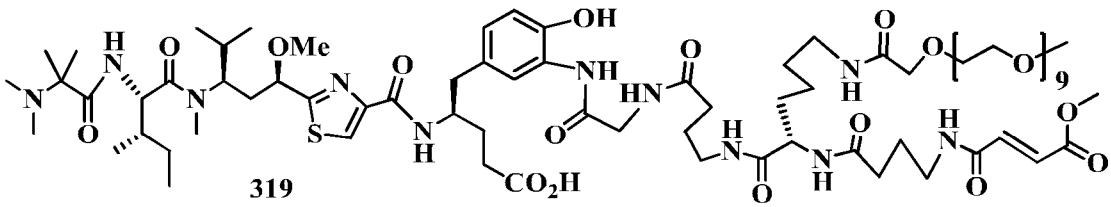
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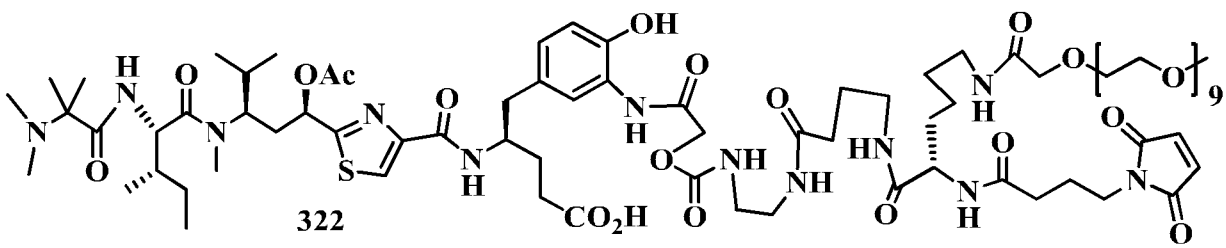
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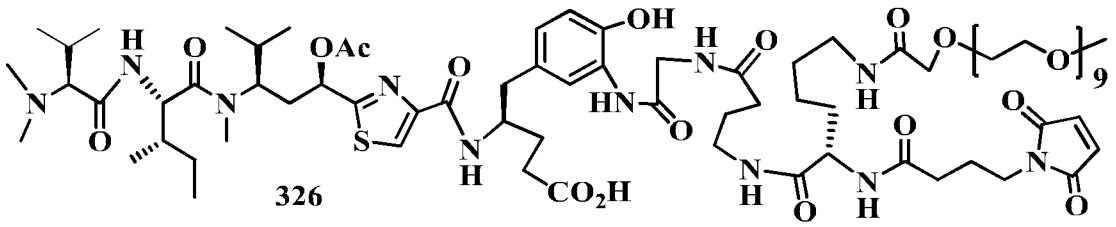
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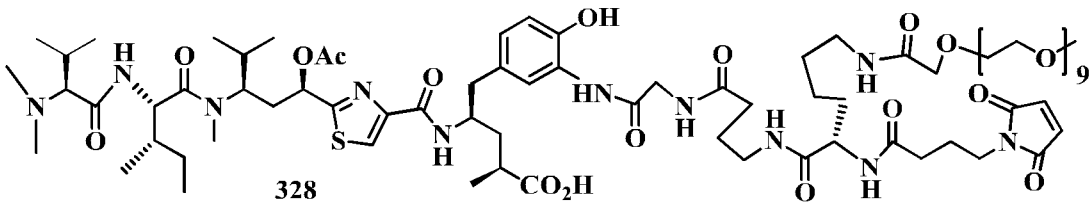
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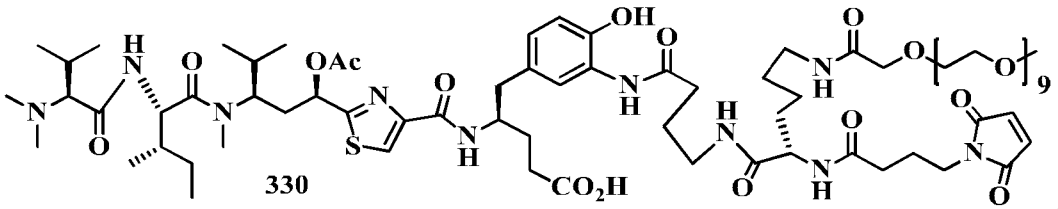
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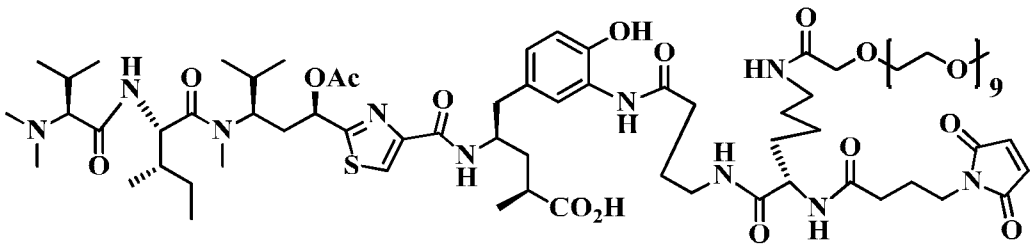
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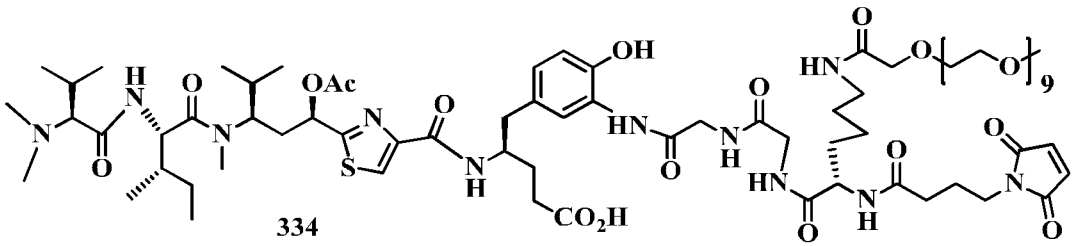
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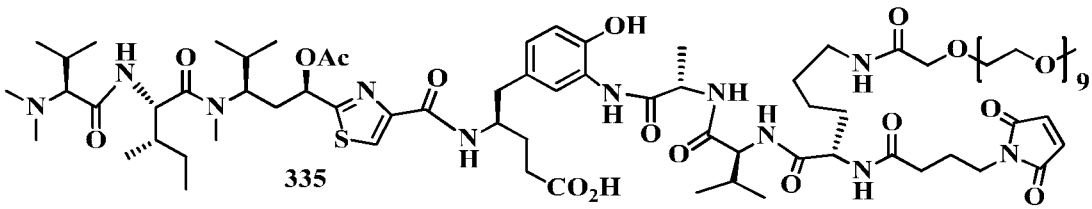
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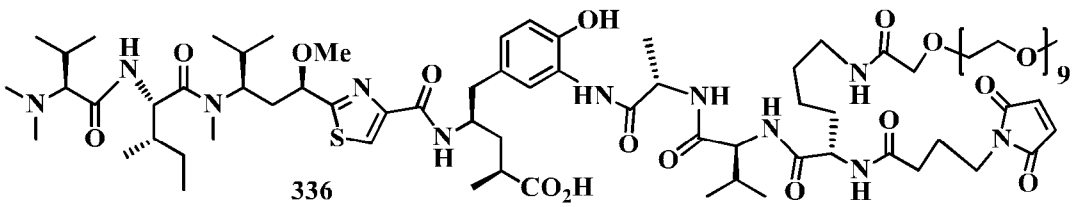
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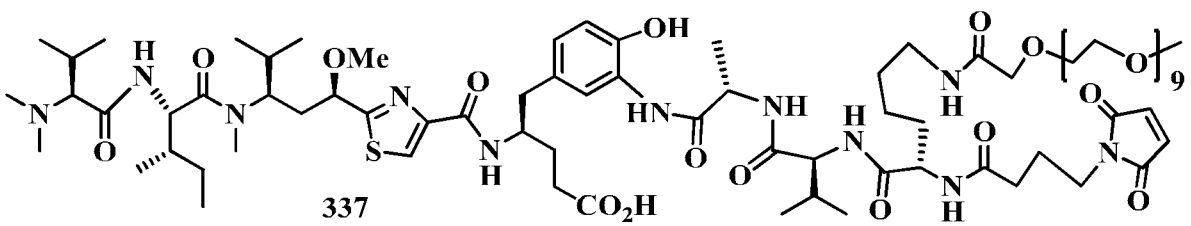
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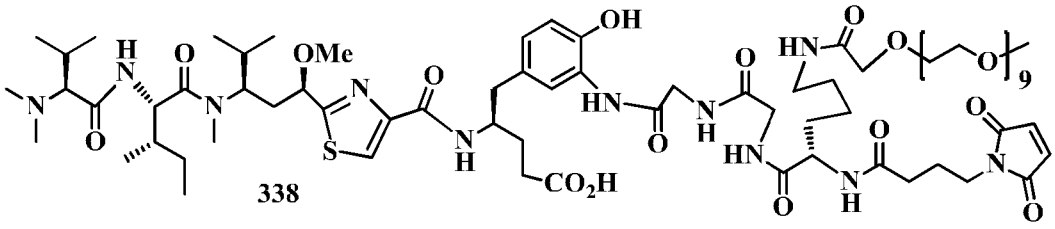
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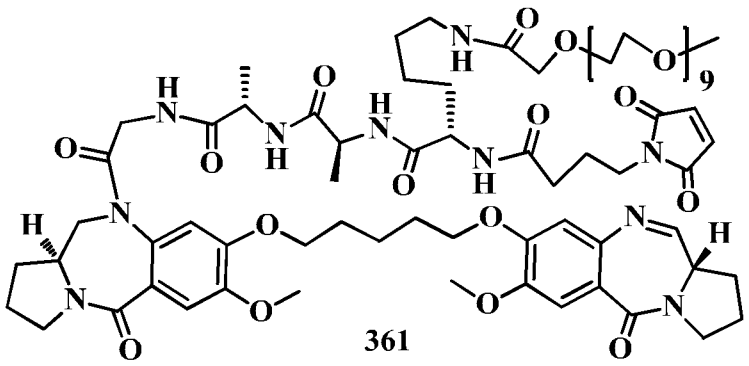
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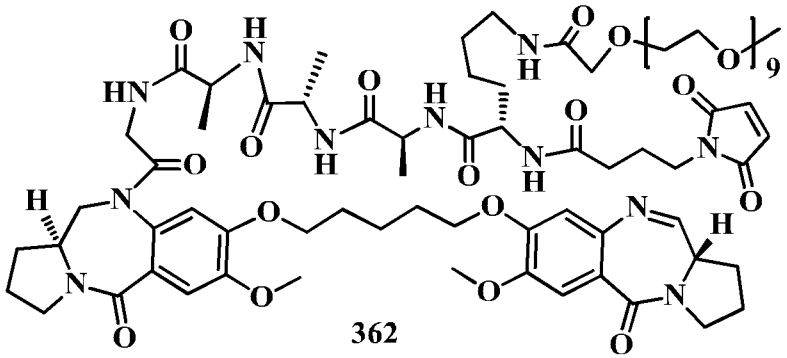
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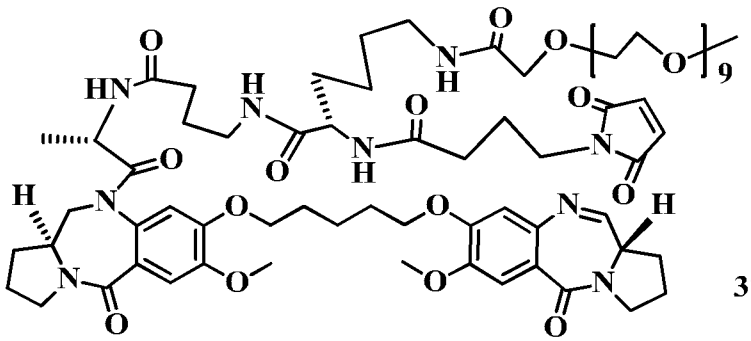
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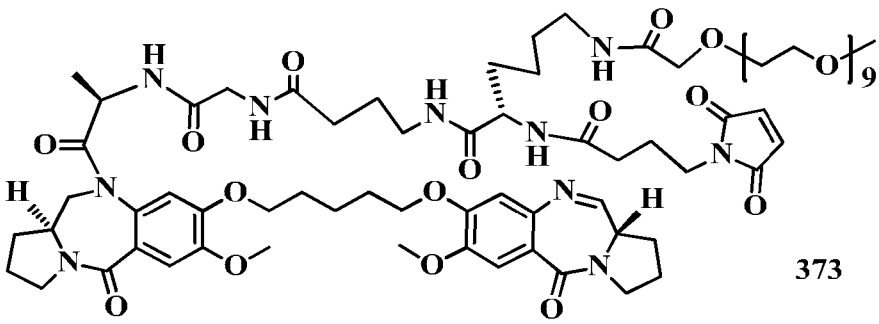
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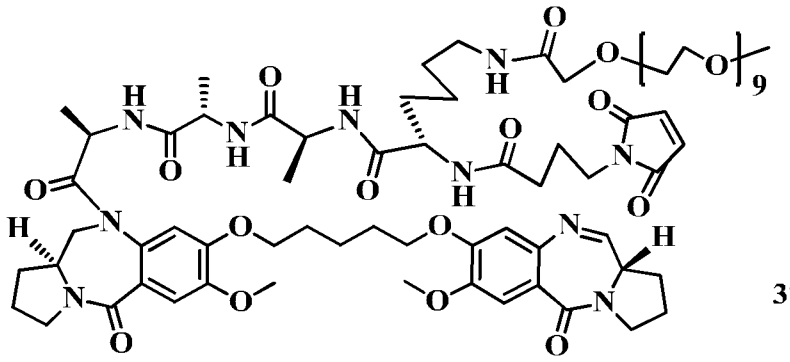
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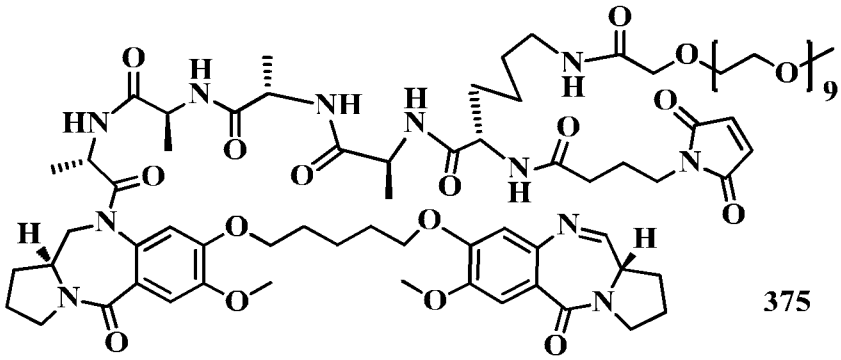


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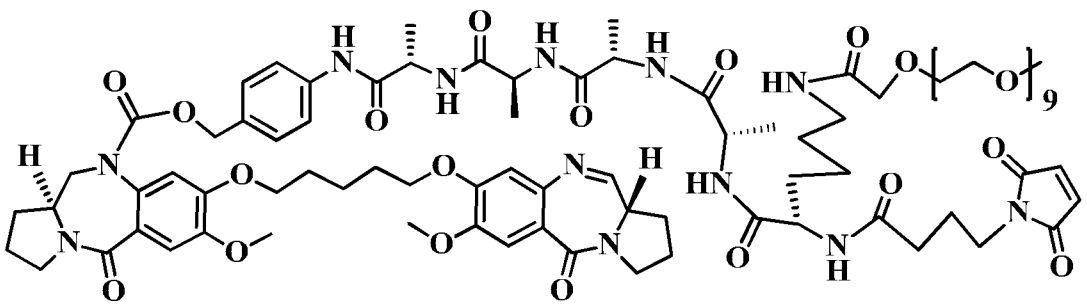
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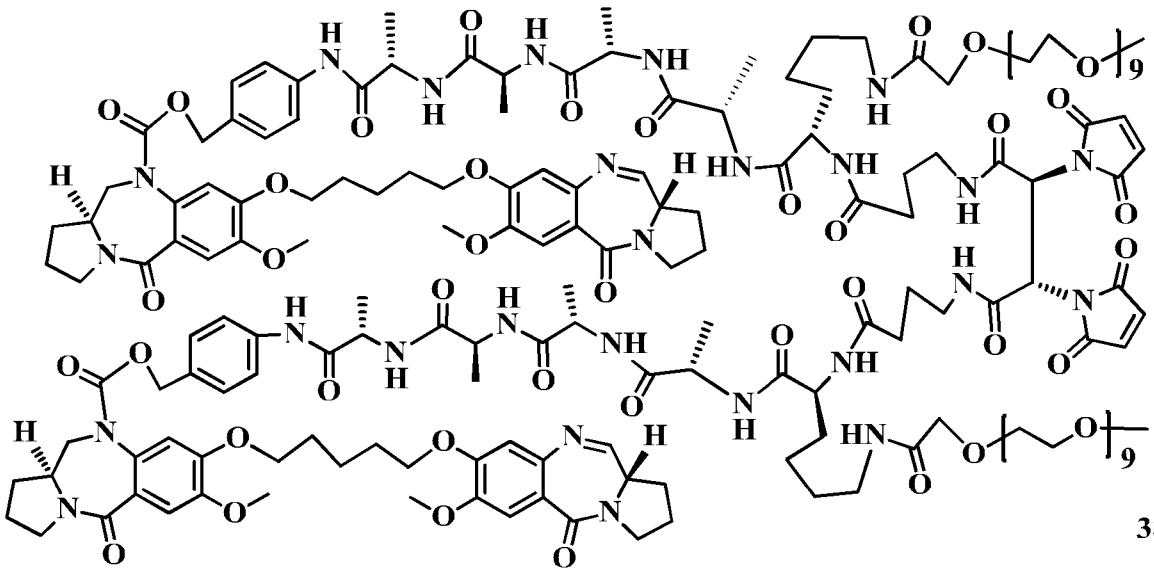
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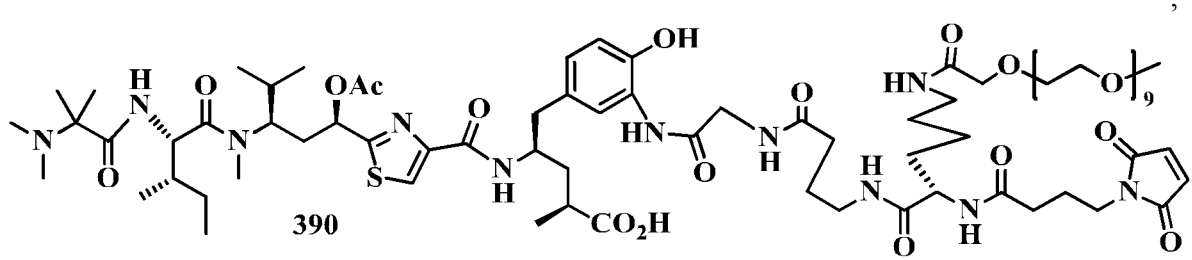
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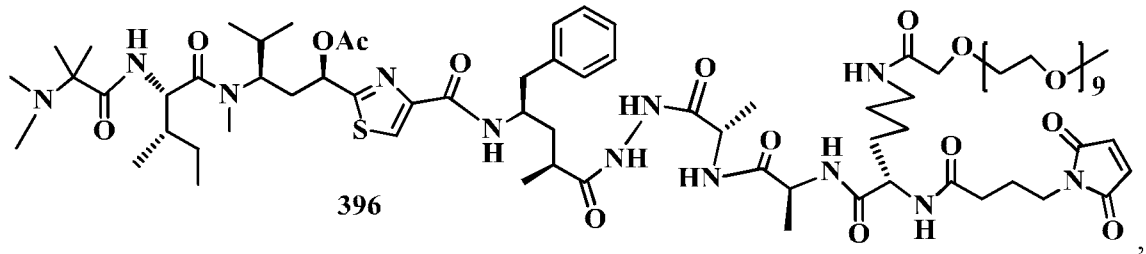
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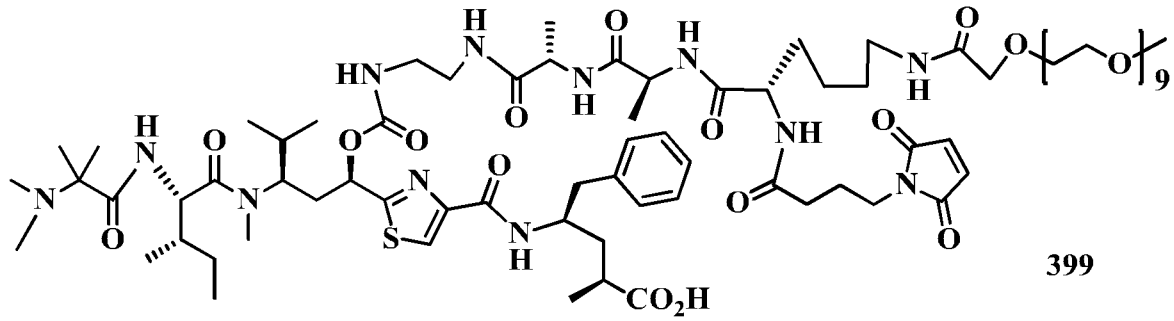


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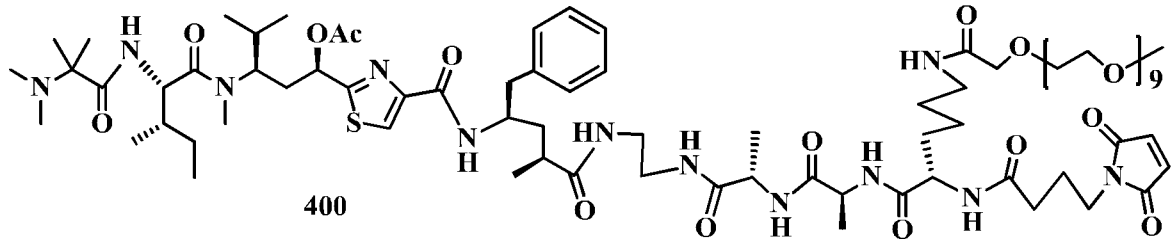
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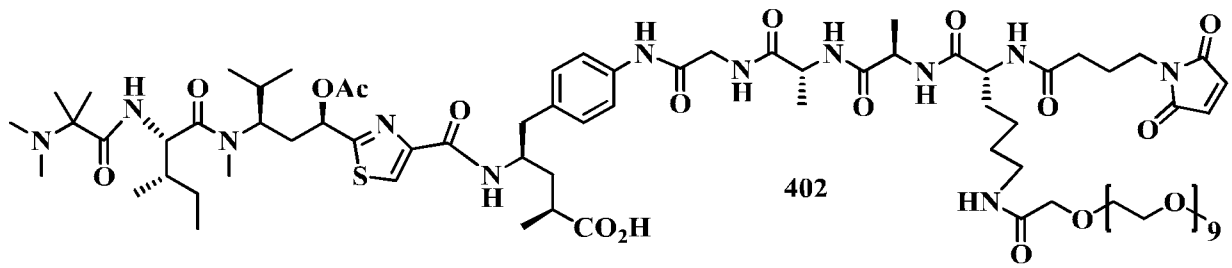
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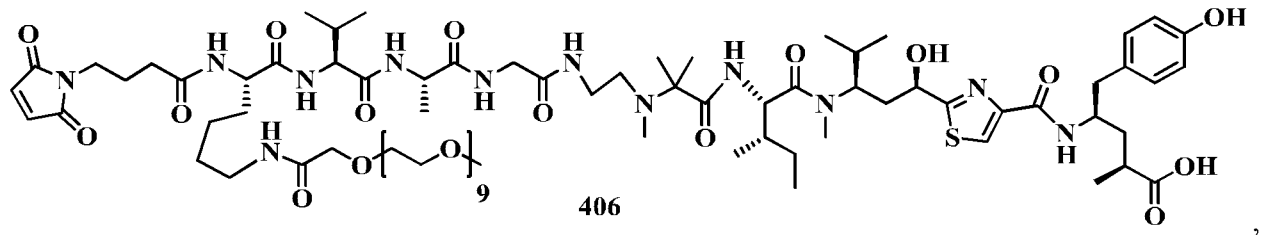
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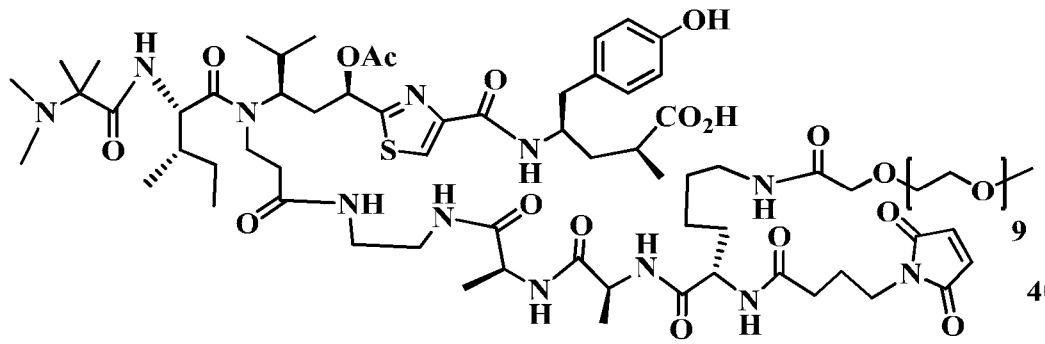
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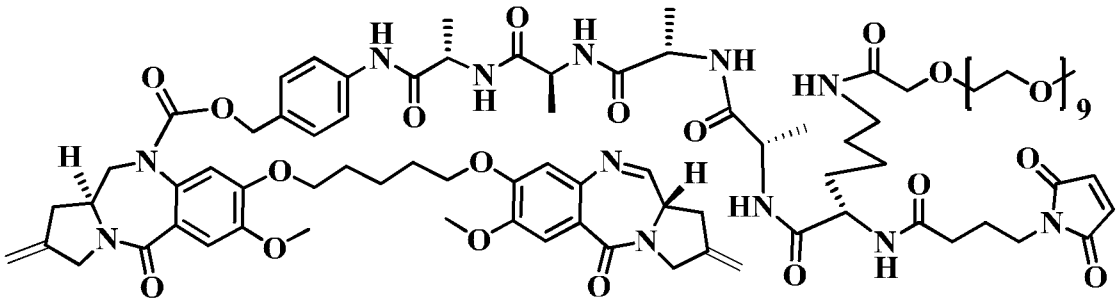
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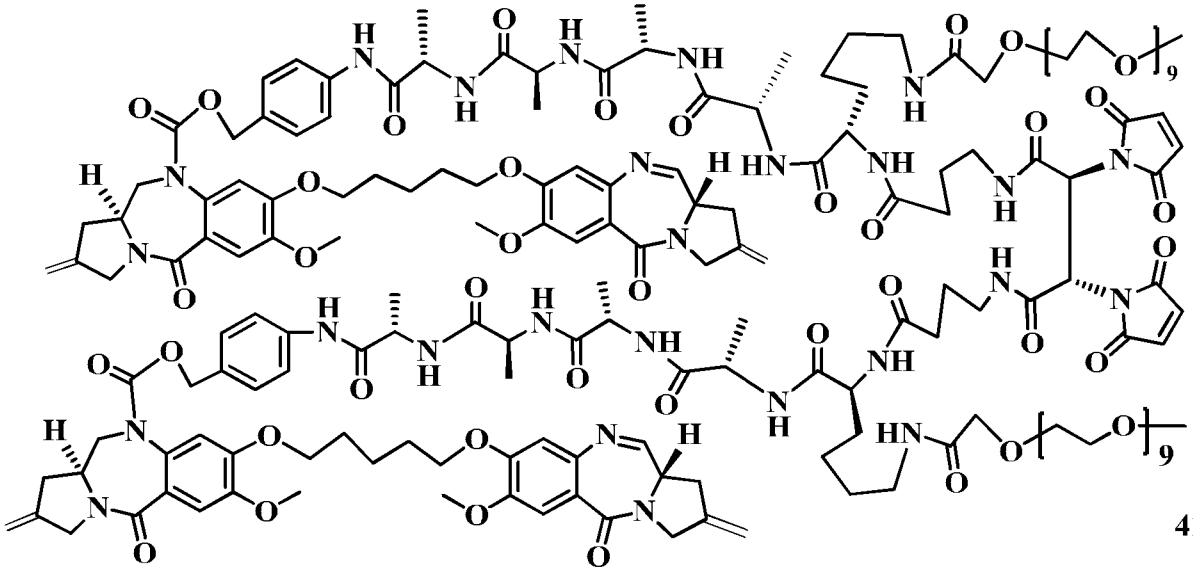


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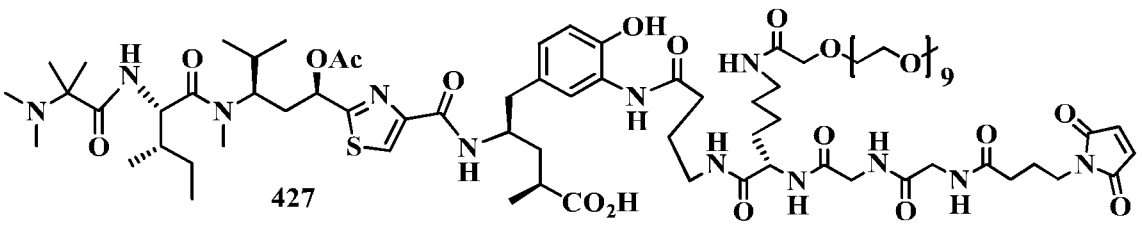


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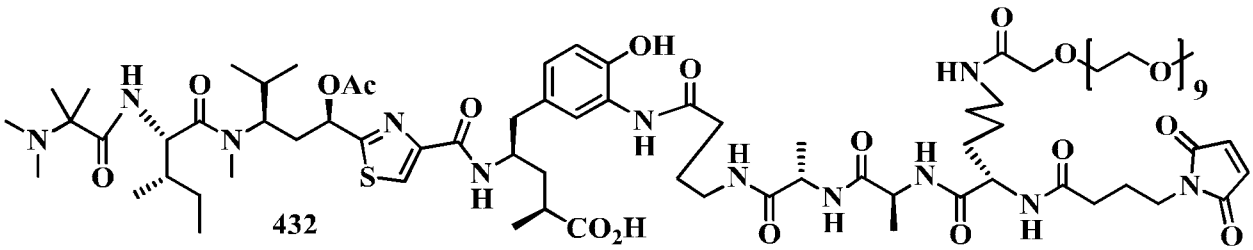


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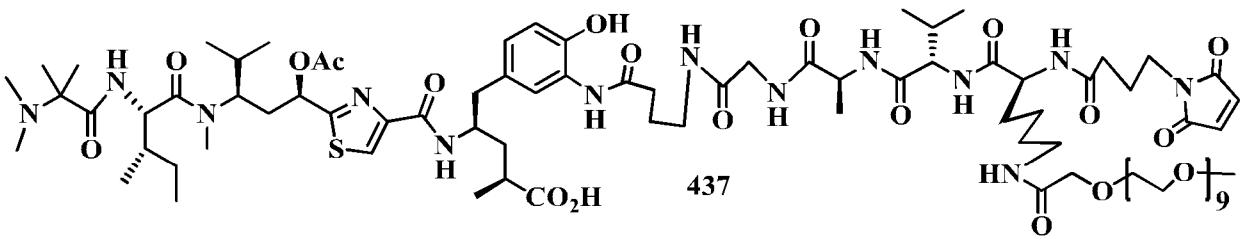
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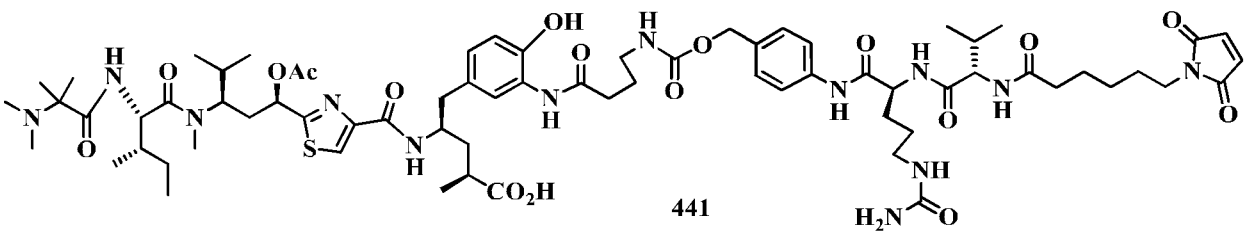
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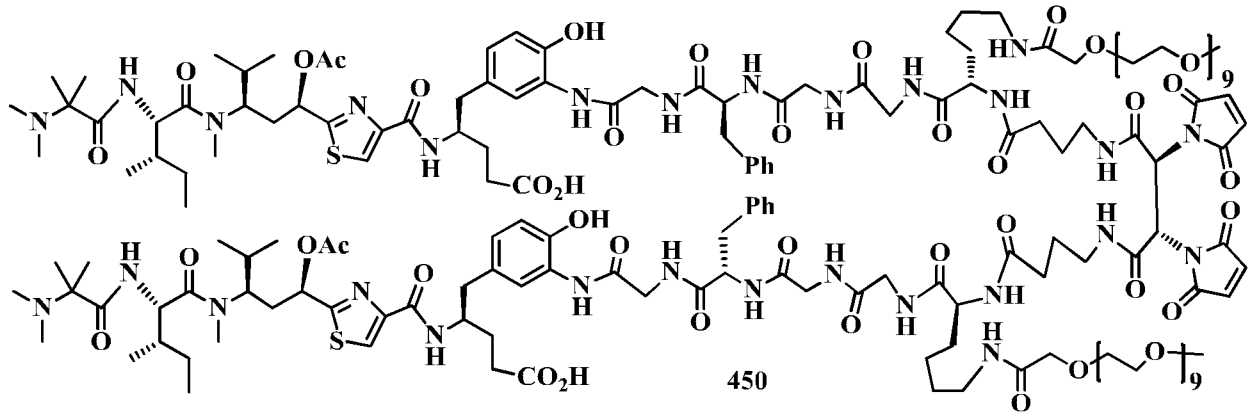
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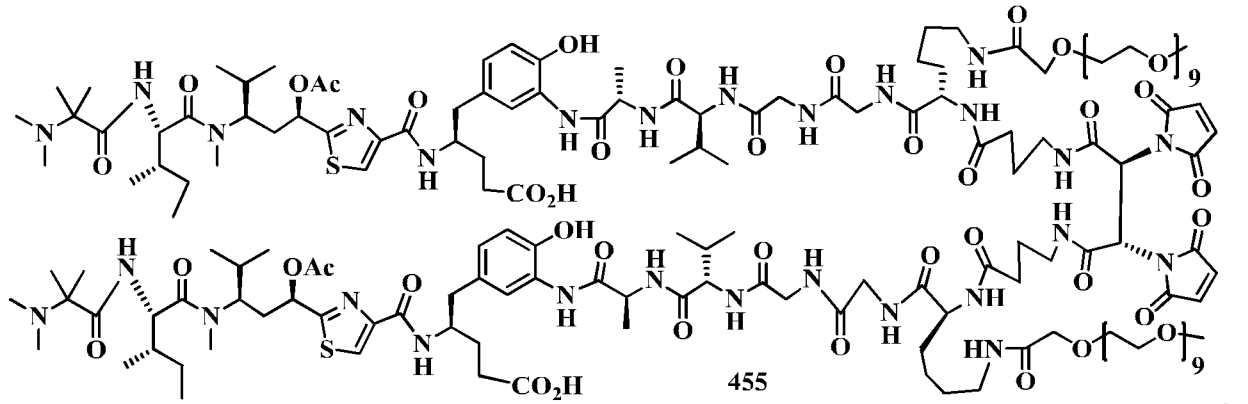


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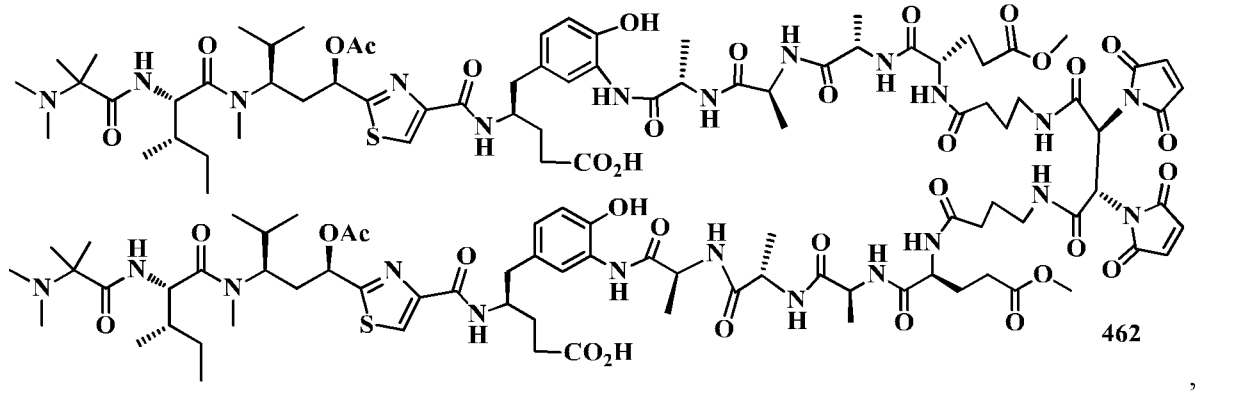
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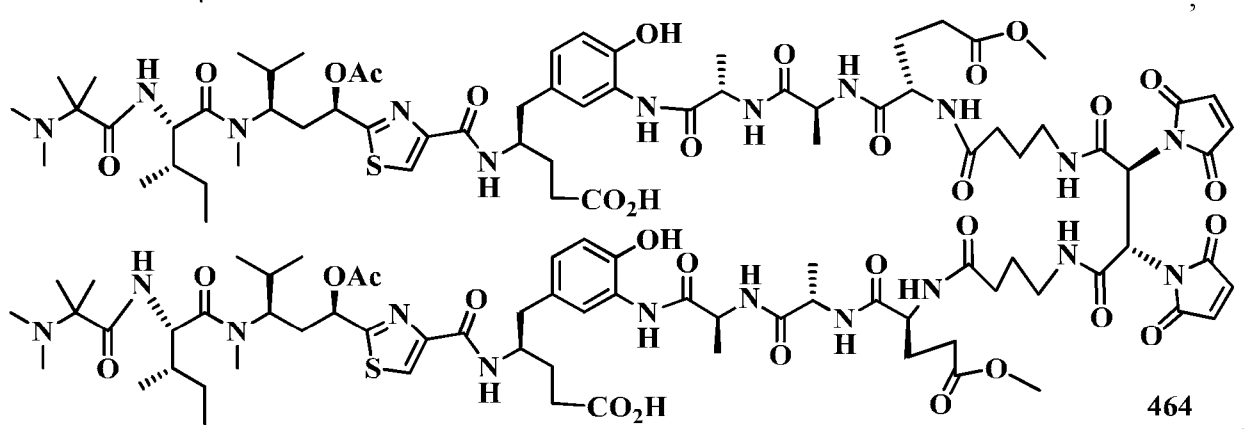
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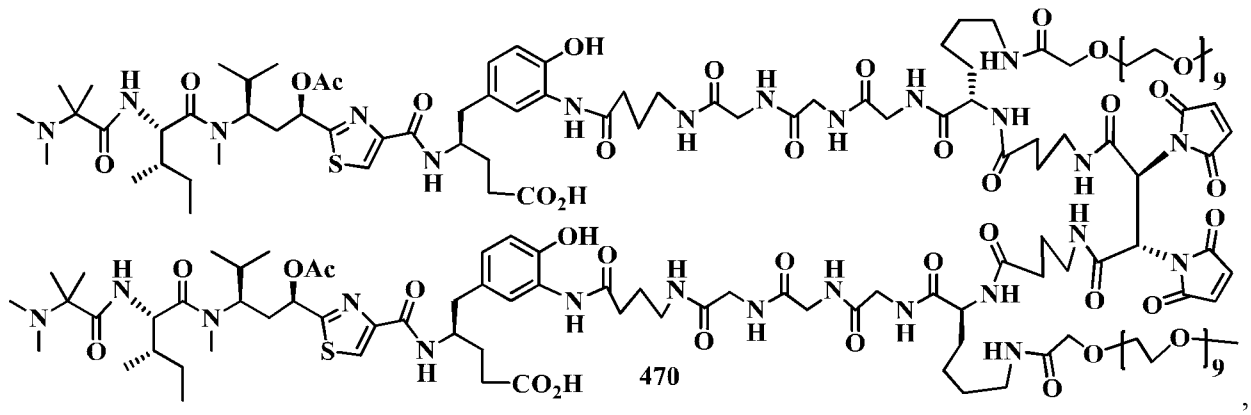
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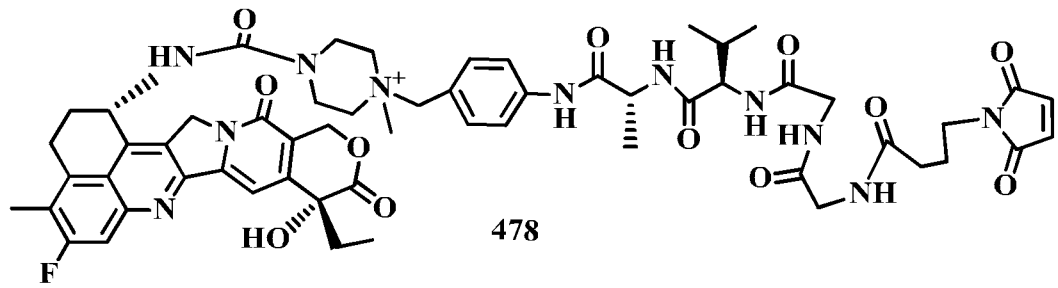
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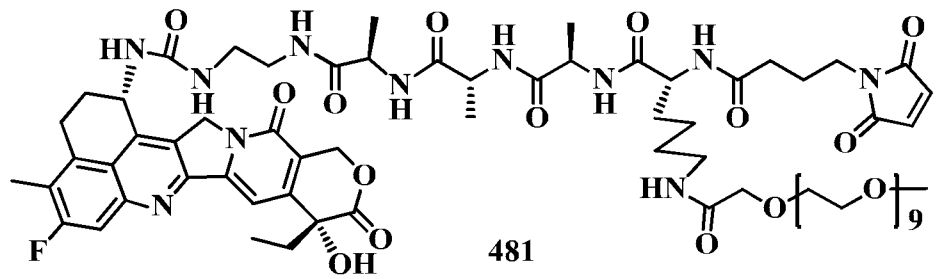
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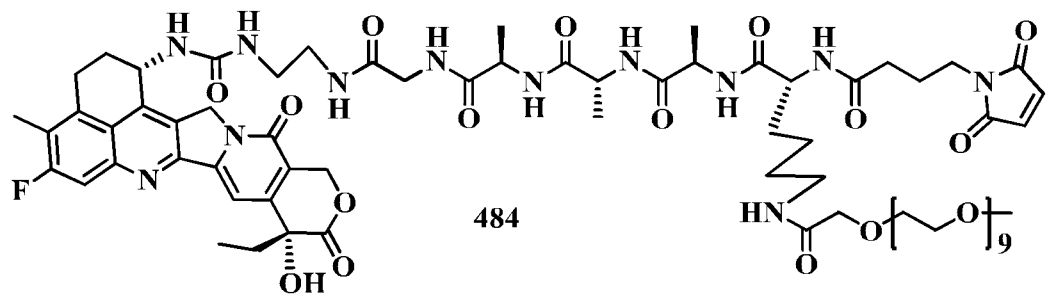
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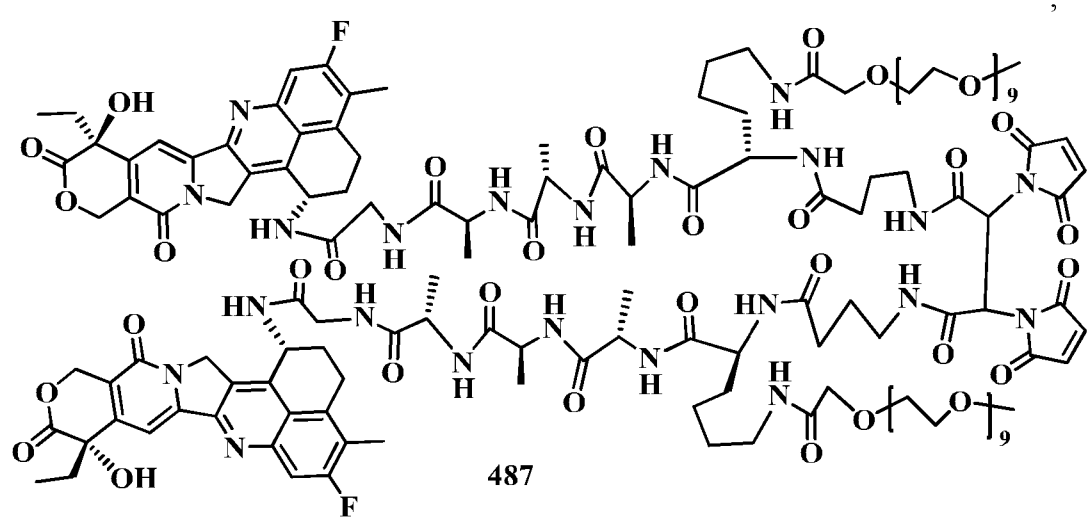
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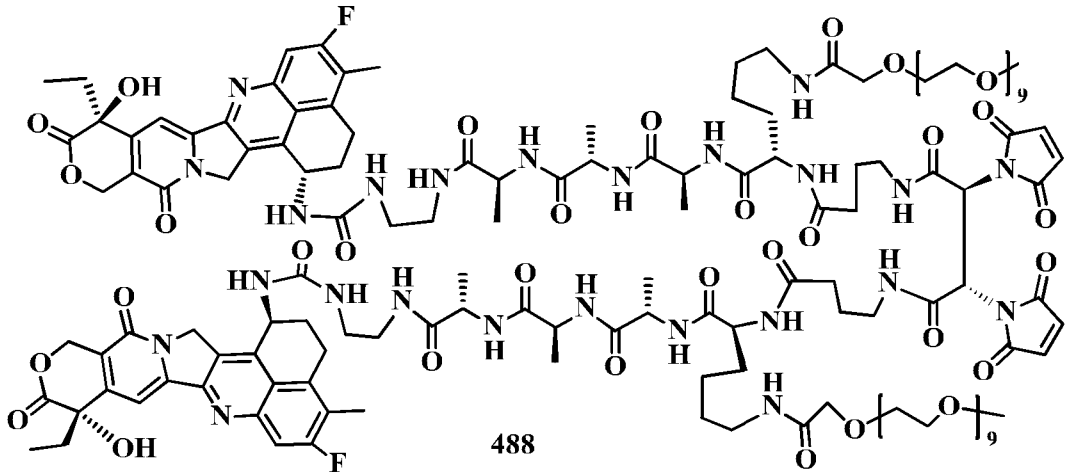
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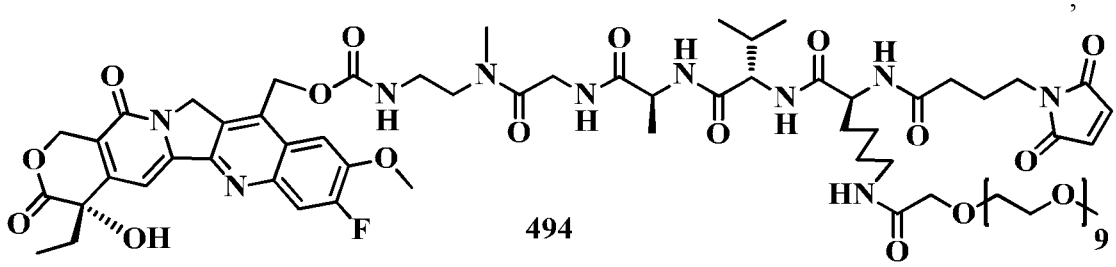
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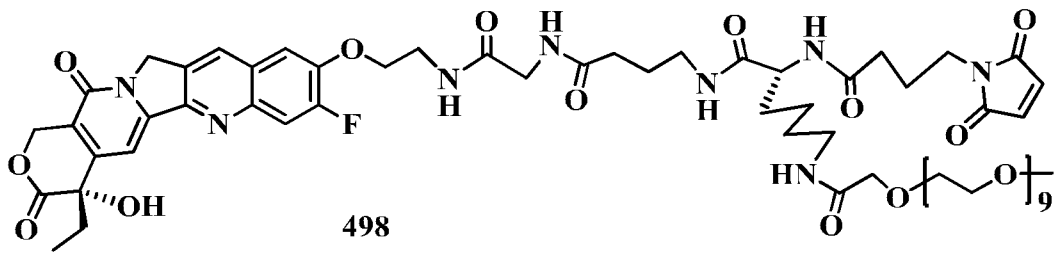
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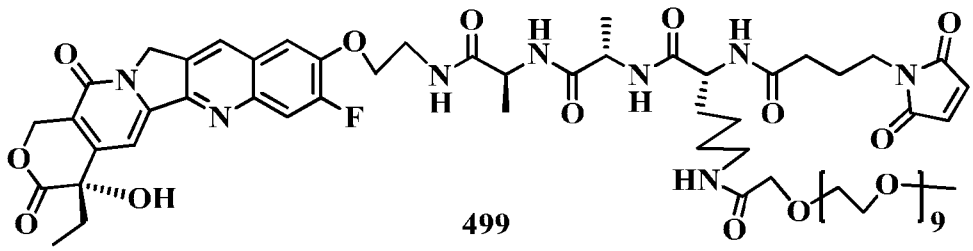
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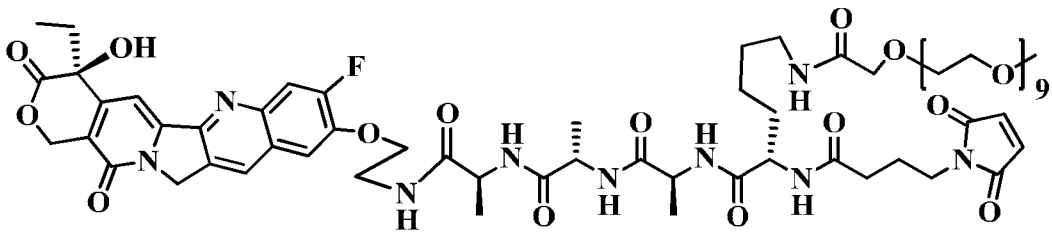
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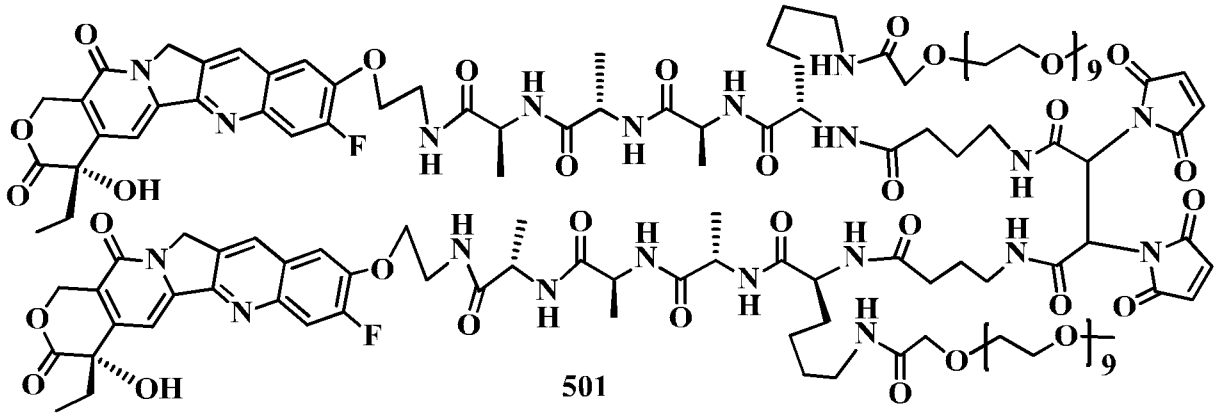
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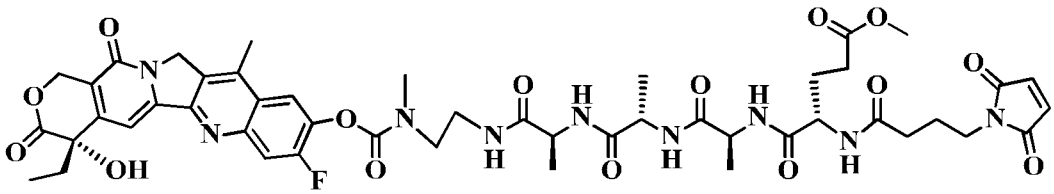


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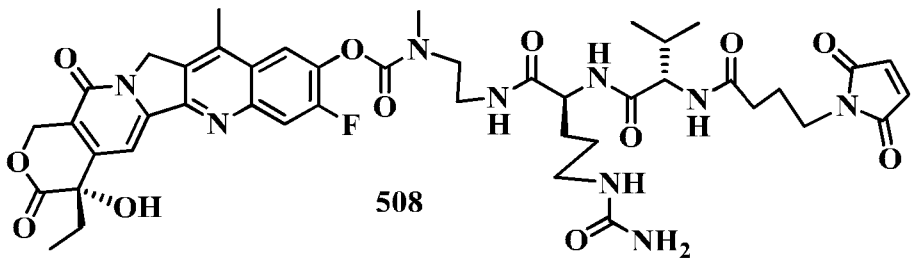
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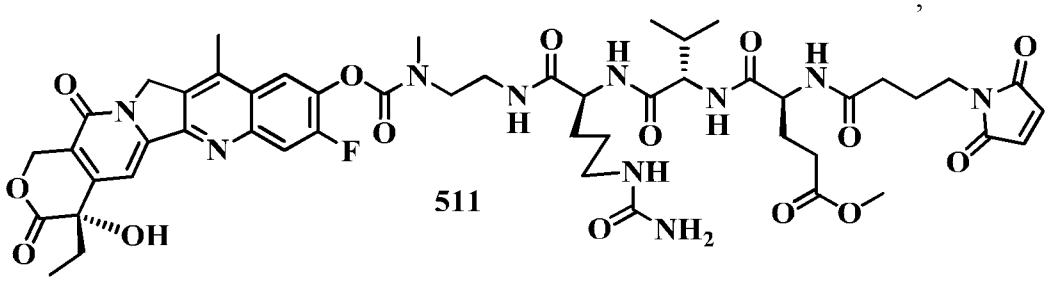
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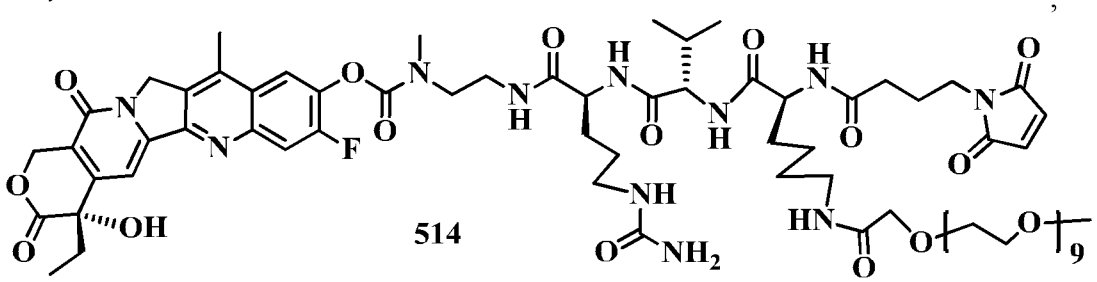
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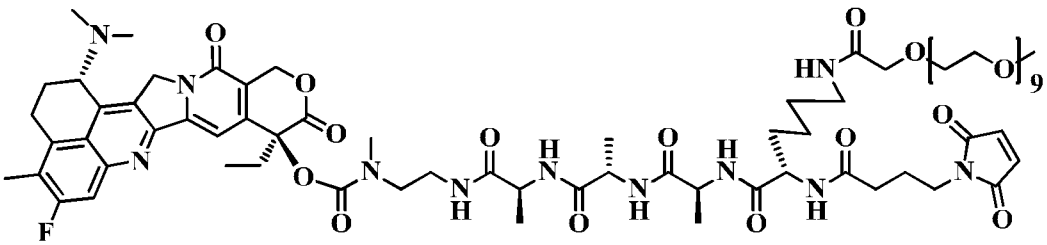
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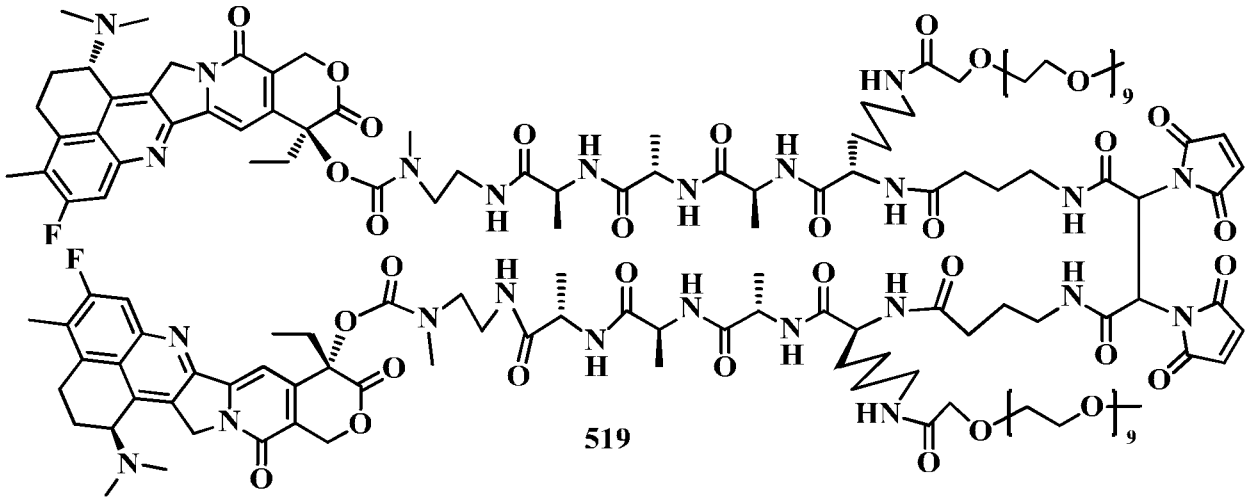
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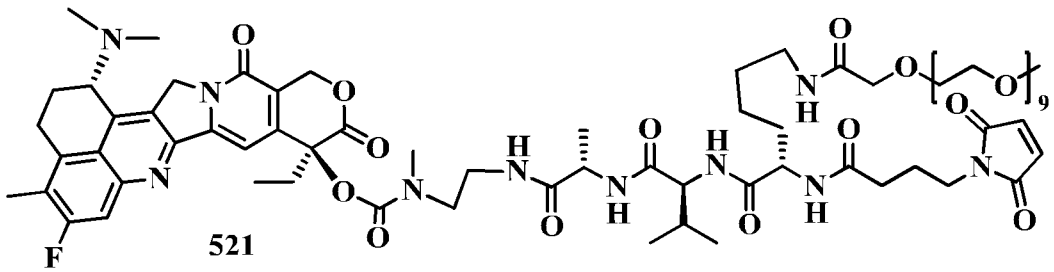


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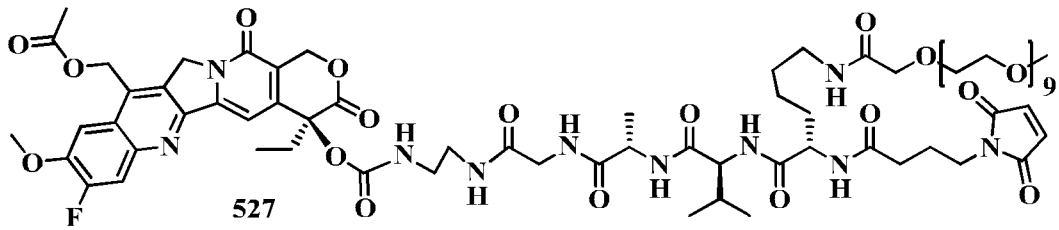
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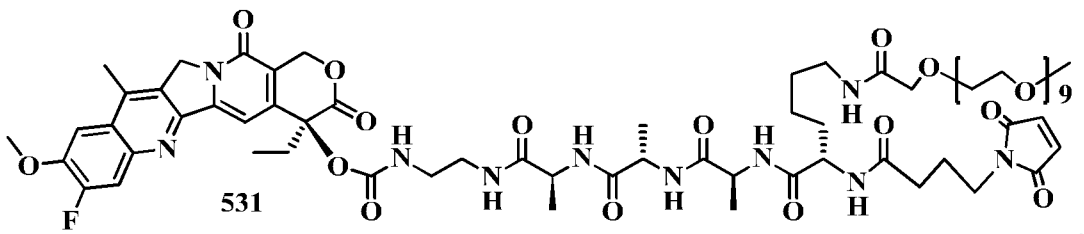
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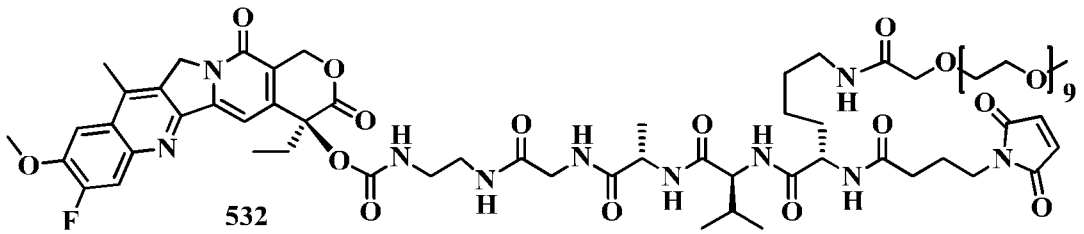
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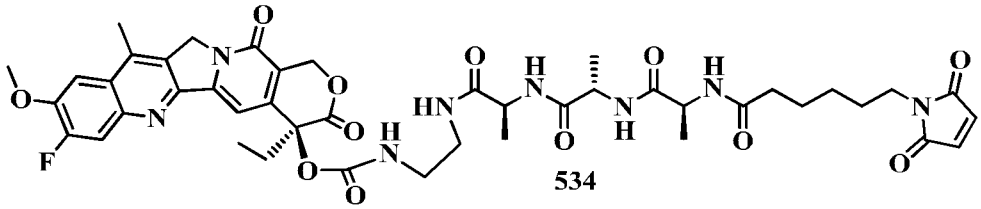
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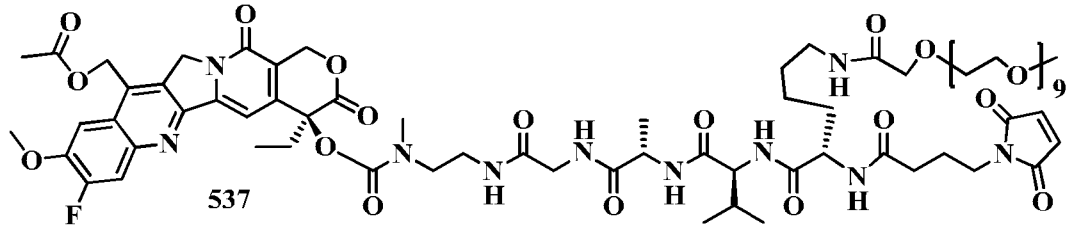
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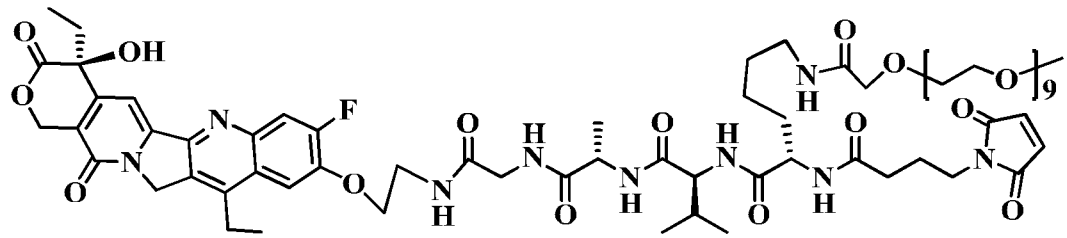
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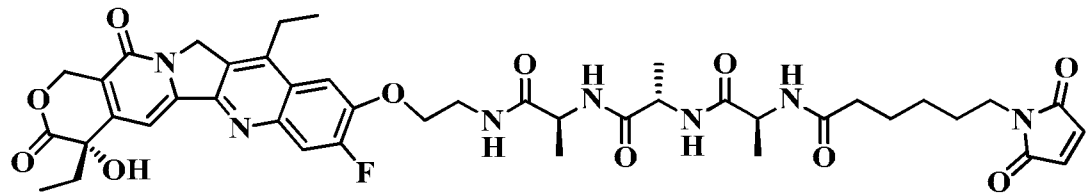
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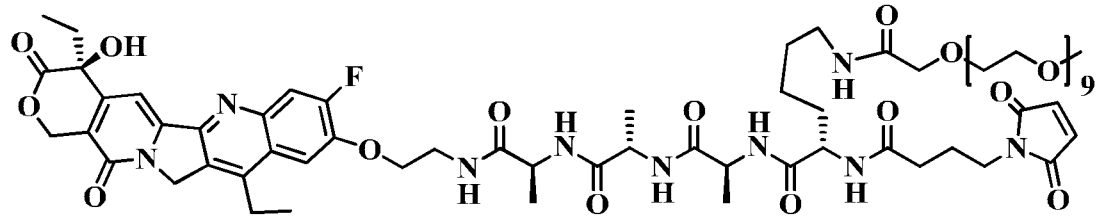
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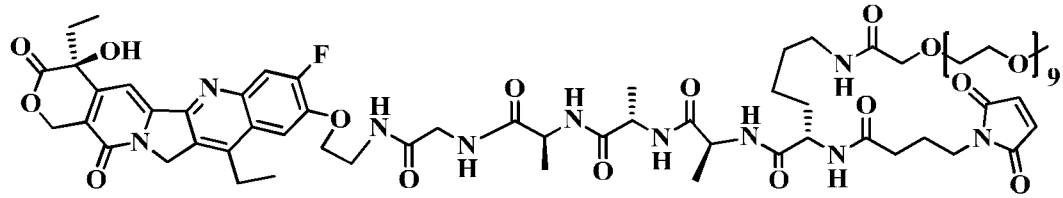
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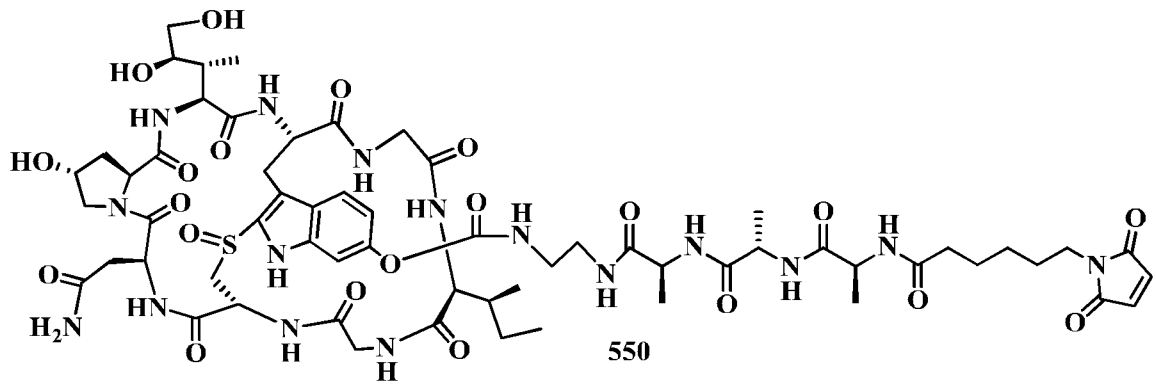
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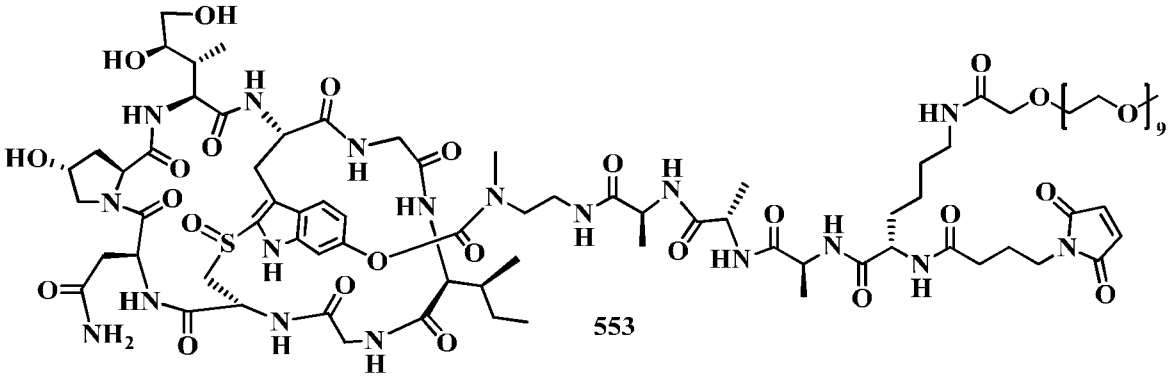
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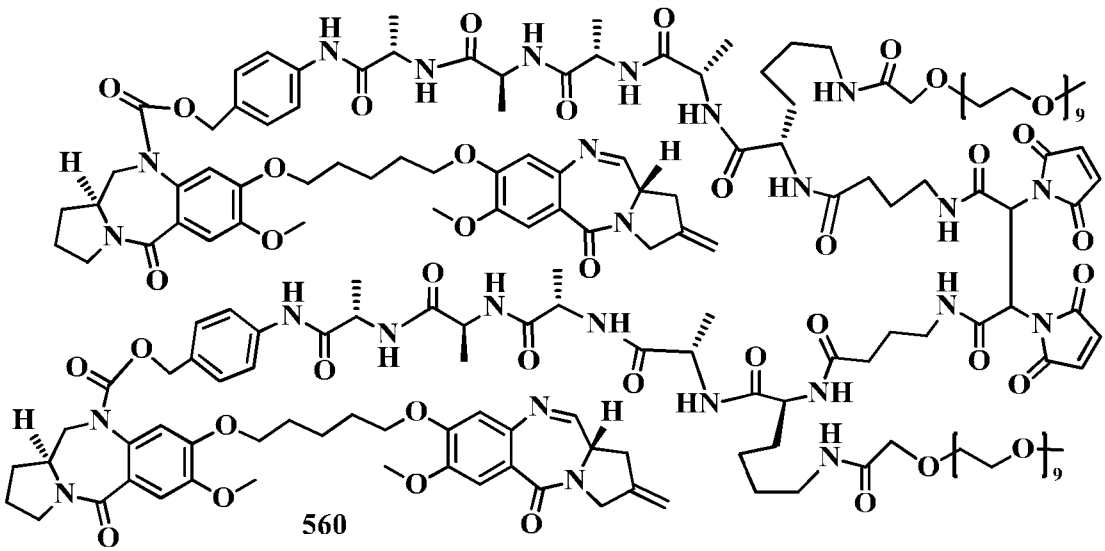


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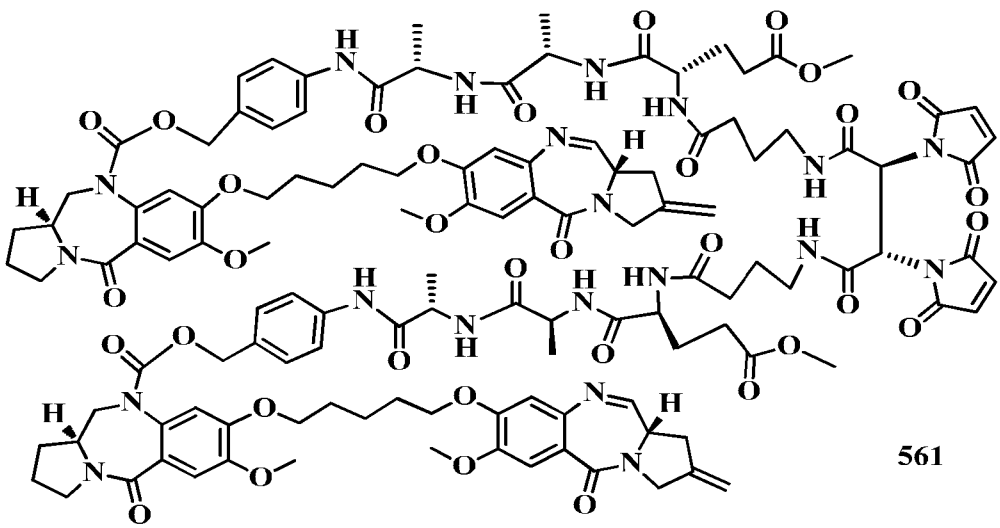
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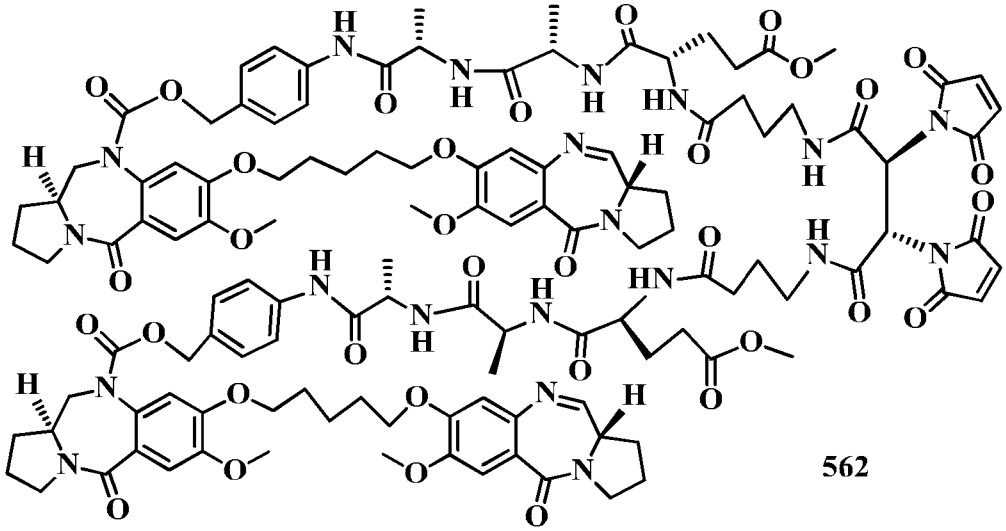
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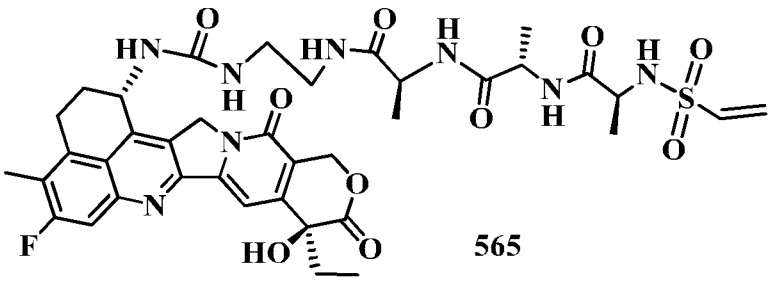
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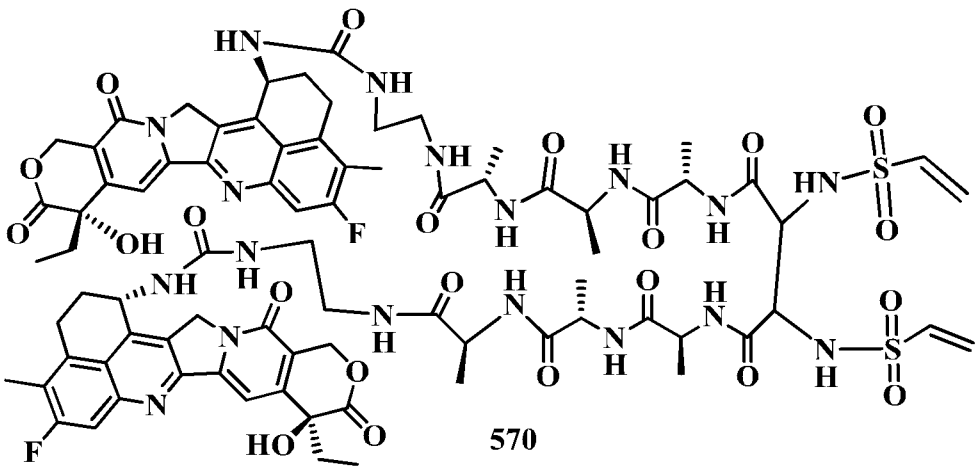


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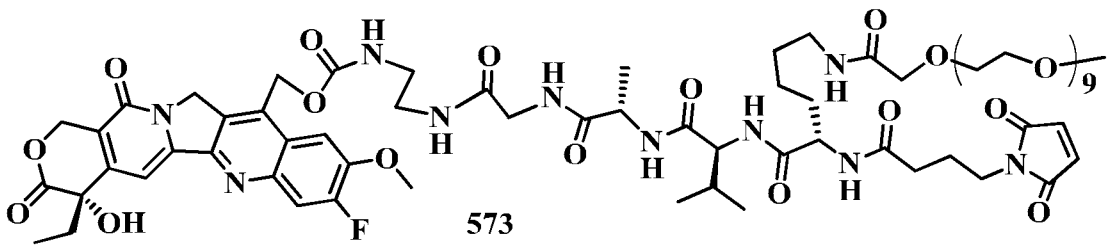


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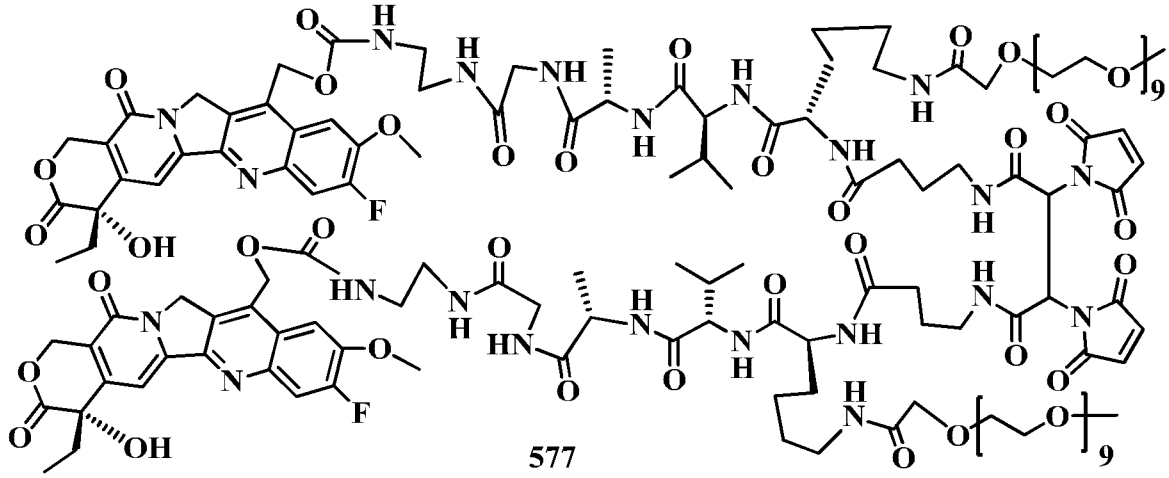
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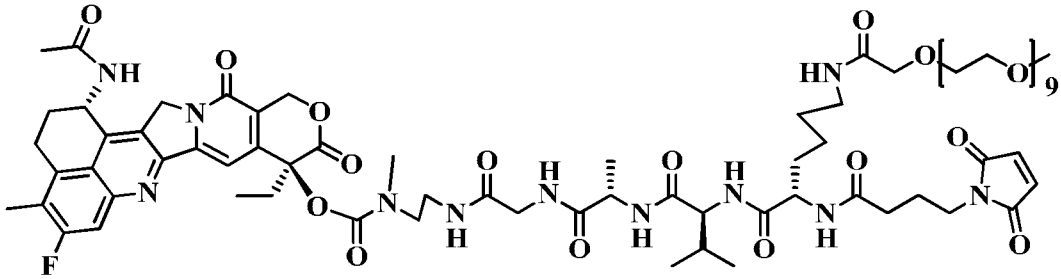


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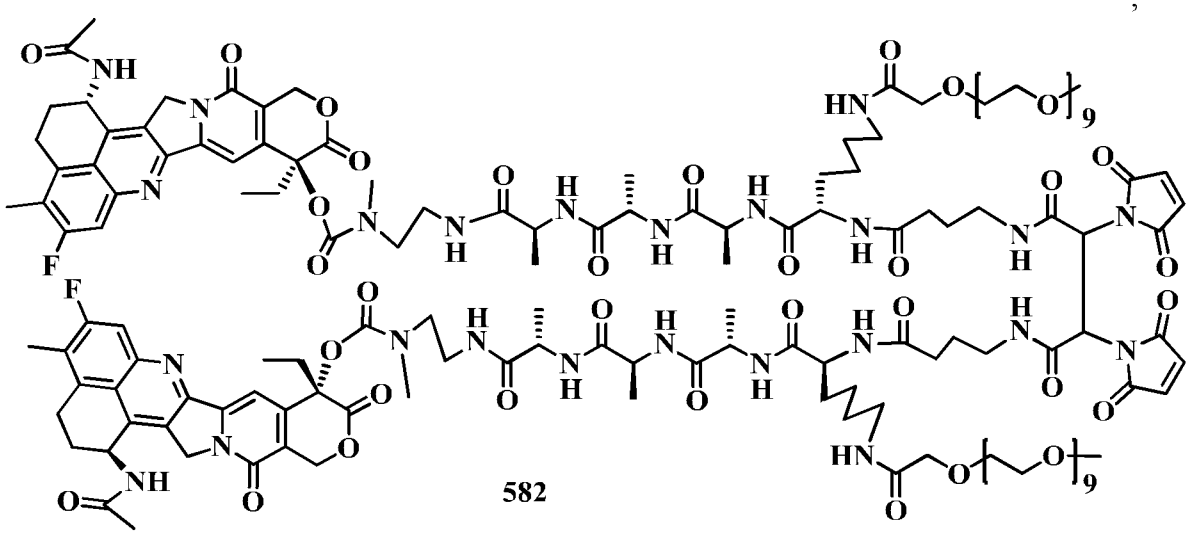
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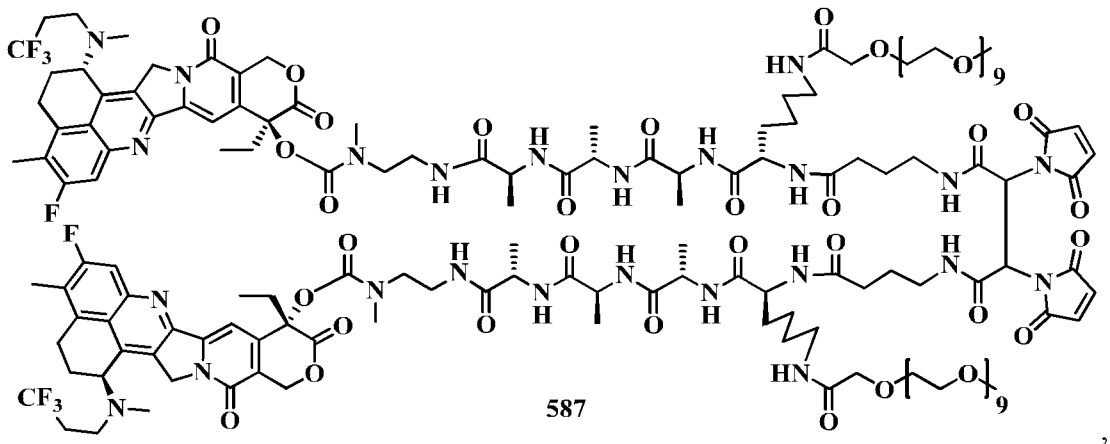
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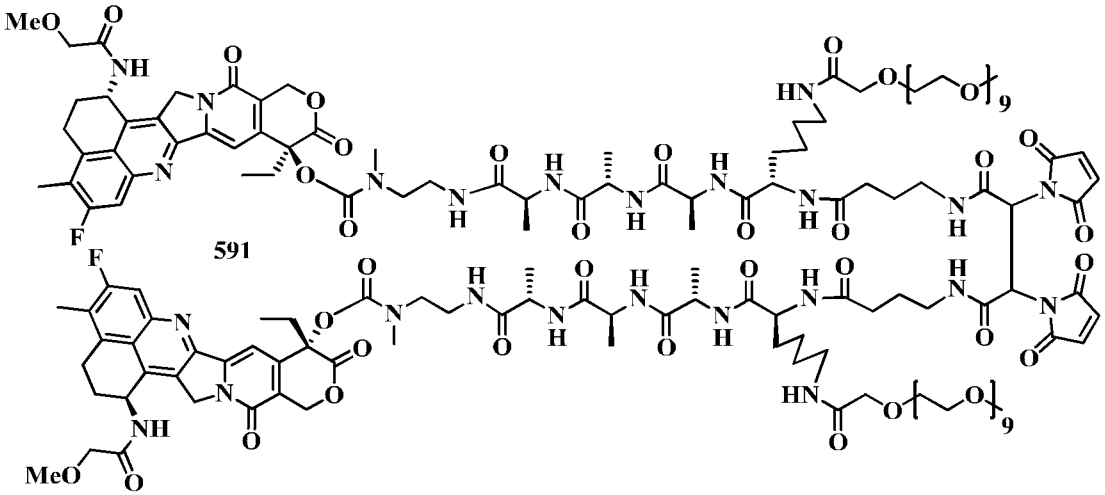
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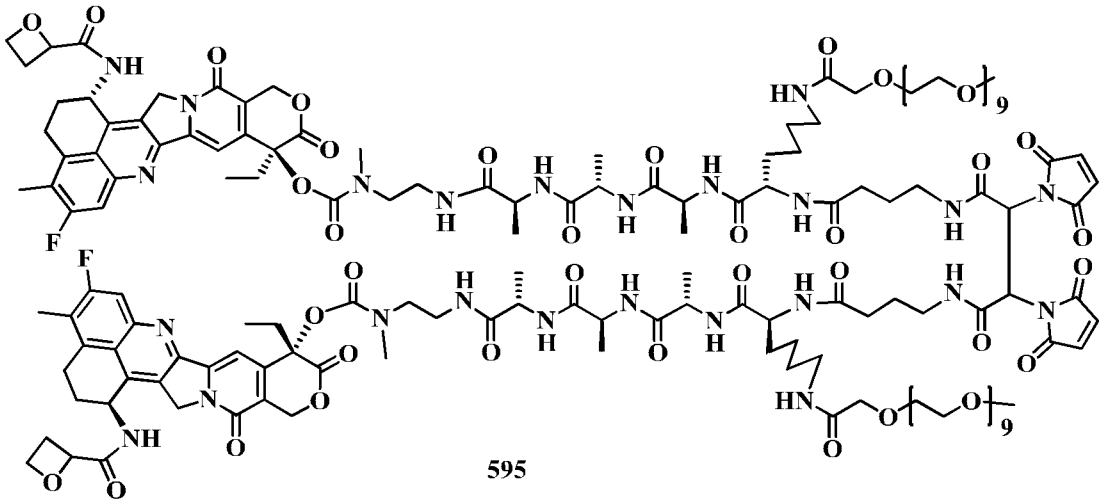
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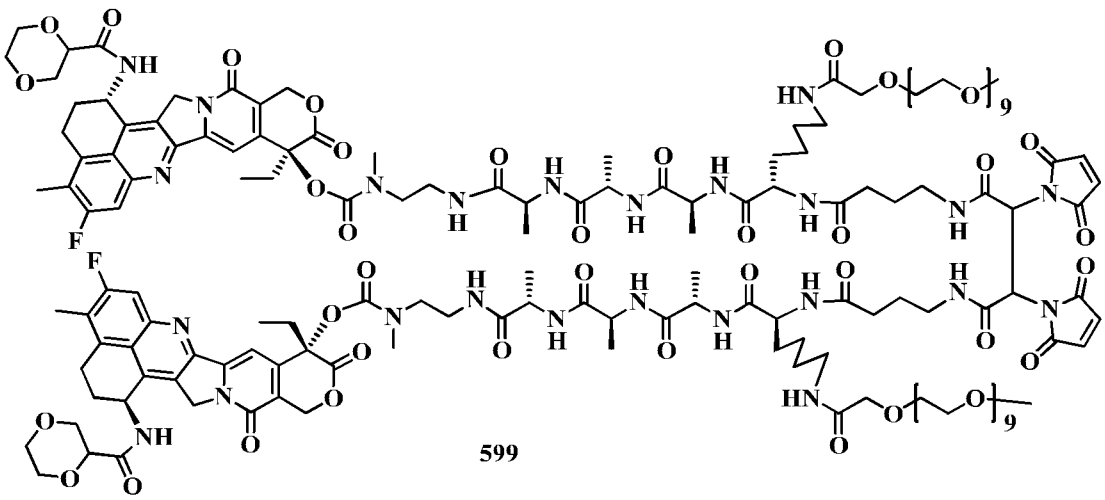
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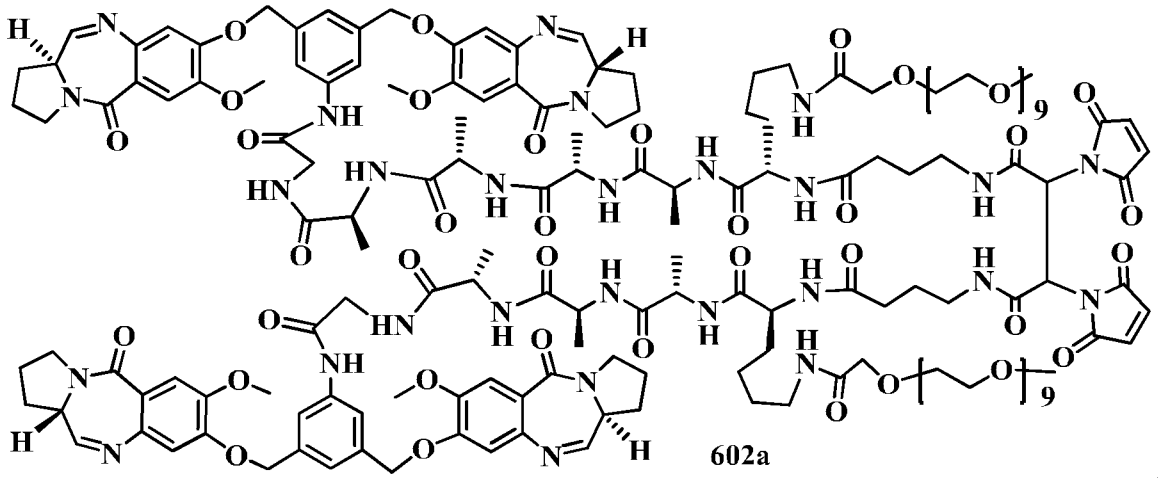
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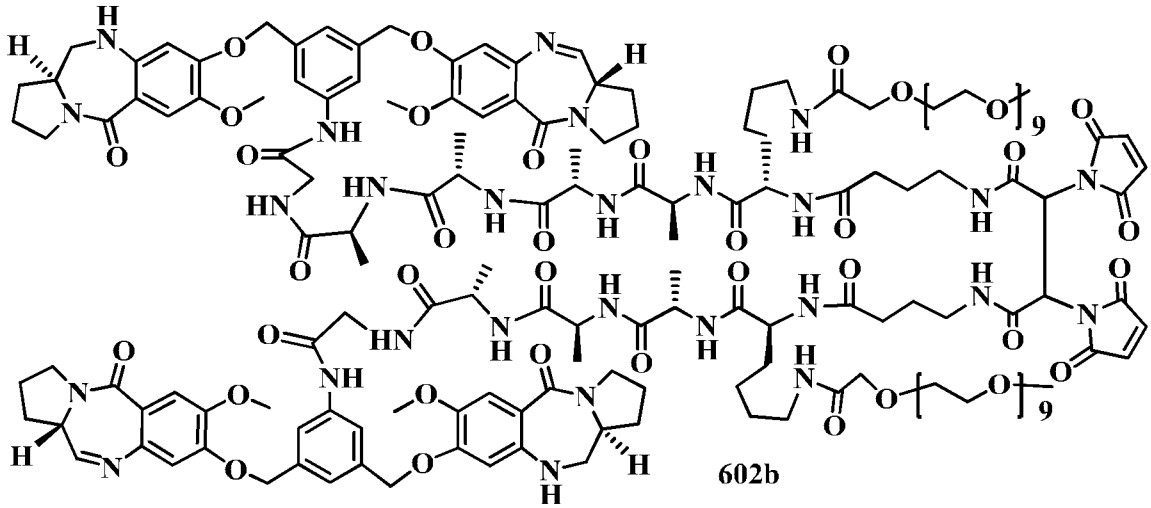


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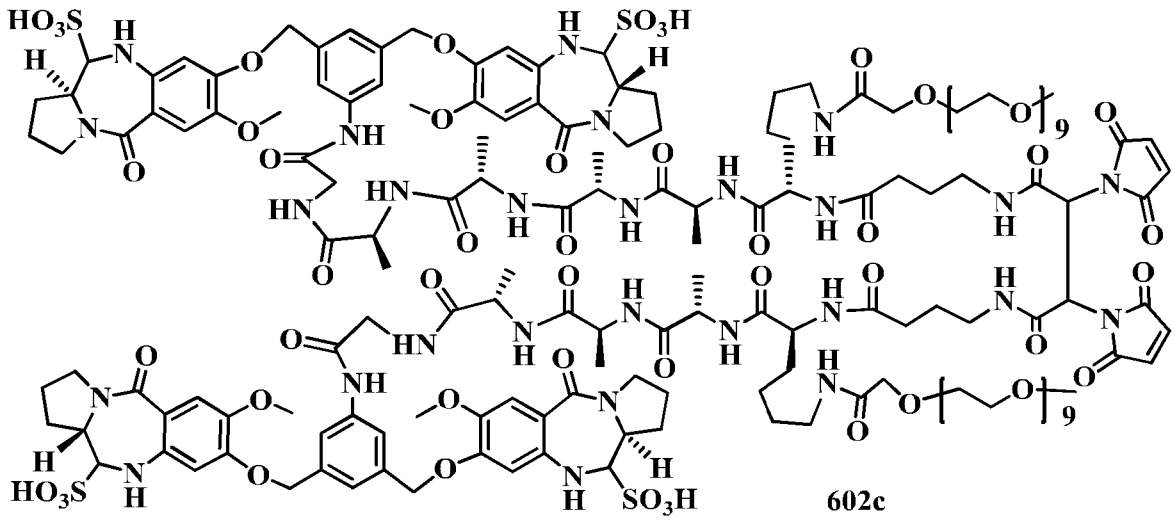


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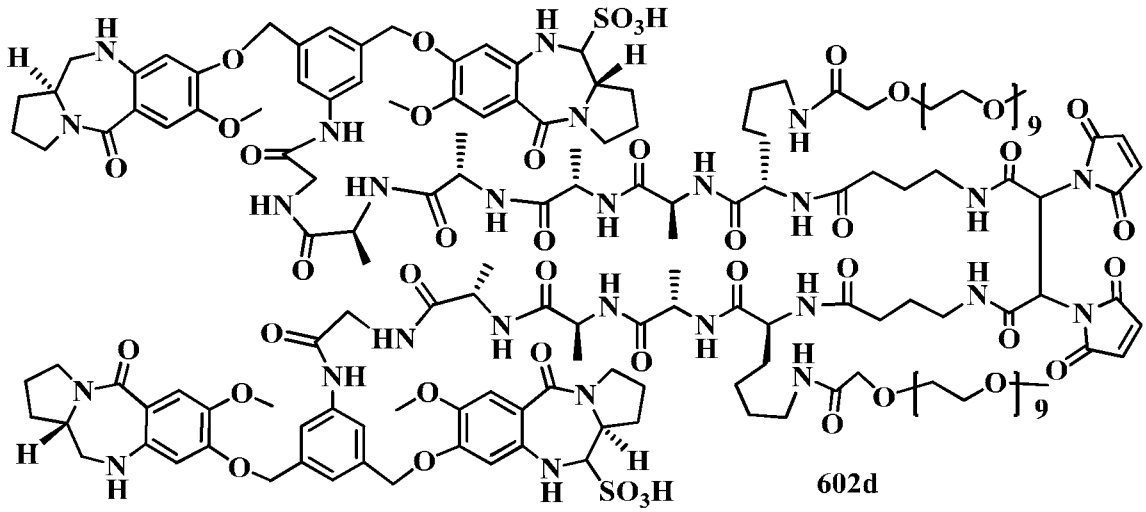
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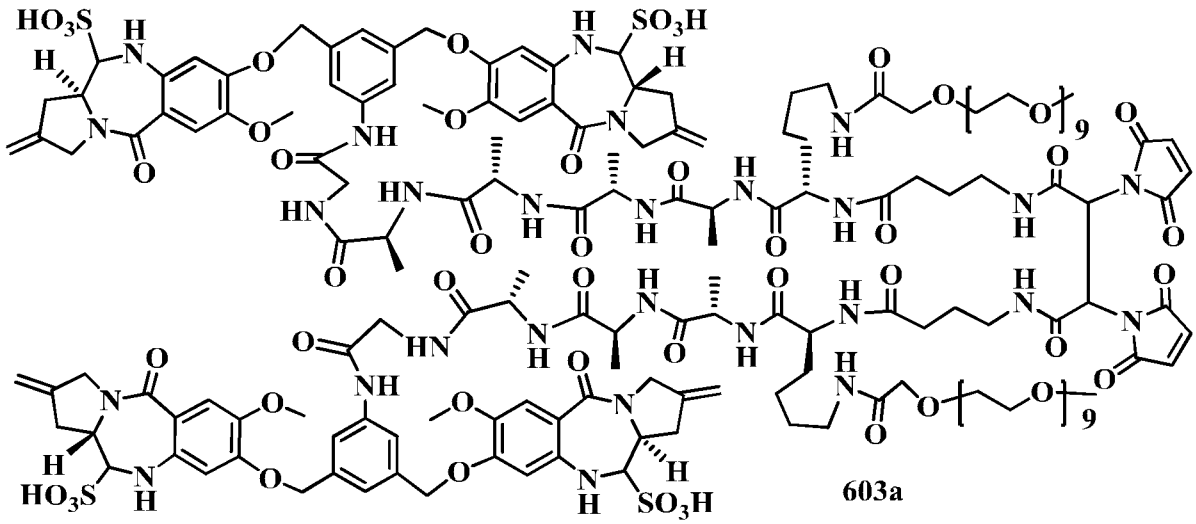
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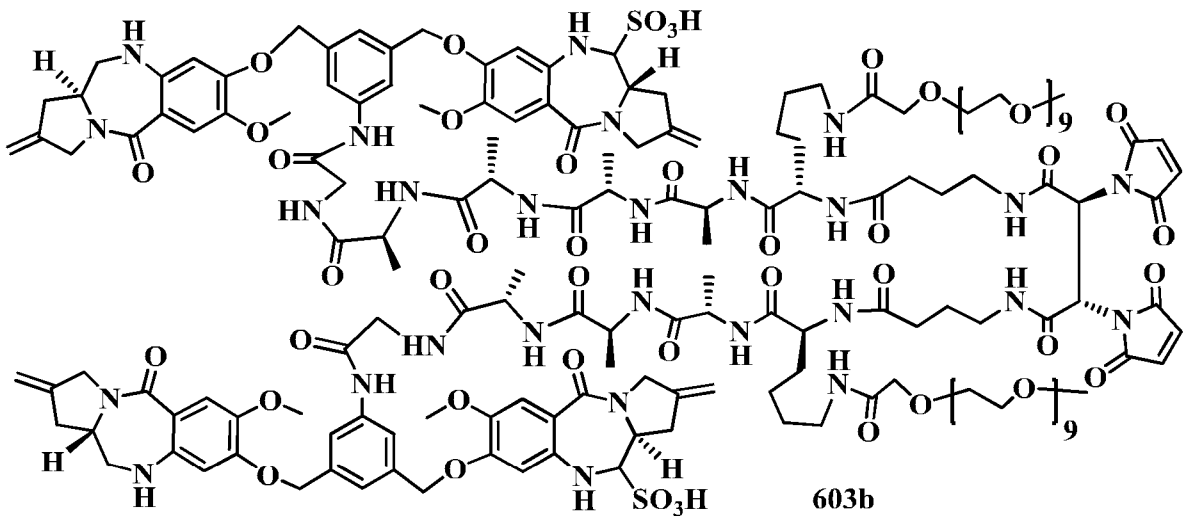


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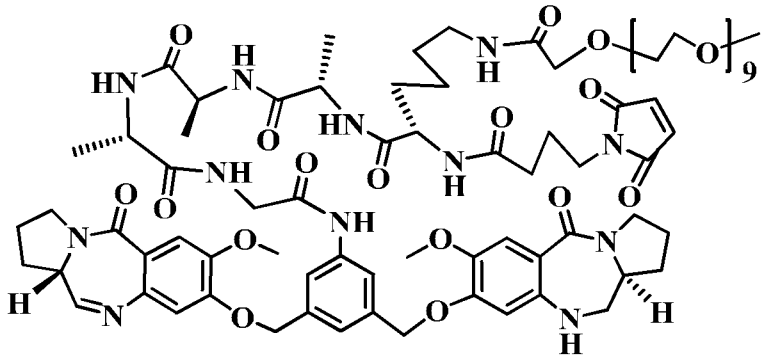
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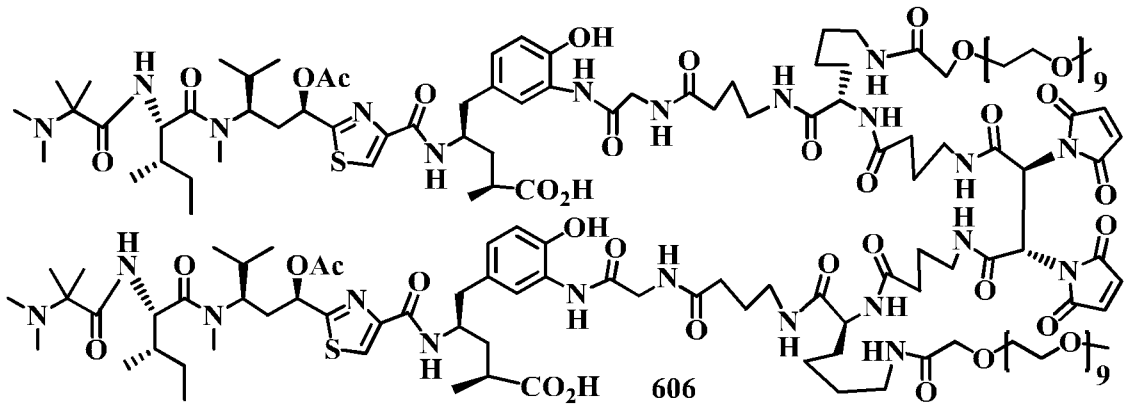


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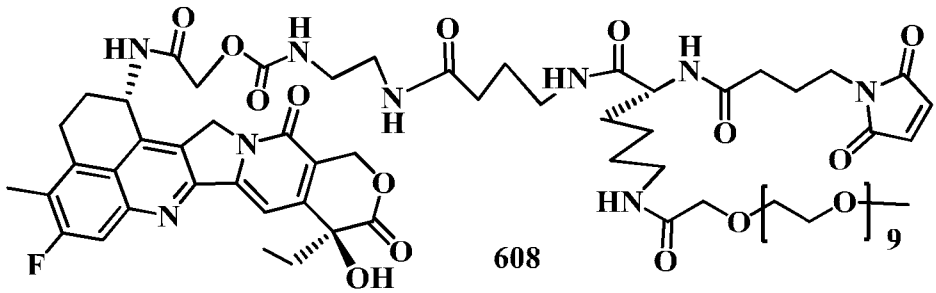


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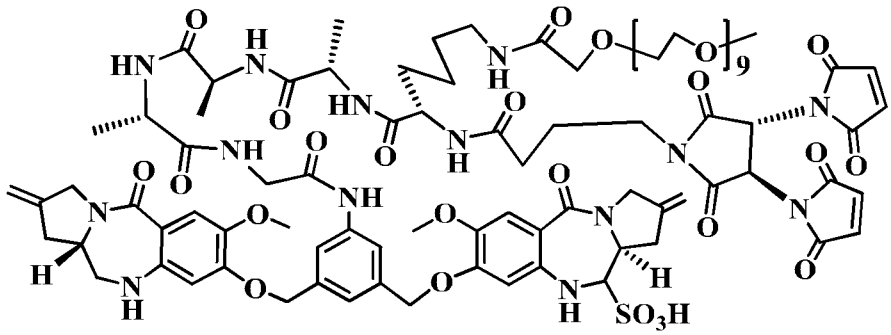
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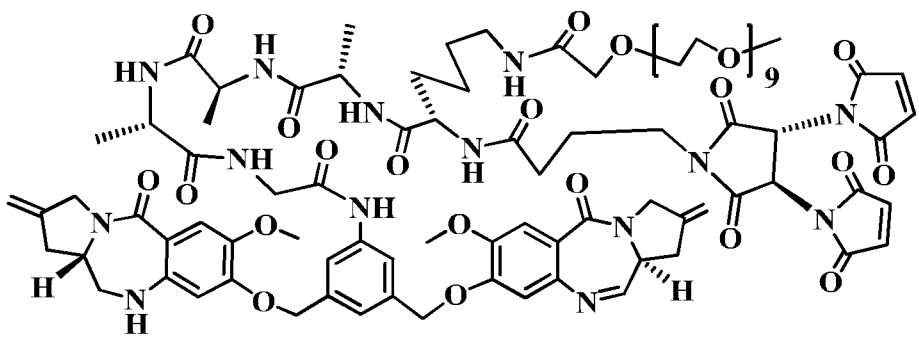


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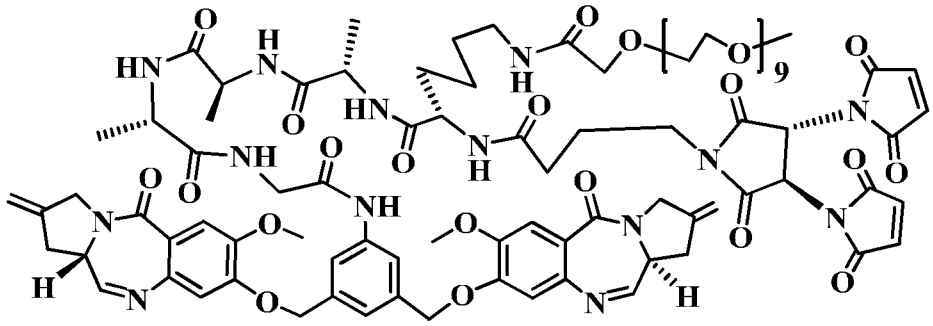
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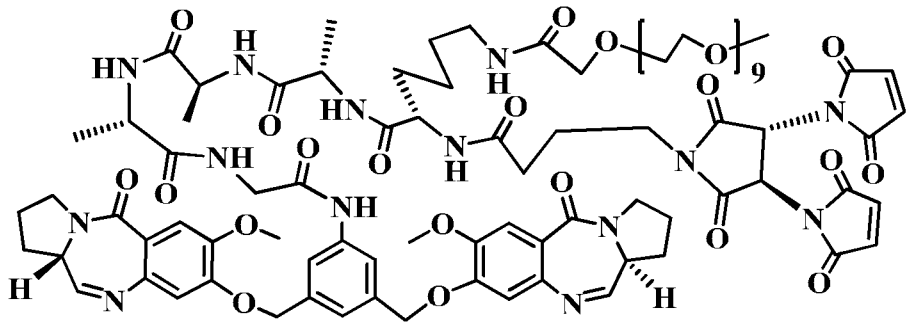
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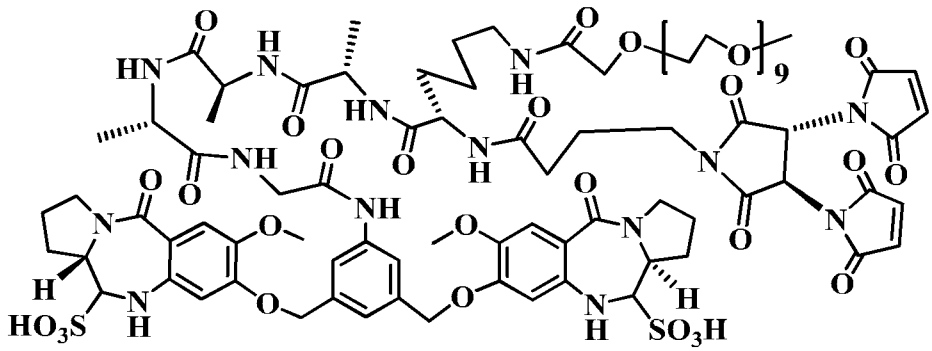
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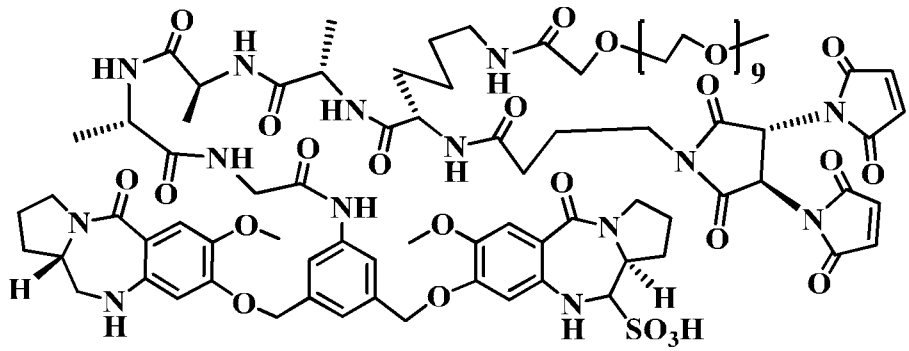
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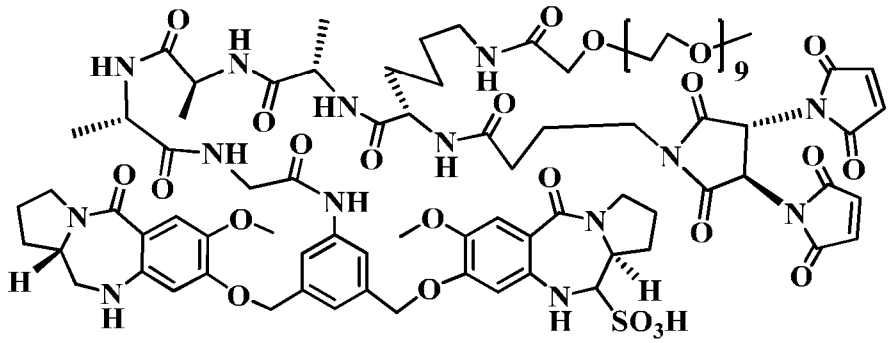
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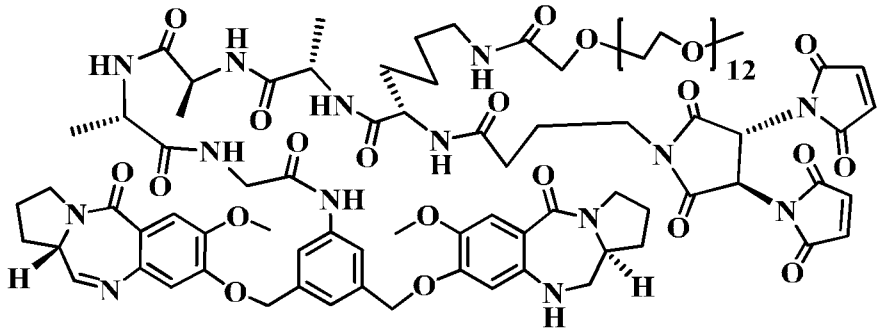
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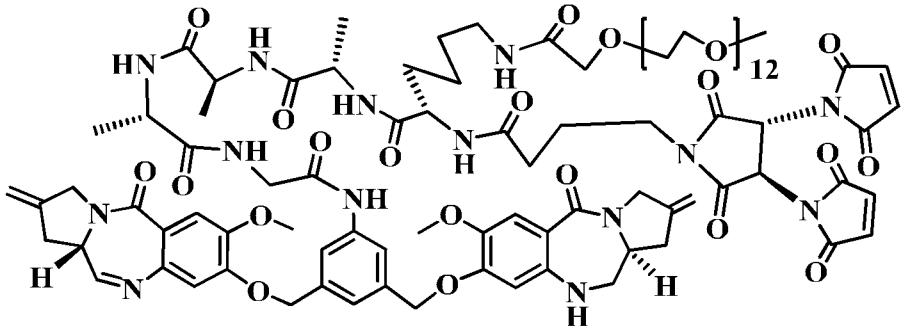
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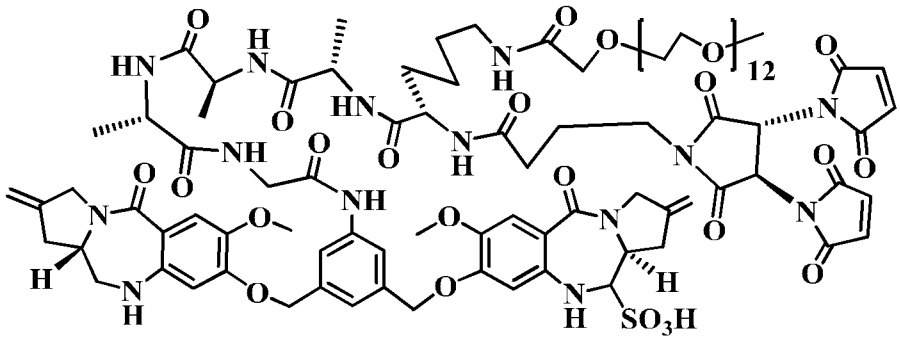
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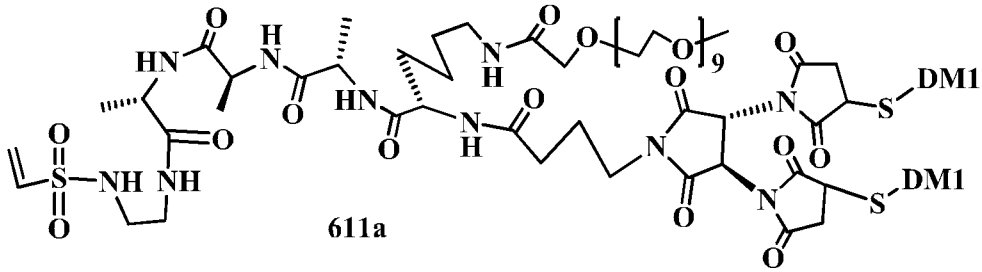
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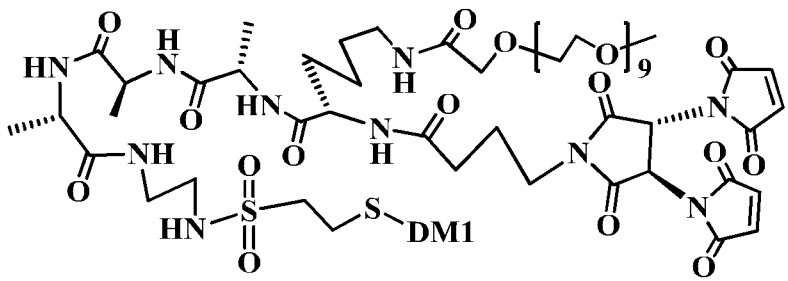


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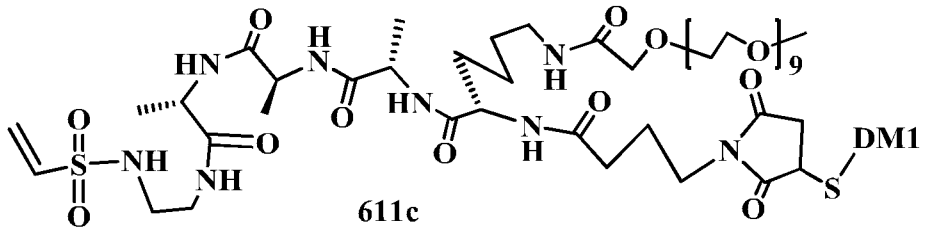
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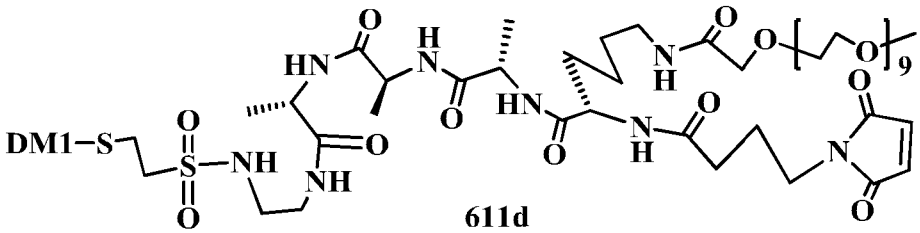


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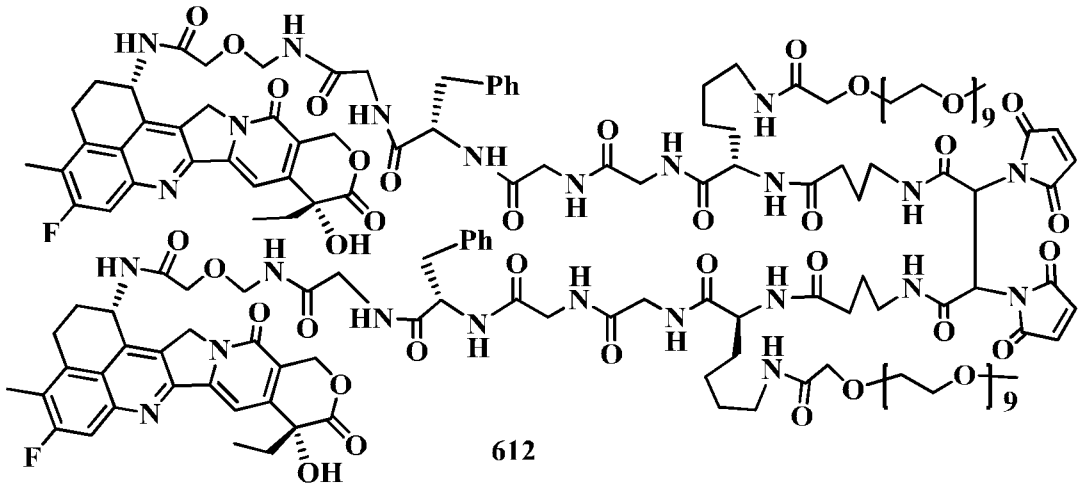
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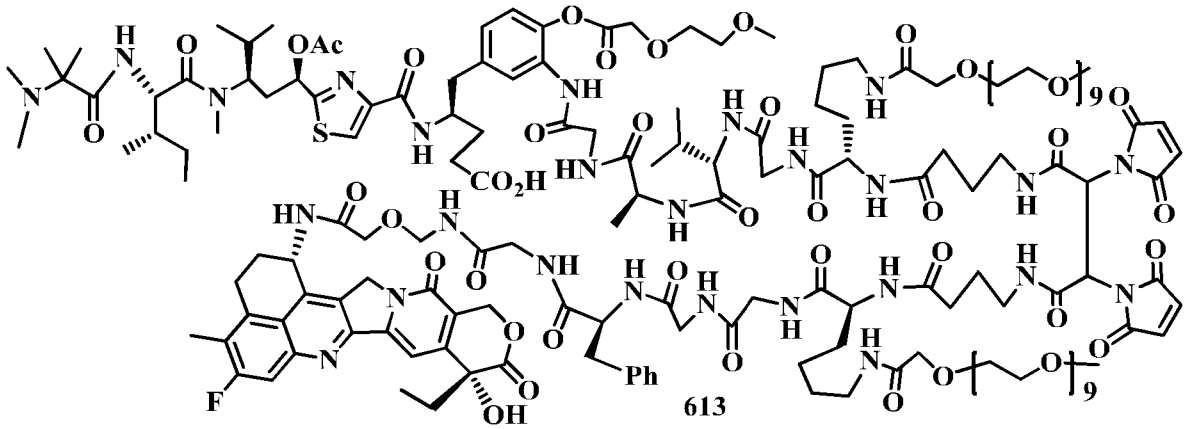
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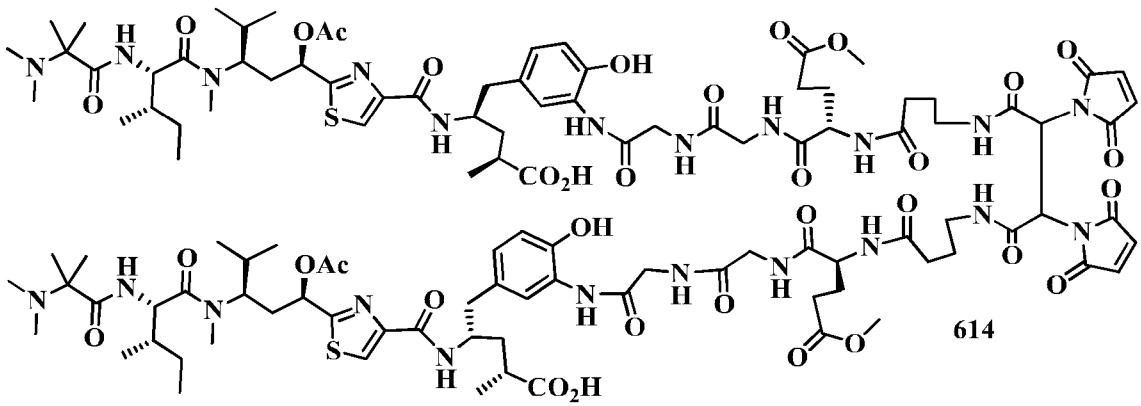
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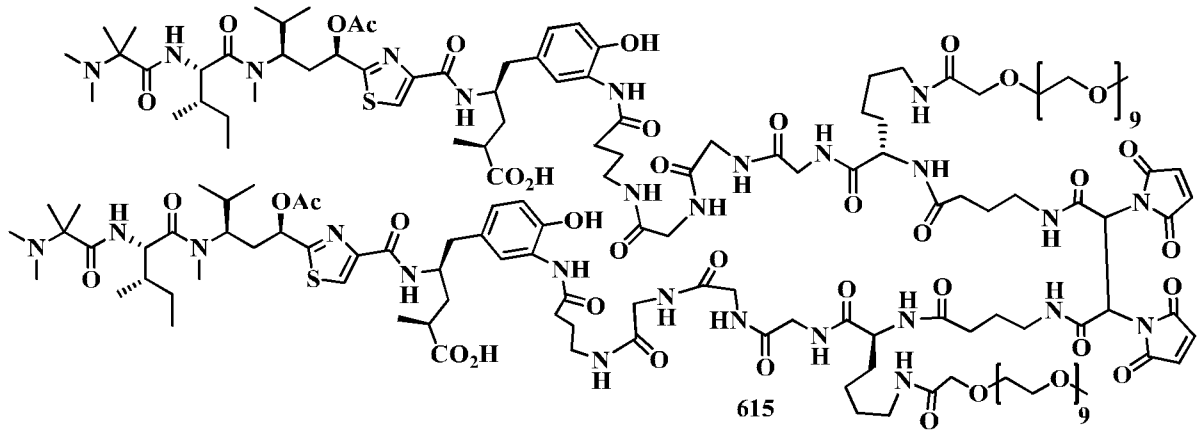
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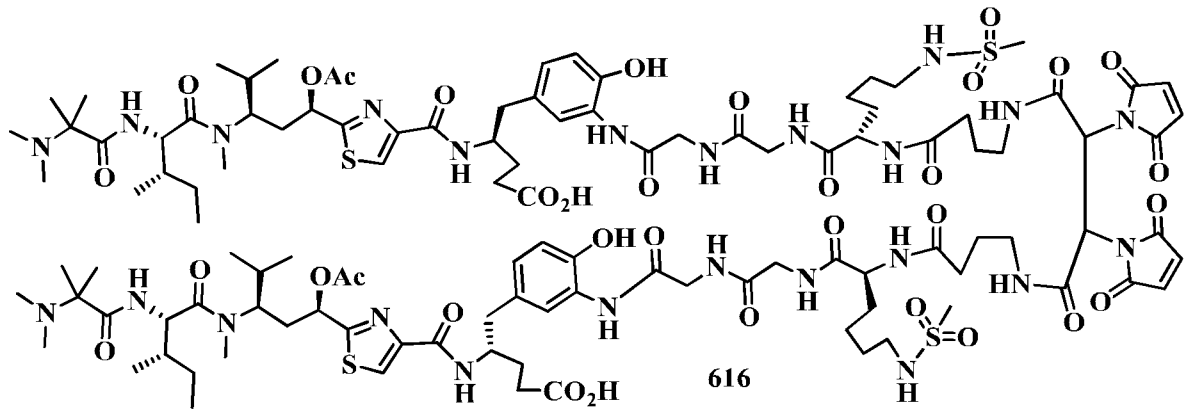
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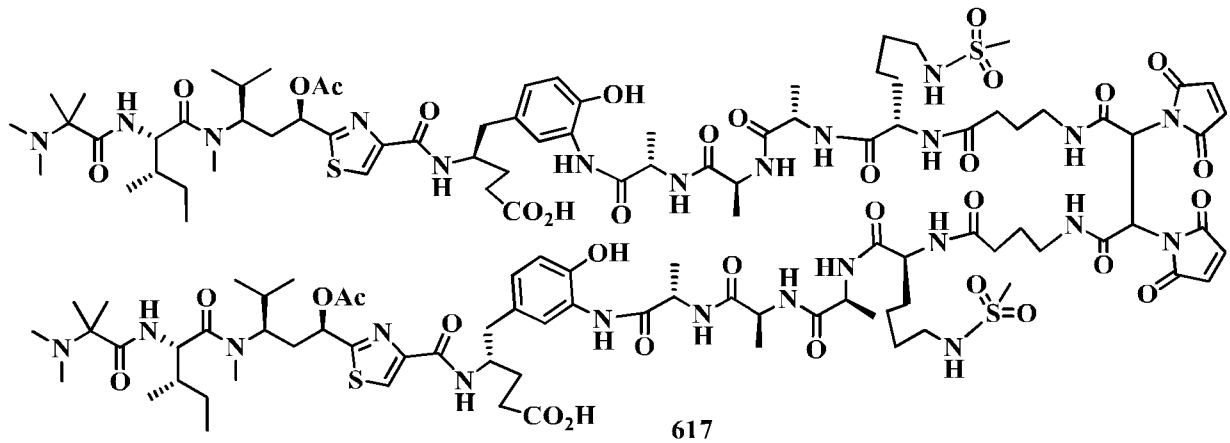
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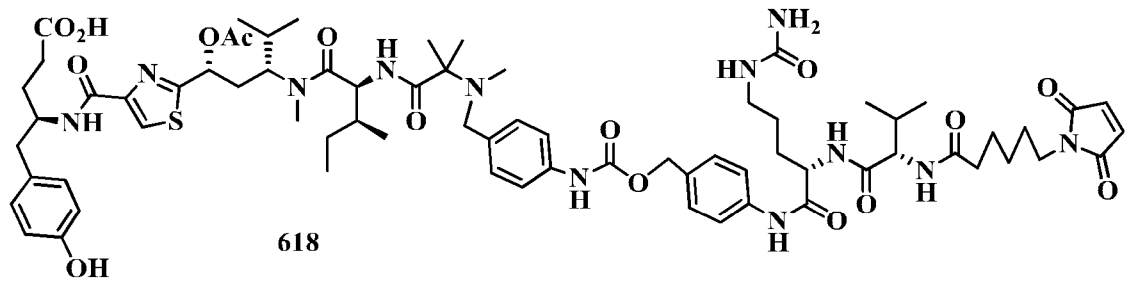
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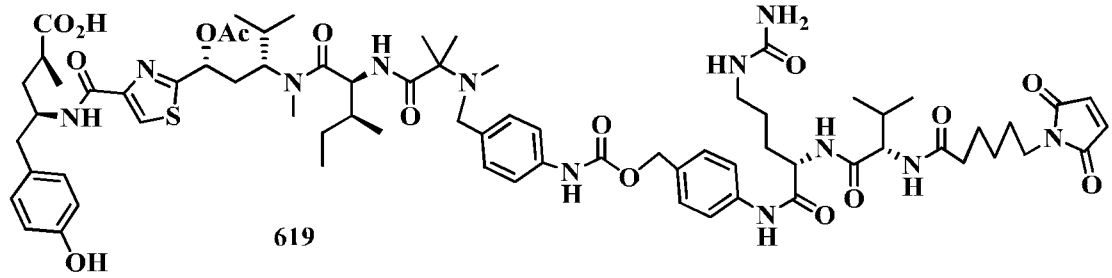
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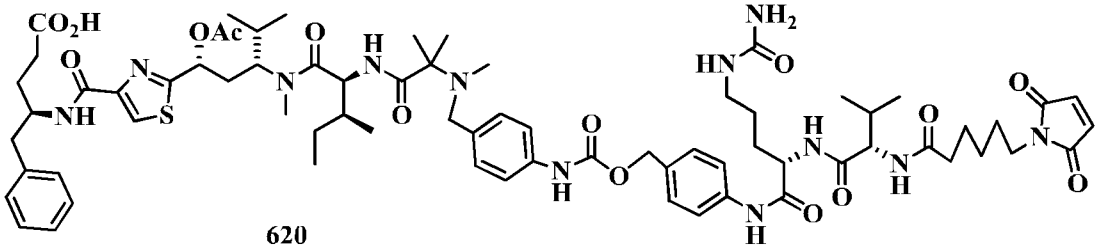
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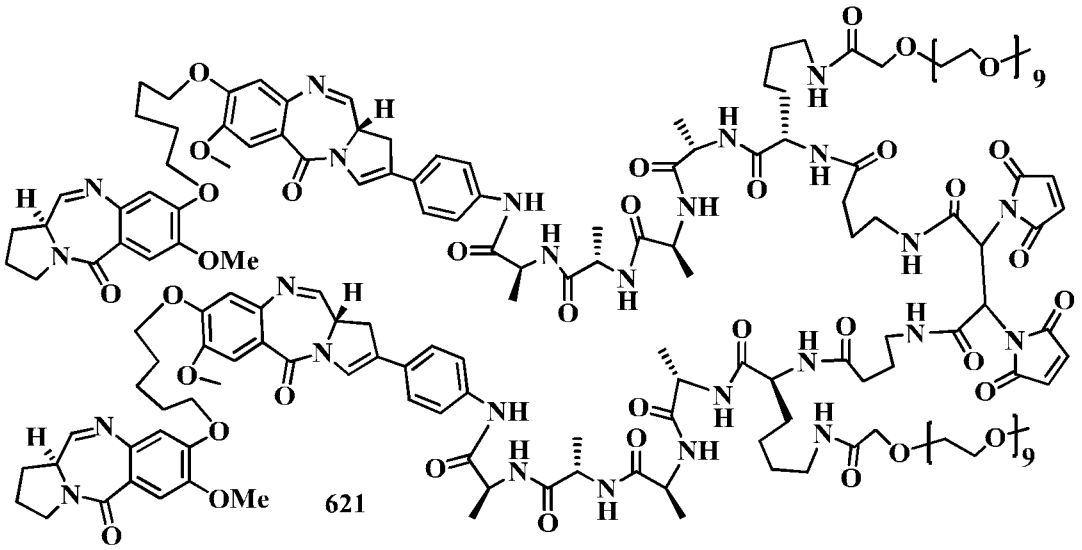
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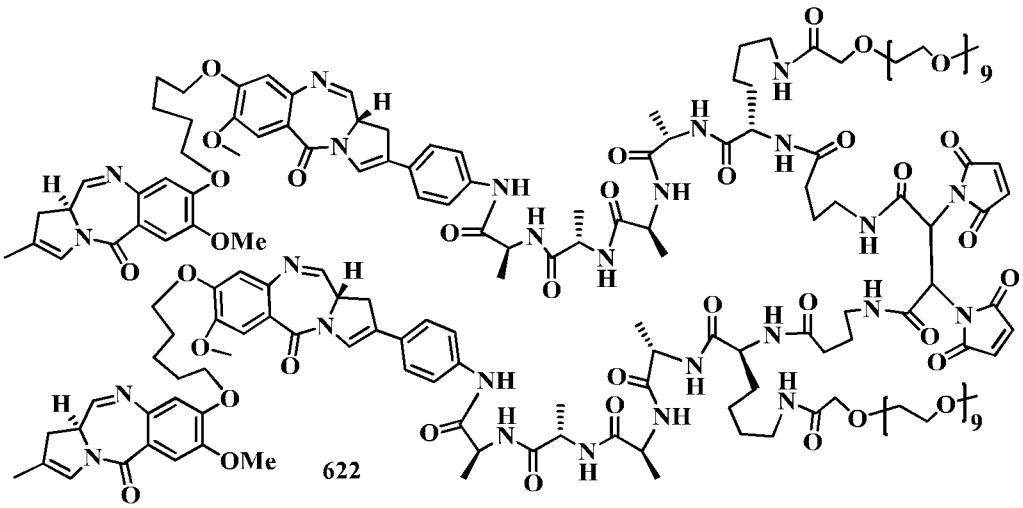
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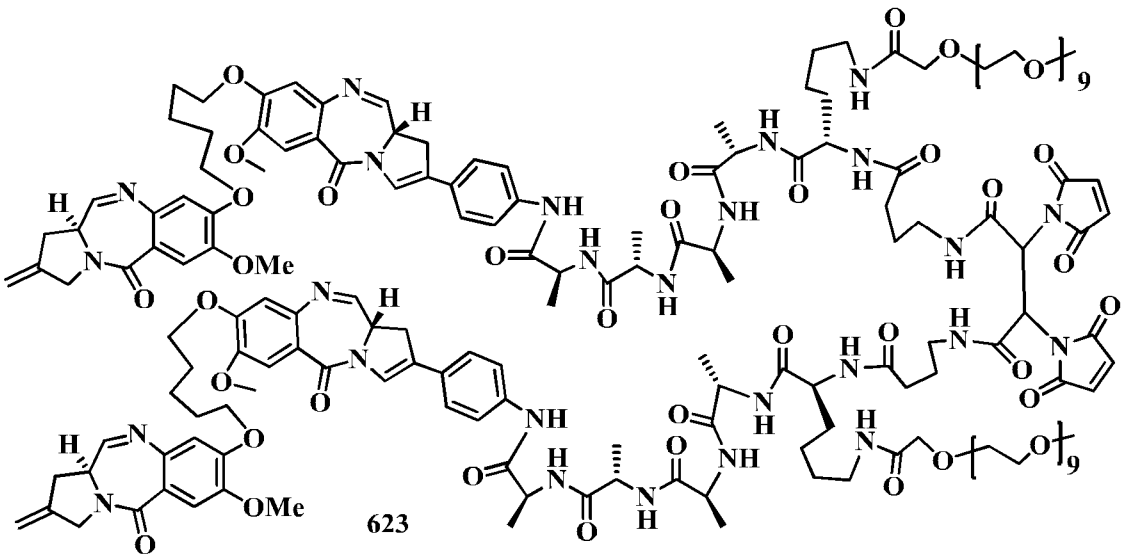
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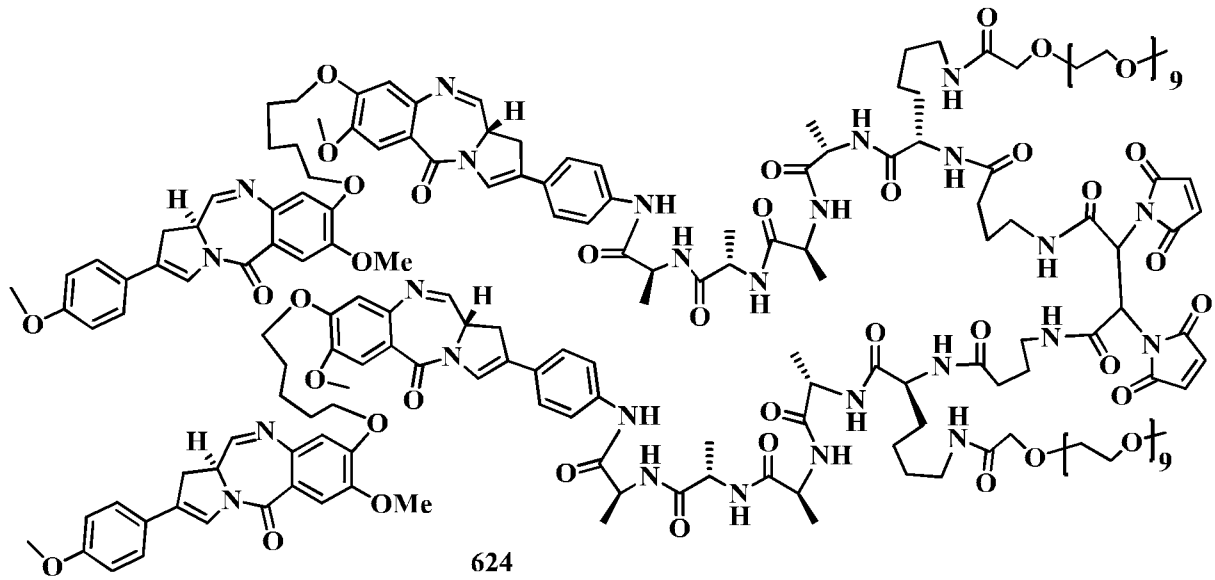
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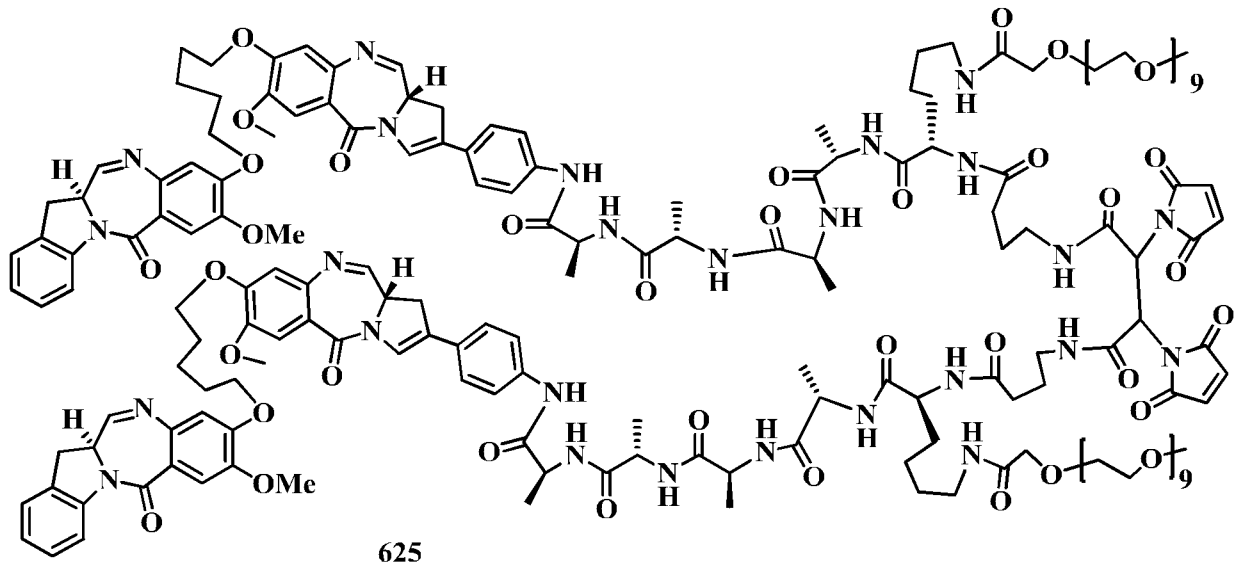
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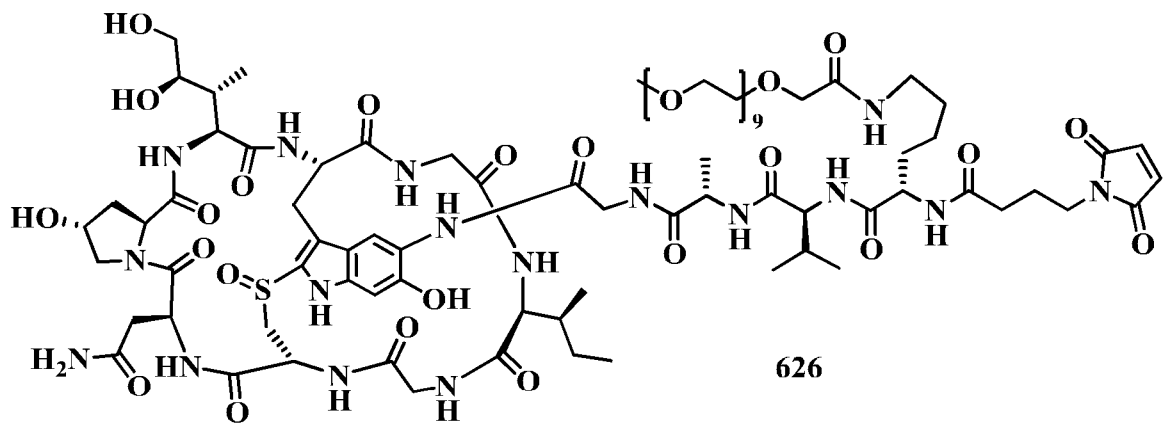
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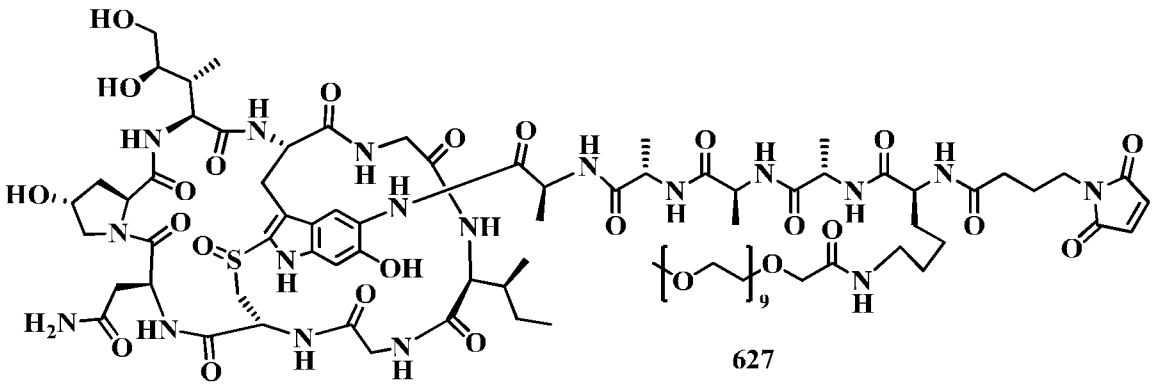
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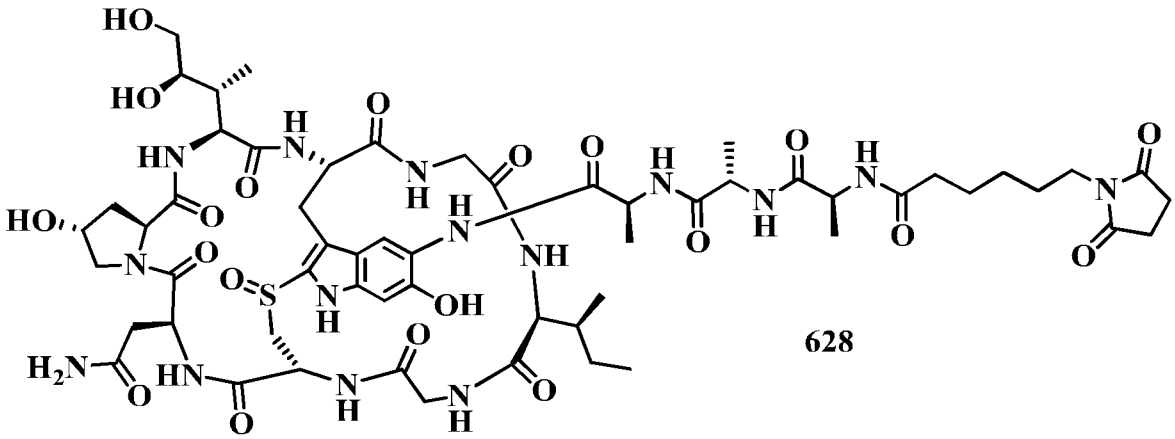
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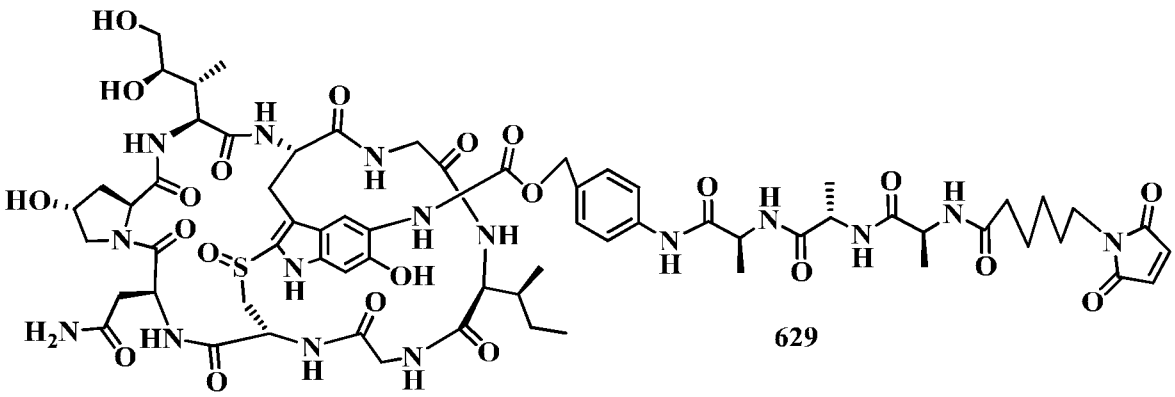
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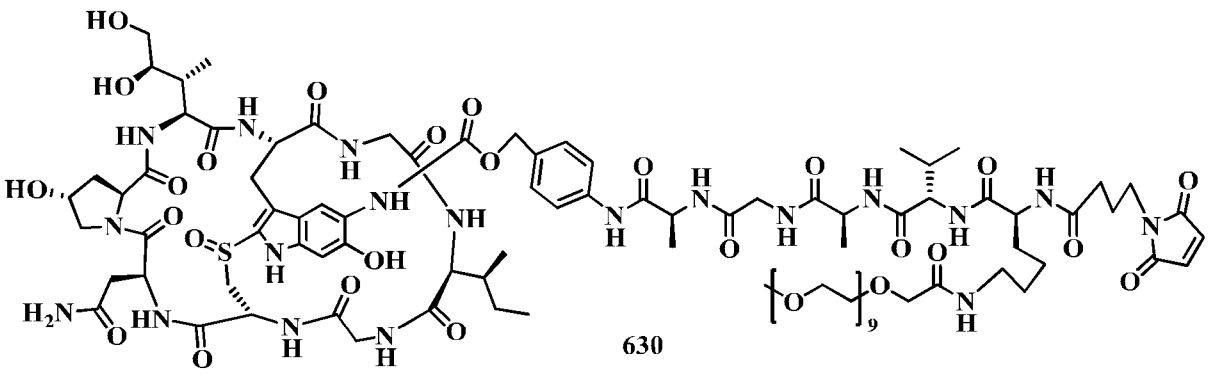
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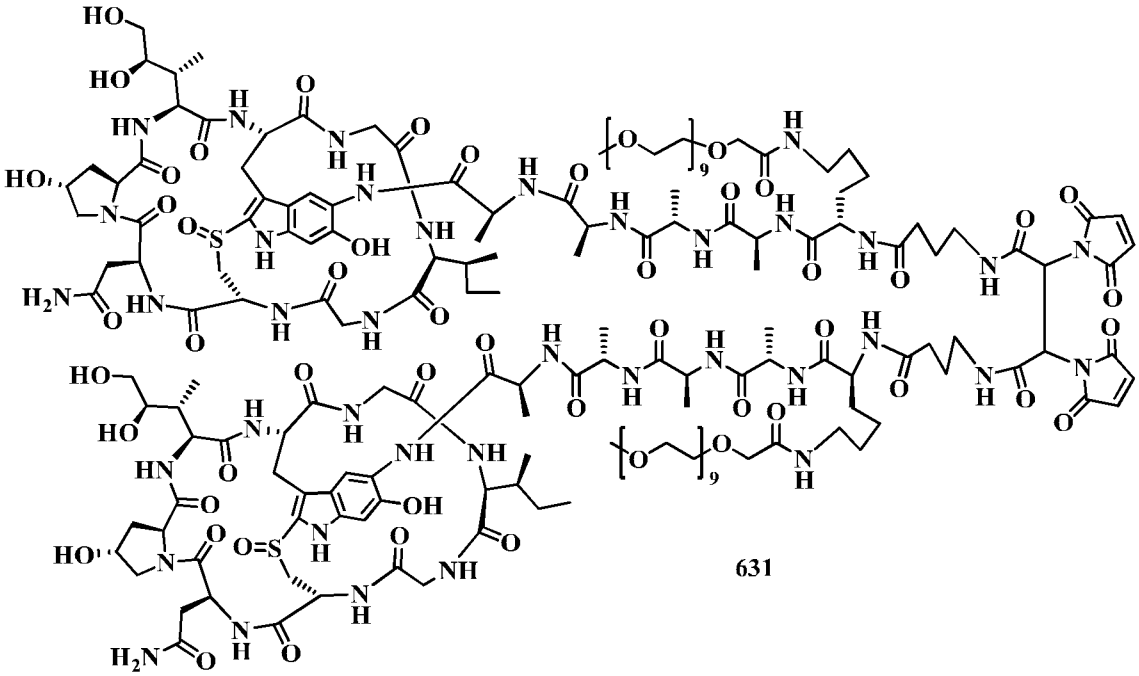
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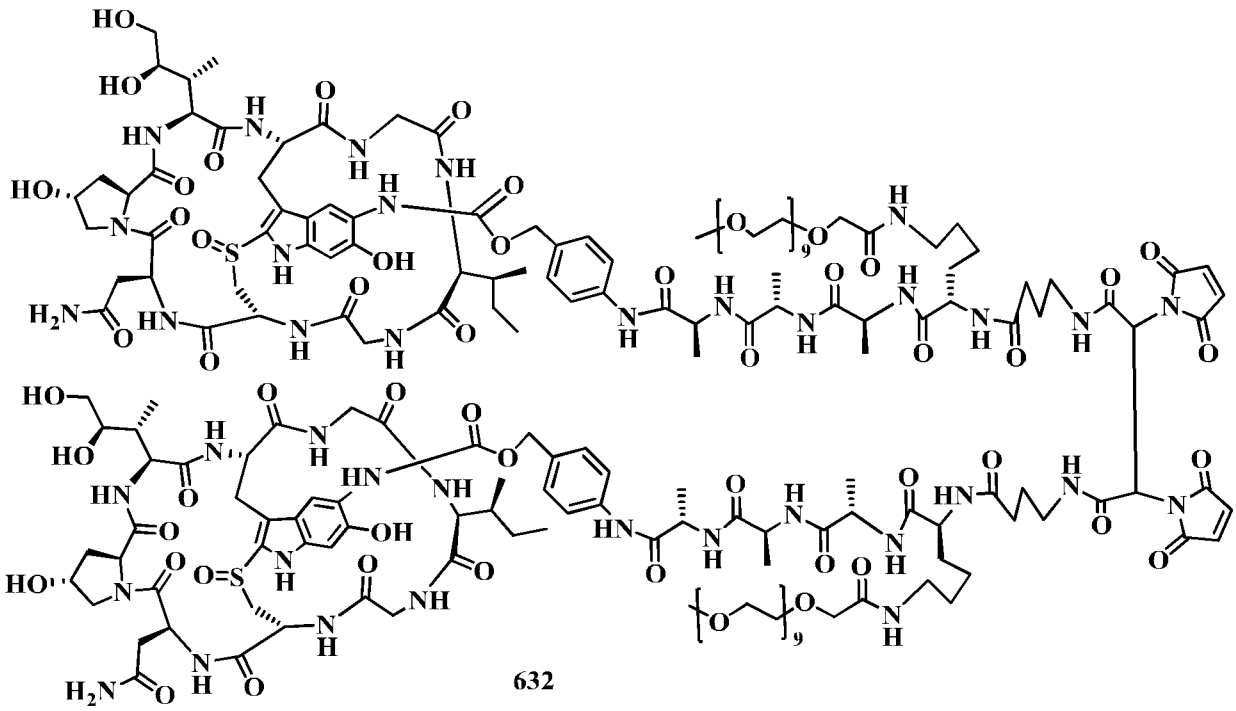
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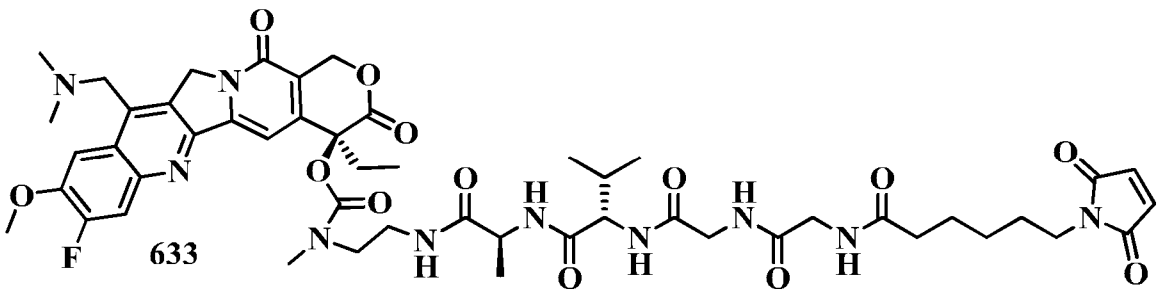
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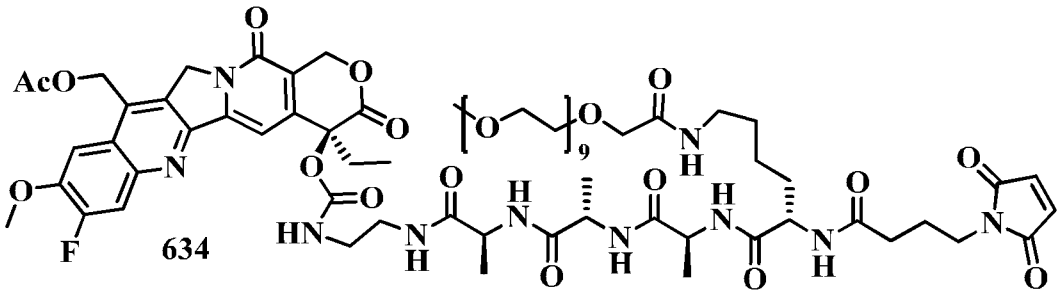
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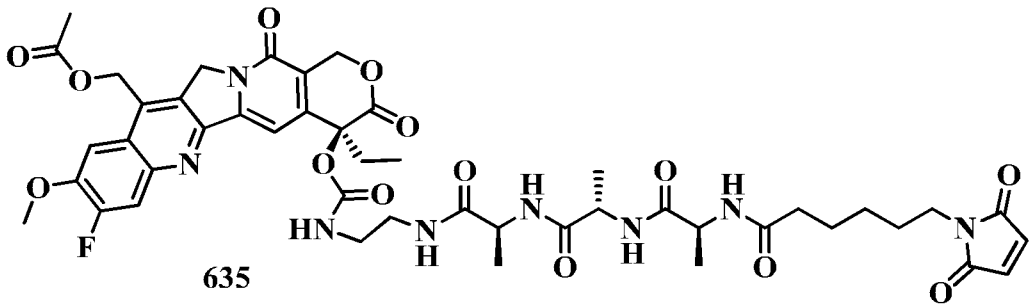
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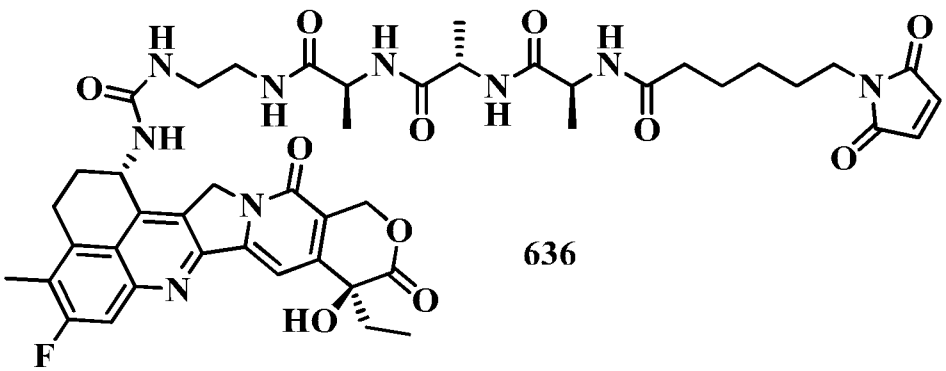
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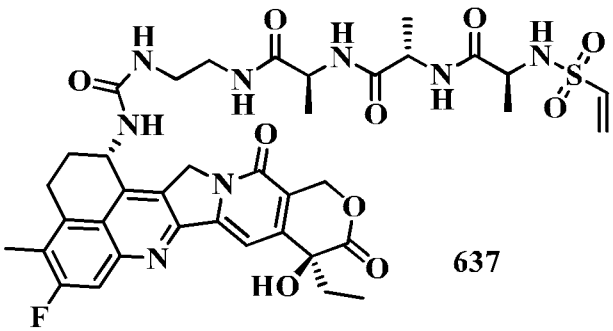
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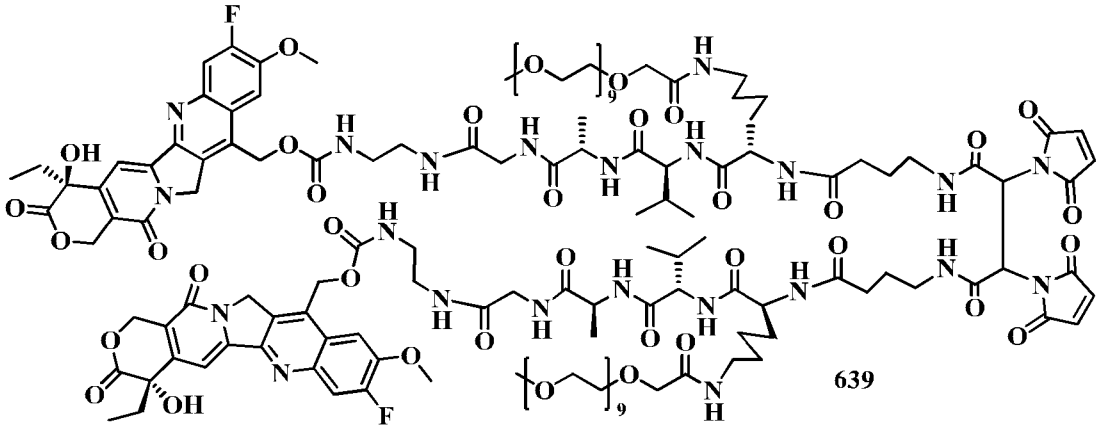
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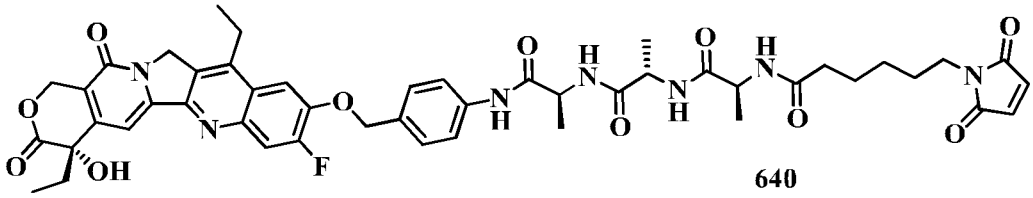
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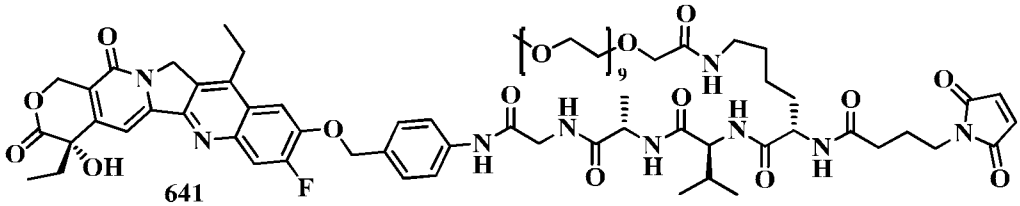
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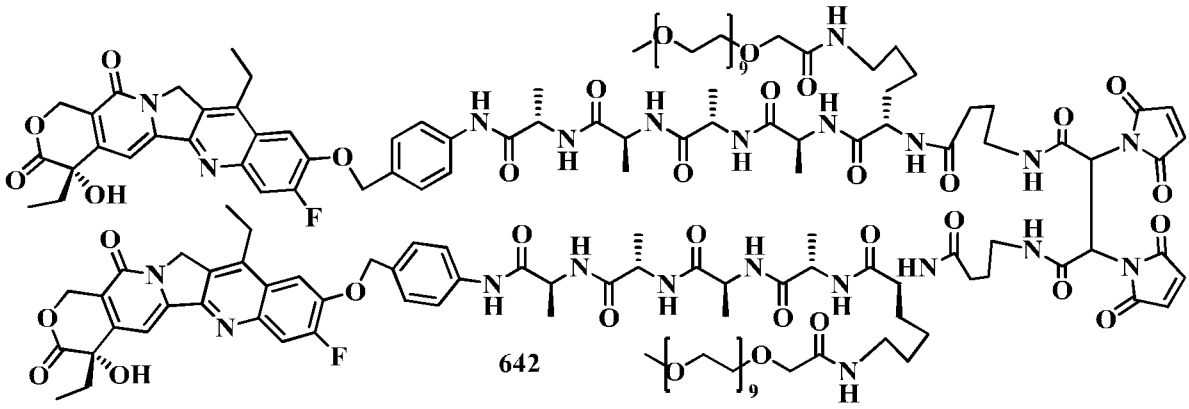
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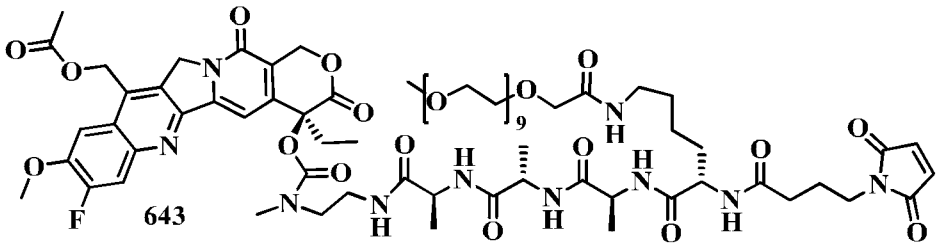
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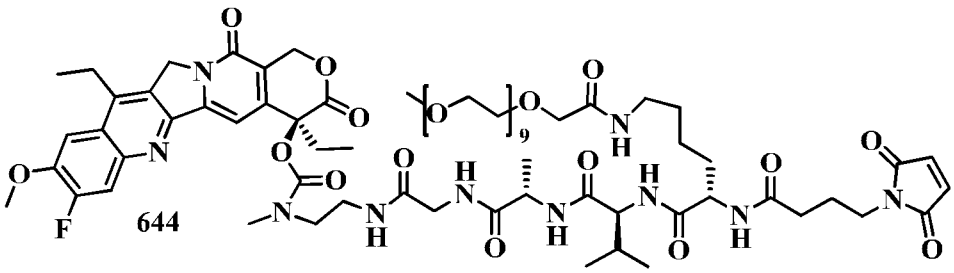
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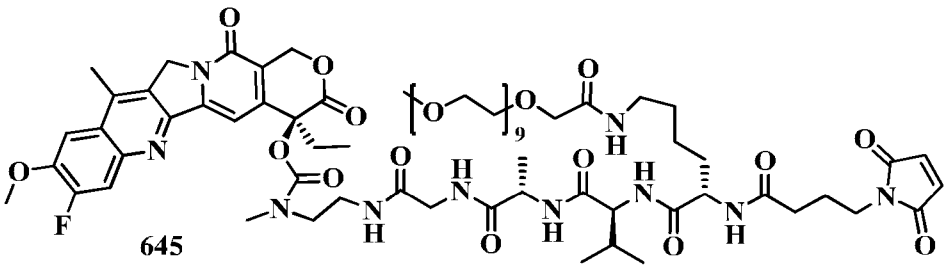
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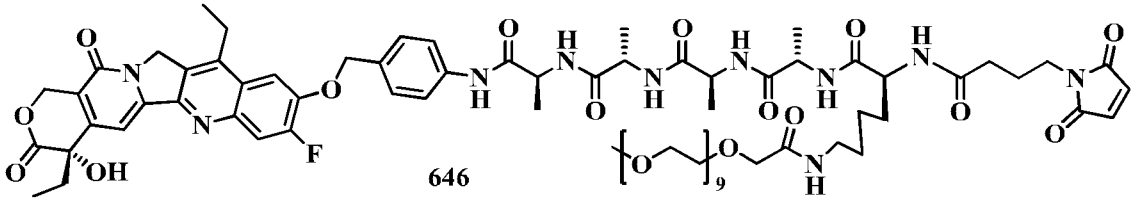
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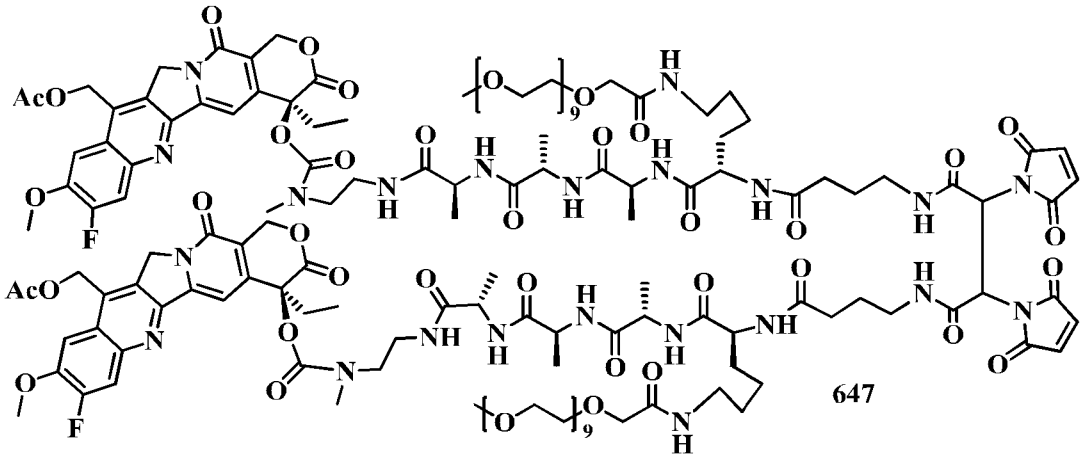
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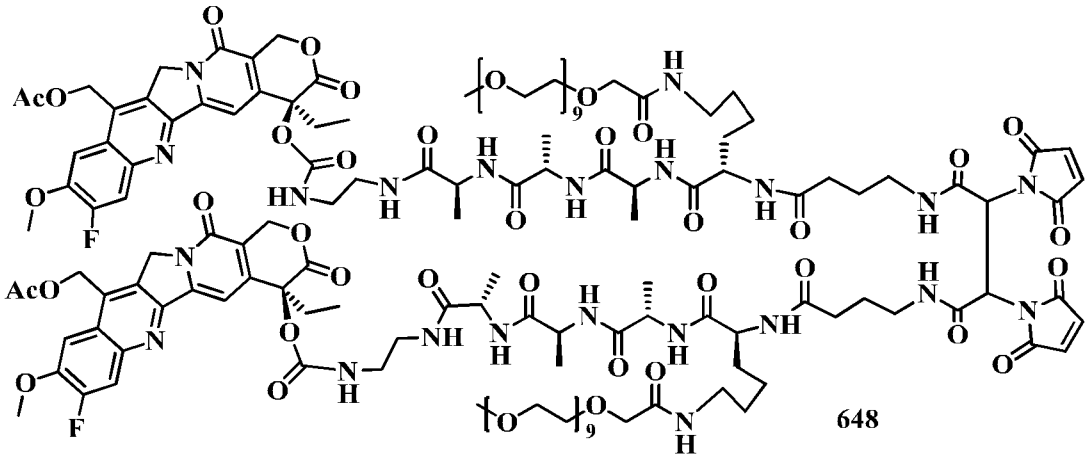
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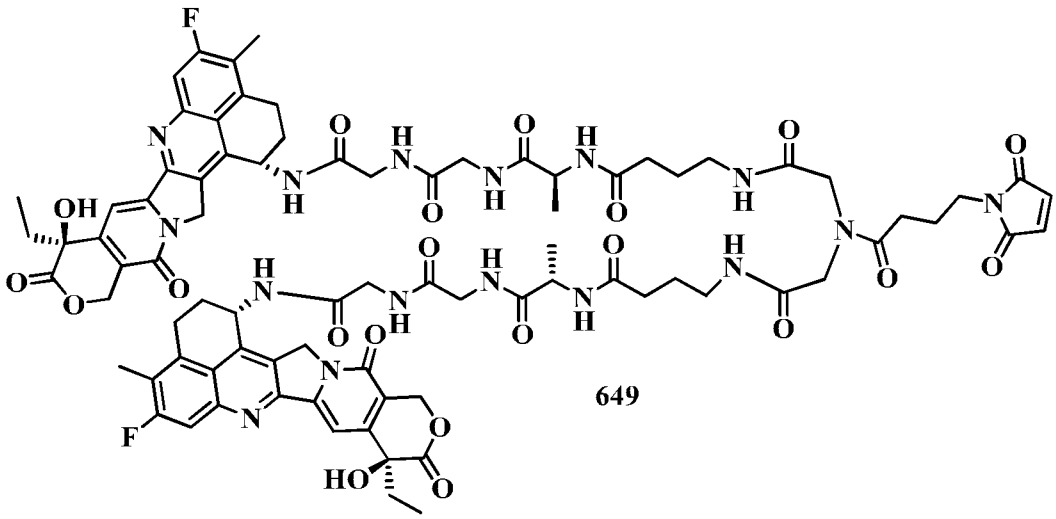
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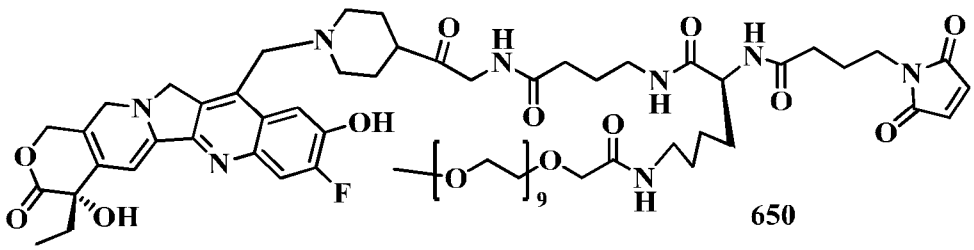
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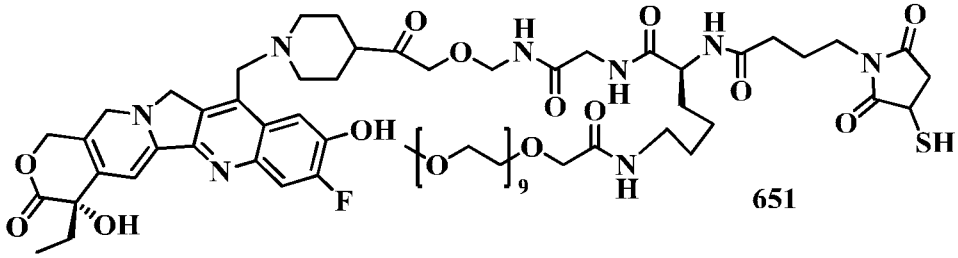
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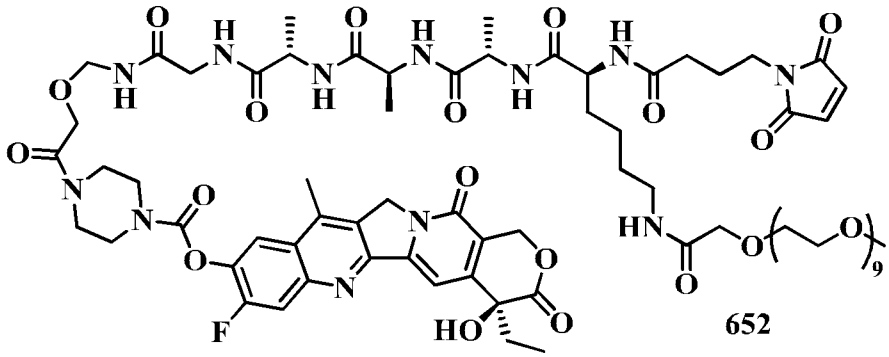
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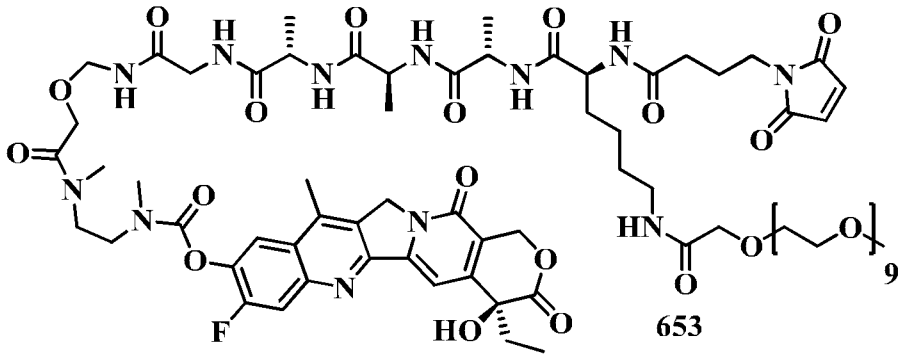
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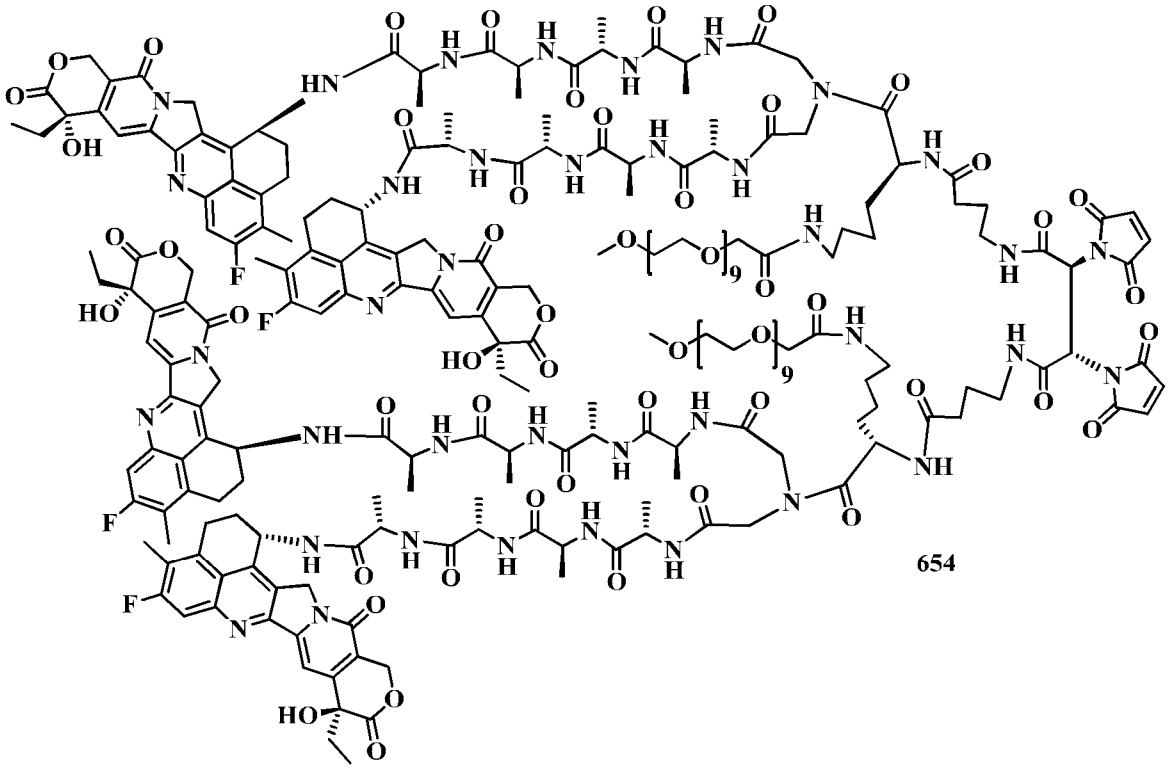
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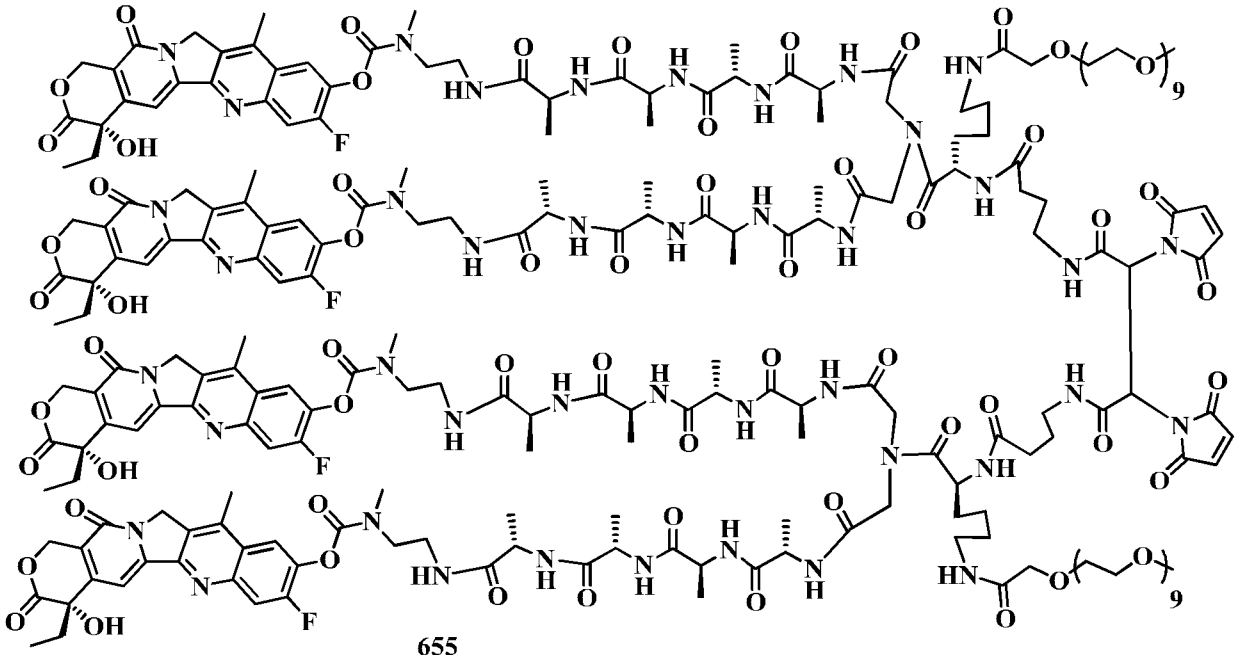
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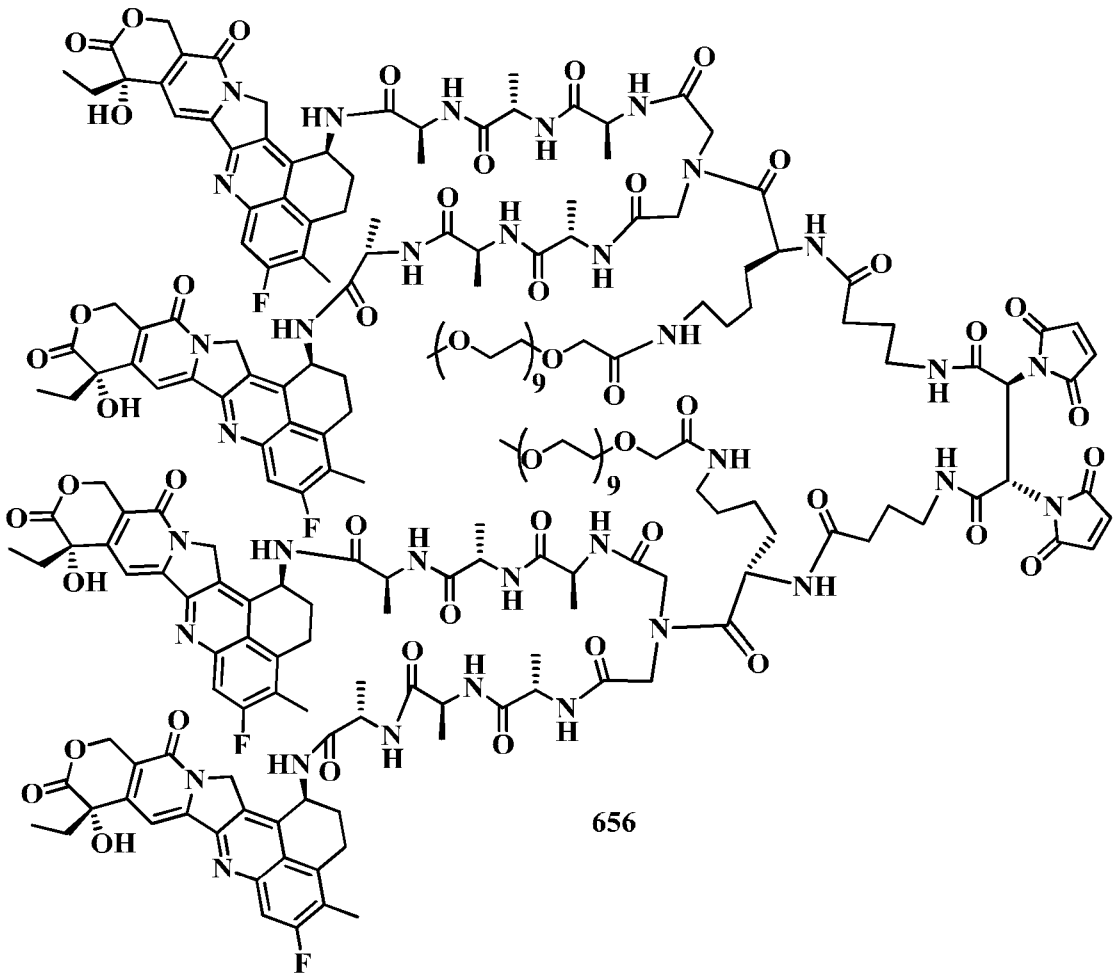
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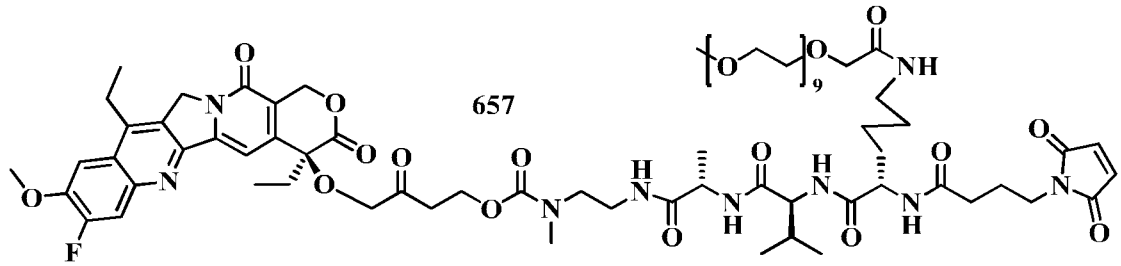
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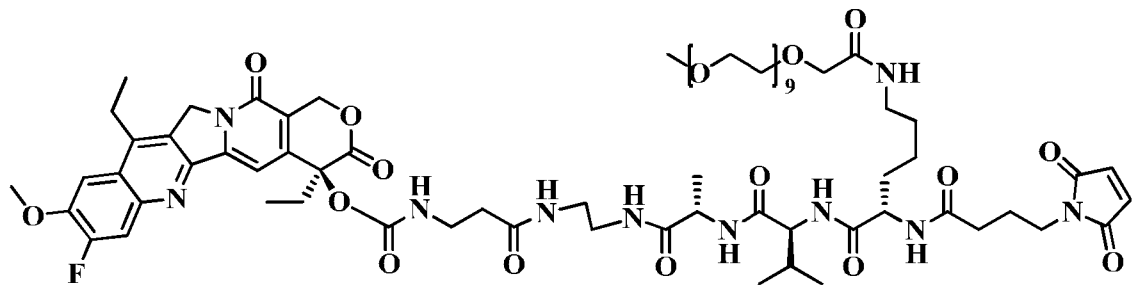
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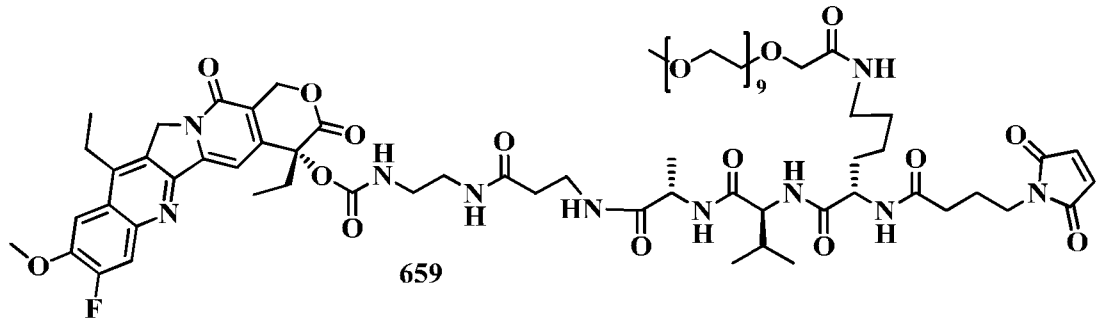
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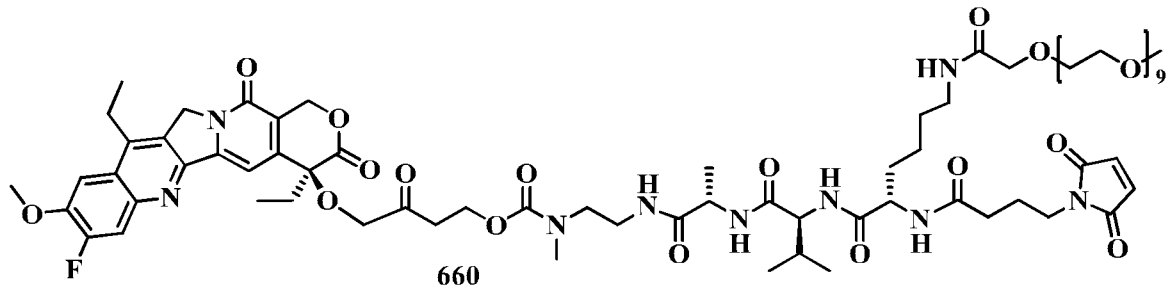
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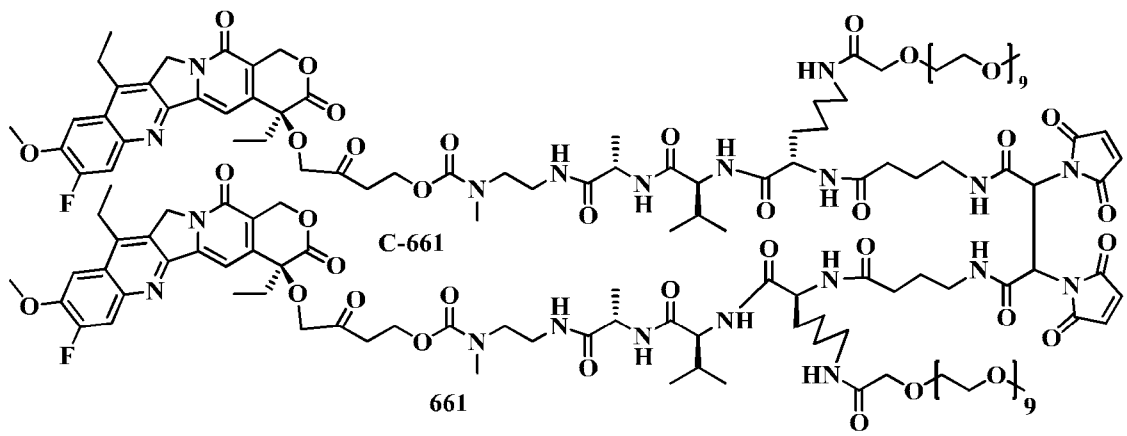
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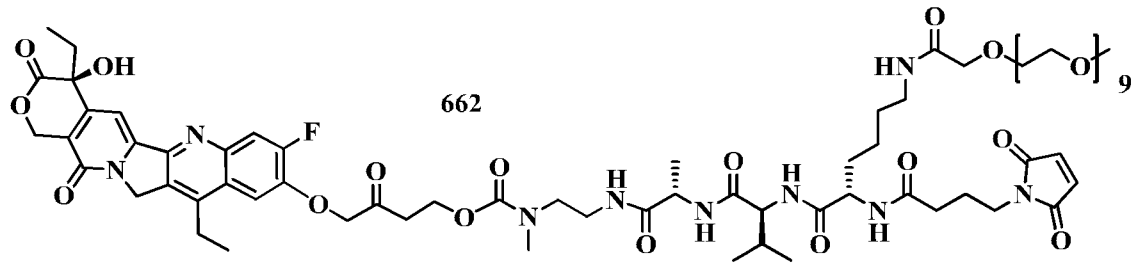


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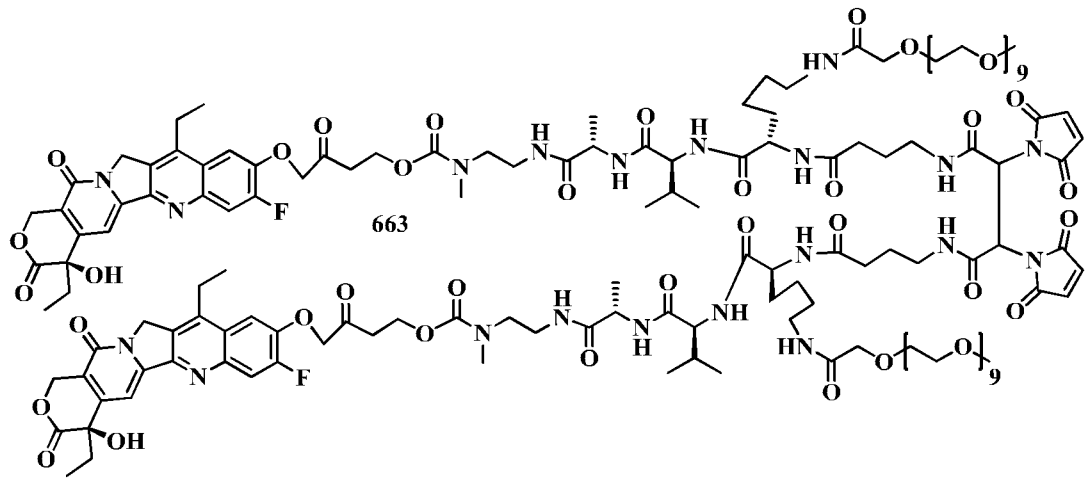


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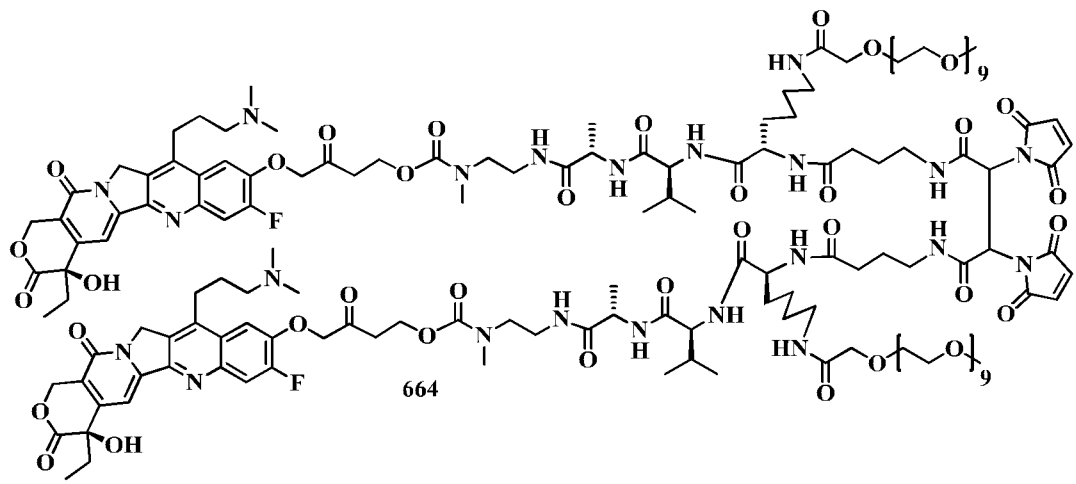
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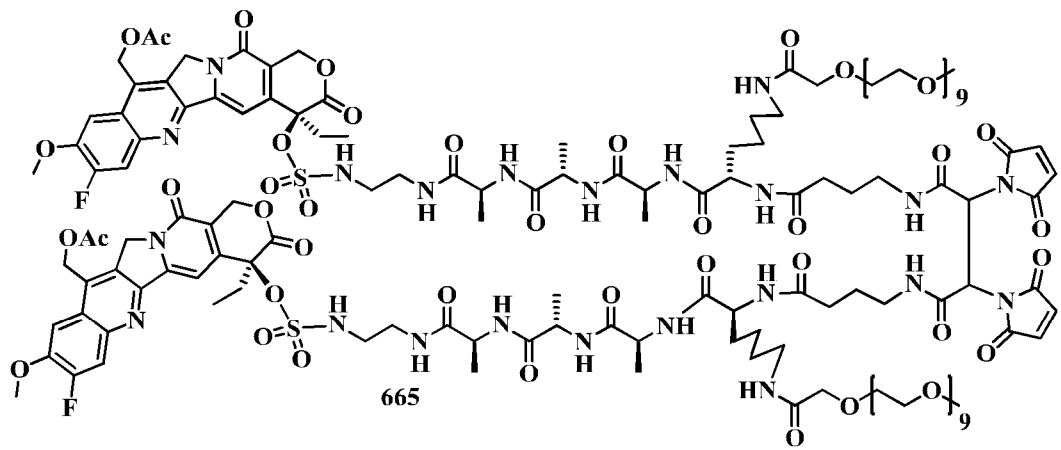
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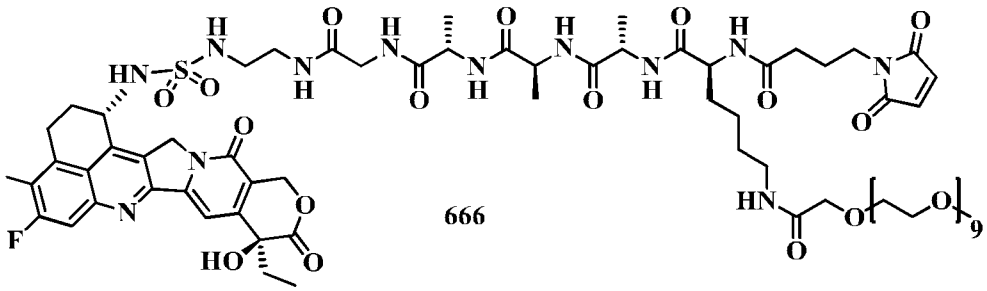
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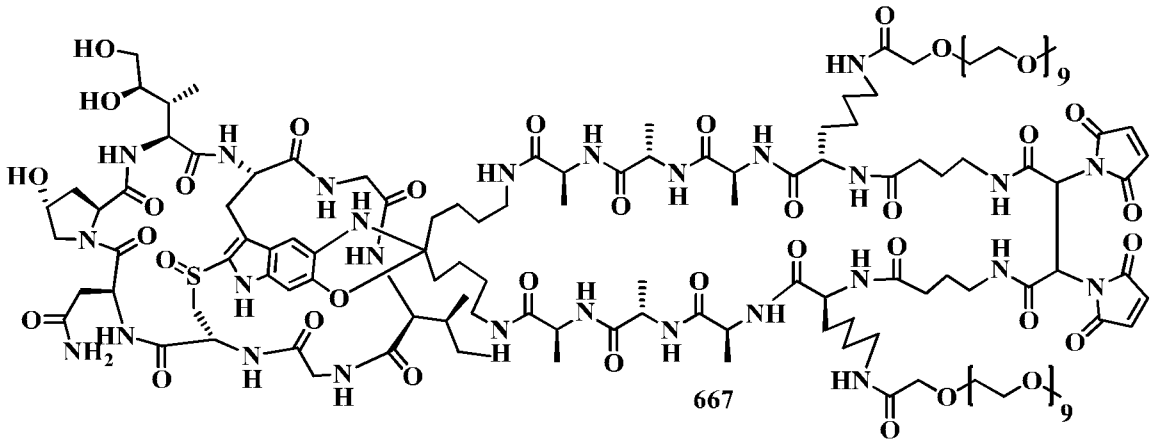
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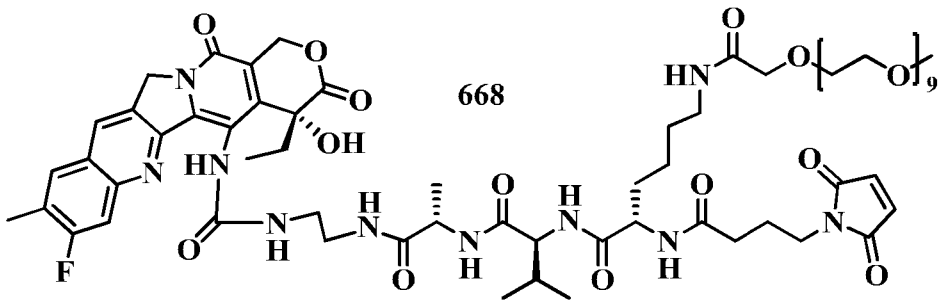
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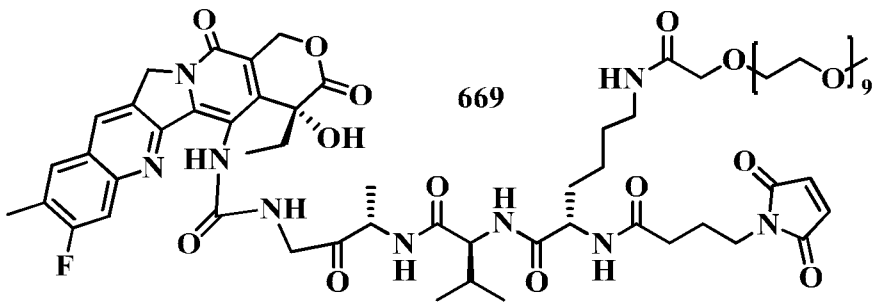
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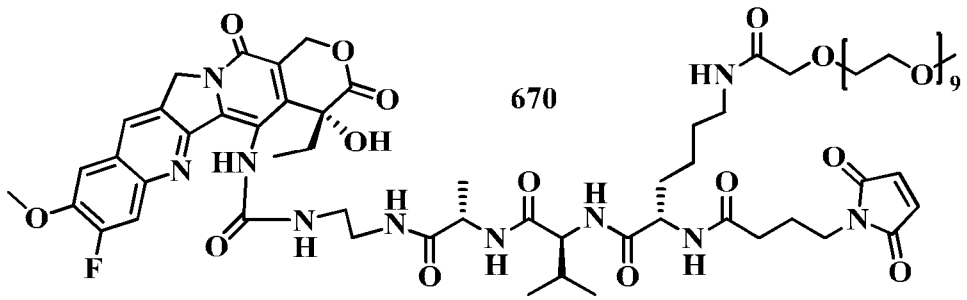
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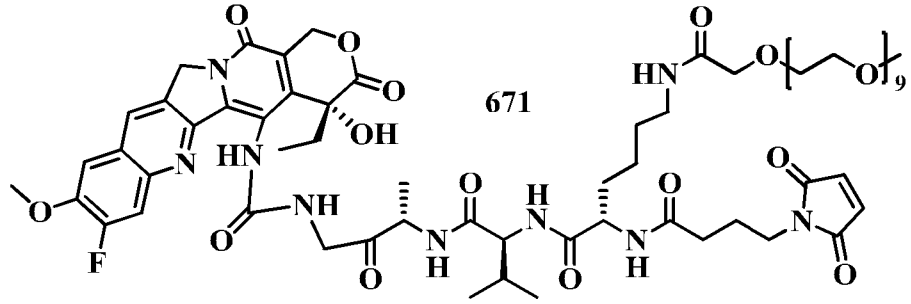
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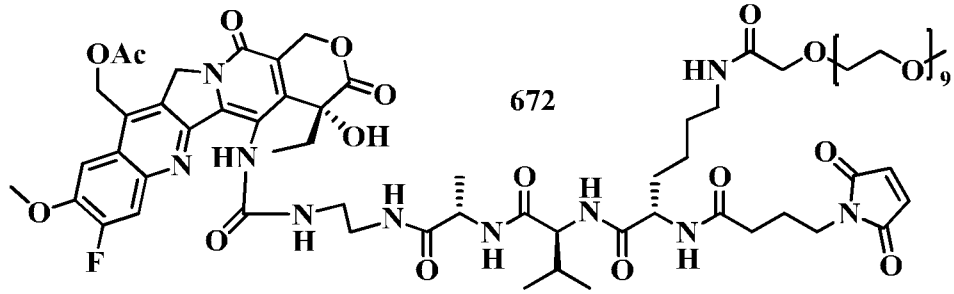
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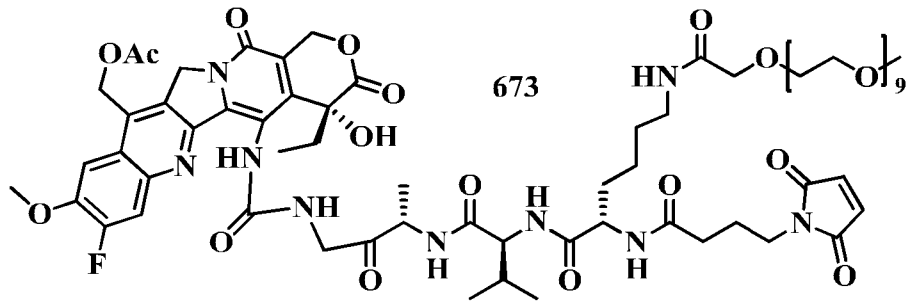
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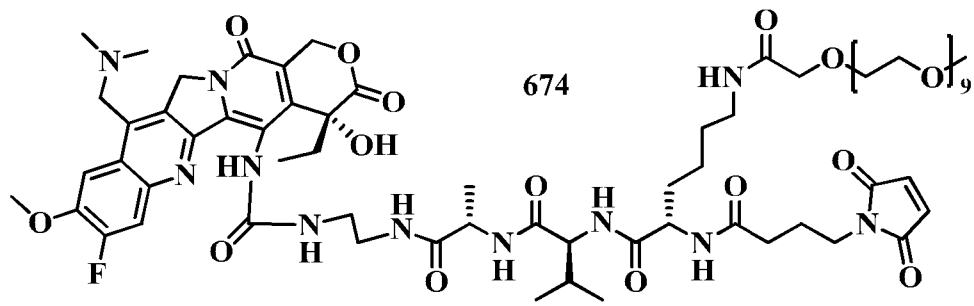
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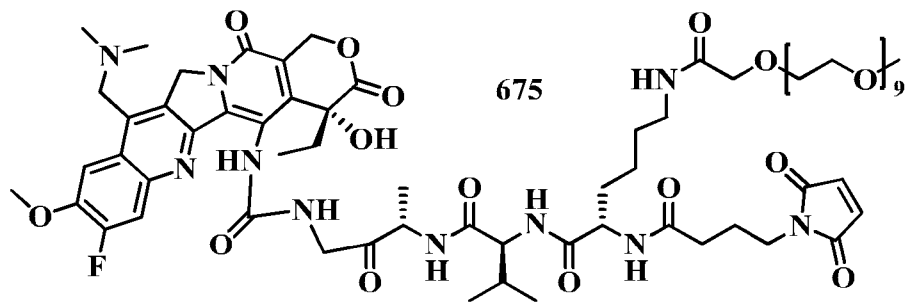
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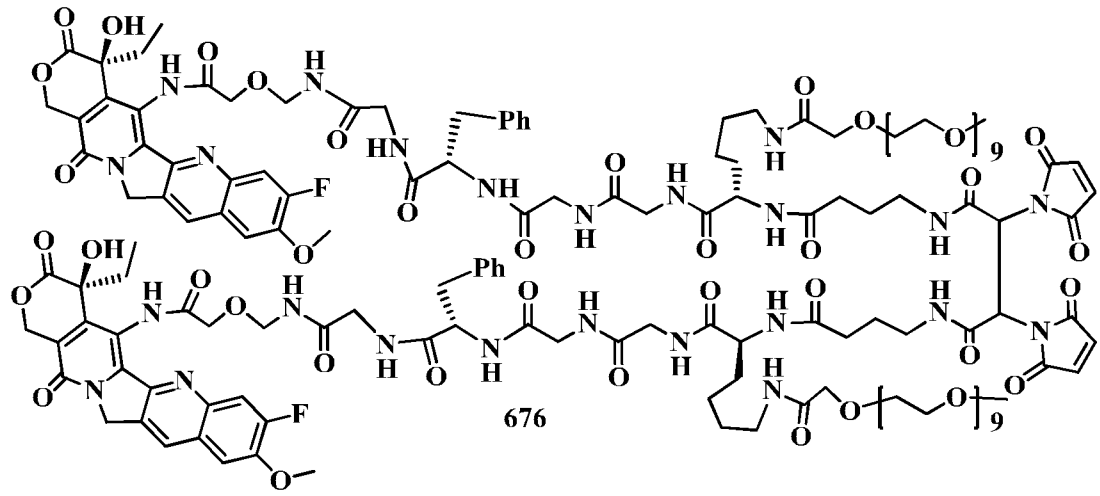
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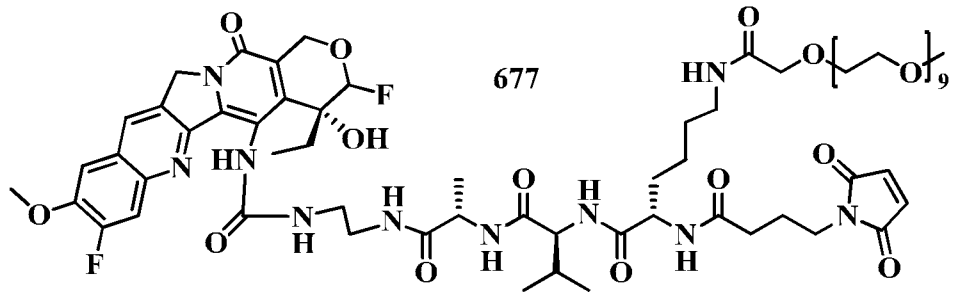
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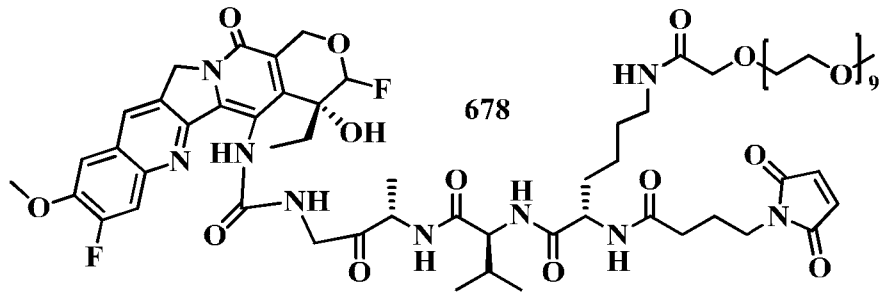
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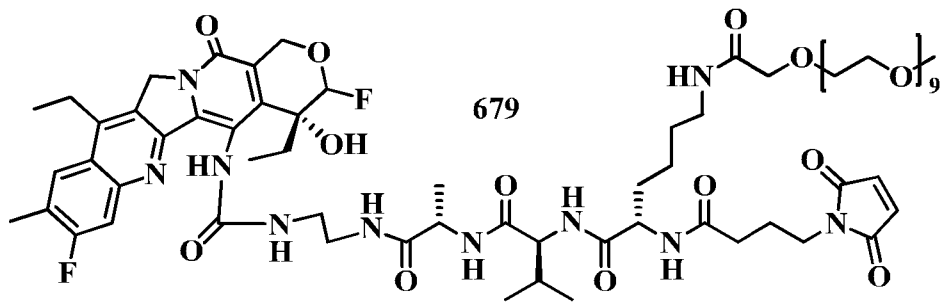
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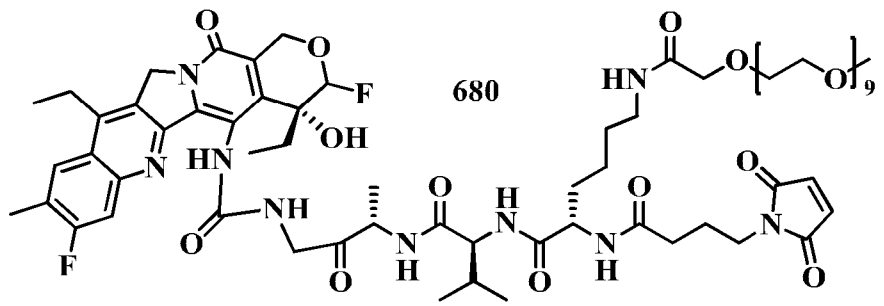
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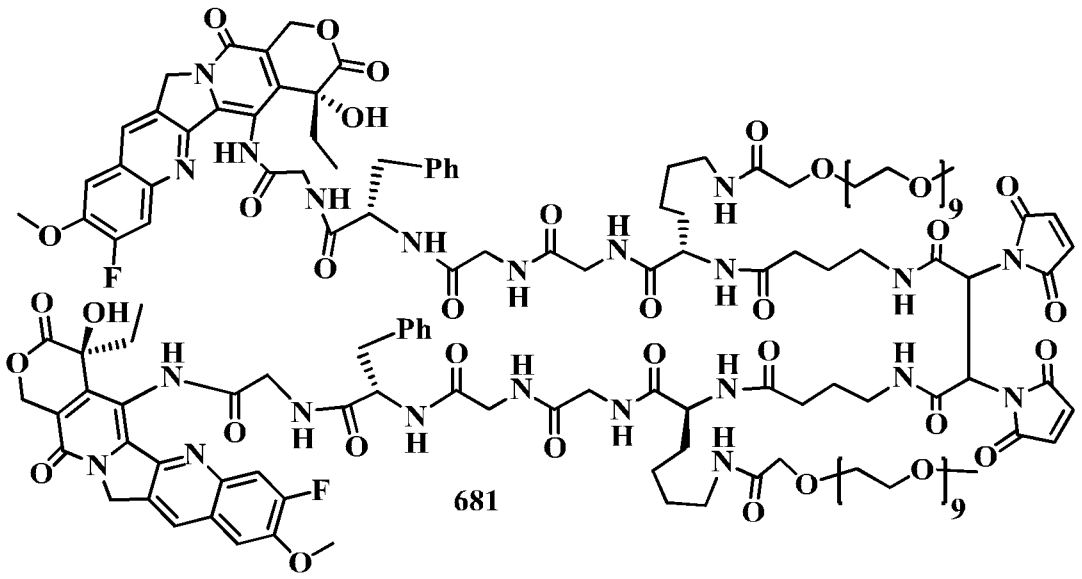
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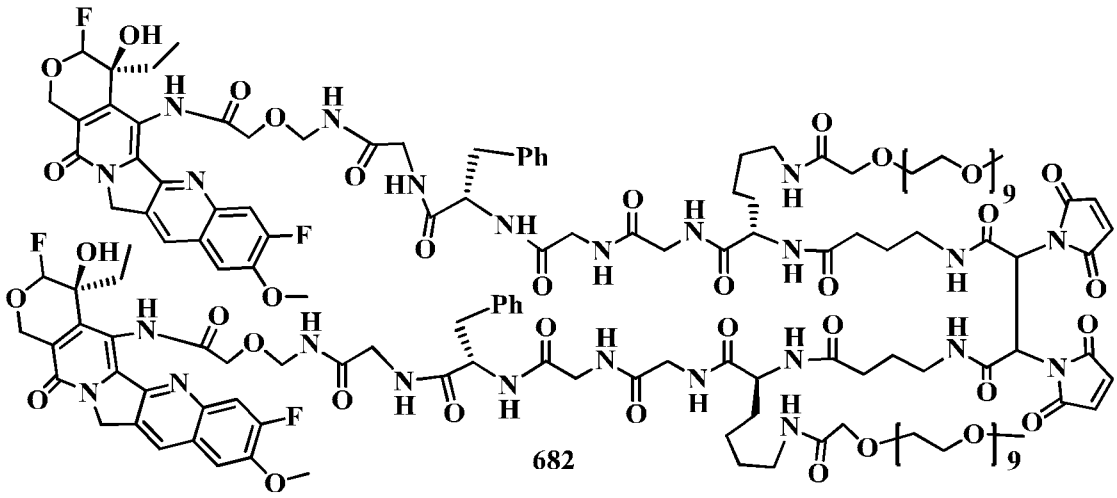
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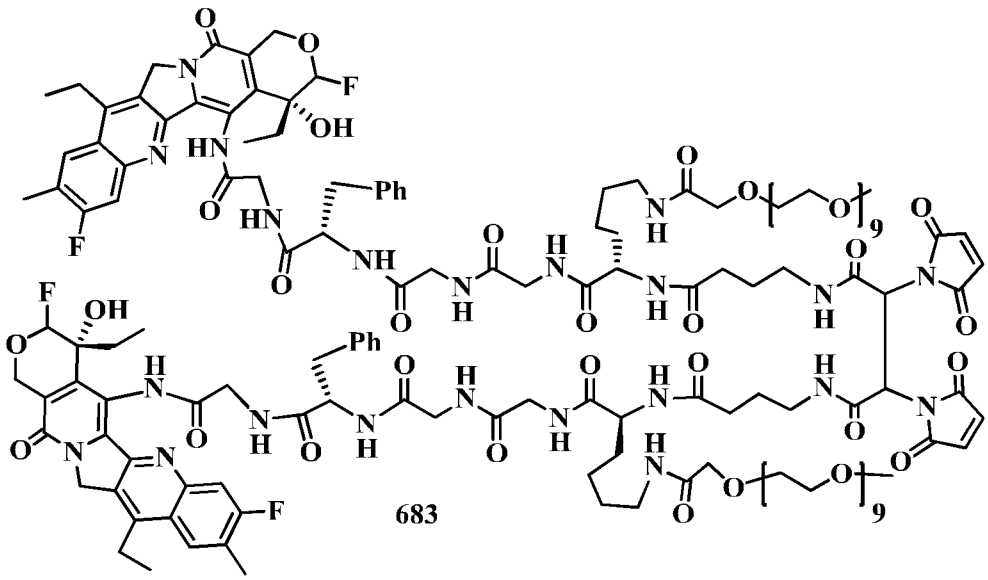
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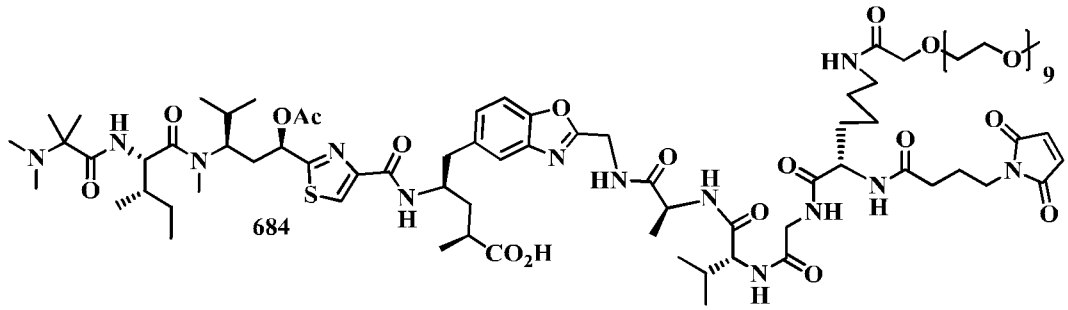
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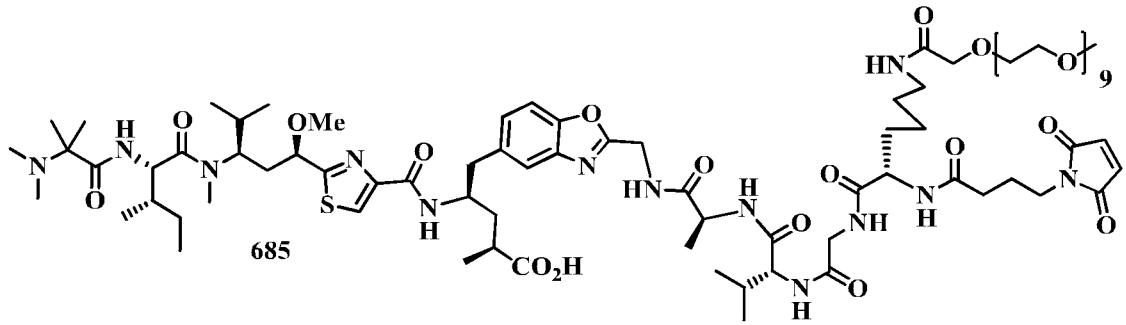
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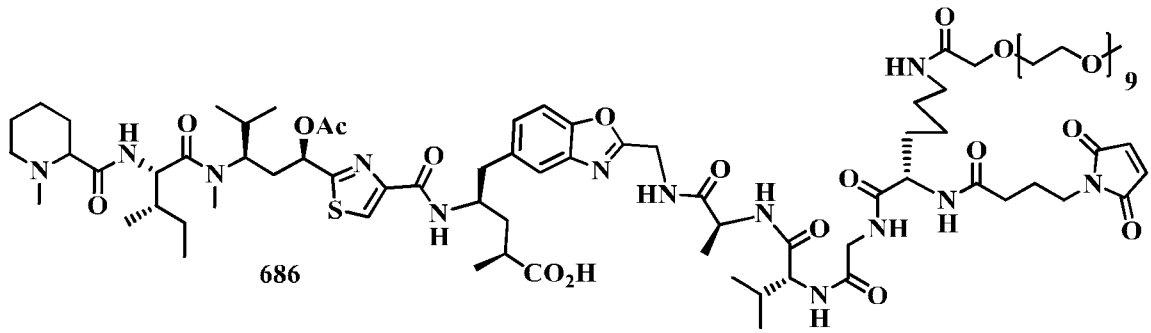
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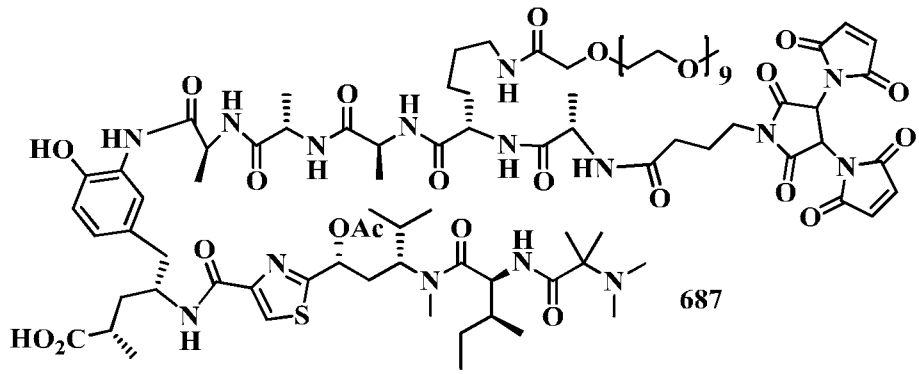
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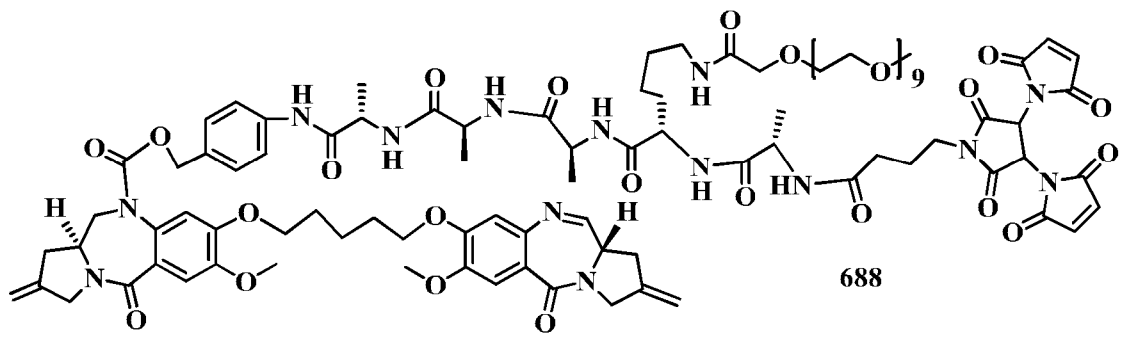
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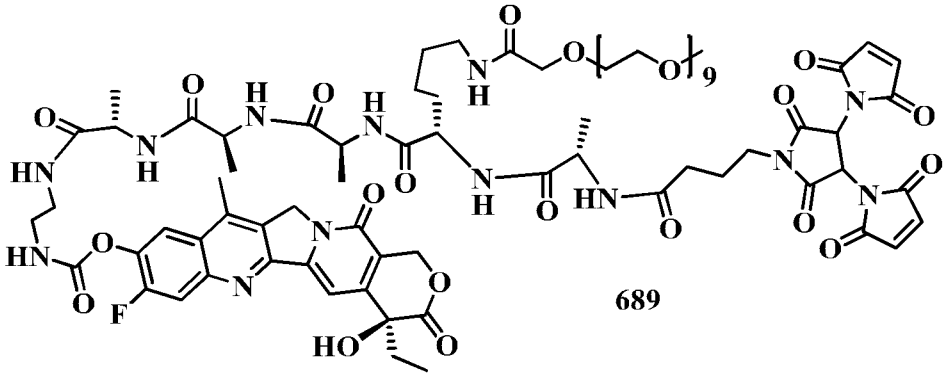
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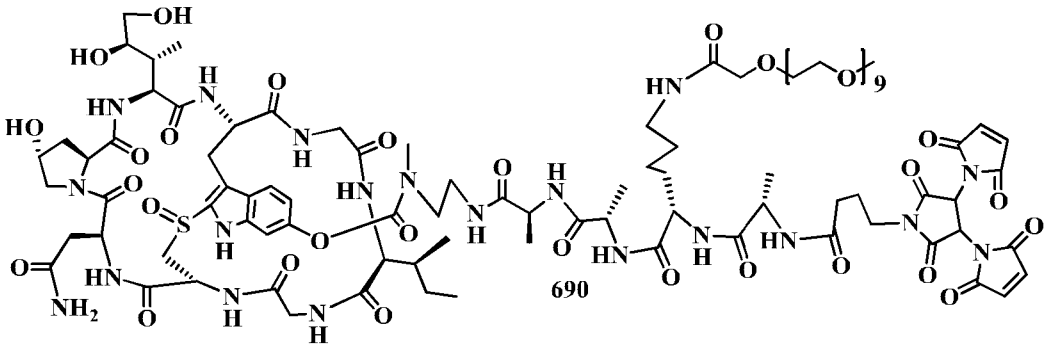
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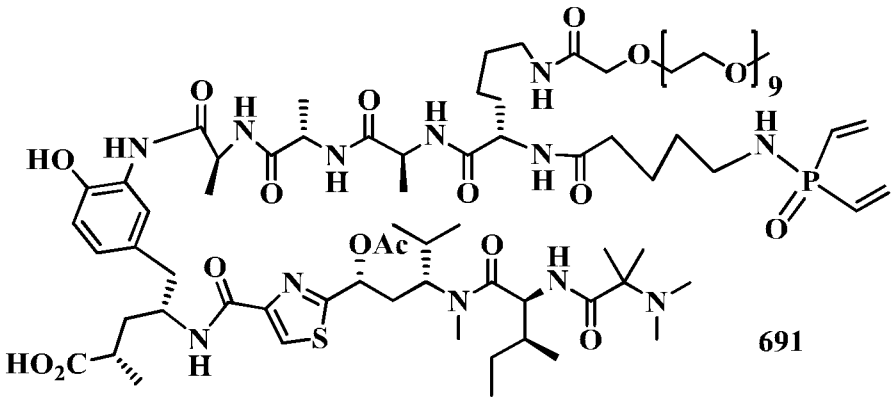
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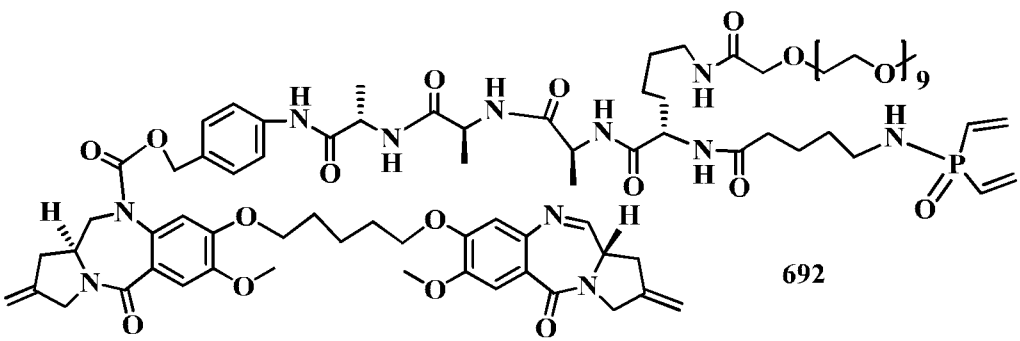
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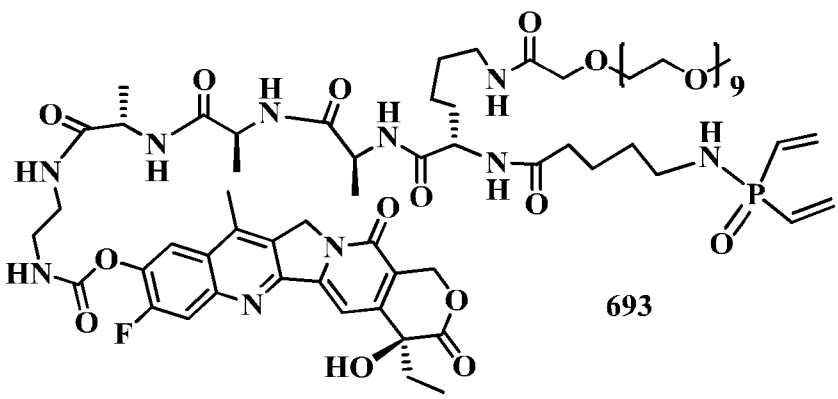
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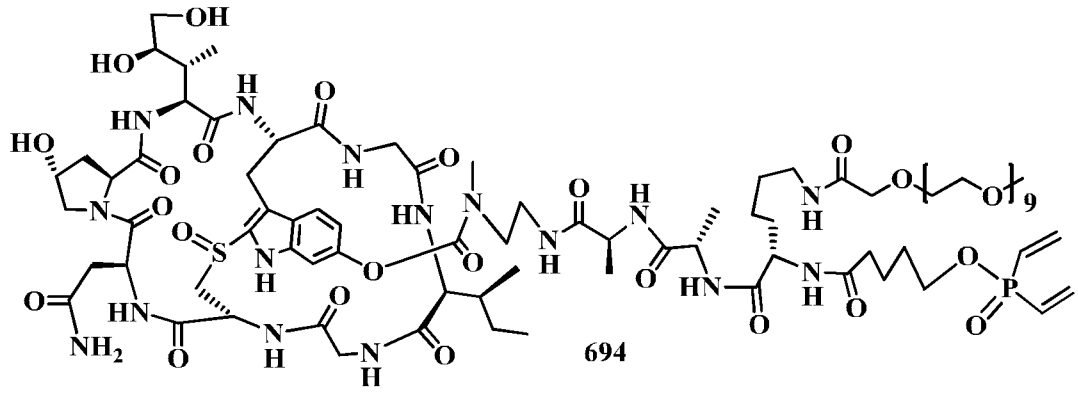
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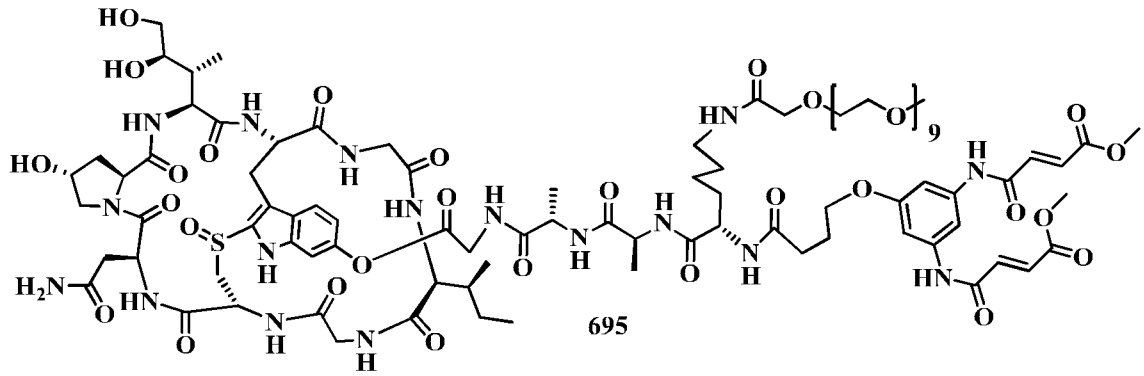
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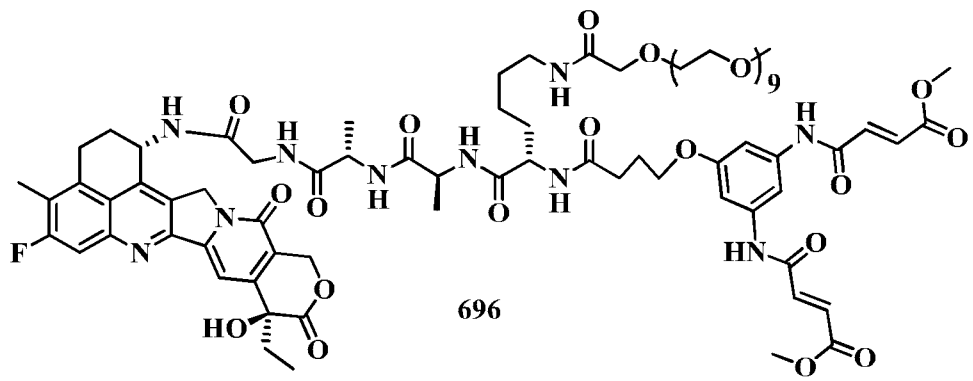
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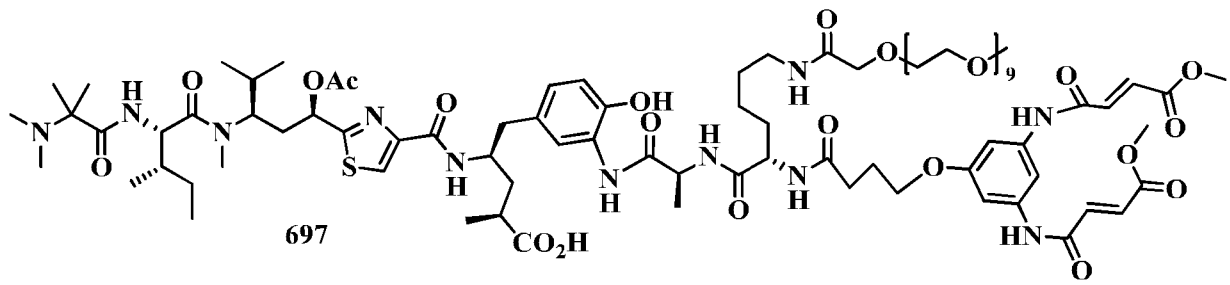
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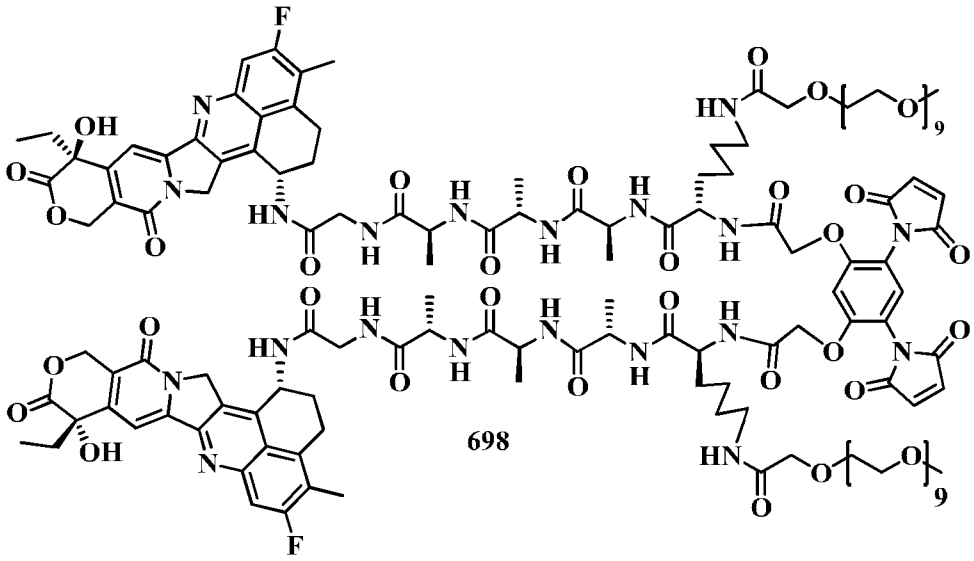
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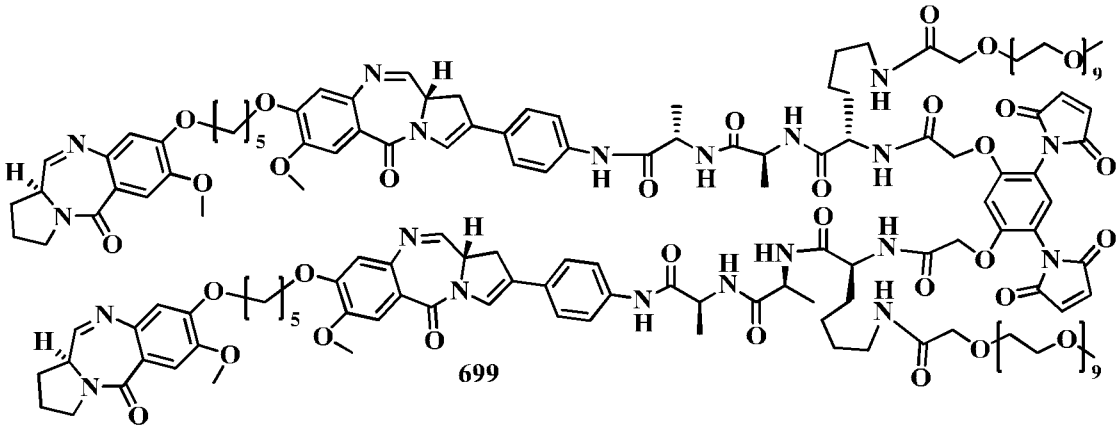
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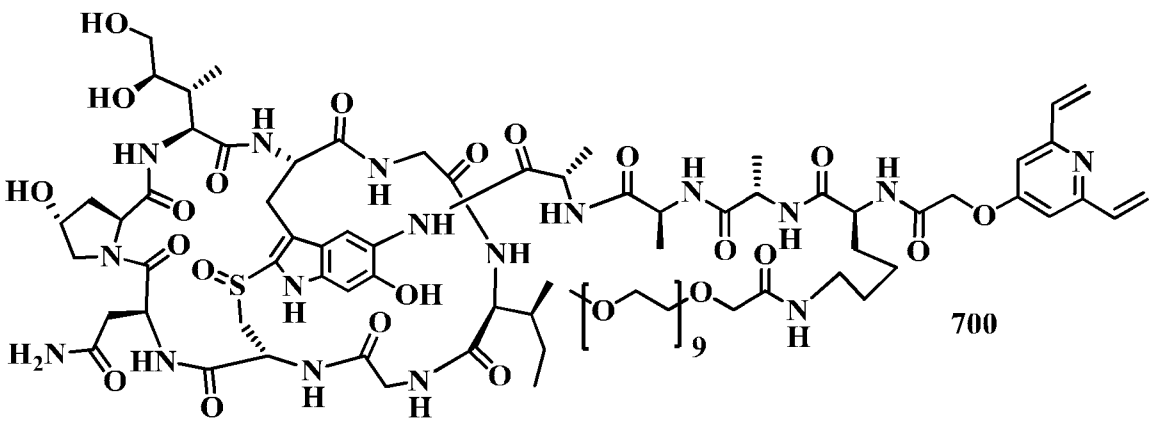
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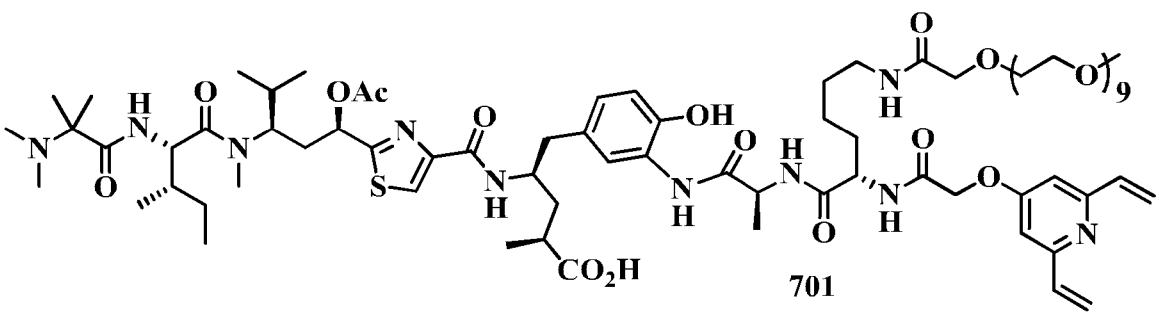
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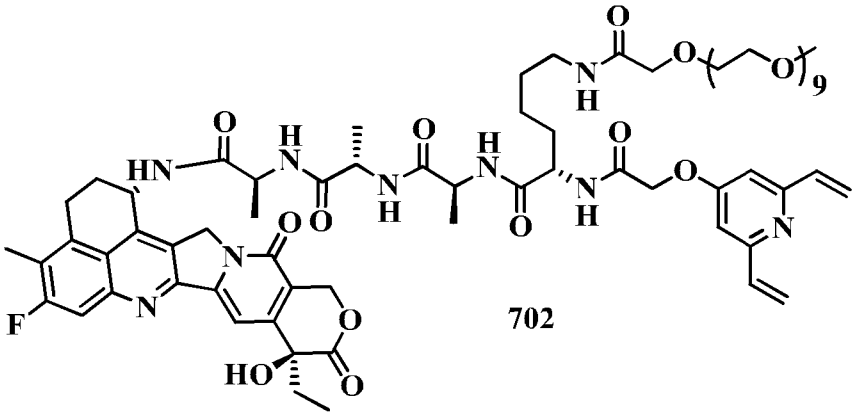
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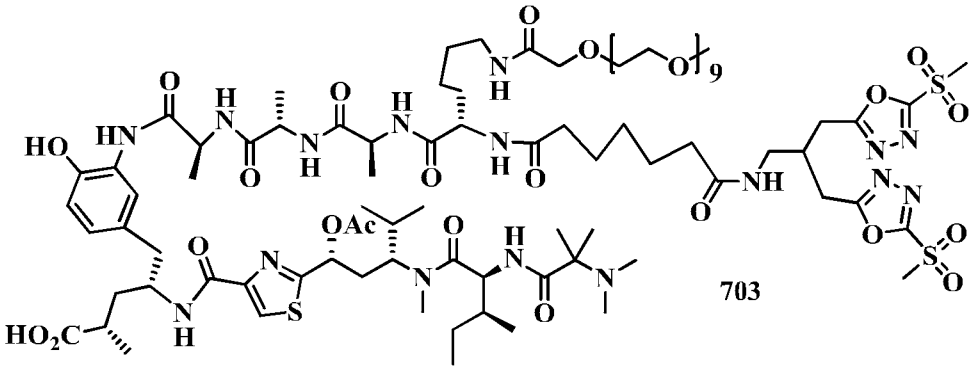
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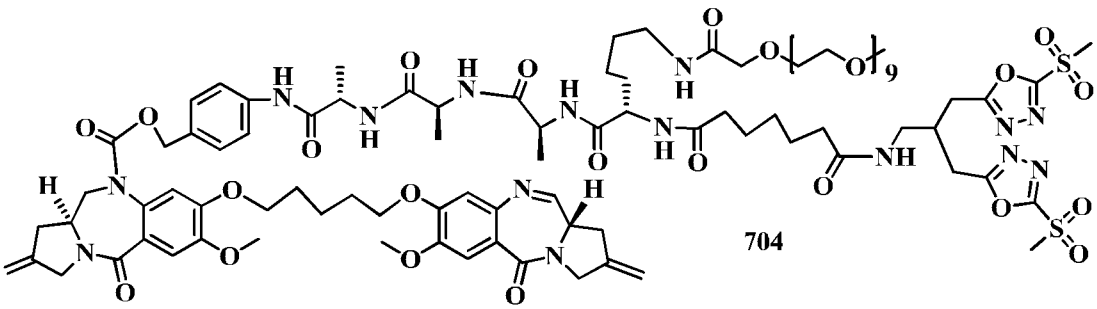
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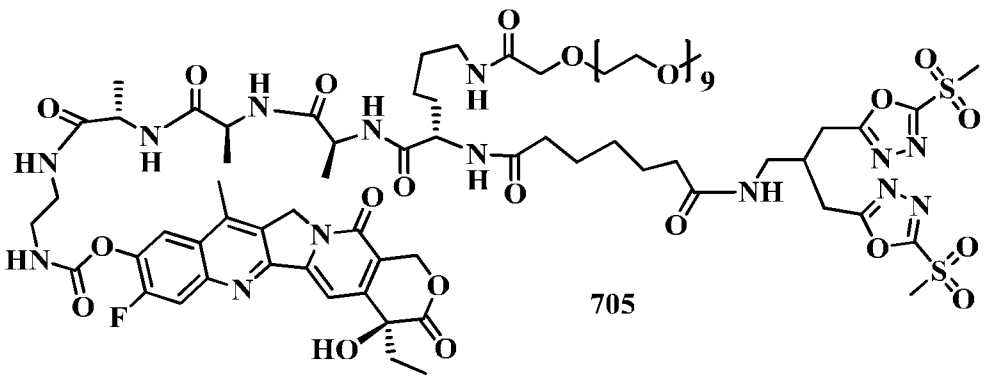
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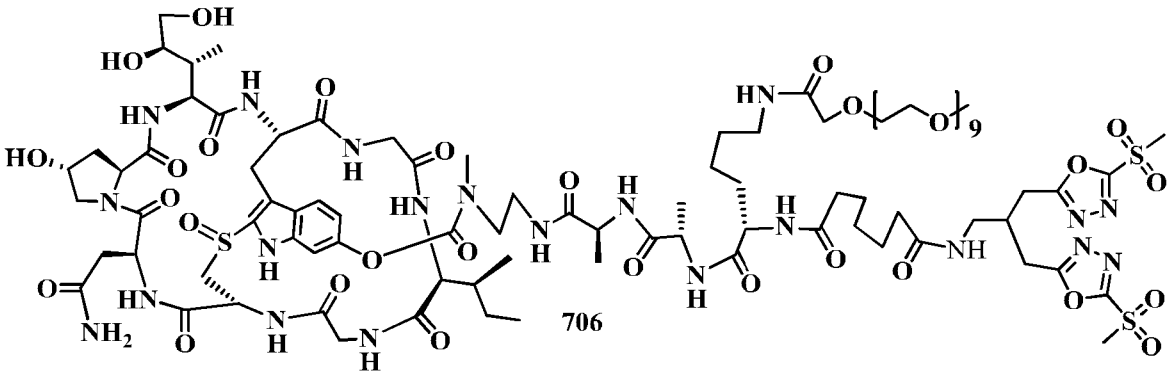
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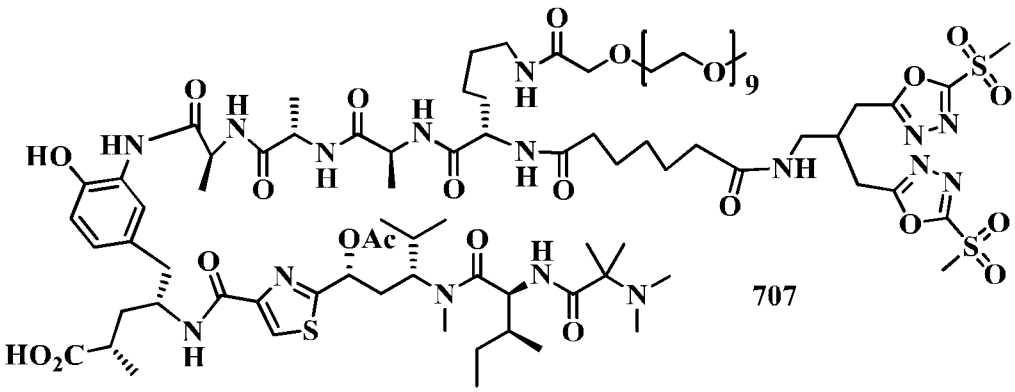
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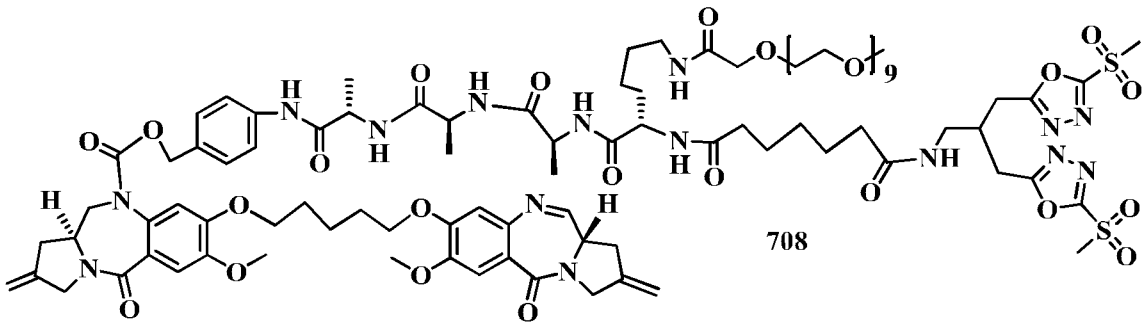
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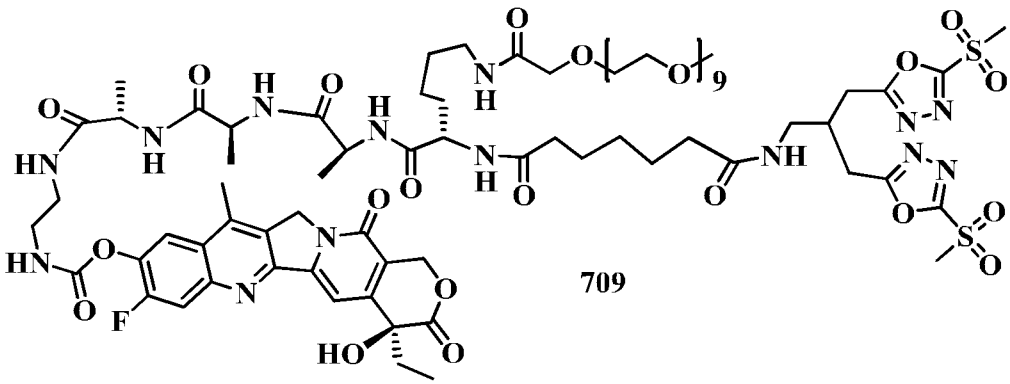
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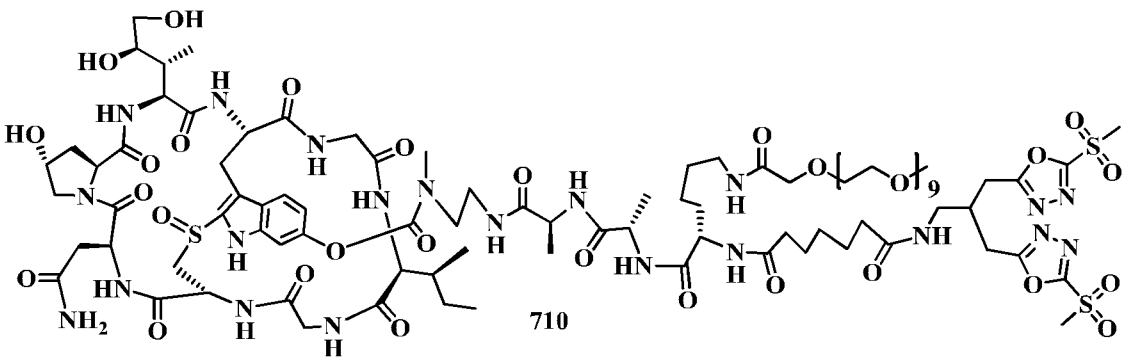
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or a derivative with one or more isotopes, a pharmaceutically acceptable salt, hydrate, or hydrated salt thereof; or a polymorphic crystalline structure thereof; or an optical isomer, racemate, diastereomer or enantiomer thereof; wherein n is 1 -10.

14. The process according to Claim 11, wherein the tumor cell is selected from the group consisting of lymphoma cells, myeloma cells, renal cells, breast cancer cells, prostate cancer cells,

ovarian cancer cells, colorectal cancer cells, gastric cancer cells, squamous cancer cells, small-cell lung cancer cells, none small-cell lung cancer cells, testicular cancer cells, malignant cells, and cells that grow and divide at an unregulated, quickened pace to cause cancers.

15. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate of Claim **4, 12** or **20,** and a pharmaceutically acceptable salt, carrier, diluent, or excipient therefor, or a combination of two or more of the conjugates, for treatment or prevention of a cancer, or an autoimmune disease, or an infectious disease.

16. The pharmaceutical composition according to Claim **15**, which is either in a liquid formula or in a formulated lyophilized solid/powder, comprising by weight of: 0.01%-99% of one or more of the conjugates of Claim **4, 12** or **20**; 0.0%-20.0% of one or more polyols; 0.0%-2.0% of one or more surfactants; 0.0%-5.0% of one or more preservatives; 0.0%-30% of one or more amino acids; 0.0%-5.0% of one or more antioxidants; 0.0%-0.3% of one or more metal chelating agents; 0.0%-30.0% of one or more buffer salts for adjusting pH of the composition to pH 4.5 - 7.5; and 0.0%-30.0% of one or more isotonic agents for adjusting osmotic pressure between from about 250 to 350 mOsm when being reconstituted for administration to a patient;

wherein the polyol is selected from the group consisting of fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose, glucose, sucrose, trehalose, sorbose, melezitose, raffinose, mannitol, xylitol, erythritol, maltitol, lactitol, erythritol, threitol, sorbitol, glycerol, or L-gluconate and metallic salts thereof;

wherein the surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 65, polysorbate 80, polysorbate 81, or polysorbate 85, poloxamer, poly(ethylene oxide)-poly(propylene oxide), polyethylene-polypropylene, Triton; sodium dodecyl sulfate (SDS), sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; dodecyl betaine, dodecyl dimethylamine oxide, cocamidopropyl betaine and coco ampho glycinate; or isostearyl ethylimidonium ethosulfate; polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol;

wherein the preservative is selected from the group consisting of benzyl alcohol, octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzetho-

nium chloride, phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol;

wherein the amino acid is selected from the group consisting of arginine, cystine, glycine, lysine, histidine, ornithine, isoleucine, leucine, alanine, glycine glutamic acid and aspartic acid;

wherein the antioxidant is selected from ascorbic acid, glutathione, cystine or and methionine;

wherein the chelating agent is EDTA or EGTA;

wherein the buffer salt is selected from the group consisting of sodium, potassium, ammonium, or trihydroxyethylamino salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid or phthalic acid; Tris or tromethamine hydrochloride, phosphate or sulfate; and arginine, glycine, glycylglycine, or histidine with anionic acetate, chloride, phosphate, sulfate, or succinate salt;

wherein the tonicity agent is selected from the group consisting of mannitol, sorbitol, sodium acetate, potassium chloride, sodium phosphate, potassium phosphate, trisodium citrate, and sodium chloride.

17. The pharmaceutical composition according to Claim 15**,** which is packed in a vial, bottle, pre-filled syringe, or pre-filled auto-injector syringe, in a form of a liquid or lyophilized solid.

18. A method for synergistical treatment or prevention of a cancer, an autoimmune disease, or an infectious disease, comprising administering the pharmaceutical composition according to Claim 15 concurrently with a synergistic agent of a chemotherapeutic agent, a radiation therapy agent, an immunotherapy agent, an autoimmune disorder agent, an anti-infectious agents or another conjugate.

19. The method according to Claim **18**, wherein the synergistic agentis one or more selected from the following drugs: Abatacept, Abiraterone acetate, Abraxane, Acetaminophen/hydrocodone, Acalabrutinib, aducanumab, Adalimumab, ADXS31-142, ADXS-HER2, Afatinib dimaleate, Aldesleukin, Alectinib, Alemtuzumab, Alitretinoin, ado-trastuzumab emtansine, Amphetamine/ dextroamphetamine, Anastrozole, Aripiprazole, anthracyclines, Aripiprazole, Atazanavir, Atezolizumab, Atorvastatin, Avelumab, Axicabtagene ciloleucel, Axitinib, Belinostat, BCG Live, Bevacizumab, Bexarotene, Blinatumomab, Bortezomib, Bosutinib, Brentuximab vedotin, Brigatinib, Budesonide, Budesonide/formoterol, Buprenorphine, Cabazitaxel, Cabozantinib, Capmatinib, Capecitabine, Carfilzomib, chimeric antigen receptor-engineered T (CAR-T) cells, Celecoxib, Ceritinib, Cetuximab, Chidamide, Ciclosporin, Cinacalcet, Crizotinib, Cobimetinib, Cosentyx, Crizotinib, CTL019, Dabigatran, Dabrafenib, Dacarbazine, Daclizumab, Dacomotinib, Daptomycin, Daratumumab, Darbepoe-

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20. The conjugate of Claim **4,** or **12**, having in vitro, in vivo or ex vivo cell killing activity.

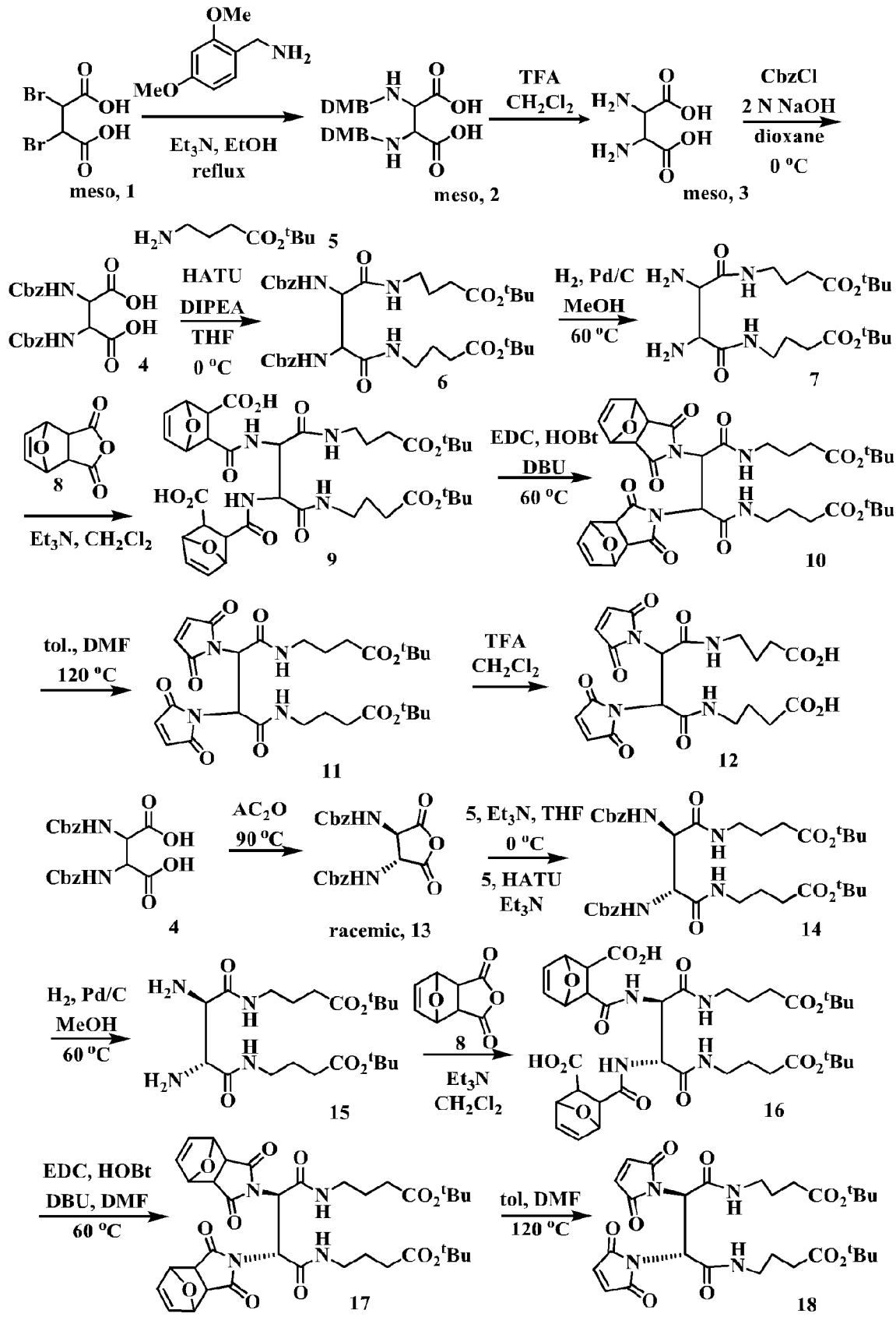

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dioxane
0 °C
H2N
CO2tBu
5
CbzHN
CbzHN
4
HATU
DIPEA
THF
0 °C
6
H2, Pd/C
MeOH
60 °C
7
8
Et3N, CH2Cl2
CO2H
HO2C
HN
9
EDC, HOBt
DBU
60 °C
10
tol., DMF
120 °C
11
TFA
CH2Cl2
CO2H
12
AC2O
90 °C
racemic, 13
5, Et3N, THF
0 °C
5, HATU
Et3N
14
H2, Pd/C
MeOH
60 °C
15
8
Et3N
CH2Cl2
16
EDC, HOBt
DBU, DMF
60 °C
17
tol, DMF
120 °C
18

Fig. 1

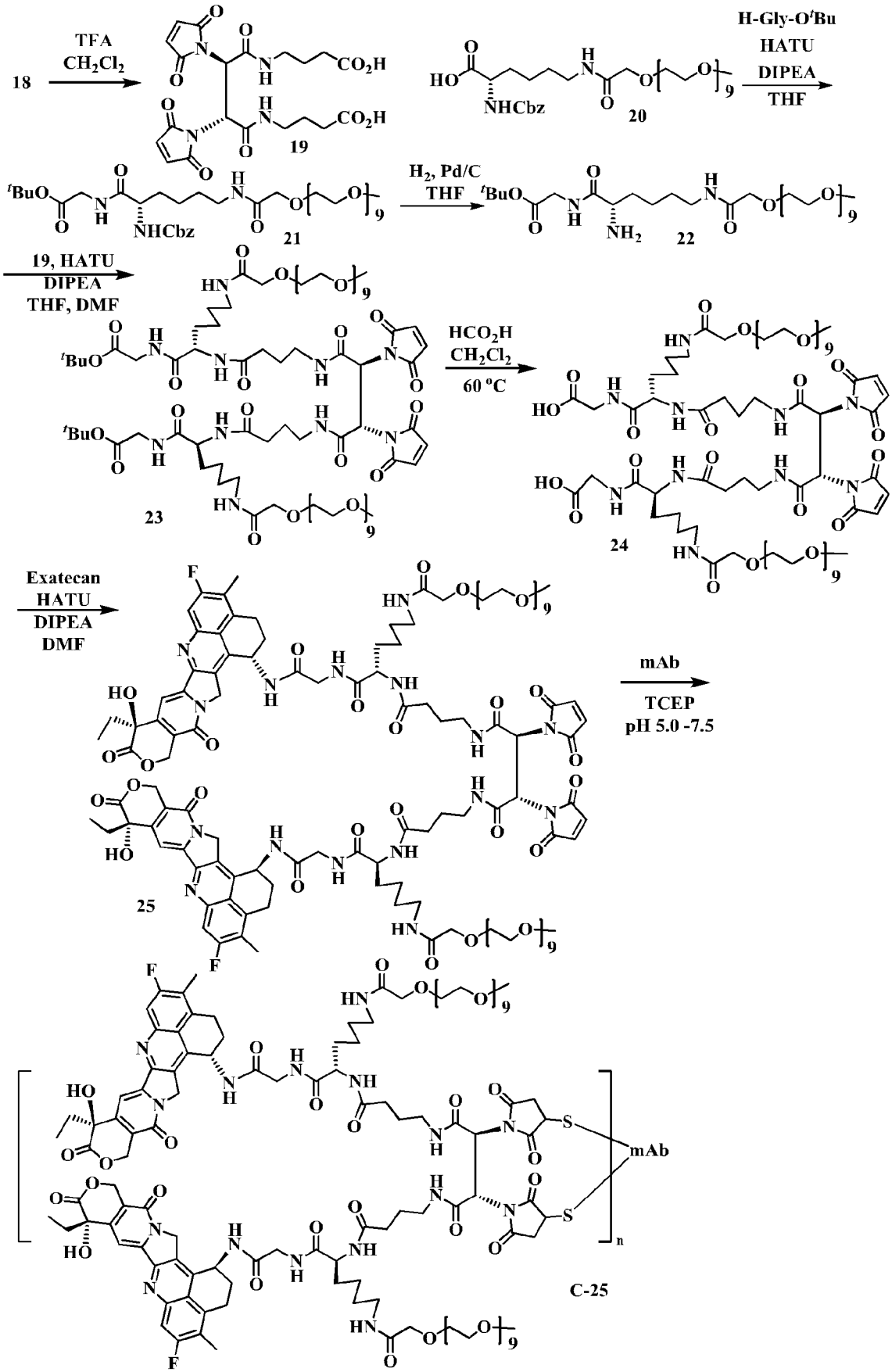

18
TFA
CH_2Cl_2
19
20
H-Gly-O^{t}Bu
HATU
DIPEA
THF
21
H_2, Pd/C
THF
22
19, HATU
DIPEA
THF, DMF
23
HCO_2H
CH_2Cl_2
60 °C
24
Exatecan
HATU
DIPEA
DMF
25
mAb
TCEP
pH 5.0 -7.5
C-25

Fig. 2.

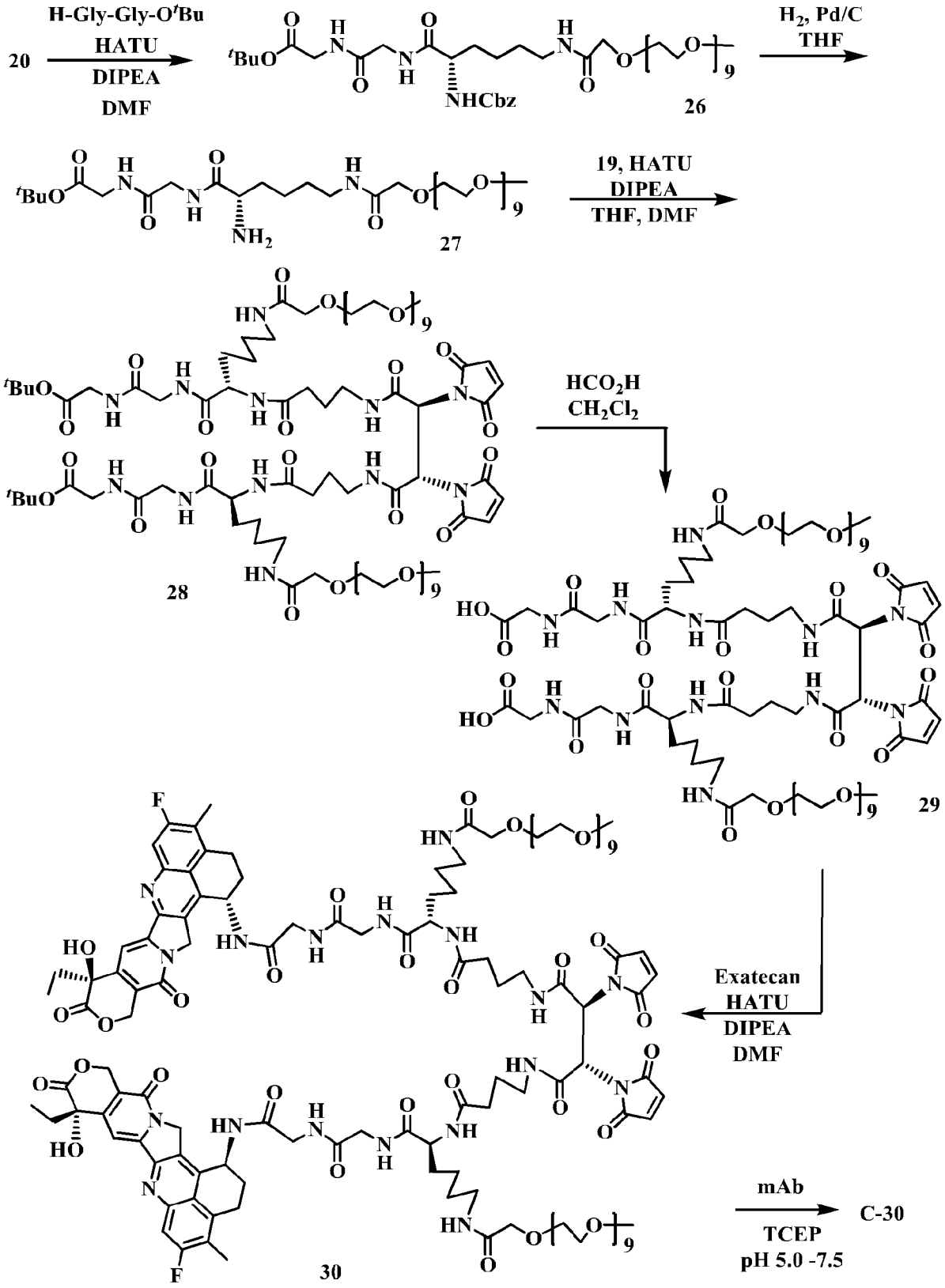

H-Gly-Gly-O^{t}Bu
HATU
DIPEA
DMF
20
^{t}BuO
NHCbz
26
H_2, Pd/C
THF
NH_2
27
19, HATU
DIPEA
THF, DMF
28
HCO_2H
CH_2Cl_2
HO
29
Exatecan
HATU
DIPEA
DMF
30
mAb
TCEP
pH 5.0 -7.5
C-30

Fig. 3

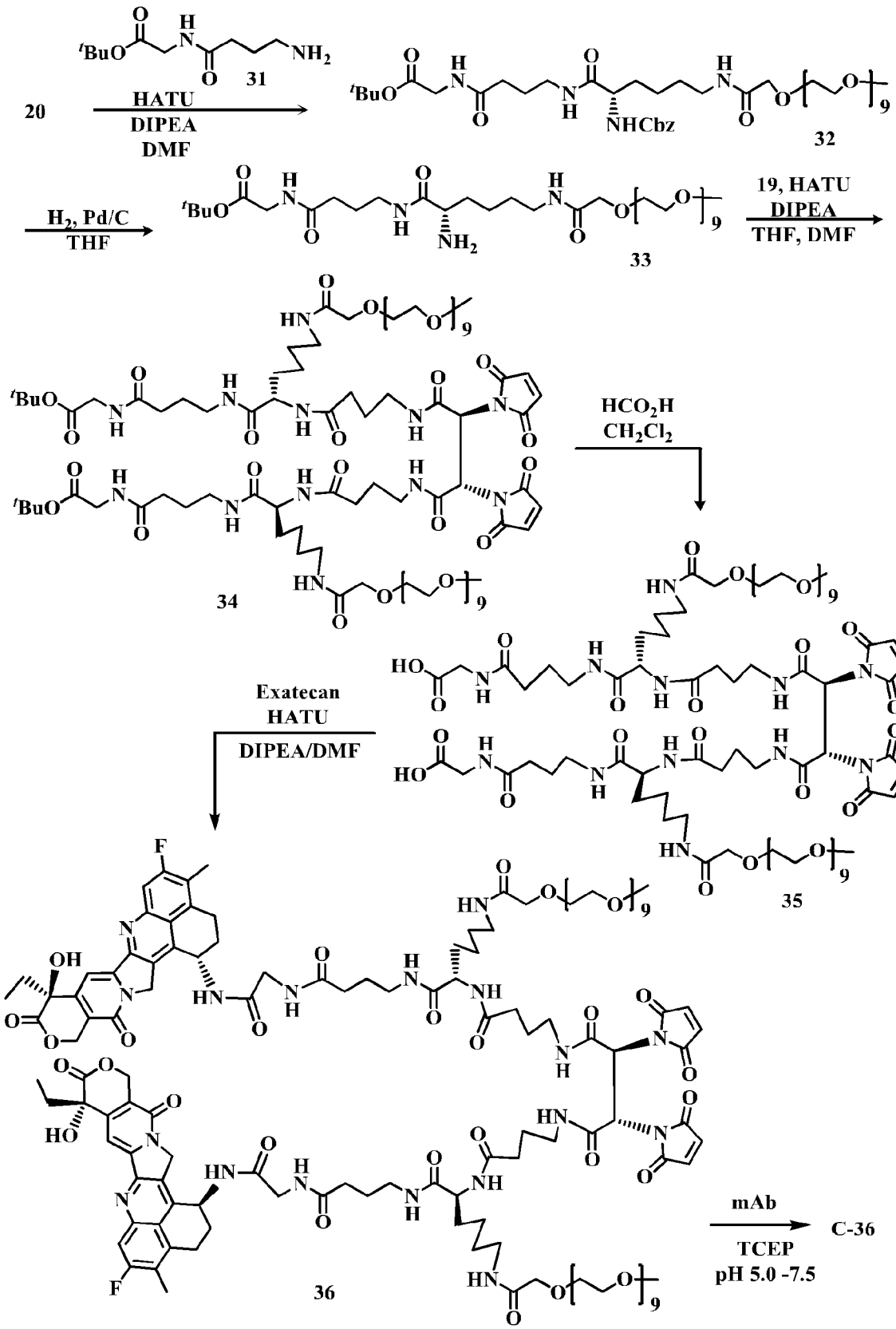

20
HATU
DIPEA
DMF
31
32
H2, Pd/C
THF
33
19, HATU
DIPEA
THF, DMF
34
HCO2H
CH2Cl2
Exatecan
HATU
DIPEA/DMF
35
36
mAb
TCEP
pH 5.0 -7.5
C-36

Fig. 4

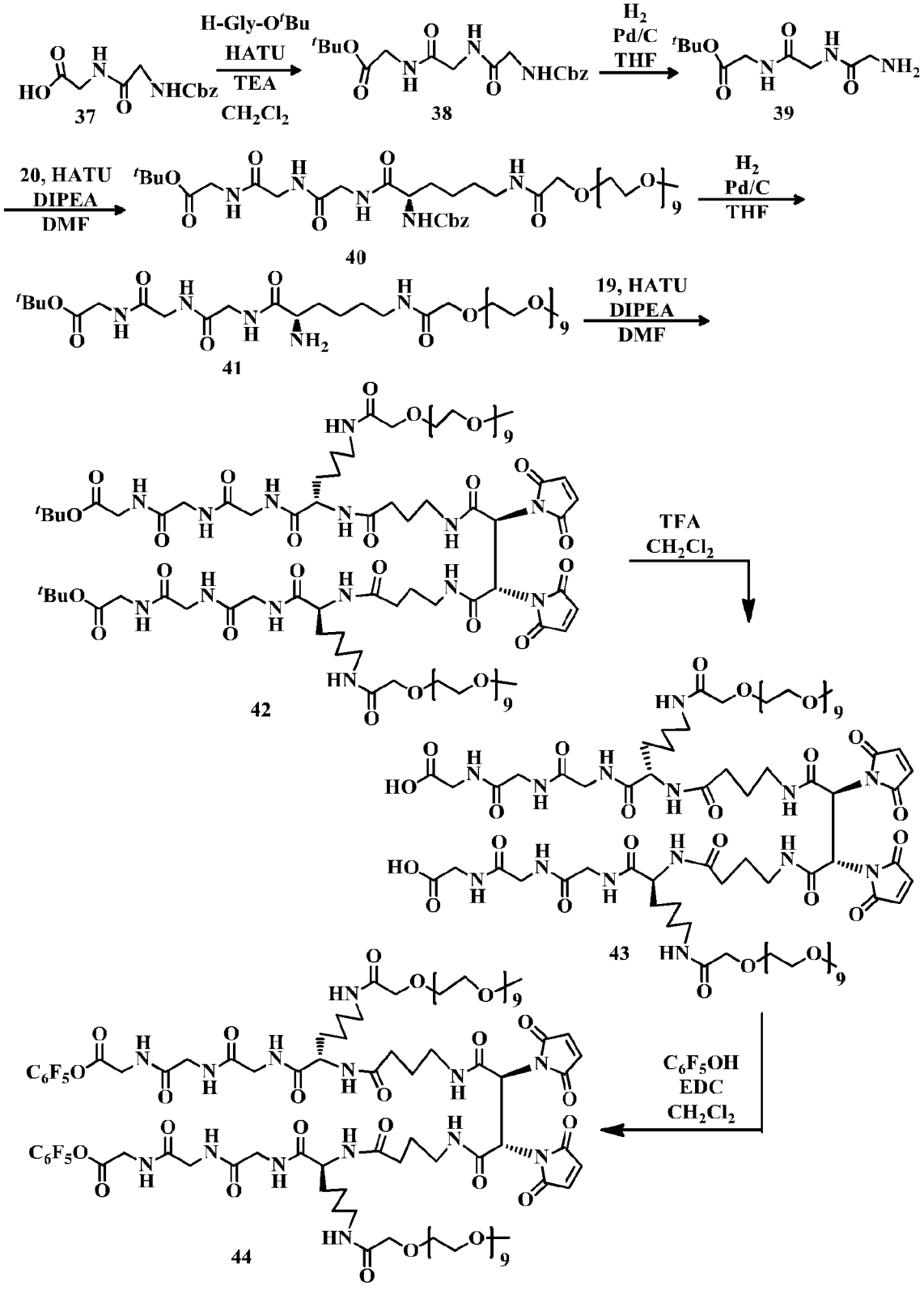

H-Gly-O^{t}Bu
HATU
TEA
CH_2Cl_2
H_2
Pd/C
THF
20, HATU
DIPEA
DMF
19, HATU
DIPEA
DMF
TFA
CH_2Cl_2
C_6F_5OH
EDC
CH_2Cl_2
37
38
39
40
41
42
43
44

Fig. 5

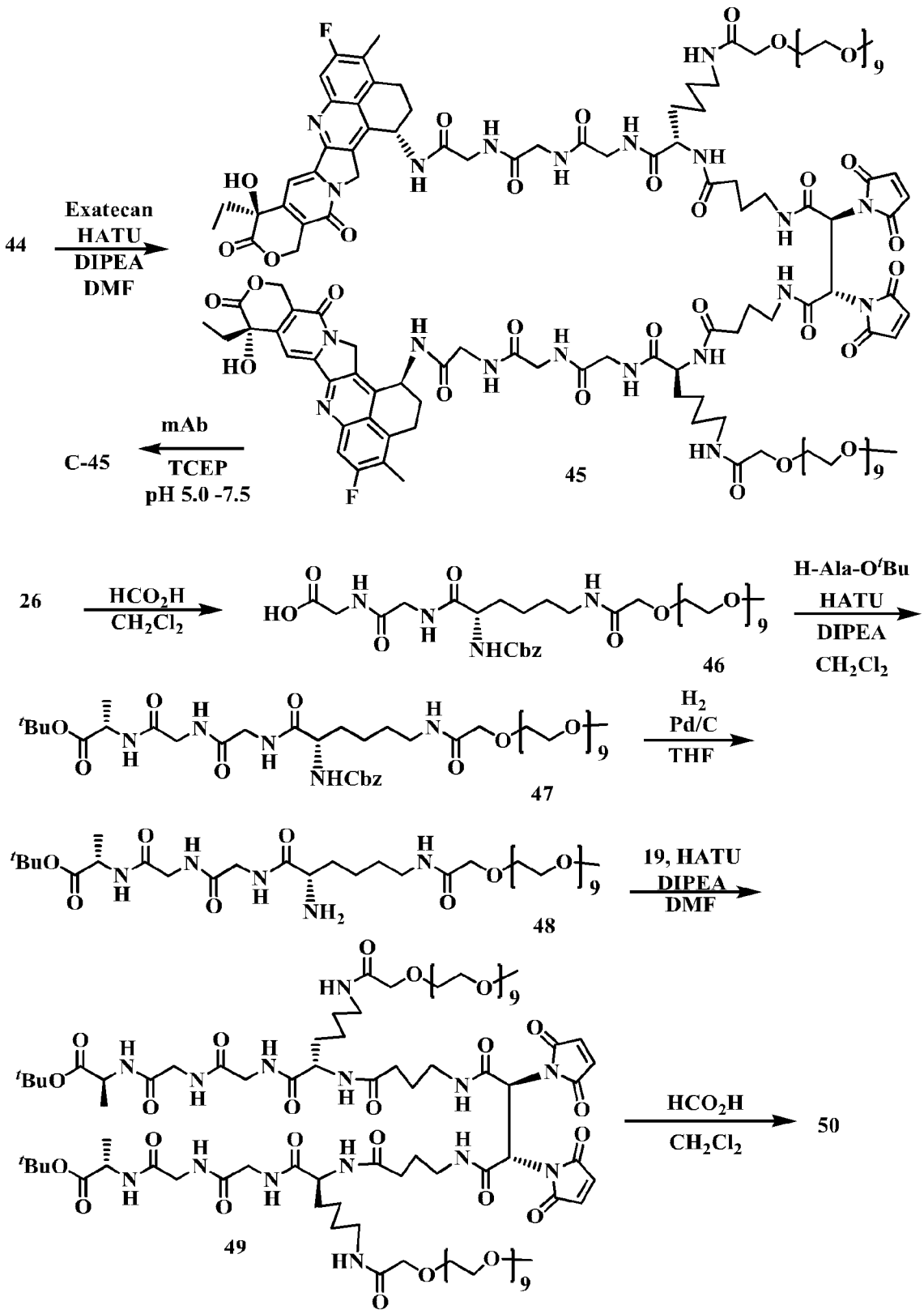

44
Exatecan
HATU
DIPEA
DMF
45
C-45
mAb
TCEP
pH 5.0 -7.5
26
HCO2H
CH2Cl2
46
H-Ala-OtBu
HATU
DIPEA
CH2Cl2
47
H2
Pd/C
THF
48
19, HATU
DIPEA
DMF
49
HCO2H
CH2Cl2
50

Fig. 6

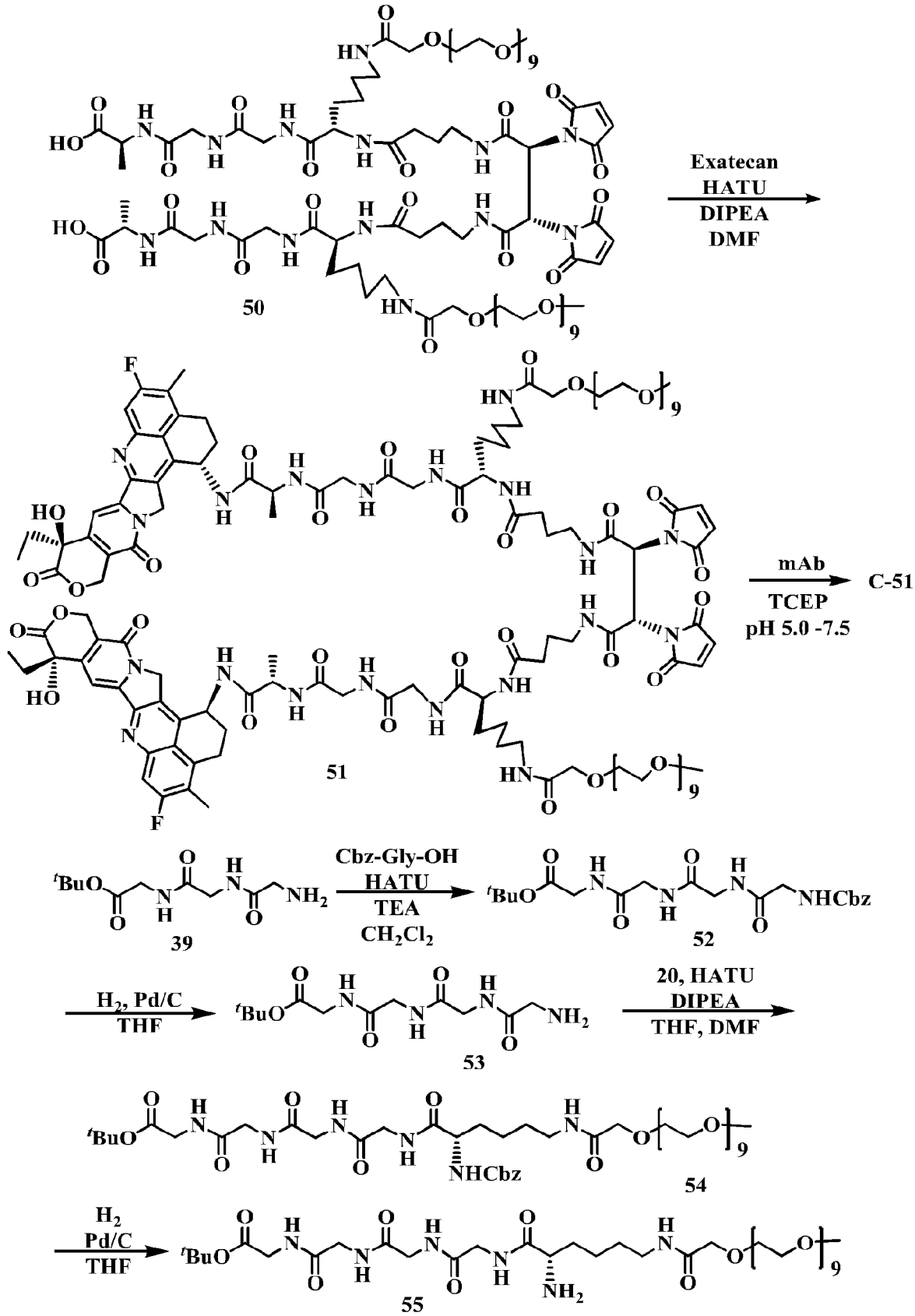

Exatecan
HATU
DIPEA
DMF
50
mAb
TCEP
pH 5.0 -7.5
C-51
51
Cbz-Gly-OH
HATU
TEA
CH_2Cl_2
39
52
H_2, Pd/C
THF
53
20, HATU
DIPEA
THF, DMF
54
H_2
Pd/C
THF
55

Fig. 7

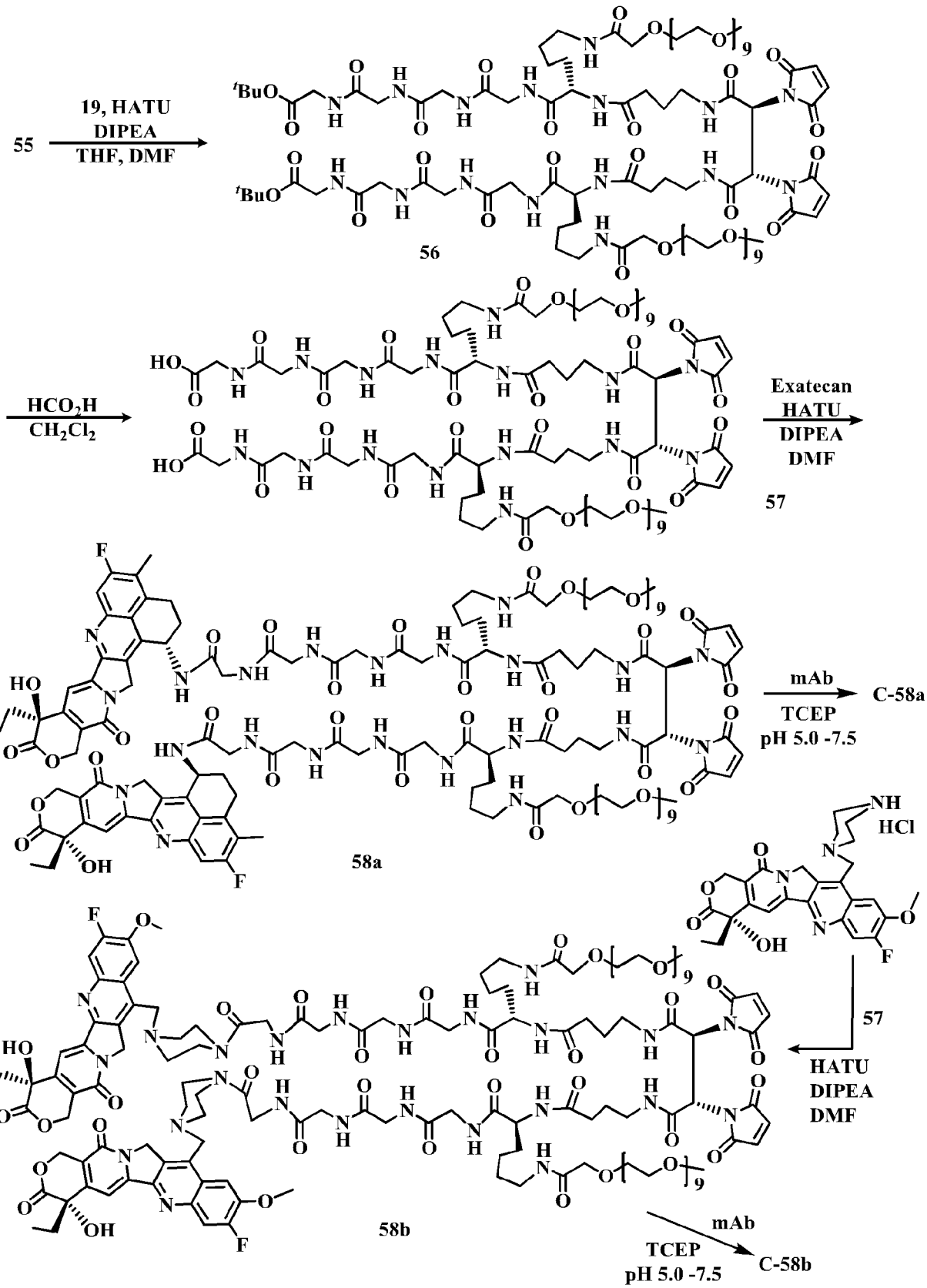

55
19, HATU
DIPEA
THF, DMF
56
HCO2H
CH2Cl2
57
Exatecan
HATU
DIPEA
DMF
mAb
TCEP
pH 5.0 -7.5
C-58a
58a
NH
HCl
57
HATU
DIPEA
DMF
58b
mAb
TCEP
pH 5.0 -7.5
C-58b

Fig. 8

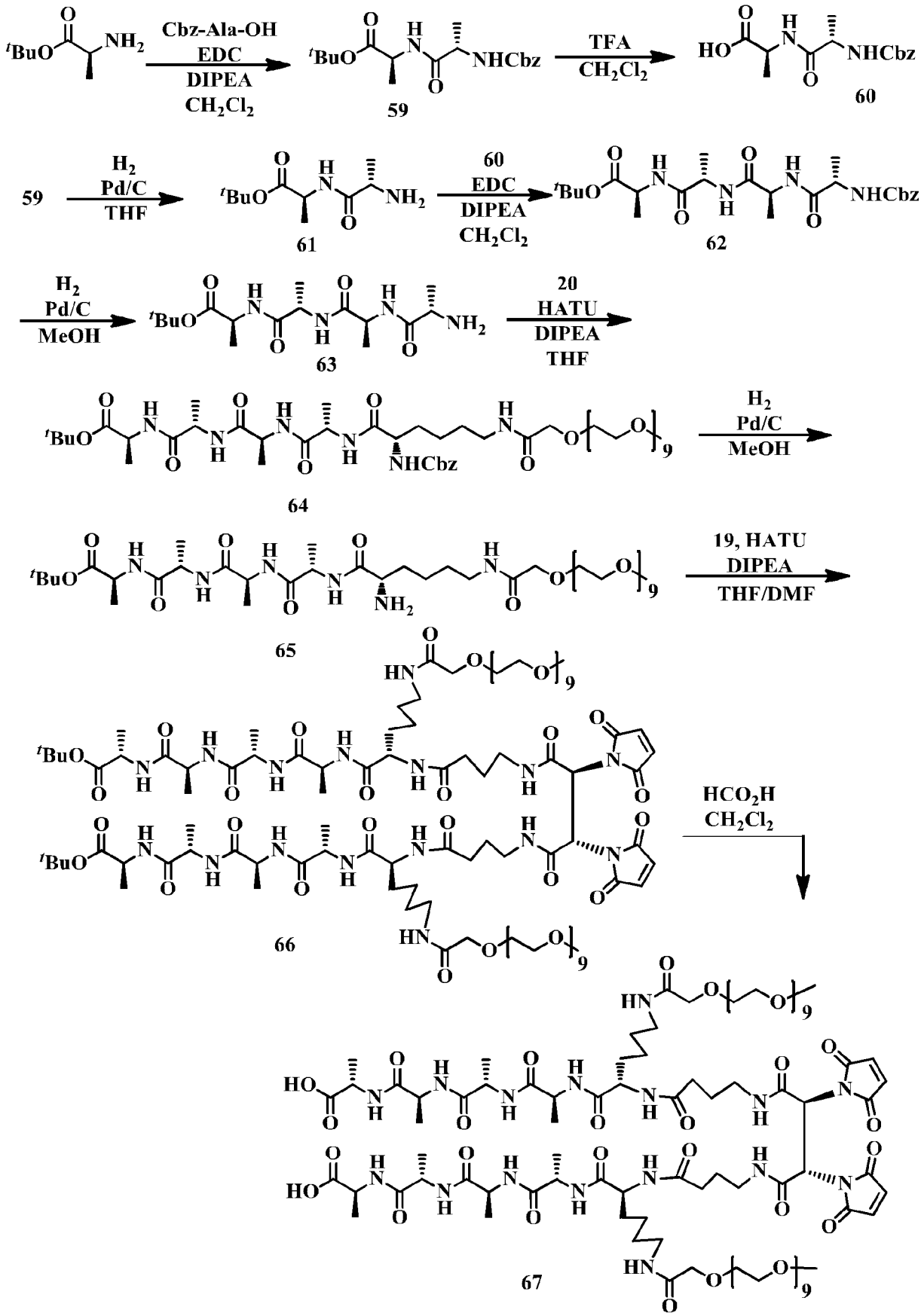

Cbz-Ala-OH
EDC
DIPEA
CH2Cl2
TFA
CH2Cl2
59
60
H2
Pd/C
THF
61
60
EDC
DIPEA
CH2Cl2
62
H2
Pd/C
MeOH
63
20
HATU
DIPEA
THF
64
H2
Pd/C
MeOH
65
19, HATU
DIPEA
THF/DMF
66
HCO2H
CH2Cl2
67

Fig. 9

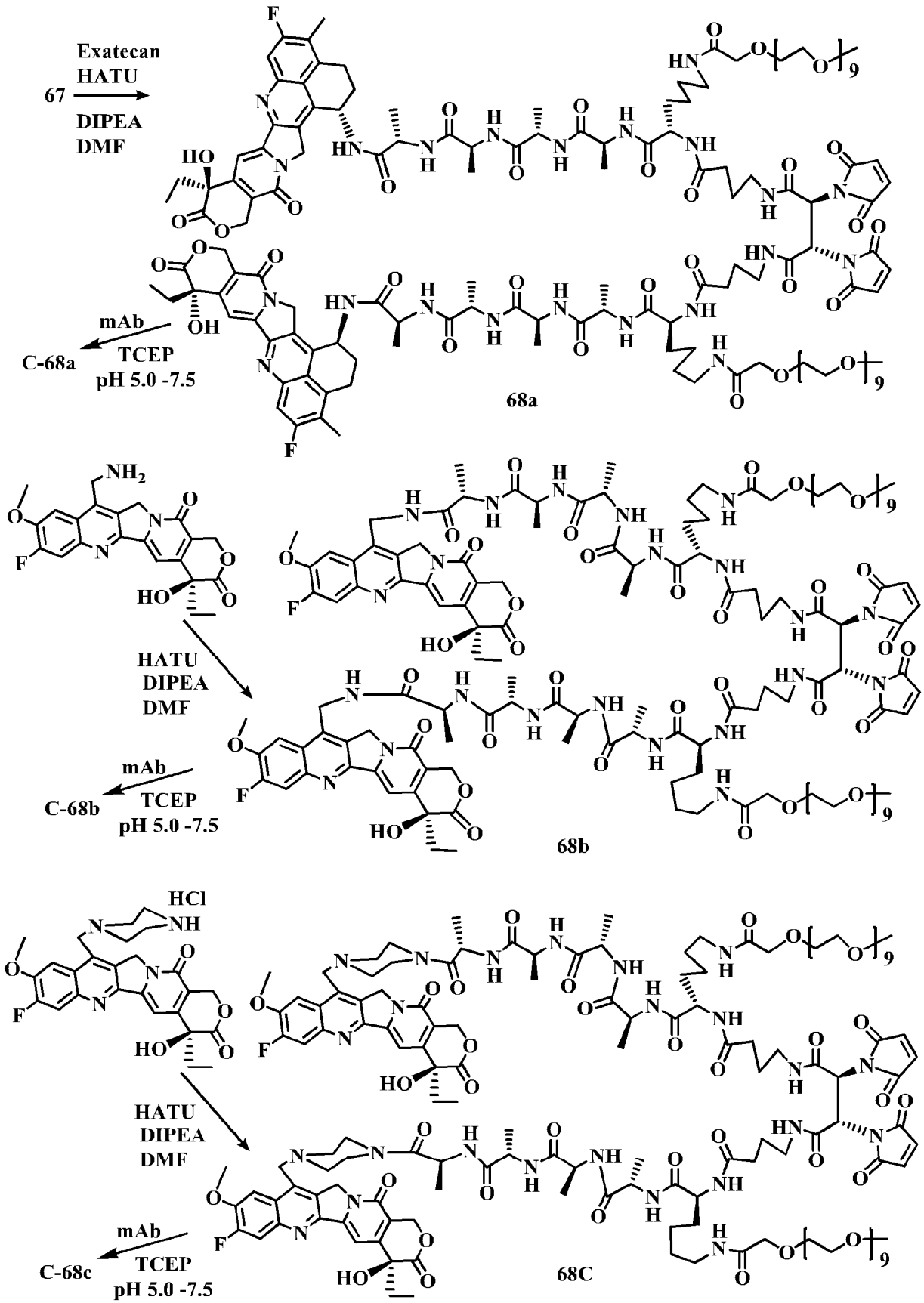

Exatecan
HATU
67
DIPEA
DMF
mAb
TCEP
C-68a
pH 5.0 -7.5
68a
HATU
DIPEA
DMF
mAb
TCEP
C-68b
pH 5.0 -7.5
68b
HATU
DIPEA
DMF
mAb
TCEP
C-68c
pH 5.0 -7.5
68C

Fig. 10

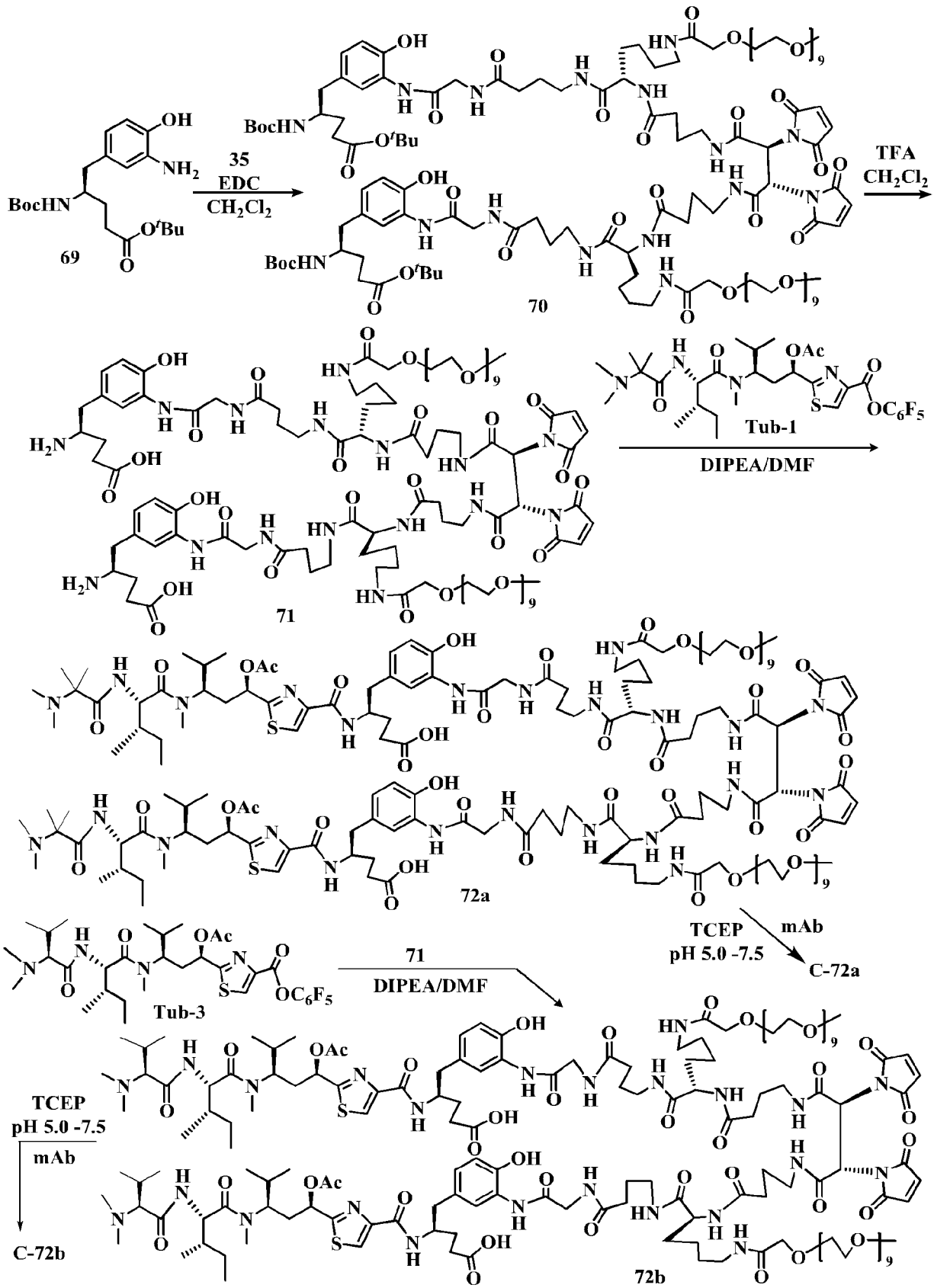

69
35
EDC
CH_2Cl_2
70
TFA
CH_2Cl_2
71
Tub-1
DIPEA/DMF
72a
TCEP
pH 5.0 -7.5
mAb
C-72a
Tub-3
71
DIPEA/DMF
TCEP
pH 5.0 -7.5
mAb
C-72b
72b

Fig. 11

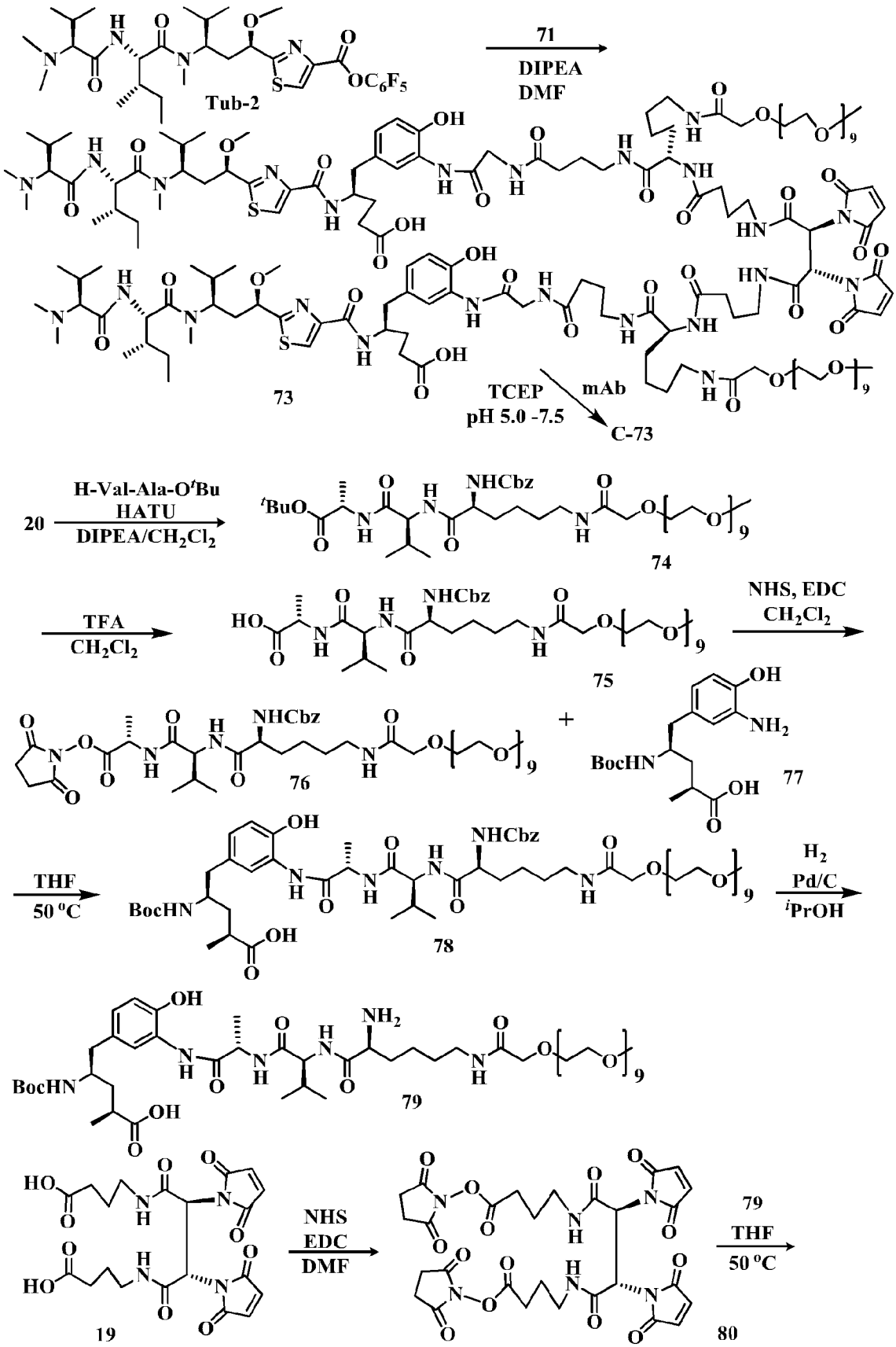

Tub-2
71
DIPEA
DMF
73
TCEP
pH 5.0 -7.5
mAb
C-73
20
H-Val-Ala-O^{t}Bu
HATU
DIPEA/CH_2Cl_2
74
TFA
CH_2Cl_2
75
NHS, EDC
CH_2Cl_2
76
77
THF
50 °C
78
H_2
Pd/C
^{i}PrOH
79
19
NHS
EDC
DMF
79
THF
50 °C
80

Fig. 12

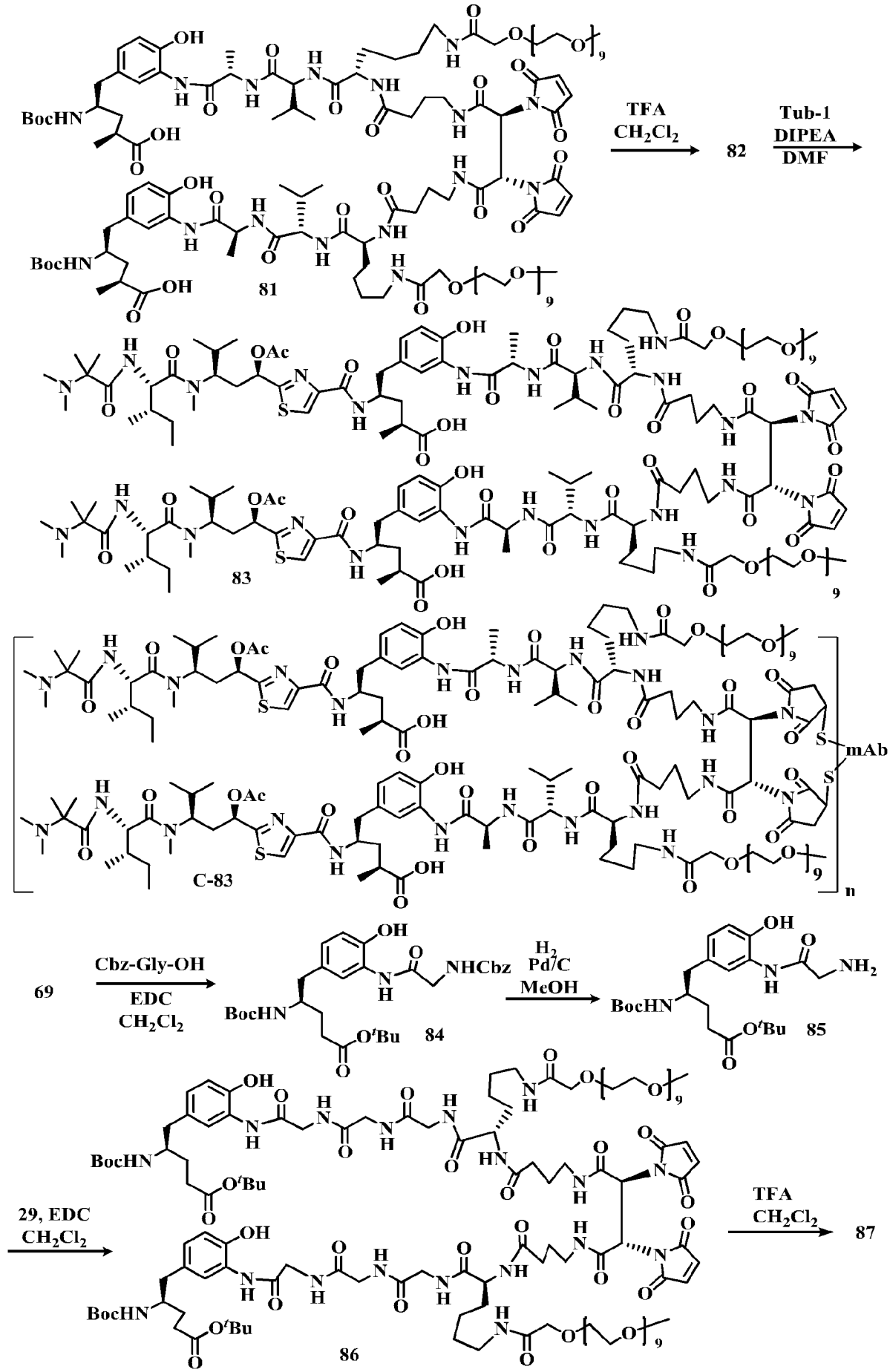

TFA
CH2Cl2
82
Tub-1
DIPEA
DMF
81
83
C-83
mAb
n
69
Cbz-Gy-OH
EDC
CH2Cl2
84
H2
Pd/C
MeOH
85
29, EDC
CH2Cl2
86
TFA
CH2Cl2
87

Fig. 13

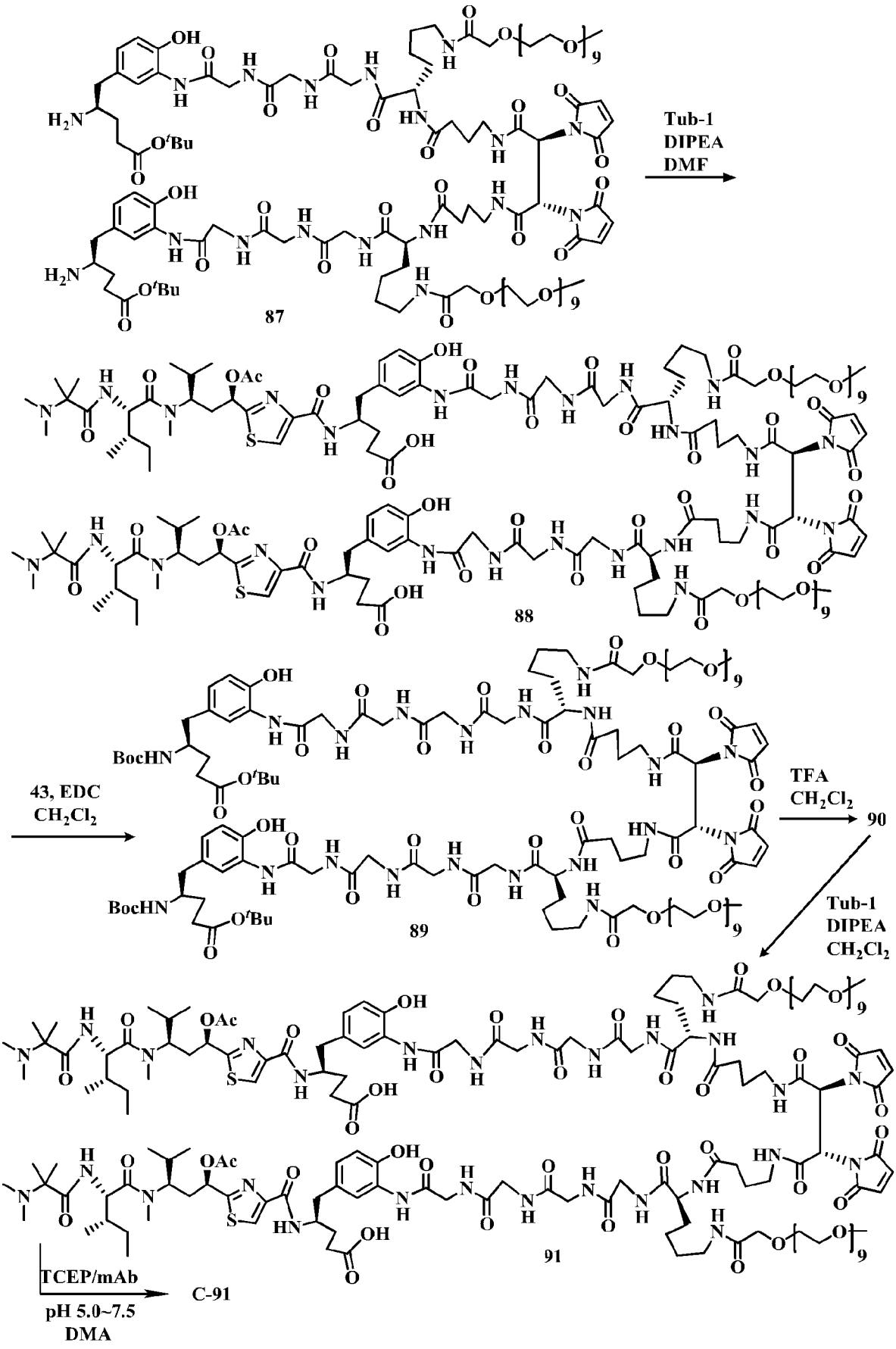

Tub-1
DIPEA
DMF
87
88
43, EDC
CH_2Cl_2
89
TFA
CH_2Cl_2
90
Tub-1
DIPEA
CH_2Cl_2
91
TCEP/mAb
pH 5.0~7.5
DMA
C-91

Fig. 14

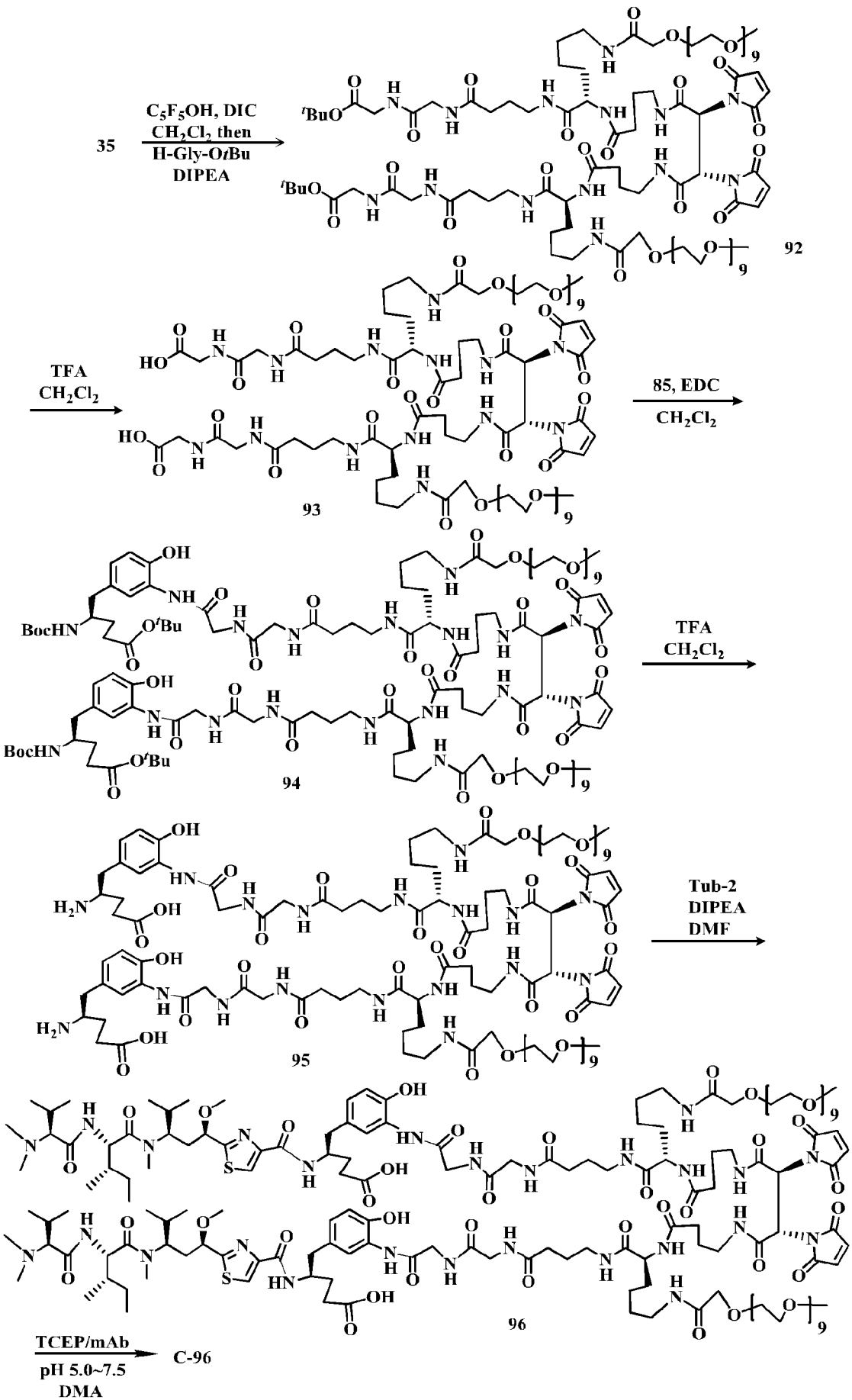

35
C5F5OH, DIC
CH2Cl2 then
H-Gly-OtBu
DIPEA
92
TFA
CH2Cl2
93
85, EDC
CH2Cl2
TFA
CH2Cl2
94
Tub-2
DIPEA
DMF
95
96
TCEP/mAb
pH 5.0~7.5
DMA
C-96

Fig. 15

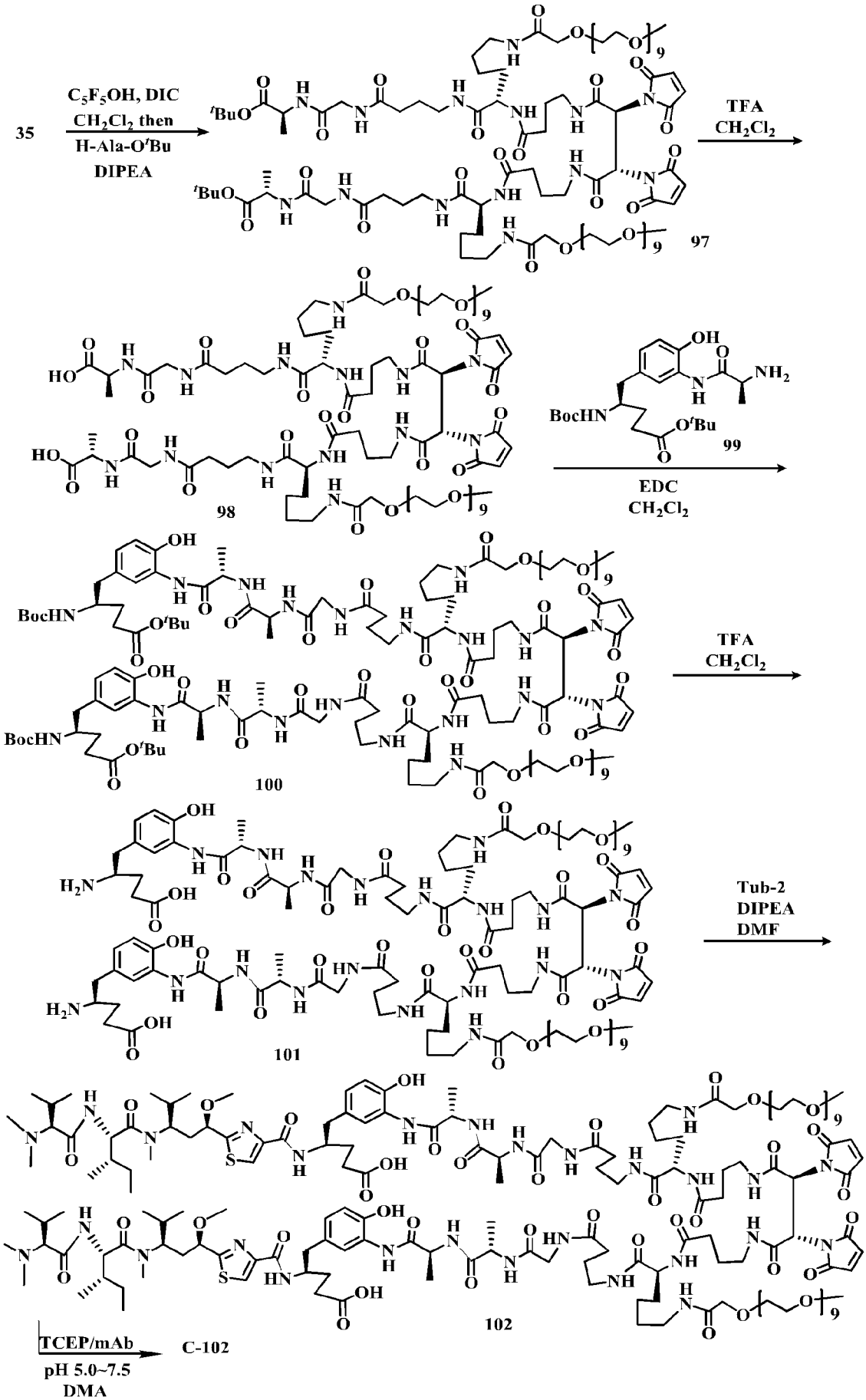

35
C5F5OH, DIC
CH2Cl2 then
H-Ala-O'Bu
DIPEA
TFA
CH2Cl2
97
98
99
EDC
CH2Cl2
100
TFA
CH2Cl2
101
Tub-2
DIPEA
DMF
102
TCEP/mAb
pH 5.0~7.5
DMA
C-102

Fig. 16

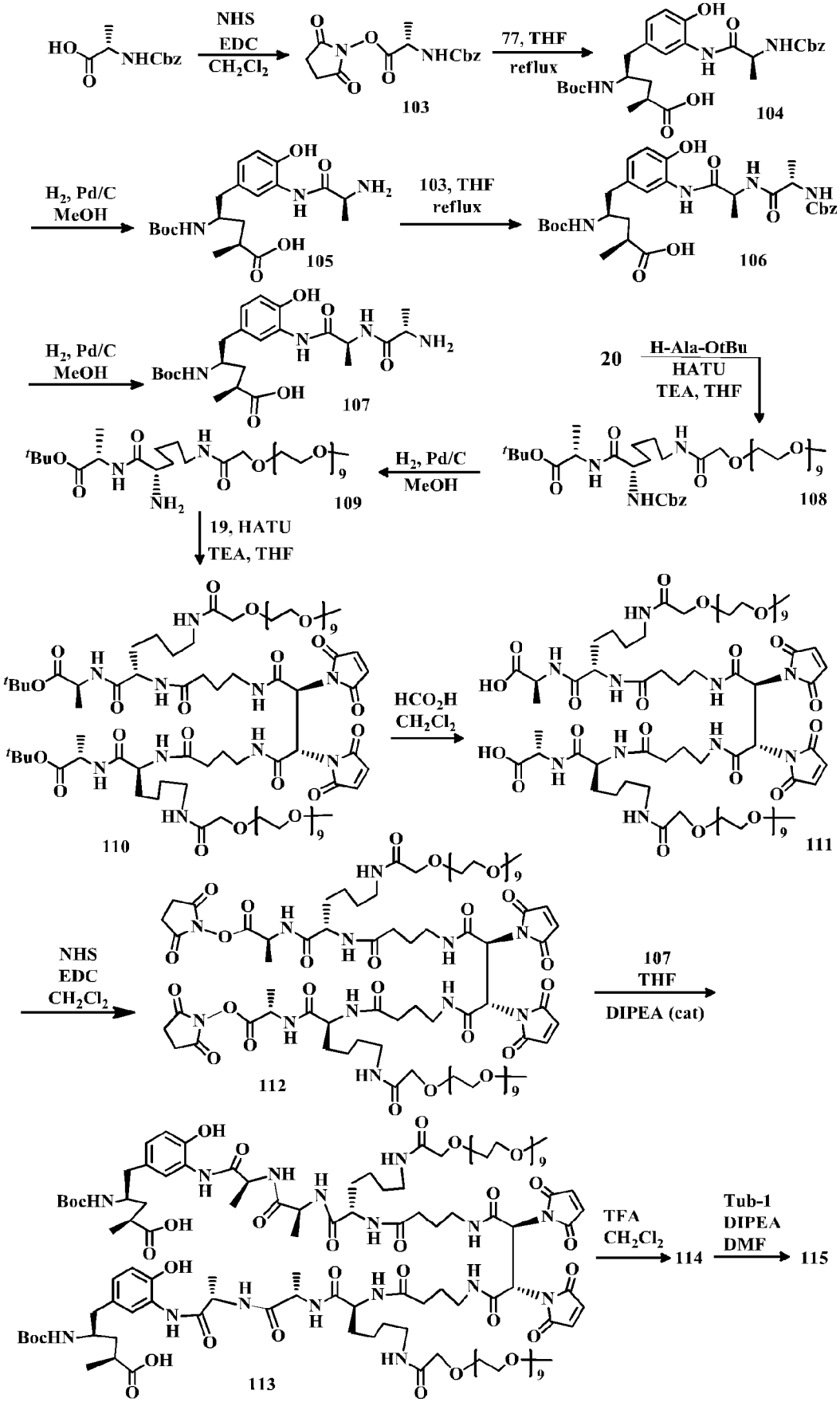

NHS
EDC
CH_2Cl_2
NHCbz
103
77, THF
reflux
104
H_2, Pd/C
MeOH
105
103, THF
reflux
106
107
20
H-Ala-OtBu
HATU
TEA, THF
108
109
19, HATU
TEA, THF
HCO_2H
CH_2Cl_2
110
111
112
107
THF
DIPEA (cat)
TFA
CH_2Cl_2
114
Tub-1
DIPEA
DMF
115
113

Fig 17.

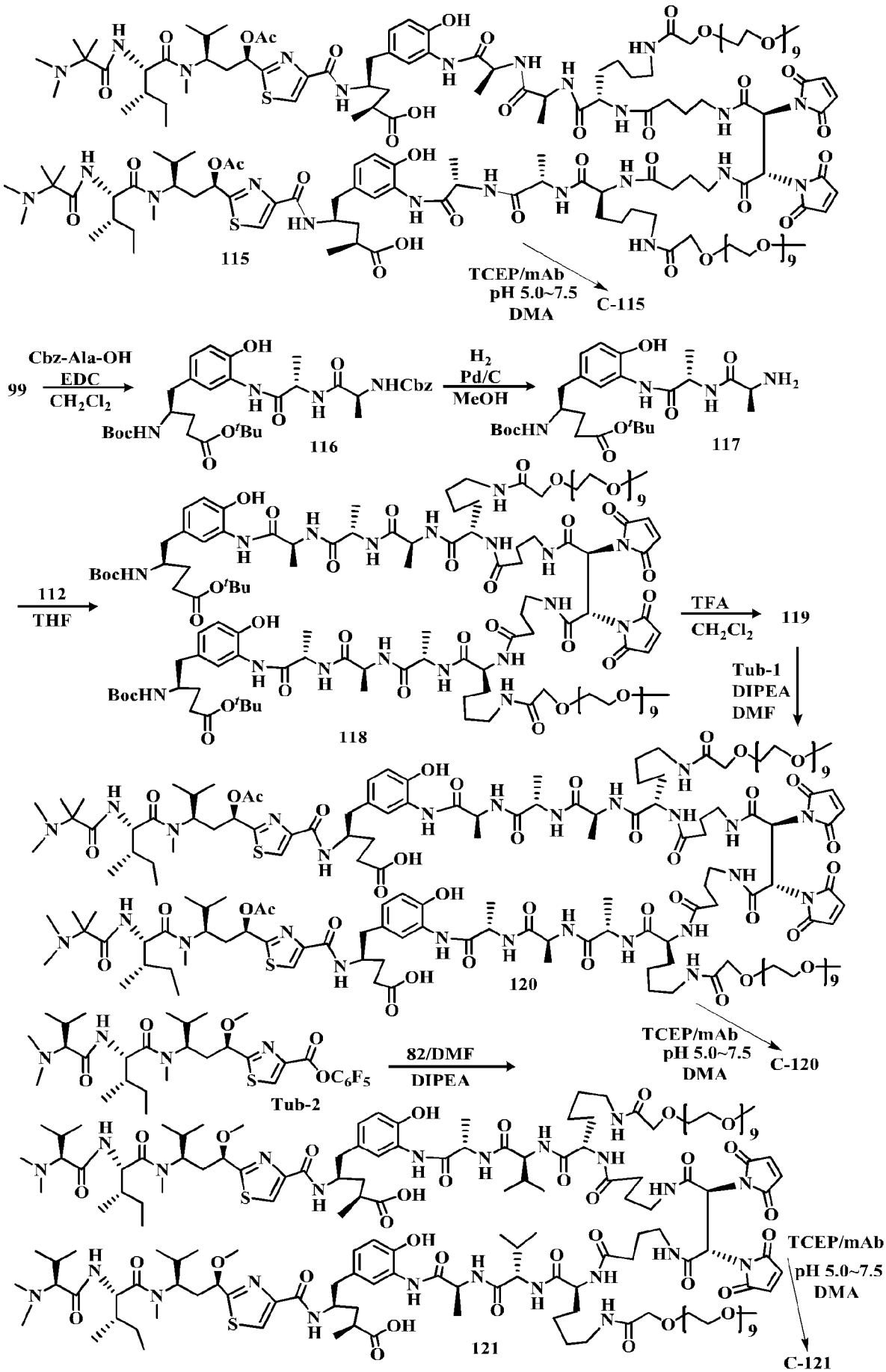

115
TCEP/mAb
pH 5.0~7.5
DMA
C-115
Cbz-Ala-OH
EDC
CH_2Cl_2
99
116
H_2
Pd/C
MeOH
117
112
THF
118
TFA
CH_2Cl_2
119
Tub-1
DIPEA
DMF
120
Tub-2
82/DMF
DIPEA
TCEP/mAb
pH 5.0~7.5
DMA
C-120
121
TCEP/mAb
pH 5.0~7.5
DMA
C-121

Fig. 18

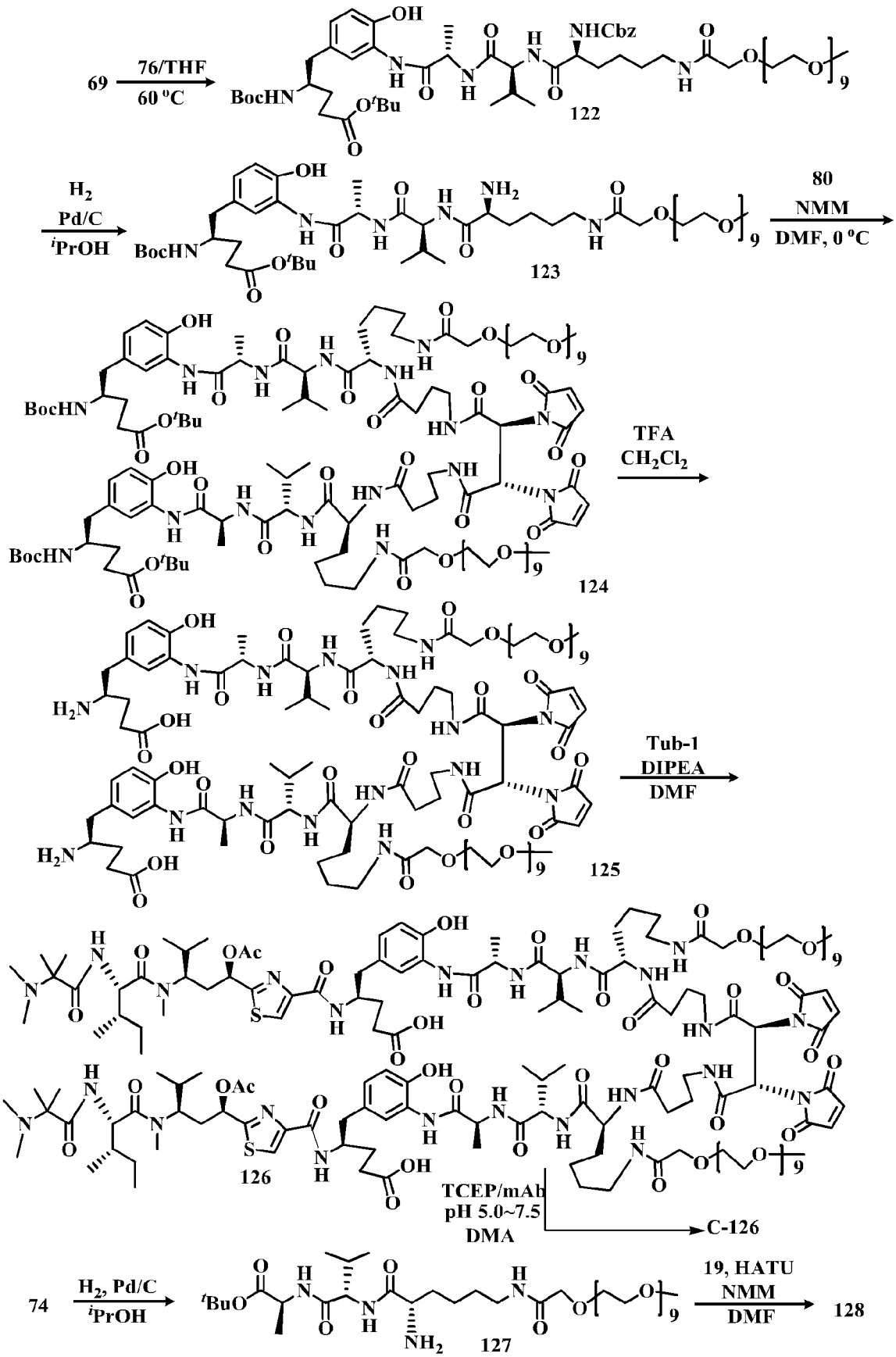

69
76/THF
60 °C
122
H2
Pd/C
iPrOH
123
80
NMM
DMF, 0 °C
TFA
CH2Cl2
124
Tub-1
DIPEA
DMF
125
126
TCEP/mAb
pH 5.0~7.5
DMA
C-126
74
H2, Pd/C
iPrOH
127
19, HATU
NMM
DMF
128

Fig. 19

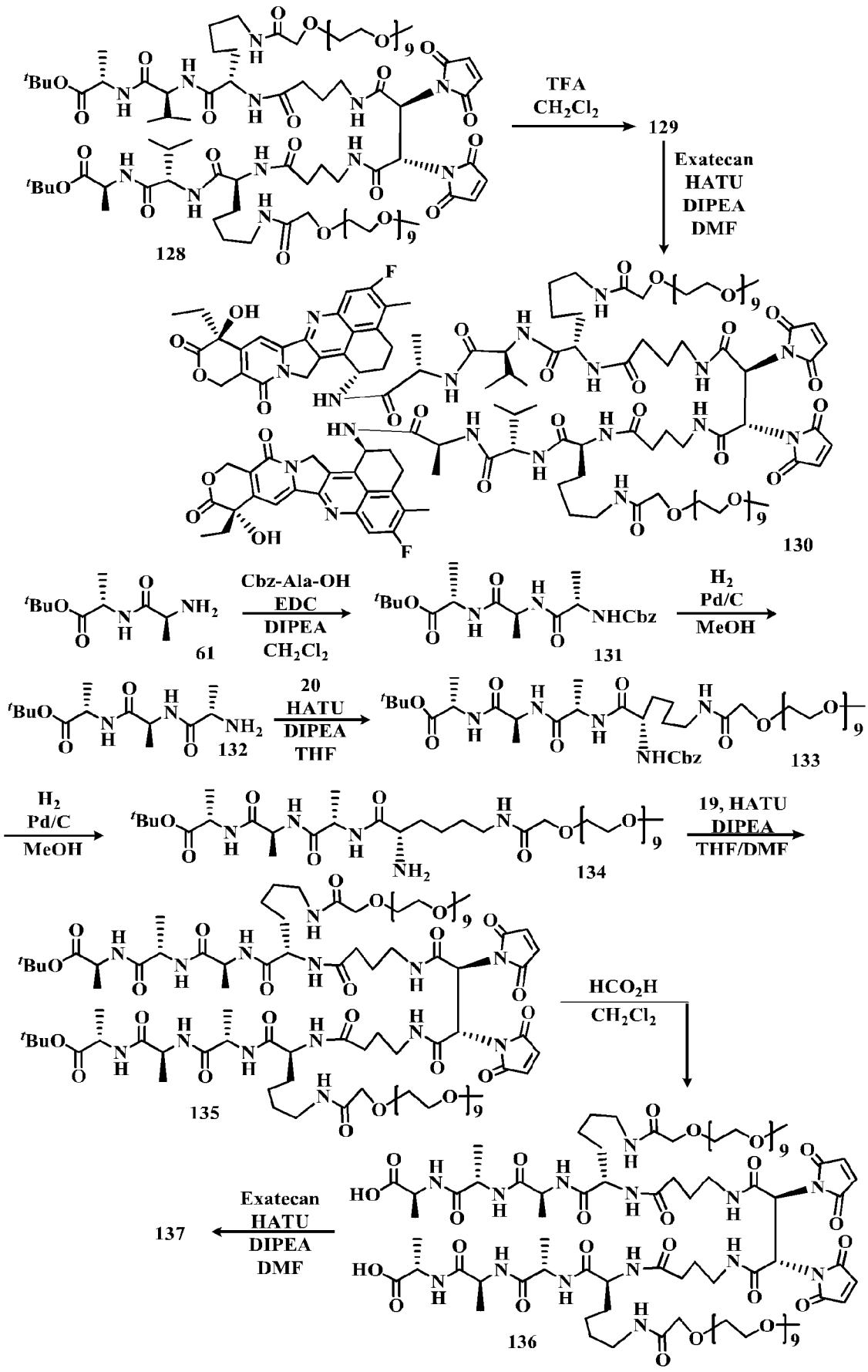

TFA
CH2Cl2
129
Exatecan
HATU
DIPEA
DMF
128
130
Cbz-Ala-OH
EDC
DIPEA
CH2Cl2
61
131
H2
Pd/C
MeOH
20
HATU
DIPEA
THF
132
133
NHCbz
H2
Pd/C
MeOH
134
19, HATU
DIPEA
THF/DMF
HCO2H
CH2Cl2
135
136
Exatecan
HATU
DIPEA
DMF
137

Fig. 20

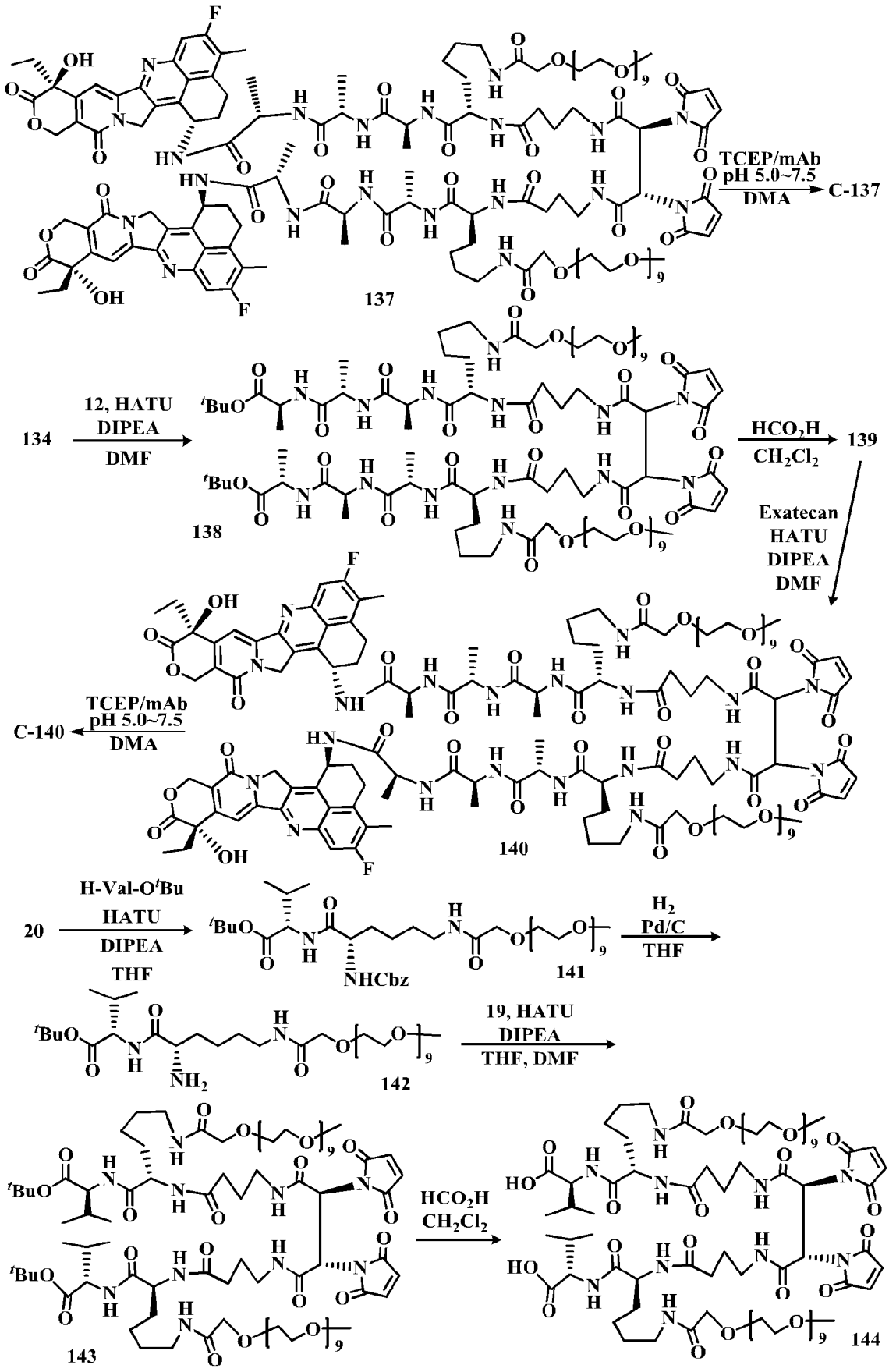

TCEP/mAb
pH 5.0~7.5
DMA
C-137
137
134
12, HATU
DIPEA
DMF
HCO2H
CH2Cl2
139
138
Exatecan
HATU
DIPEA
DMF
TCEP/mAb
pH 5.0~7.5
DMA
C-140
140
20
H-Val-OtBu
HATU
DIPEA
THF
141
H2
Pd/C
THF
142
19, HATU
DIPEA
THF, DMF
HCO2H
CH2Cl2
143
144

Fig. 21

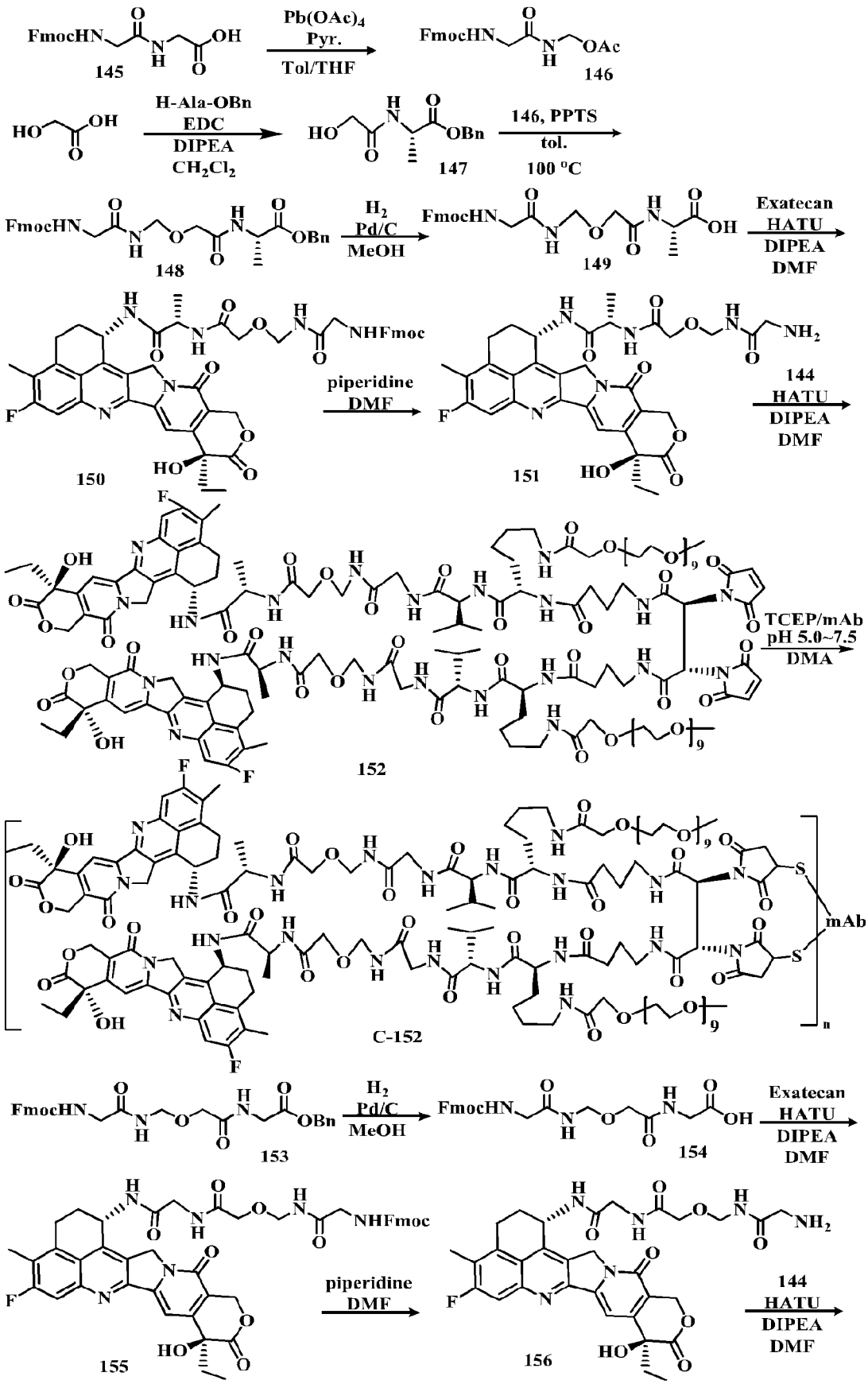

FmocHN
OH
145
Pb(OAc)4
Pyr.
Tol/THF
FmocHN
OAc
146
HO
OH
H-Ala-OBn
EDC
DIPEA
CH2Cl2
HO
OBn
147
146, PPTS
tol.
100 °C
FmocHN
OBn
148
H2
Pd/C
MeOH
FmocHN
OH
149
Exatecan
HATU
DIPEA
DMF
NHFmoc
150
piperidine
DMF
NH2
151
144
HATU
DIPEA
DMF
152
TCEP/mAb
pH 5.0~7.5
DMA
mAb
C-152
FmocHN
OBn
153
H2
Pd/C
MeOH
FmocHN
OH
154
Exatecan
HATU
DIPEA
DMF
NHFmoc
155
piperidine
DMF
NH2
156
144
HATU
DIPEA
DMF

Fig. 22

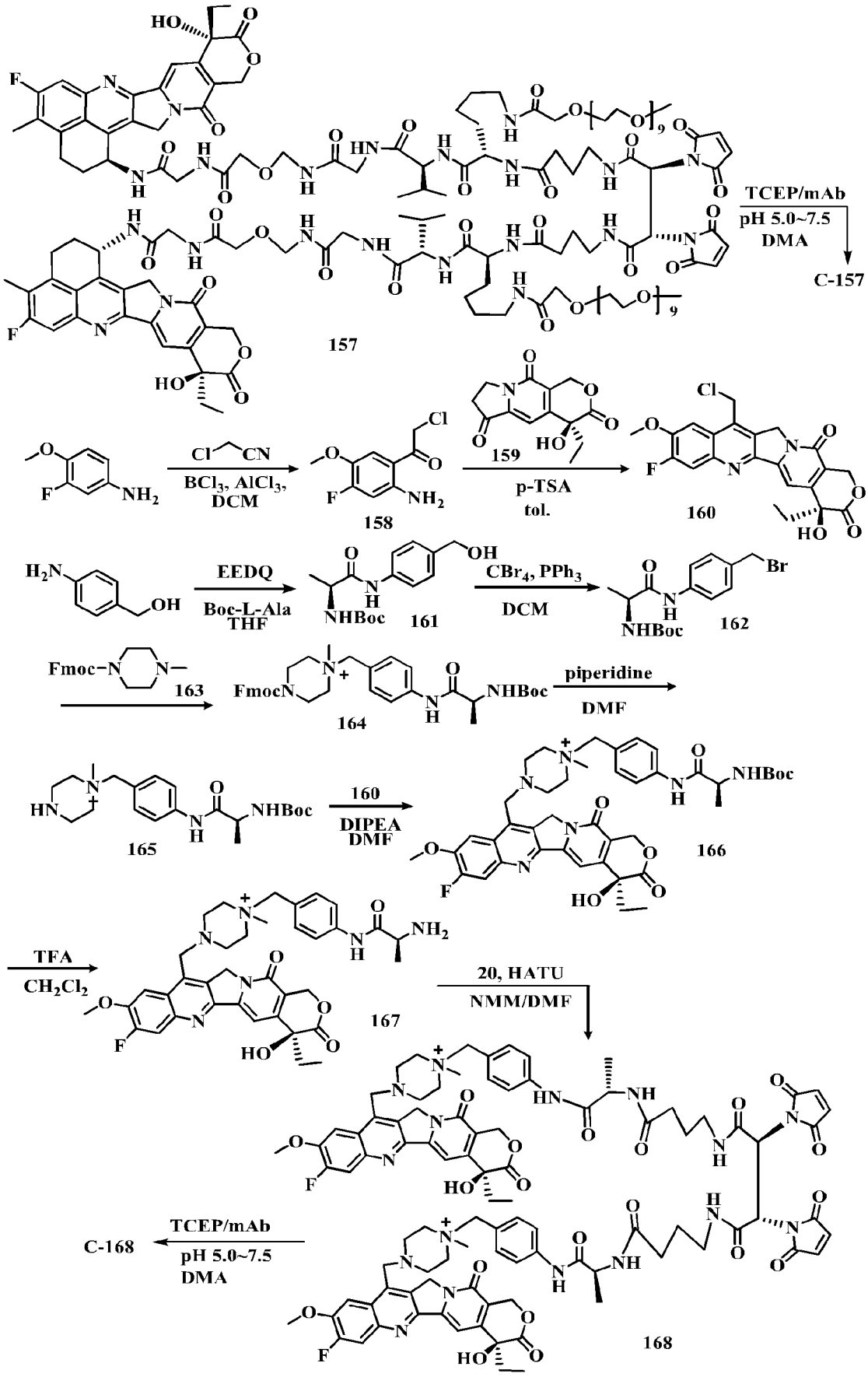

TCEP/mAb
pH 5.0~7.5
DMA
C-157
157
Cl—CN
BCl3, AlCl3,
DCM
158
159
p-TSA
tol.
160
EEDQ
Boc-L-Ala
THF
161
CBr4, PPh3
DCM
162
Fmoc
163
piperidine
DMF
164
165
160
DIPEA
DMF
166
TFA
CH2Cl2
167
20, HATU
NMM/DMF
TCEP/mAb
pH 5.0~7.5
DMA
C-168
168

Fig. 23

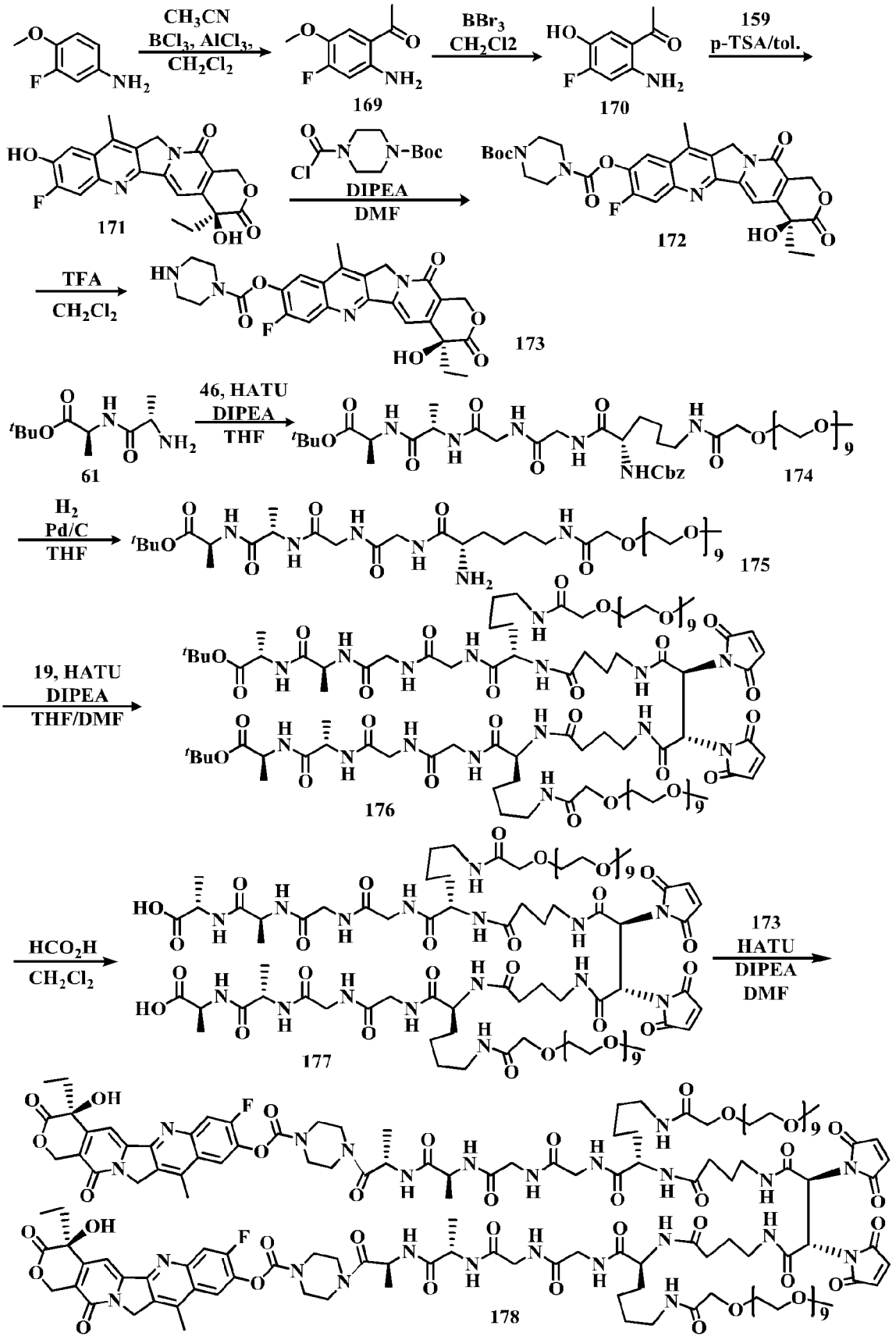

CH3CN
BCl3, AlCl3,
CH2Cl2
BBr3
CH2Cl2
159
p-TSA/tol.
169
170
171
DIPEA
DMF
172
TFA
CH2Cl2
173
46, HATU
DIPEA
THF
61
174
H2
Pd/C
THF
175
19, HATU
DIPEA
THF/DMF
176
HCO2H
CH2Cl2
177
173
HATU
DIPEA
DMF
178

Fig. 24

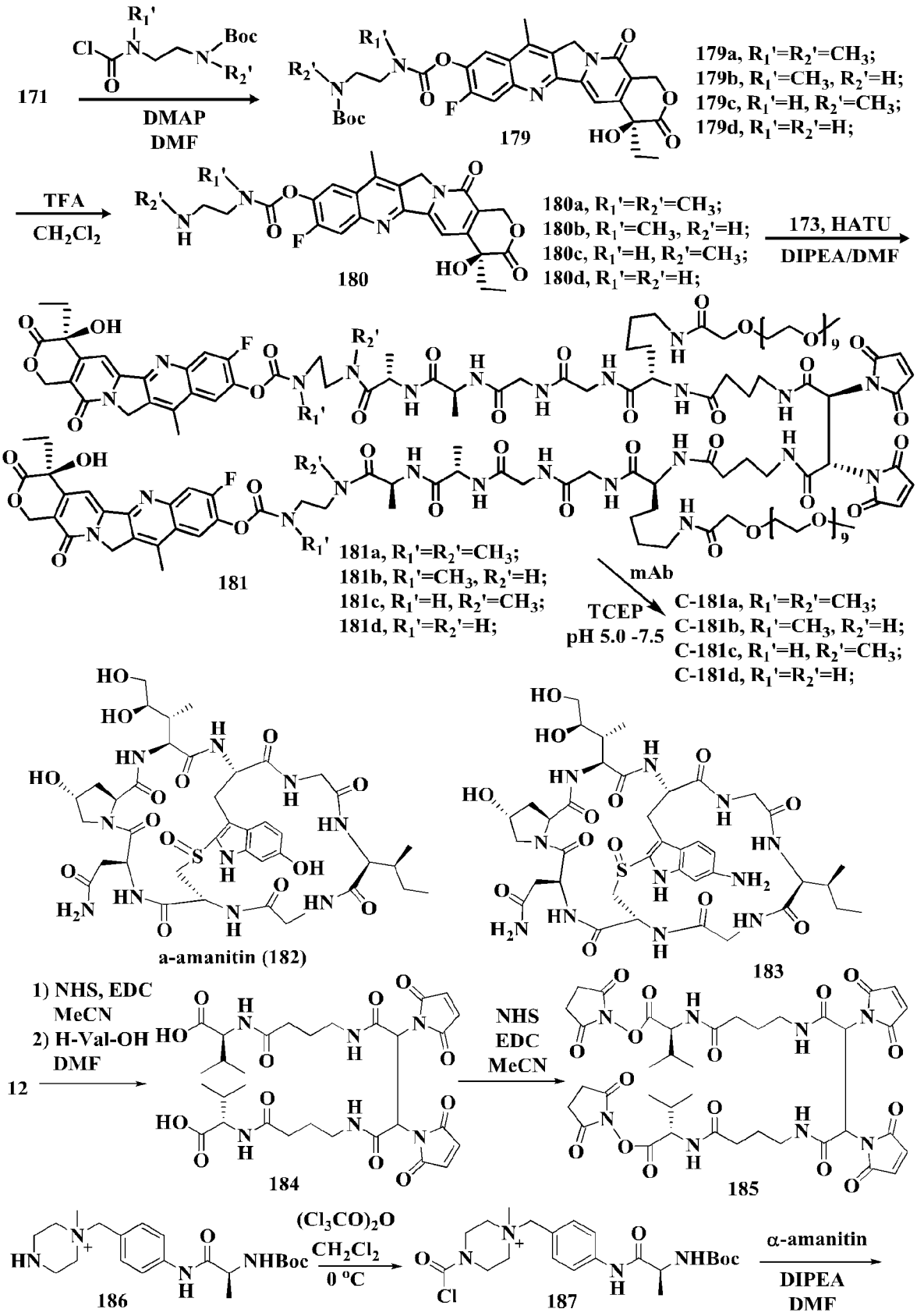

171
DMAP
DMF
179
179a, R1'=R2'=CH3;
179b, R1'=CH3, R2'=H;
179c, R1'=H, R2'=CH3;
179d, R1'=R2'=H;
TFA
CH2Cl2
180
180a, R1'=R2'=CH3;
180b, R1'=CH3, R2'=H;
180c, R1'=H, R2'=CH3;
180d, R1'=R2'=H;
173, HATU
DIPEA/DMF
181
181a, R1'=R2'=CH3;
181b, R1'=CH3, R2'=H;
181c, R1'=H, R2'=CH3;
181d, R1'=R2'=H;
mAb
TCEP
pH 5.0 -7.5
C-181a, R1'=R2'=CH3;
C-181b, R1'=CH3, R2'=H;
C-181c, R1'=H, R2'=CH3;
C-181d, R1'=R2'=H;
a-amanitin (182)
183
1) NHS, EDC
MeCN
2) H-Val-OH
DMF
12
184
NHS
EDC
MeCN
185
(Cl3CO)2O
CH2Cl2
0 °C
186
187
α-amanitin
DIPEA
DMF

Fig. 25

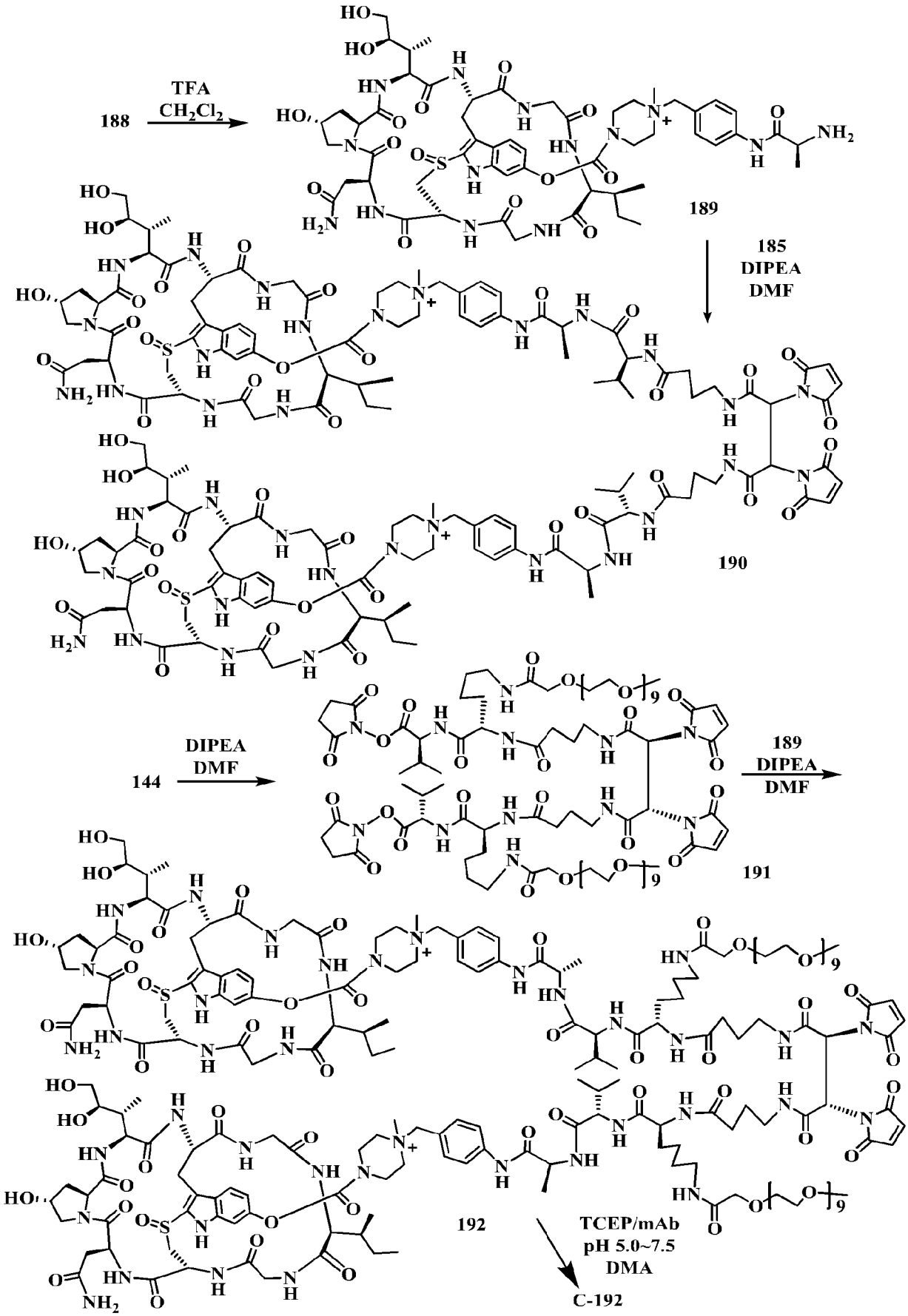

188
TFA
CH_2Cl_2
189
185
DIPEA
DMF
190
144
DIPEA
DMF
189
DIPEA
DMF
191
192
TCEP/mAb
pH 5.0~7.5
DMA
C-192

Fig. 26

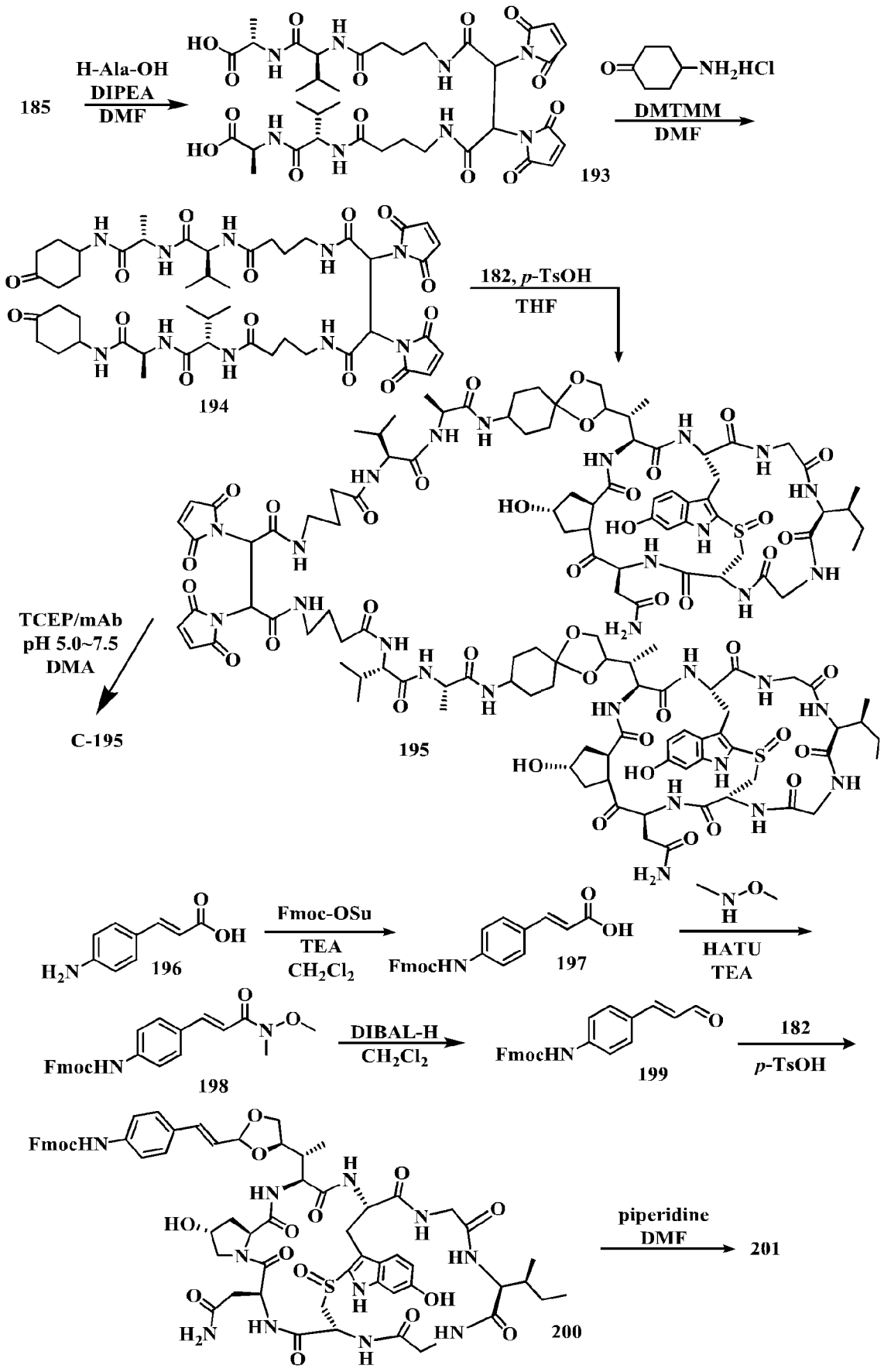

185
H-Ala-OH
DIPEA
DMF
193
NH2HCl
DMTMM
DMF
194
182, p-TsOH
THF
TCEP/mAb
pH 5.0~7.5
DMA
C-195
195
196
Fmoc-OSu
TEA
CH2Cl2
197
HATU
TEA
198
DIBAL-H
CH2Cl2
199
182
p-TsOH
200
piperidine
DMF
201

Fig. 27

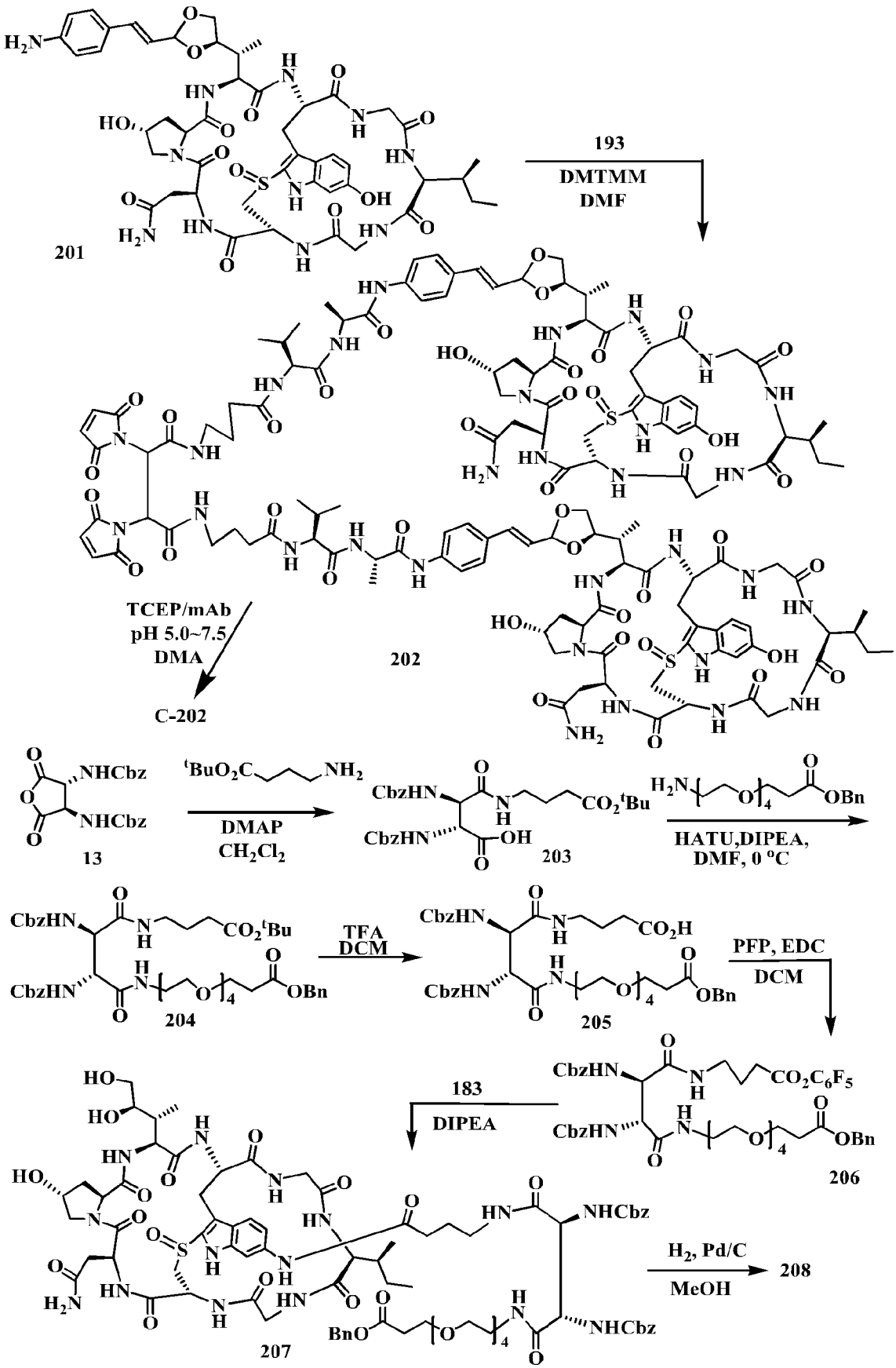

201
193
DMTMM
DMF
202
TCEP/mAb
pH 5.0~7.5
DMA
C-202
13
DMAP
CH2Cl2
203
HATU,DIPEA,
DMF, 0 °C
204
TFA
DCM
205
PFP, EDC
DCM
206
183
DIPEA
207
H2, Pd/C
MeOH
208

Fig. 28

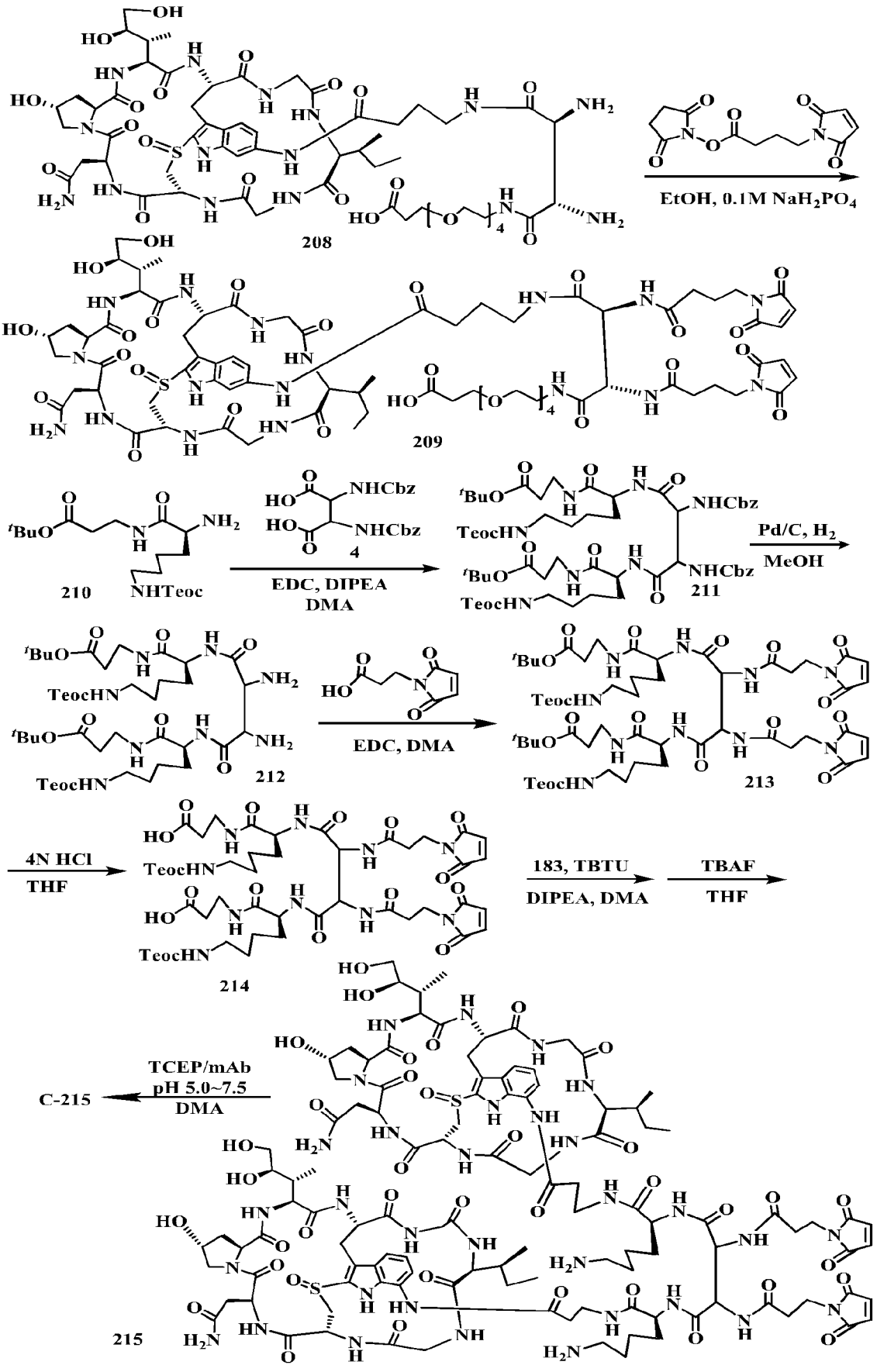

208
EtOH, 0.1M NaH2PO4
209
210
NHTeoc
4
EDC, DIPEA
DMA
211
Pd/C, H2
MeOH
212
EDC, DMA
213
4N HCl
THF
214
183, TBTU
DIPEA, DMA
TBAF
THF
TCEP/mAb
pH 5.0~7.5
DMA
C-215
215

Fig. 29

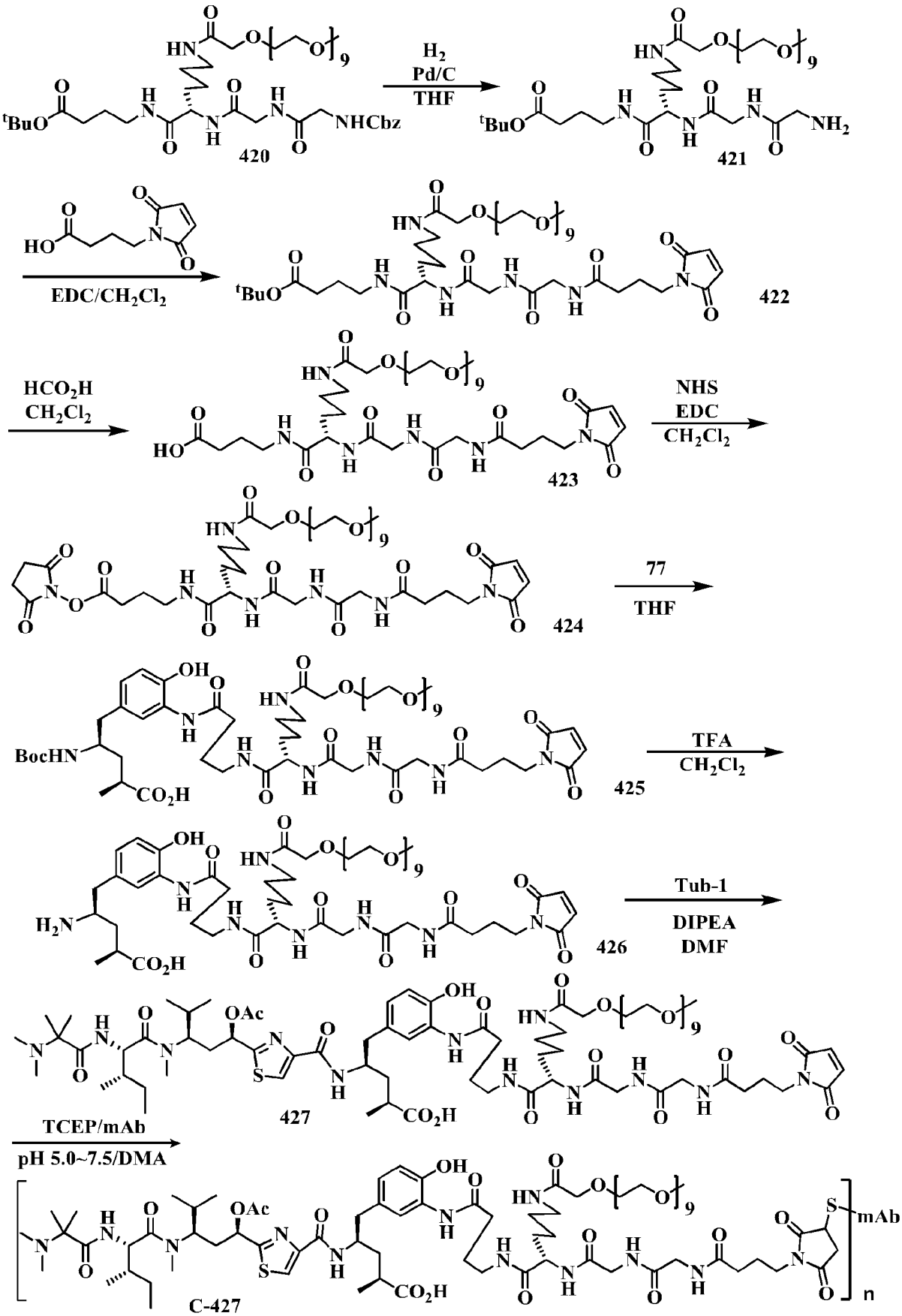

H2
Pd/C
THF
420
421
EDC/CH2Cl2
422
HCO2H
CH2Cl2
423
NHS
EDC
CH2Cl2
424
77
THF
425
TFA
CH2Cl2
426
Tub-1
DIPEA
DMF
427
TCEP/mAb
pH 5.0~7.5/DMA
C-427
mAb

Fig. 30

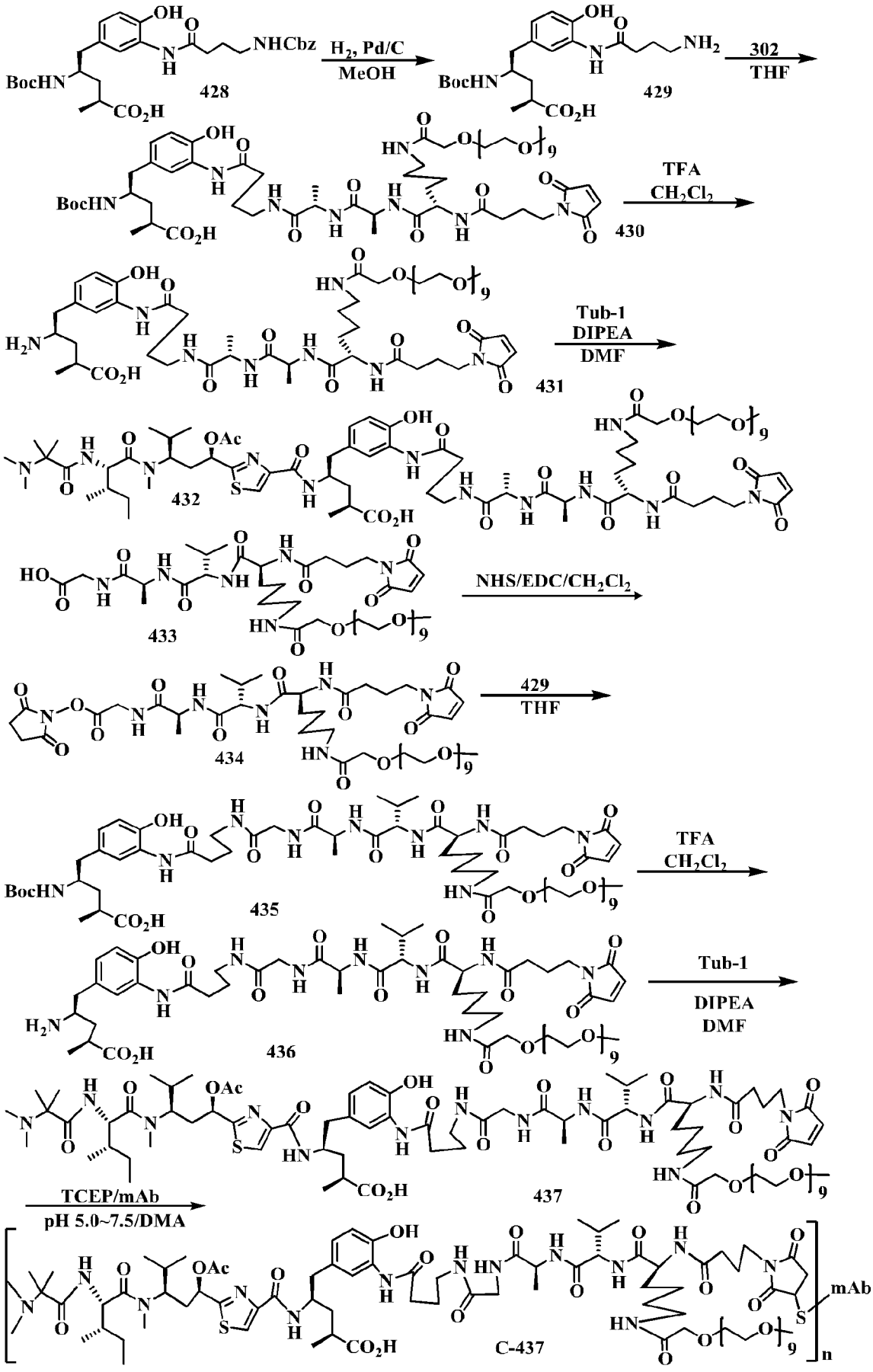

428
H2, Pd/C
MeOH
429
302
THF
TFA
CH2Cl2
430
Tub-1
DIPEA
DMF
431
432
433
NHS/EDC/CH2Cl2
434
429
THF
435
TFA
CH2Cl2
436
Tub-1
DIPEA
DMF
437
TCEP/mAb
pH 5.0~7.5/DMA
C-437
mAb

Fig. 31

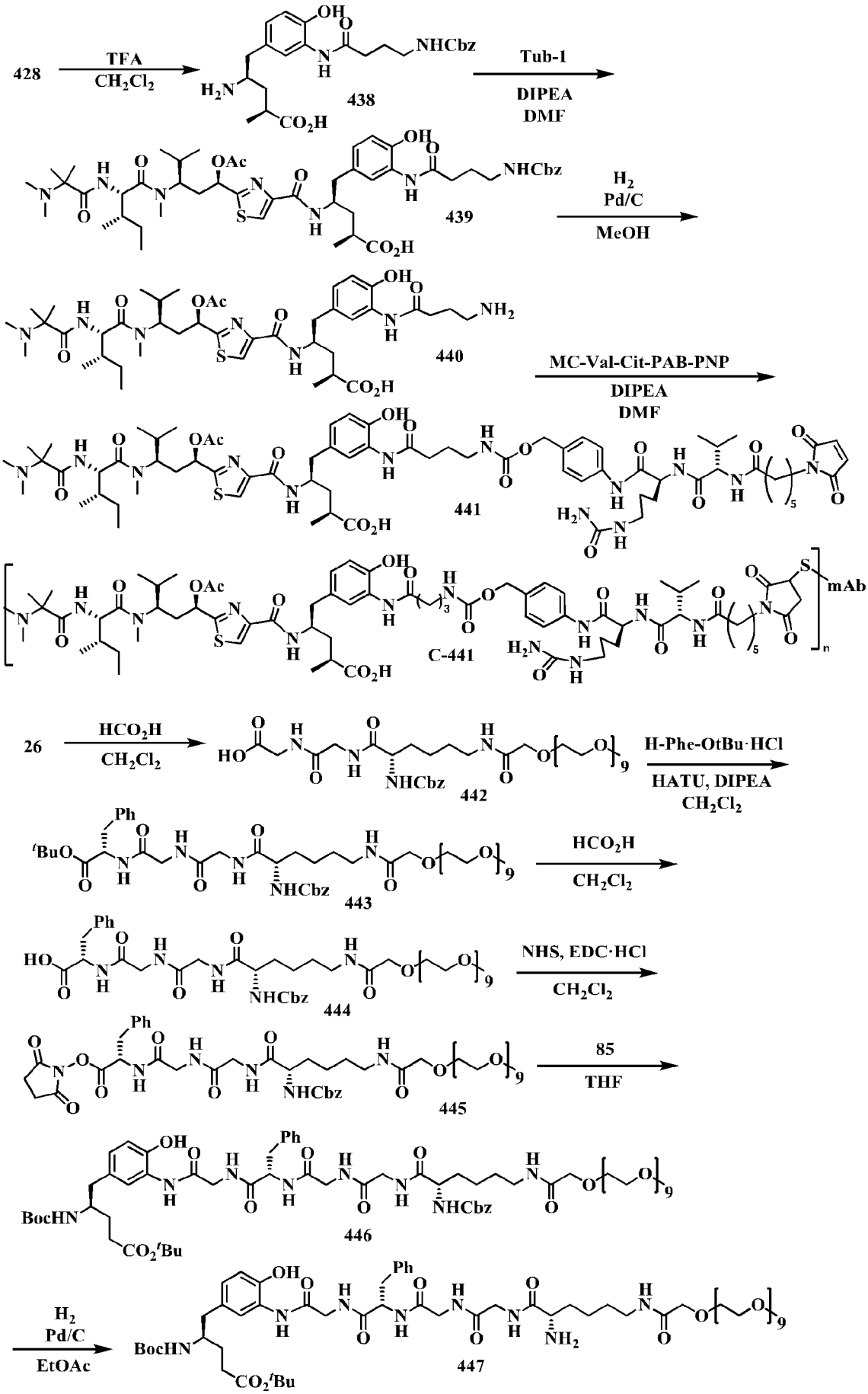

428
TFA
CH2Cl2
438
Tub-1
DIPEA
DMF
439
H2
Pd/C
MeOH
440
MC-Val-Cit-PAB-PNP
DIPEA
DMF
441
C-441
mAb
26
HCO2H
CH2Cl2
442
H-Phe-OtBu·HCl
HATU, DIPEA
CH2Cl2
443
HCO2H
CH2Cl2
444
NHS, EDC·HCl
CH2Cl2
445
85
THF
446
H2
Pd/C
EtOAc
447

Fig. 32

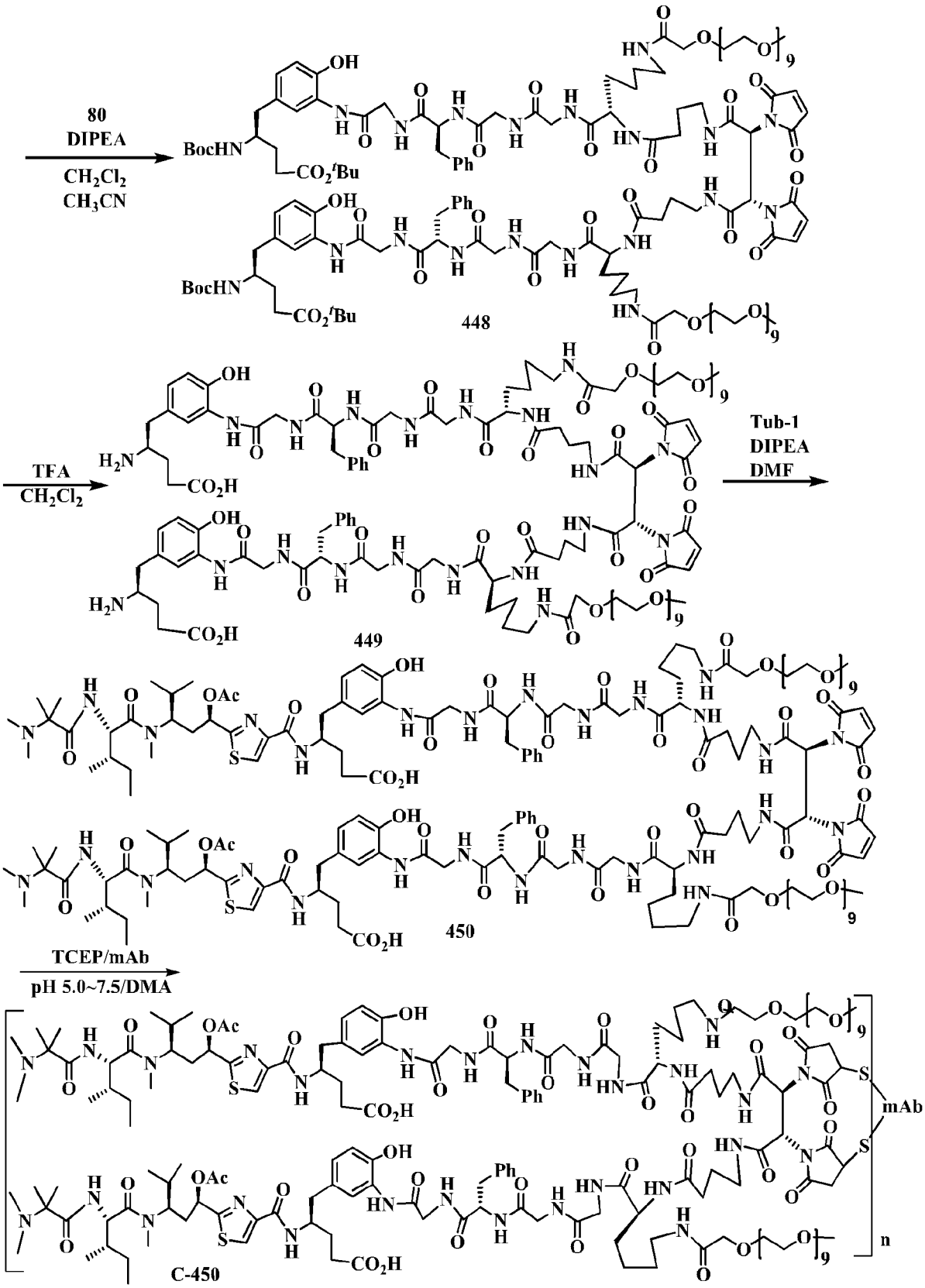

80
DIPEA
CH2Cl2
CH3CN
BocHN
CO2tBu
448
TFA
CH2Cl2
H2N
CO2H
449
Tub-1
DIPEA
DMF
OAc
450
TCEP/mAb
pH 5.0~7.5/DMA
mAb
C-450

Fig. 33

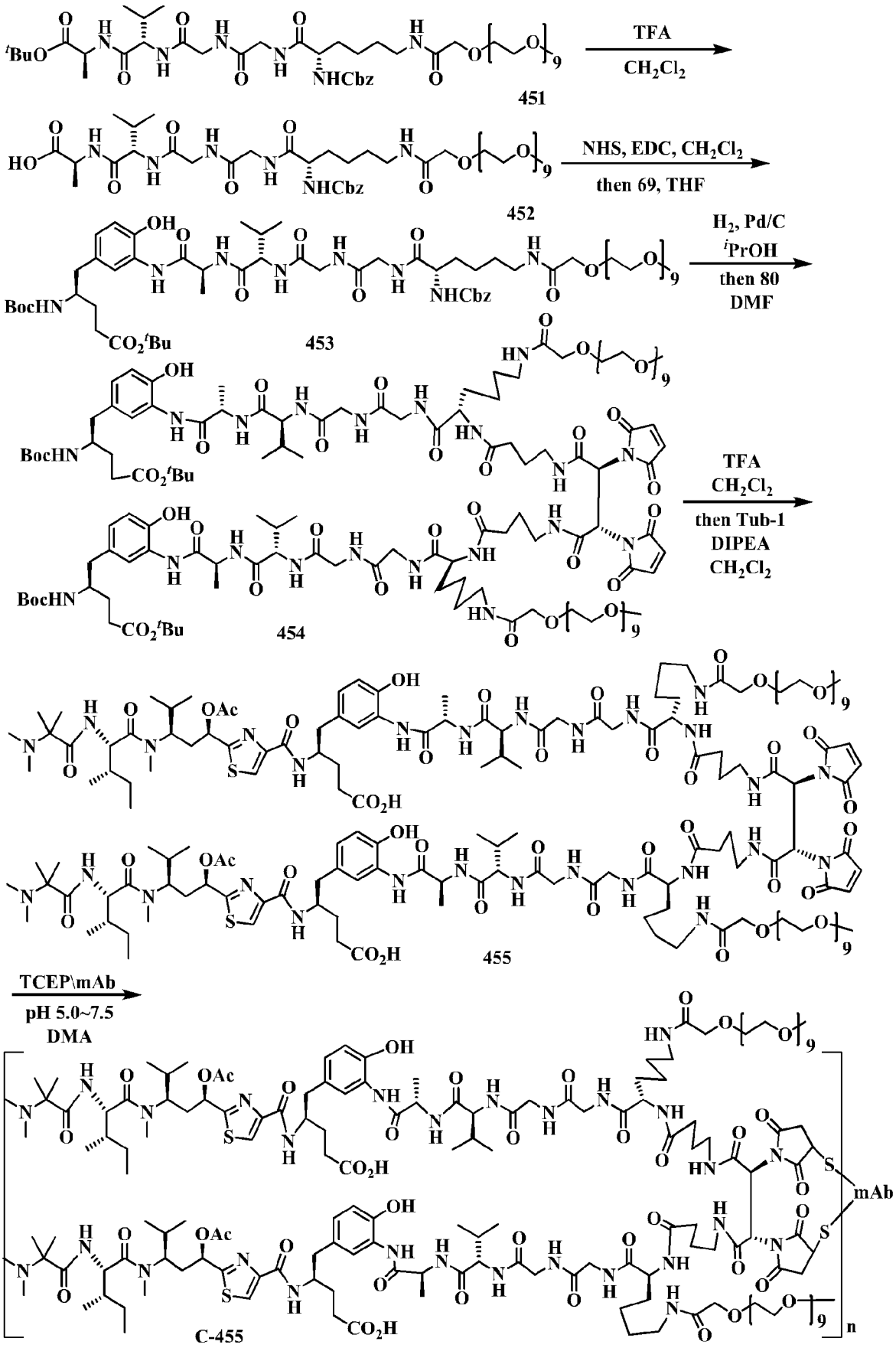

TFA
CH2Cl2
451
NHS, EDC, CH2Cl2
then 69, THF
452
H2, Pd/C
iPrOH
then 80
DMF
453
TFA
CH2Cl2
then Tub-1
DIPEA
CH2Cl2
454
455
TCEP\mAb
pH 5.0~7.5
DMA
C-455
mAb

Fig. 34

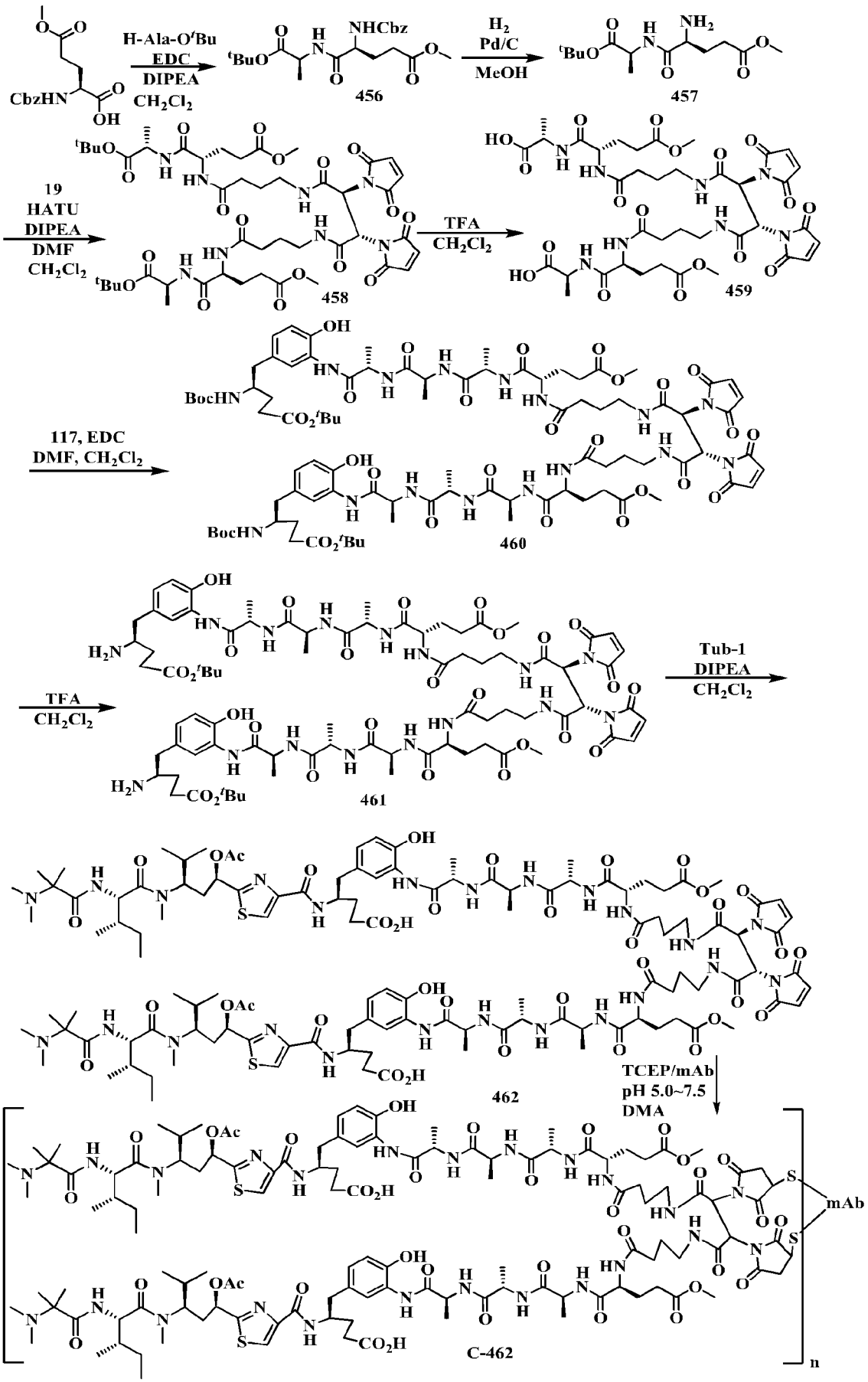

H-Ala-O^{t}Bu
EDC
DIPEA
CH_2Cl_2
456
H_2
Pd/C
MeOH
457
19
HATU
DIPEA
DMF
CH_2Cl_2
458
TFA
CH_2Cl_2
459
117, EDC
DMF, CH_2Cl_2
460
TFA
CH_2Cl_2
461
Tub-1
DIPEA
CH_2Cl_2
462
TCEP/mAb
pH 5.0~7.5
DMA
C-462
mAb
n

Fig. 35

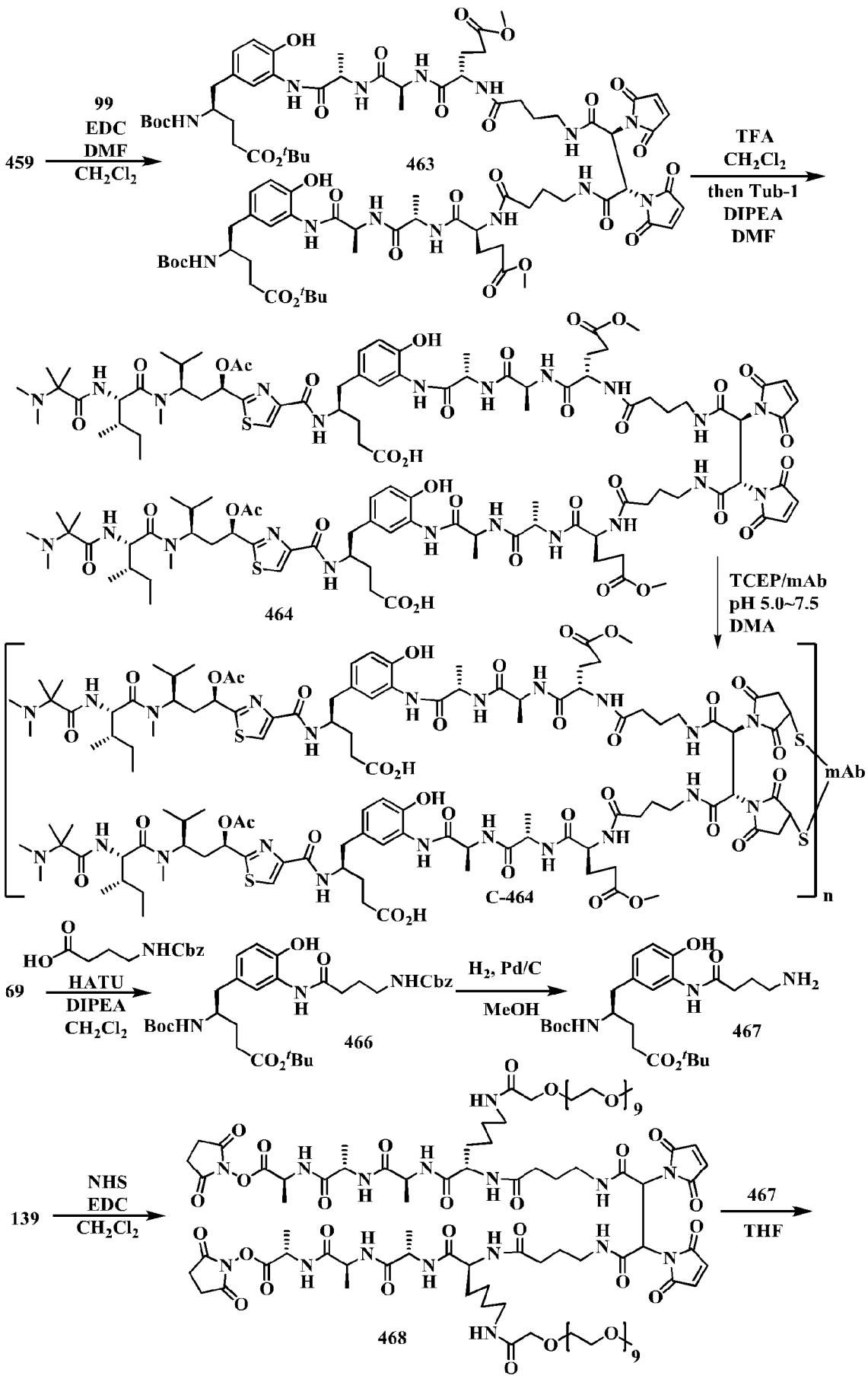

99
EDC
DMF
CH_2Cl_2
459
463
TFA
CH_2Cl_2
then Tub-1
DIPEA
DMF
464
TCEP/mAb
pH 5.0~7.5
DMA
C-464
mAb
n
69
HATU
DIPEA
CH_2Cl_2
466
H_2, Pd/C
MeOH
467
139
NHS
EDC
CH_2Cl_2
468
467
THF

Fig. 36

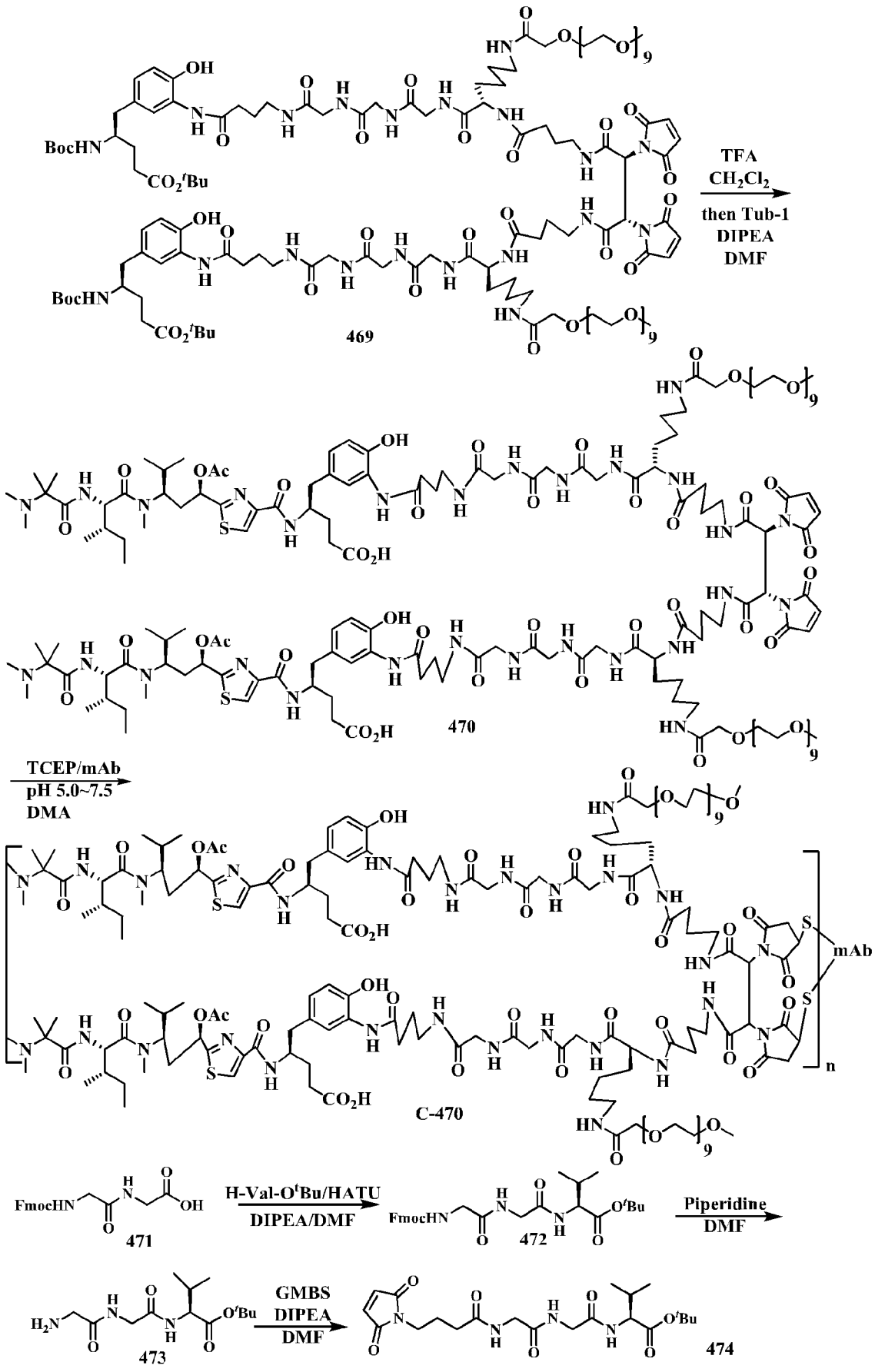

TFA
CH2Cl2
then Tub-1
DIPEA
DMF
469
470
TCEP/mAb
pH 5.0~7.5
DMA
C-470
mAb
FmocHN
471
H-Val-OtBu/HATU
DIPEA/DMF
472
Piperidine
DMF
473
GMBS
DIPEA
DMF
474

Fig. 37

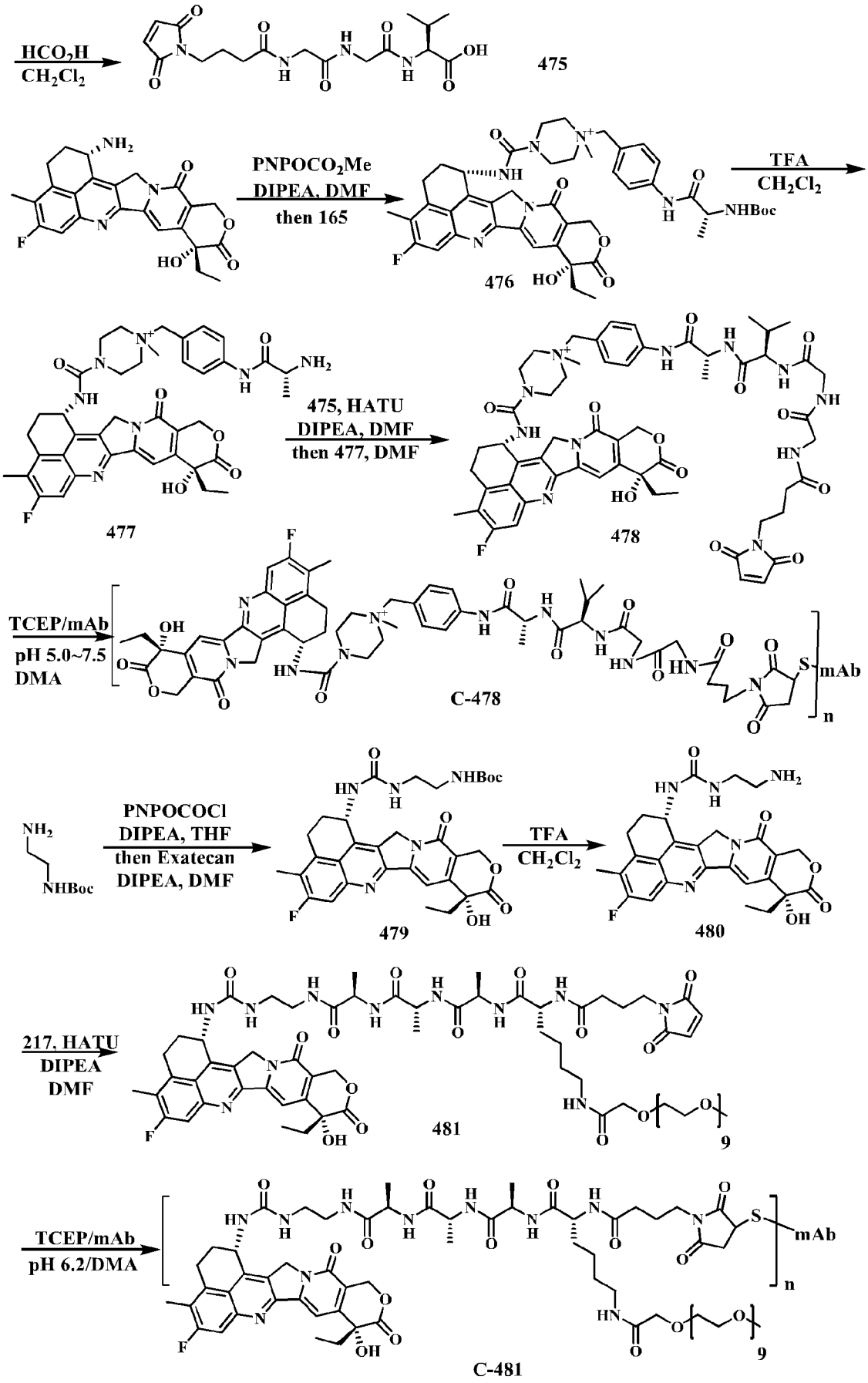

HCO2H
CH2Cl2
475
PNPOCO2Me
DIPEA, DMF
then 165
476
TFA
CH2Cl2
477
475, HATU
DIPEA, DMF
then 477, DMF
478
TCEP/mAb
pH 5.0~7.5
DMA
C-478
PNPOCOCl
DIPEA, THF
then Exatecan
DIPEA, DMF
479
TFA
CH2Cl2
480
217, HATU
DIPEA
DMF
481
9
TCEP/mAb
pH 6.2/DMA
C-481

Fig. 38

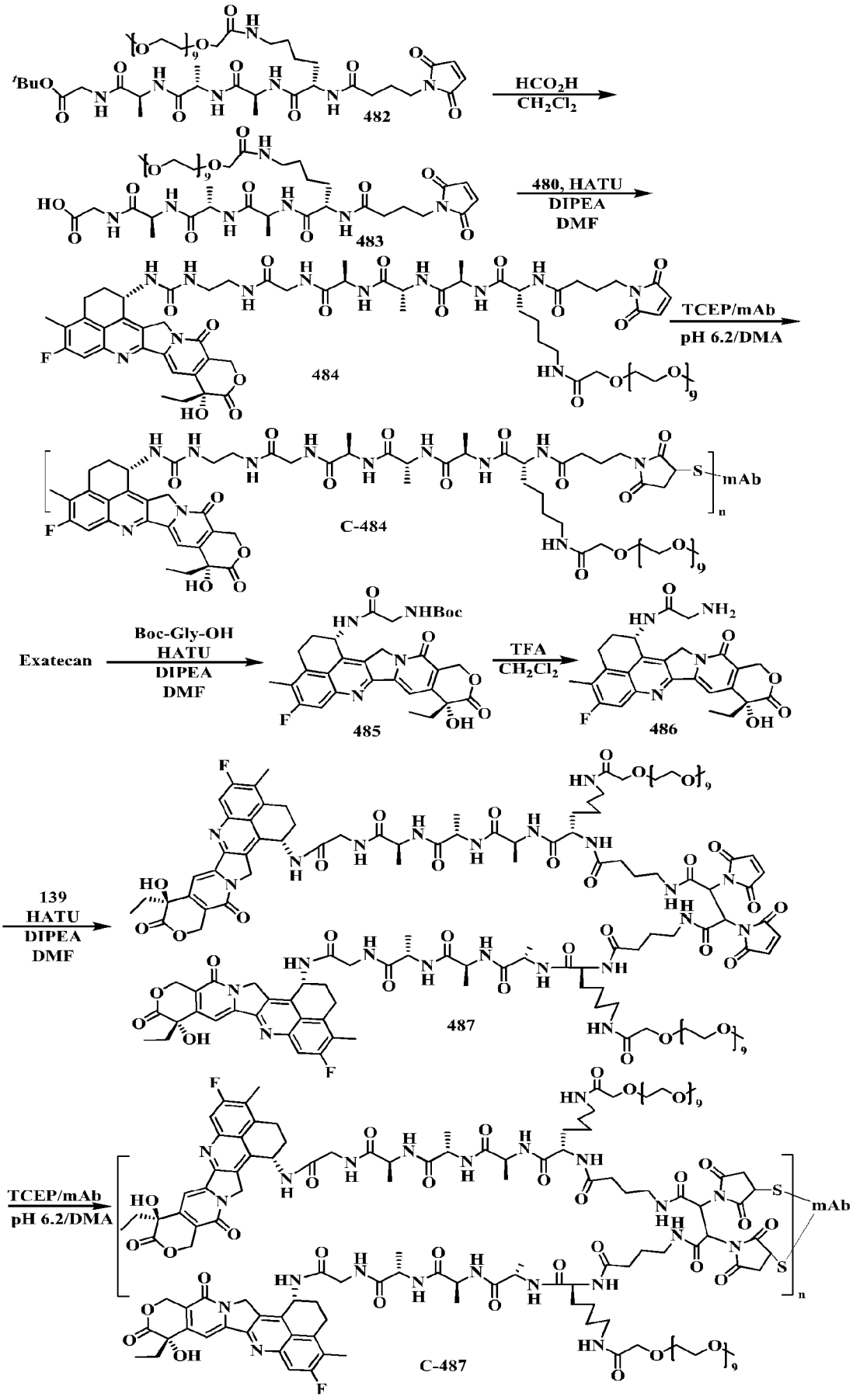

HCO2H
CH2Cl2
482
480, HATU
DIPEA
DMF
483
TCEP/mAb
pH 6.2/DMA
484
C-484
mAb
Exatecan
Boc-Gly-OH
HATU
DIPEA
DMF
485
TFA
CH2Cl2
486
139
HATU
DIPEA
DMF
487
TCEP/mAb
pH 6.2/DMA
C-487

Fig. 39

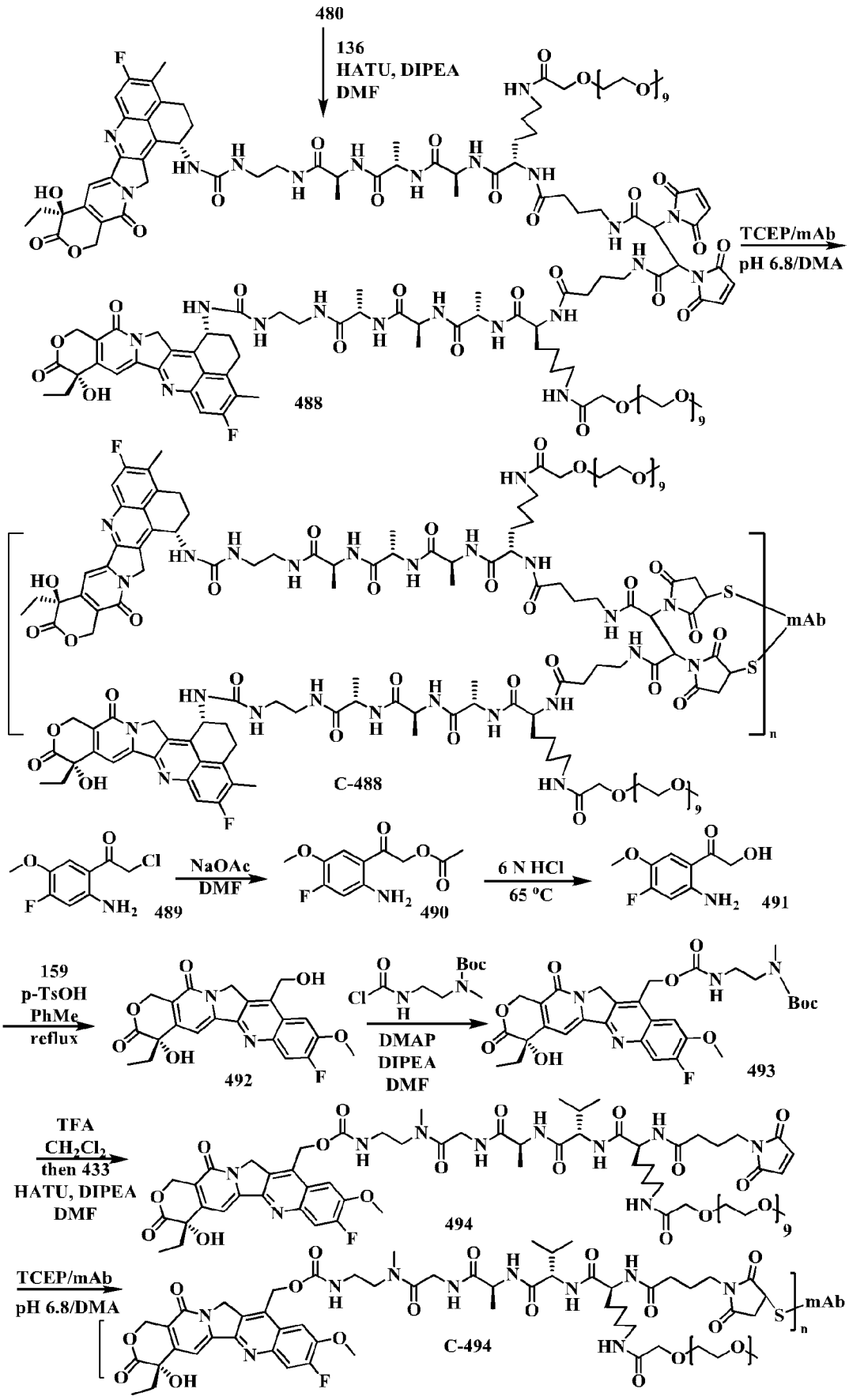

480
136
HATU, DIPEA
DMF
TCEP/mAb
pH 6.8/DMA
488
C-488
mAb
NaOAc
DMF
6 N HCl
65 °C
489
490
491
159
p-TsOH
PhMe
reflux
492
DMAP
DIPEA
DMF
493
TFA
CH2Cl2
then 433
HATU, DIPEA
DMF
494
TCEP/mAb
pH 6.8/DMA
C-494

Fig. 40

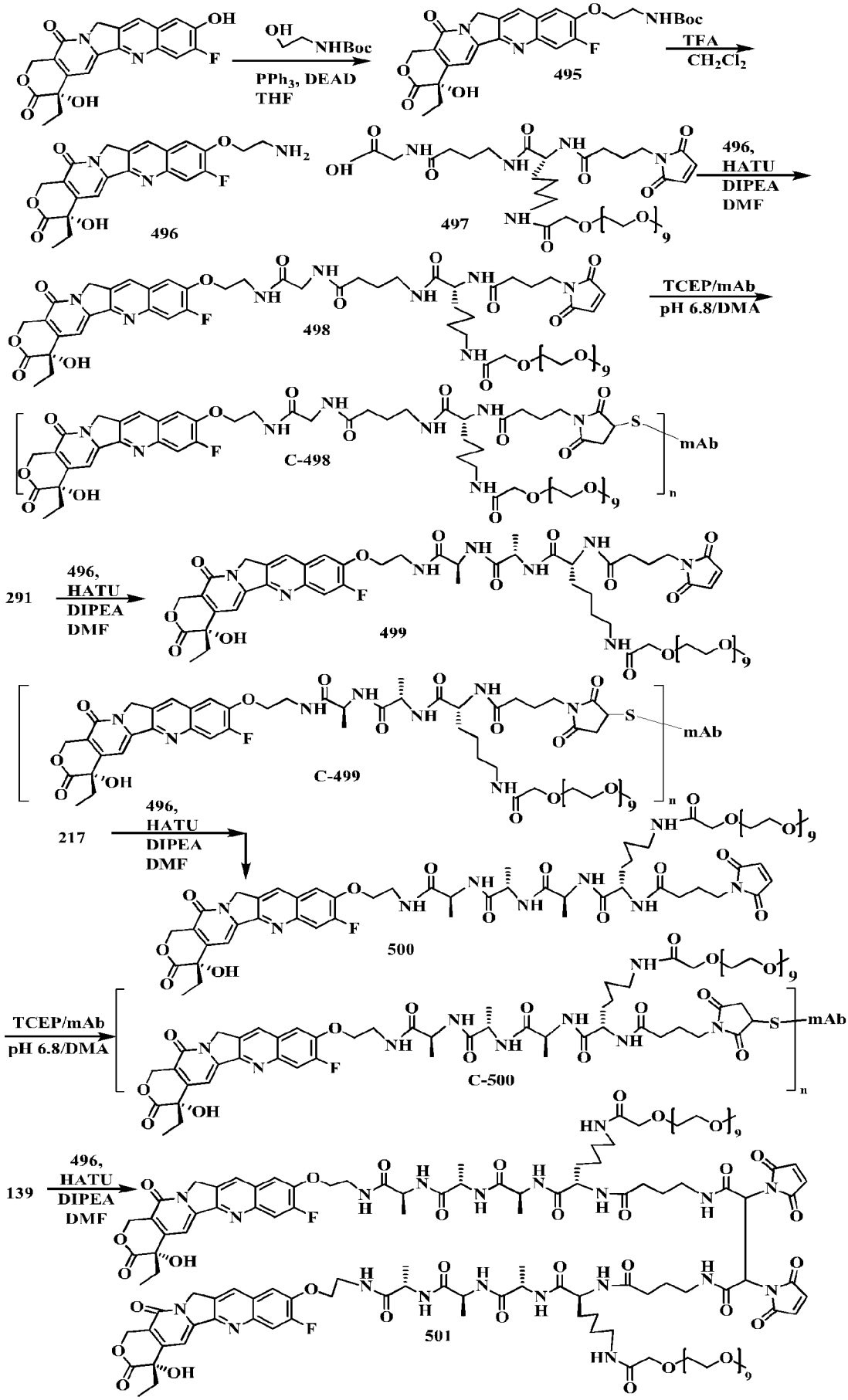

PPh3, DEAD
THF
TFA
CH2Cl2
495
496
497
496,
HATU
DIPEA
DMF
498
TCEP/mAb
pH 6.8/DMA
C-498
mAb
291
499
C-499
217
500
C-500
139
501

Fig. 41

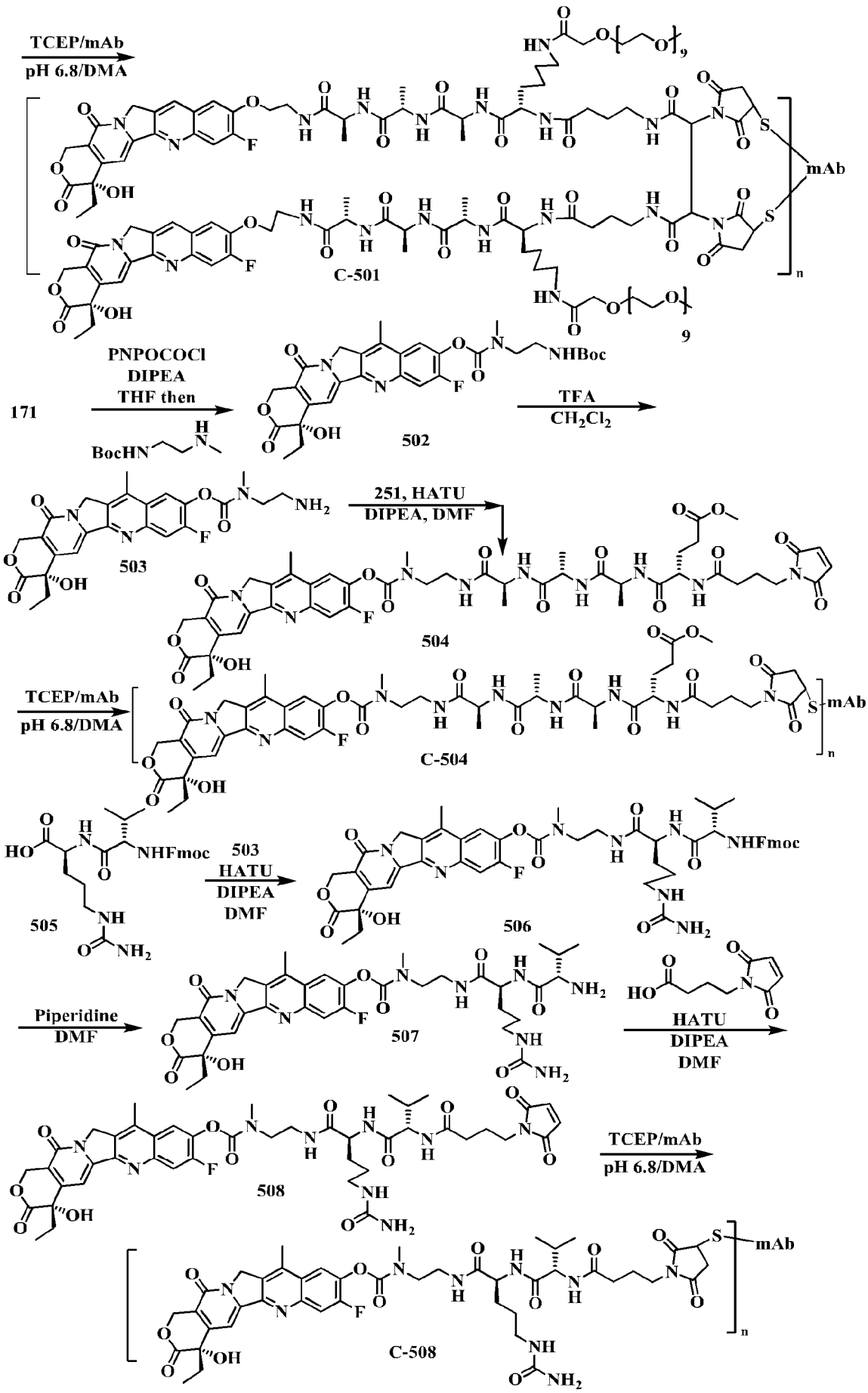

TCEP/mAb
pH 6.8/DMA
C-501
mAb
PNPOCOCl
DIPEA
THF then
171
BocHN
502
NHBoc
TFA
CH2Cl2
503
NH2
251, HATU
DIPEA, DMF
504
TCEP/mAb
pH 6.8/DMA
C-504
505
NHFmoc
503
HATU
DIPEA
DMF
506
Piperidine
DMF
507
HATU
DIPEA
DMF
508
TCEP/mAb
pH 6.8/DMA
C-508

Fig. 42

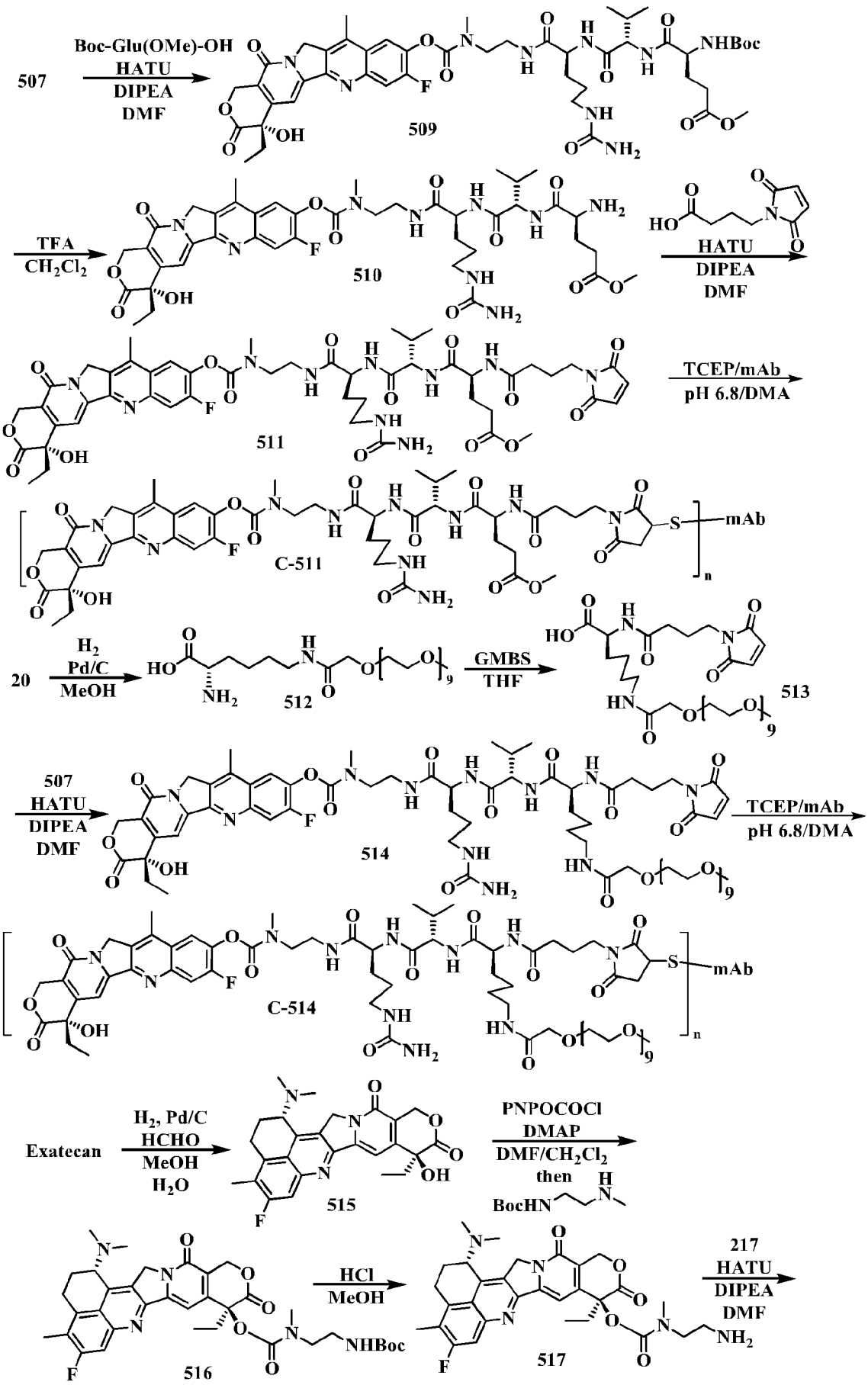

507
Boc-Glu(OMe)-OH
HATU
DIPEA
DMF
509
TFA
CH_2Cl_2
510
HATU
DIPEA
DMF
511
TCEP/mAb
pH 6.8/DMA
C-511
mAb
20
H_2
Pd/C
MeOH
512
GMBS
THF
513
507
HATU
DIPEA
DMF
514
TCEP/mAb
pH 6.8/DMA
C-514
mAb
Exatecan
H_2, Pd/C
HCHO
MeOH
H_2O
515
PNPOCOCl
DMAP
DMF/CH_2Cl_2
then
BocHN
HCl
MeOH
516
217
HATU
DIPEA
DMF
517

Fig. 43

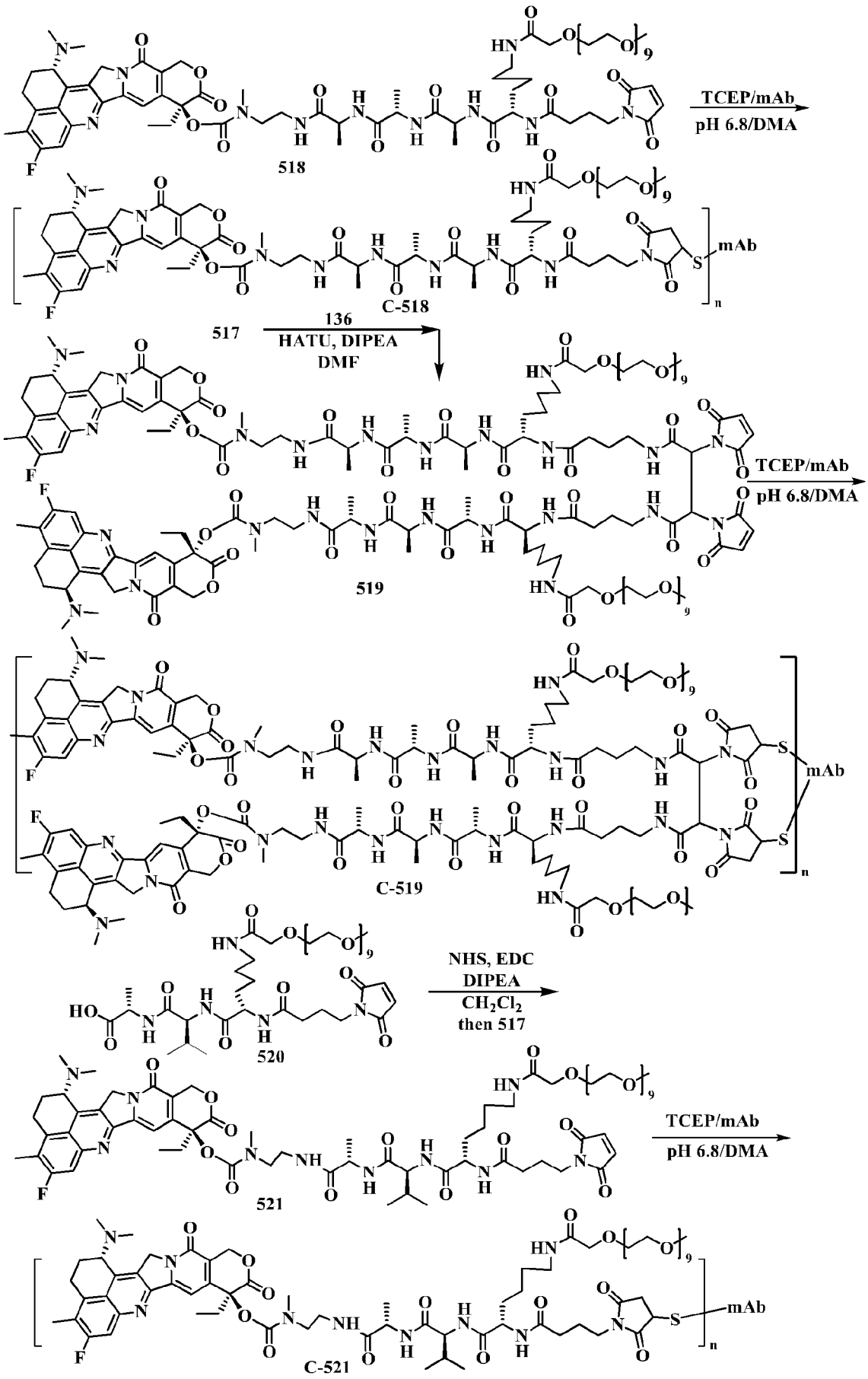

518
TCEP/mAb
pH 6.8/DMA
C-518
517
136
HATU, DIPEA
DMF
519
TCEP/mAb
pH 6.8/DMA
C-519
520
NHS, EDC
DIPEA
CH_2Cl_2
then 517
521
TCEP/mAb
pH 6.8/DMA
C-521

Fig. 44

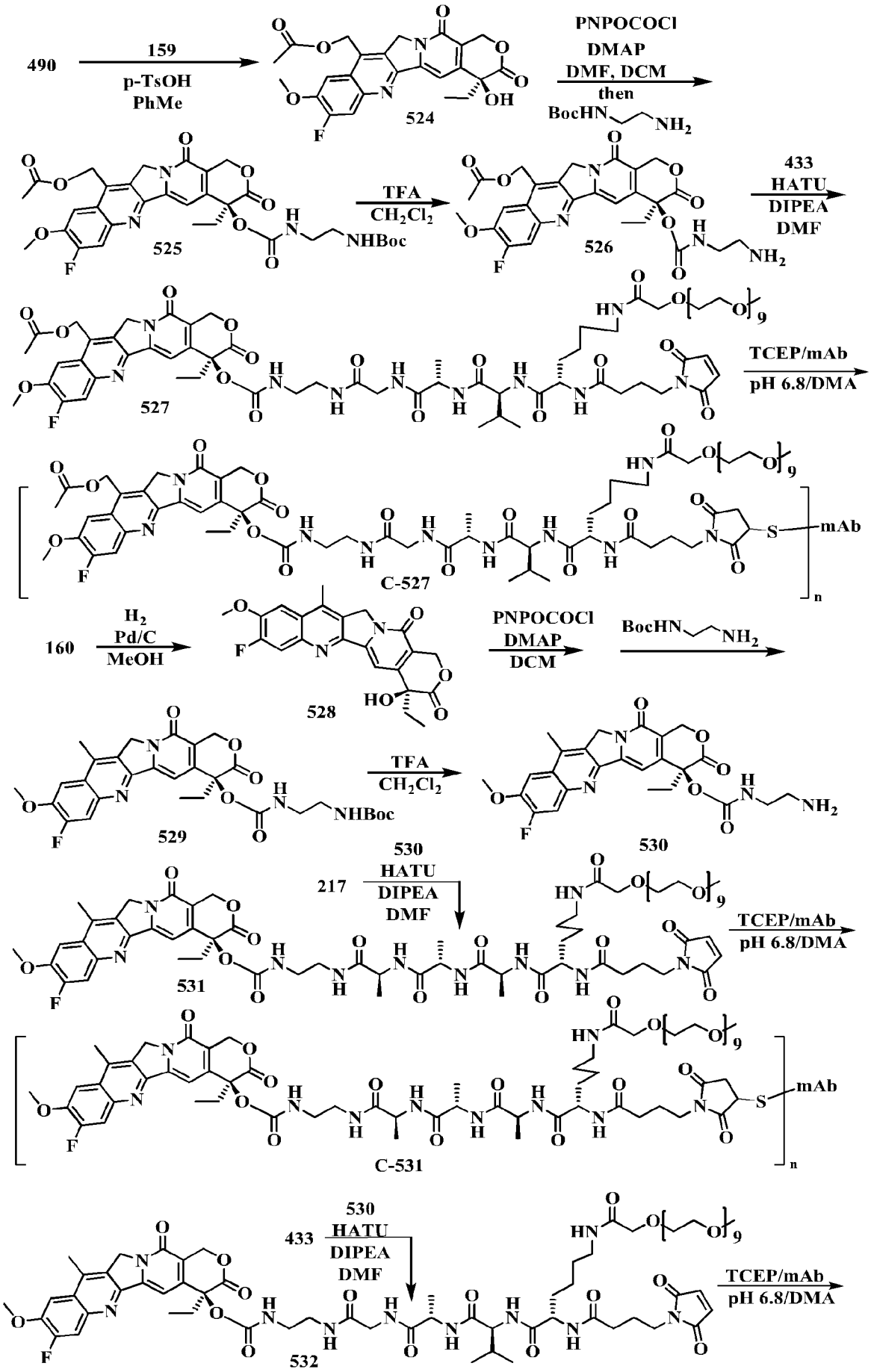

490
159
p-TsOH
PhMe
524
PNPOCOCl
DMAP
DMF, DCM
then
BocHN
NH2
525
TFA
CH2Cl2
NHBoc
526
433
HATU
DIPEA
DMF
527
TCEP/mAb
pH 6.8/DMA
C-527
mAb
160
H2
Pd/C
MeOH
528
PNPOCOCl
DMAP
DCM
529
530
217
531
C-531
532

Fig. 45

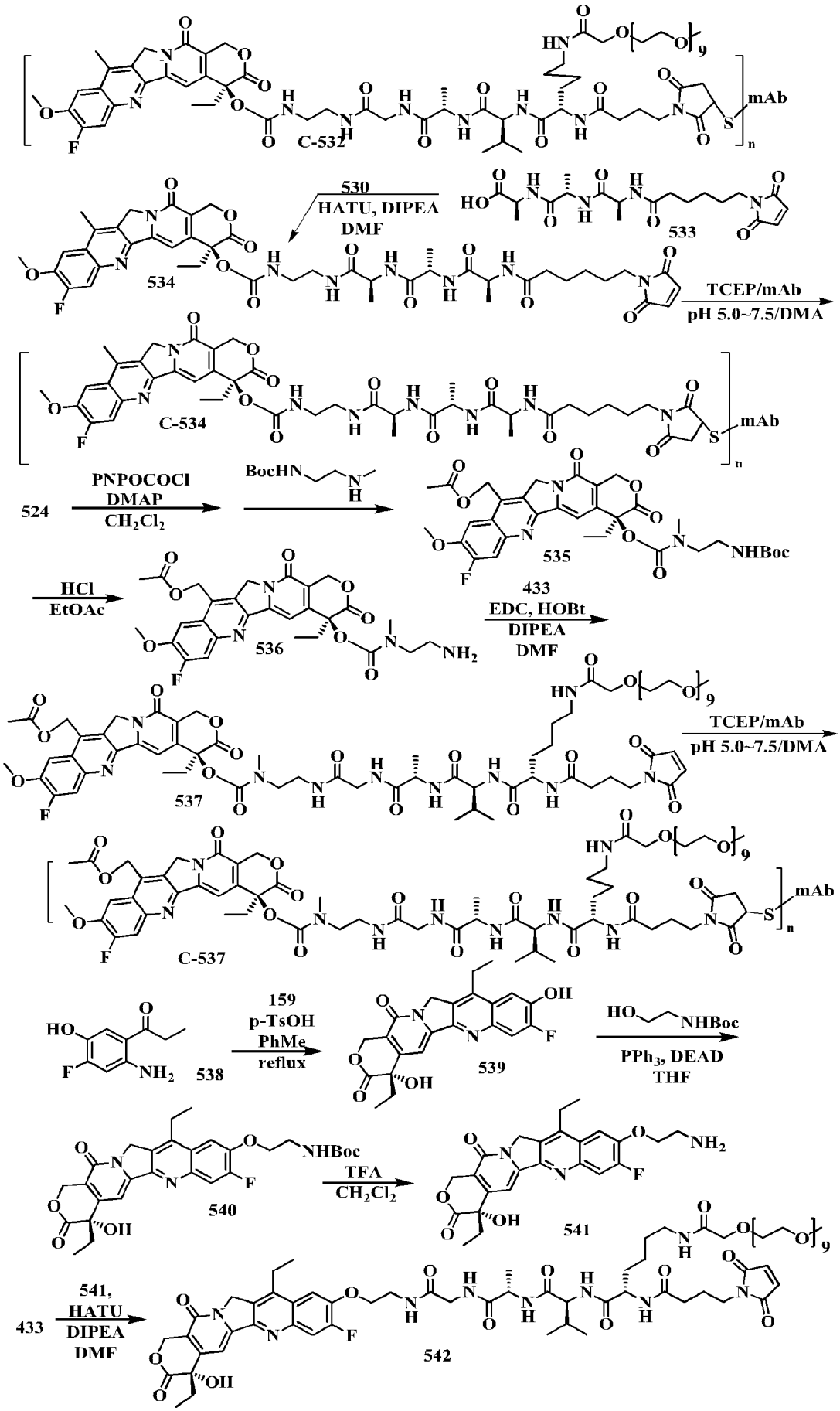

C-532
530
HATU, DIPEA
DMF
533
534
TCEP/mAb
pH 5.0~7.5/DMA
C-534
524
PNPOCOCl
DMAP
CH2Cl2
BocHN
535
HCl
EtOAc
536
433
EDC, HOBt
DIPEA
DMF
537
TCEP/mAb
pH 5.0~7.5/DMA
C-537
159
p-TsOH
PhMe
reflux
538
539
PPh3, DEAD
THF
540
TFA
CH2Cl2
541
541,
HATU
DIPEA
DMF
433
542

Fig. 46

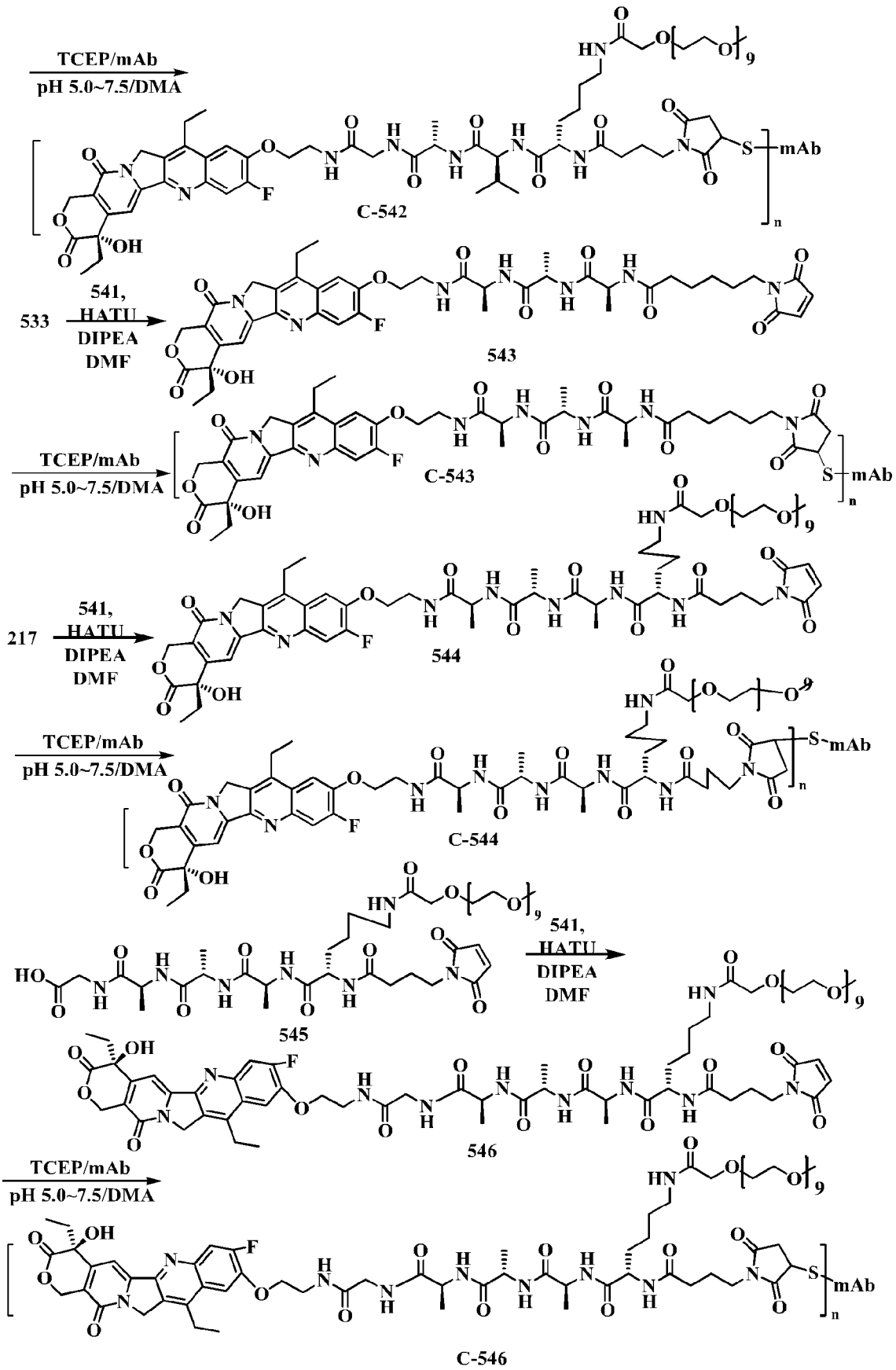

TCEP/mAb
pH 5.0~7.5/DMA
C-542
533
541,
HATU
DIPEA
DMF
543
TCEP/mAb
pH 5.0~7.5/DMA
C-543
217
541,
HATU
DIPEA
DMF
544
TCEP/mAb
pH 5.0~7.5/DMA
C-544
545
541,
HATU
DIPEA
DMF
546
TCEP/mAb
pH 5.0~7.5/DMA
C-546

Fig. 47

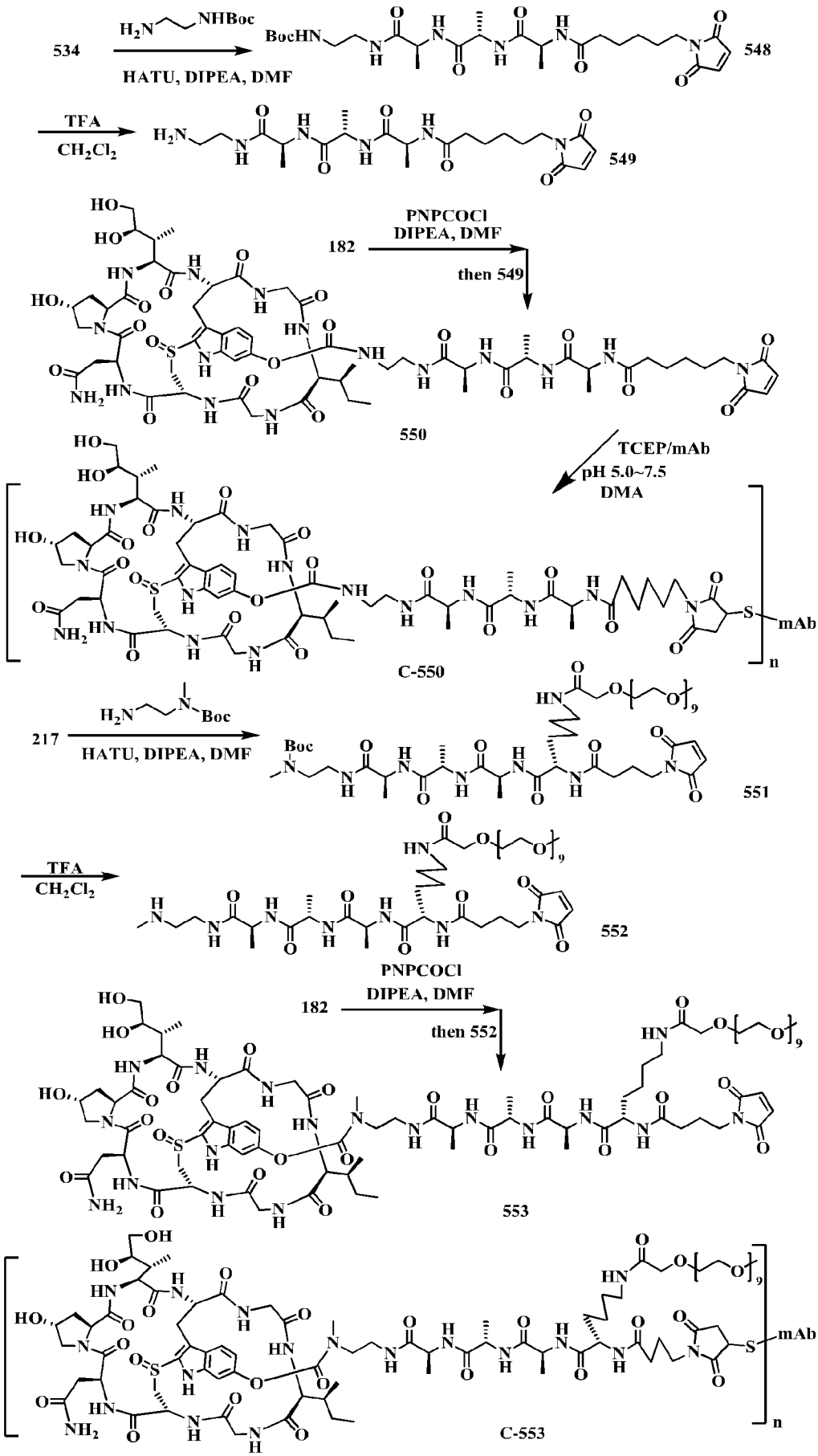

534
HATU, DIPEA, DMF
548
TFA
CH2Cl2
549
182
PNPCOCl
DIPEA, DMF
then 549
550
TCEP/mAb
pH 5.0~7.5
DMA
C-550
217
HATU, DIPEA, DMF
551
TFA
CH2Cl2
552
PNPCOCl
182
DIPEA, DMF
then 552
553
C-553

Fig. 48

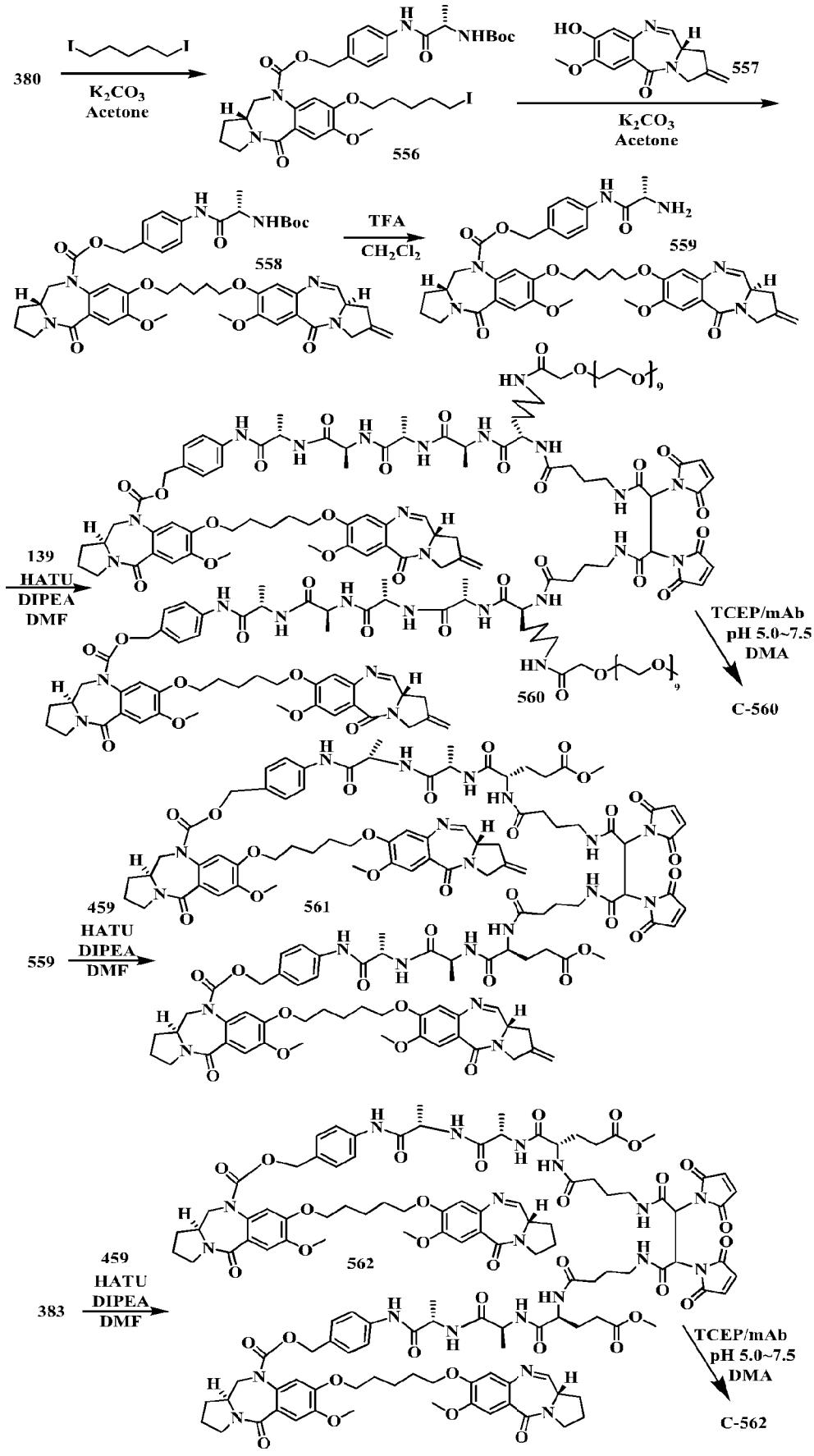

380
K2CO3
Acetone
556
557
K2CO3
Acetone
558
TFA
CH2Cl2
559
139
HATU
DIPEA
DMF
560
TCEP/mAb
pH 5.0~7.5
DMA
C-560
459
HATU
DIPEA
DMF
561
559
562
383
C-562

Fig. 49

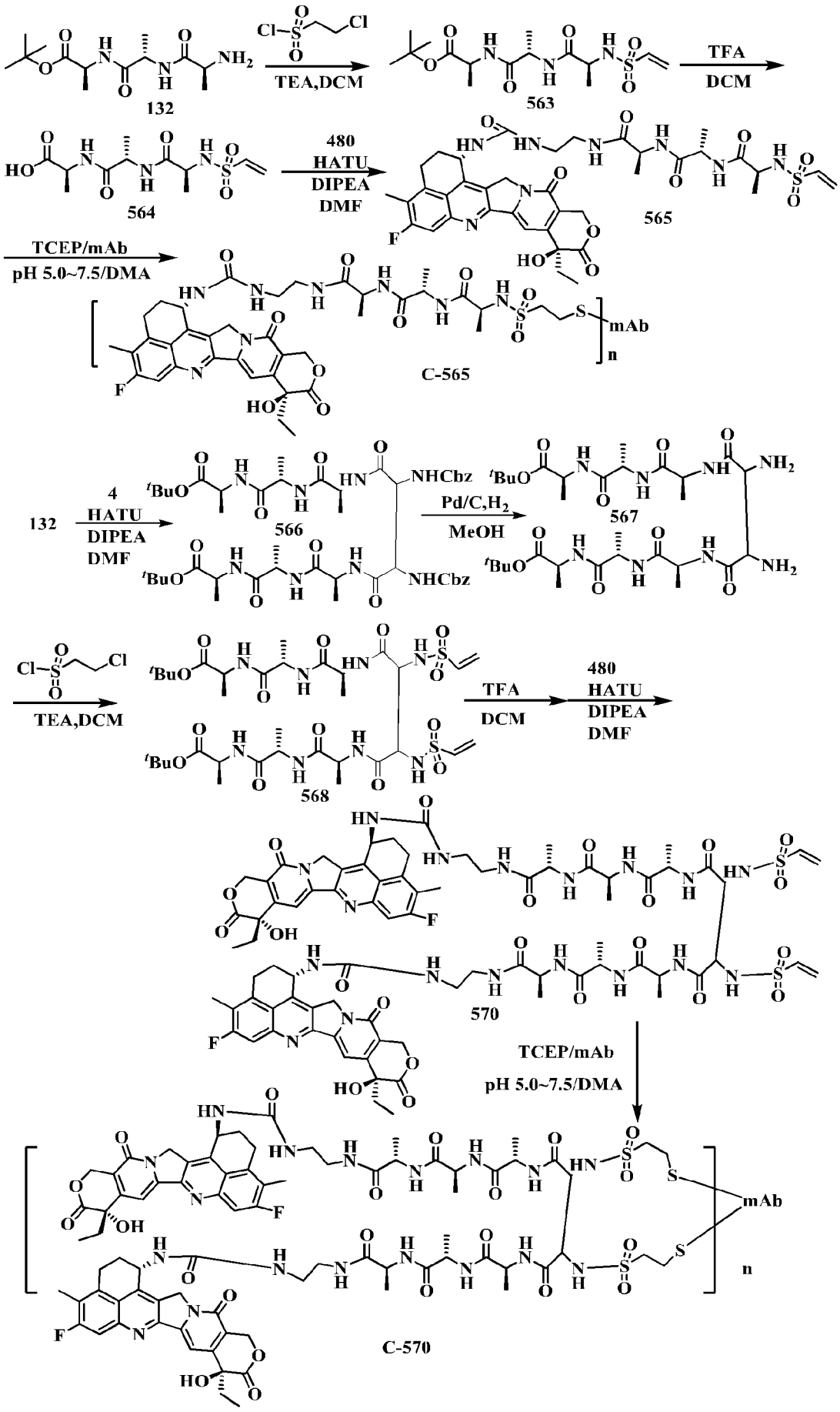

132
TEA,DCM
563
TFA
DCM
564
480
HATU
DIPEA
DMF
565
TCEP/mAb
pH 5.0~7.5/DMA
C-565
mAb
n
4
132
HATU
DIPEA
DMF
566
Pd/C,H2
MeOH
567
TEA,DCM
568
TFA
DCM
480
HATU
DIPEA
DMF
570
TCEP/mAb
pH 5.0~7.5/DMA
C-570

Fig. 50

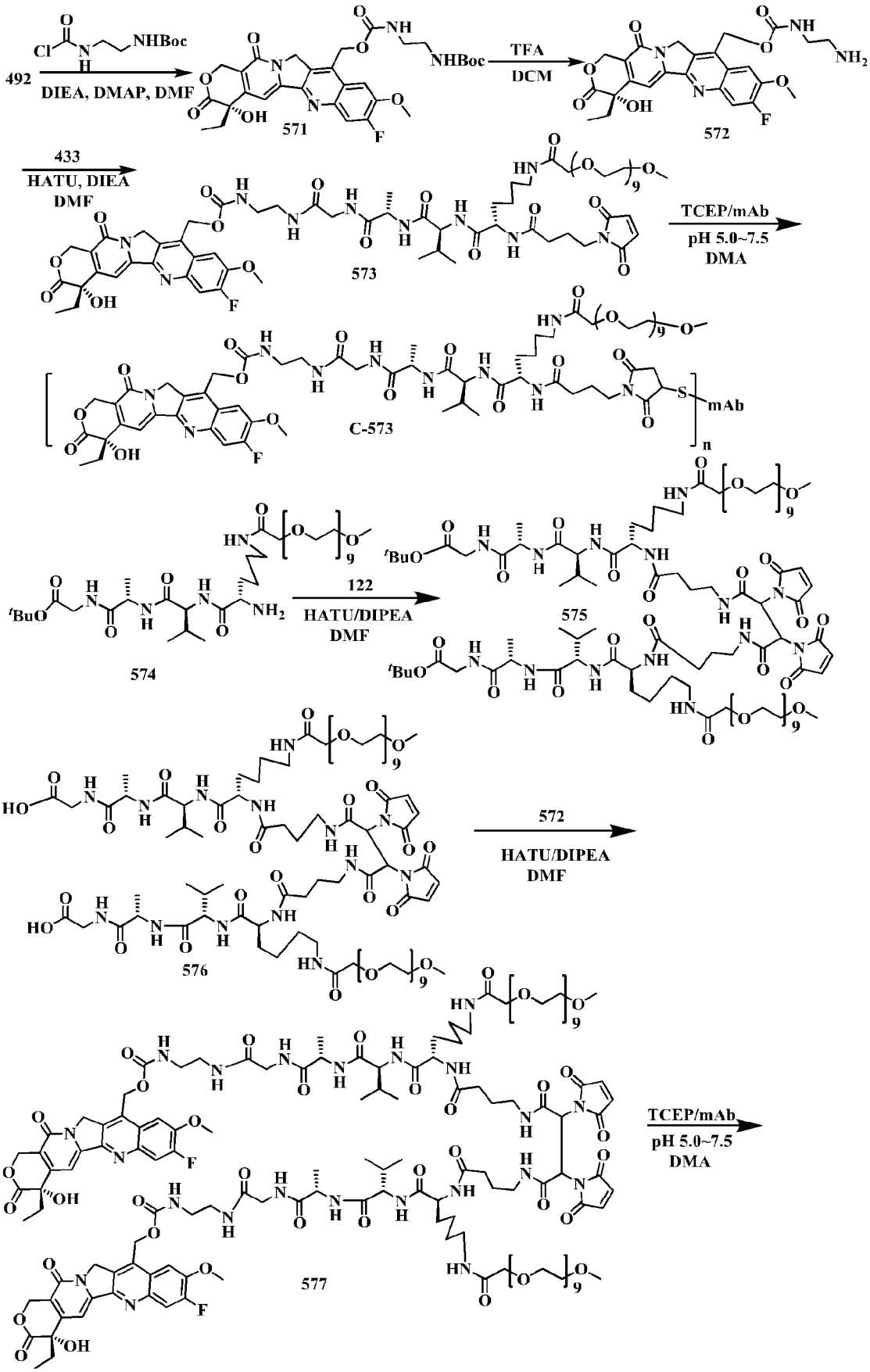

492
DIEA, DMAP, DMF
571
TFA
DCM
572
433
HATU, DIEA
DMF
573
TCEP/mAb
pH 5.0~7.5
DMA
C-573
mAb
n
574
122
HATU/DIPEA
DMF
575
572
HATU/DIPEA
DMF
576
TCEP/mAb
pH 5.0~7.5
DMA
577

Fig. 51

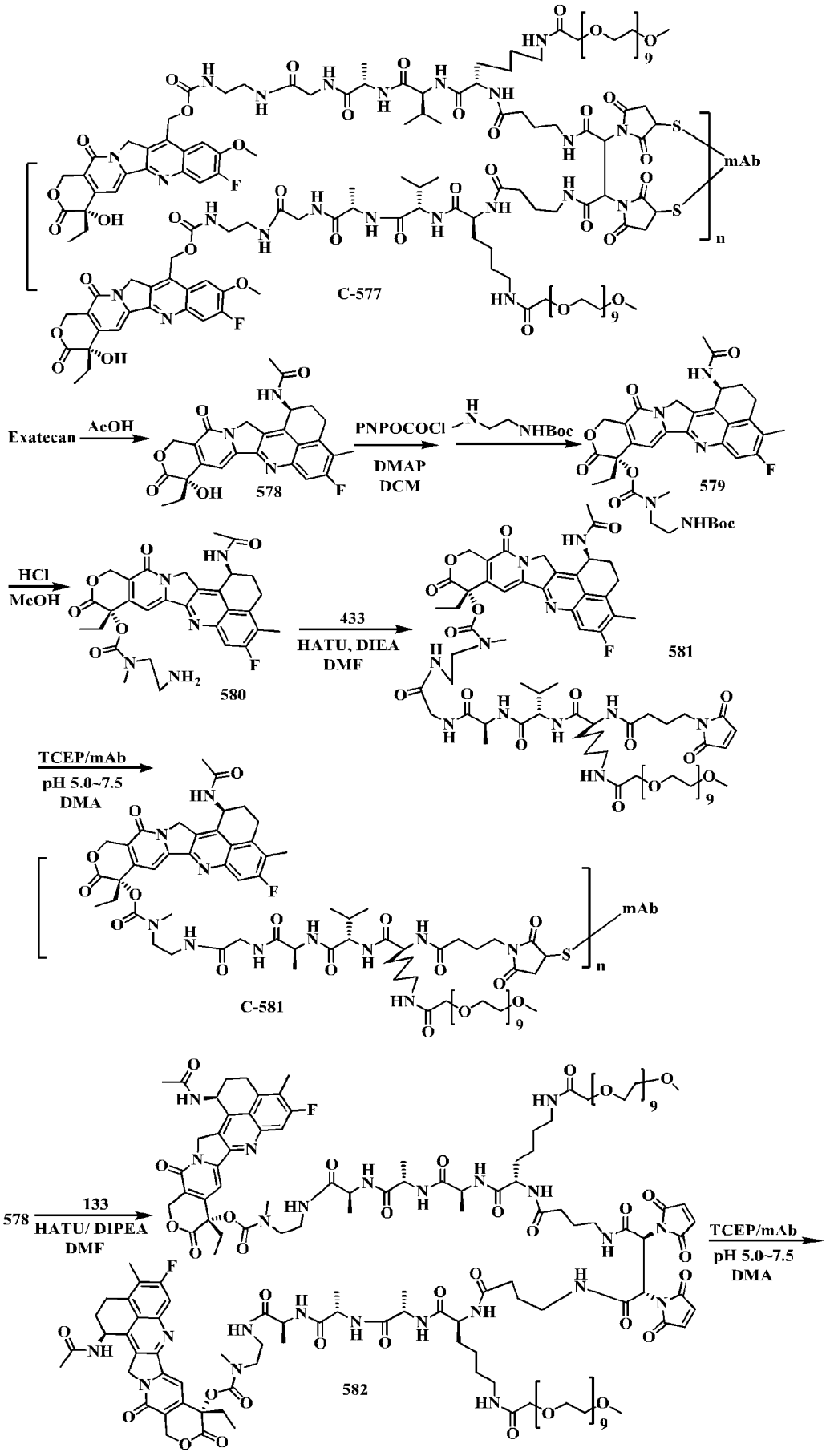

C-577
Exatecan
AcOH
578
PNPOCOCl
DMAP
DCM
NHBoc
579
HCl
MeOH
580
433
HATU, DIEA
DMF
581
TCEP/mAb
pH 5.0~7.5
DMA
C-581
578
133
HATU/ DIPEA
DMF
582
TCEP/mAb
pH 5.0~7.5
DMA

Fig. 52

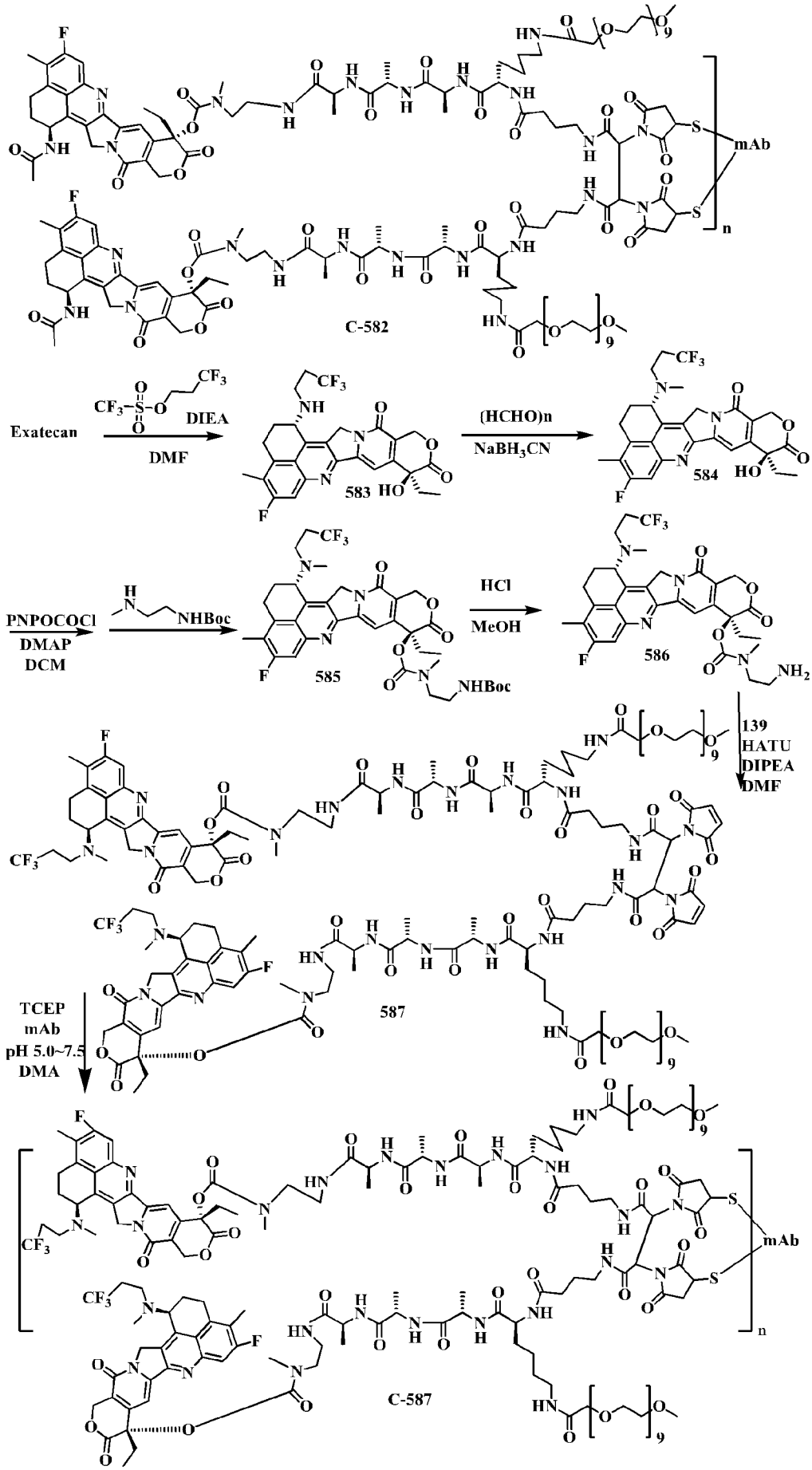

C-582
Exatecan
DIEA
DMF
583
(HCHO)n
NaBH3CN
584
PNPOCOCl
DMAP
DCM
585
HCl
MeOH
586
139
HATU
DIPEA
DMF
587
TCEP
mAb
pH 5.0~7.5
DMA
C-587

Fig. 53

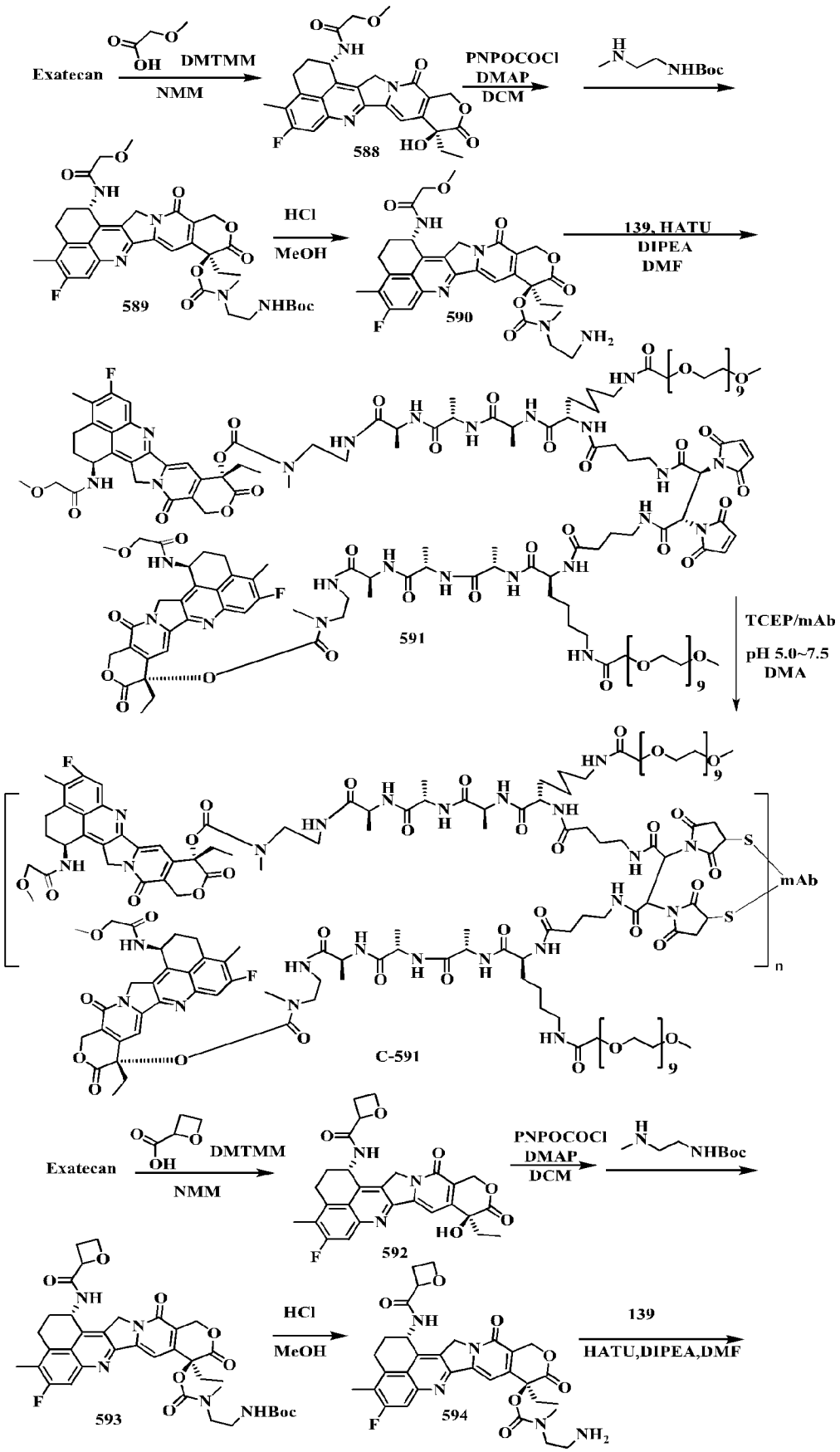

Exatecan
DMTMM
NMM
588
PNPOCOCl
DMAP
DCM
NHBoc
589
HCl
MeOH
590
139, HATU
DIPEA
DMF
591
TCEP/mAb
pH 5.0~7.5
DMA
mAb
C-591
Exatecan
DMTMM
NMM
592
PNPOCOCl
DMAP
DCM
NHBoc
593
HCl
MeOH
594
139
HATU,DIPEA,DMF

Fig. 54

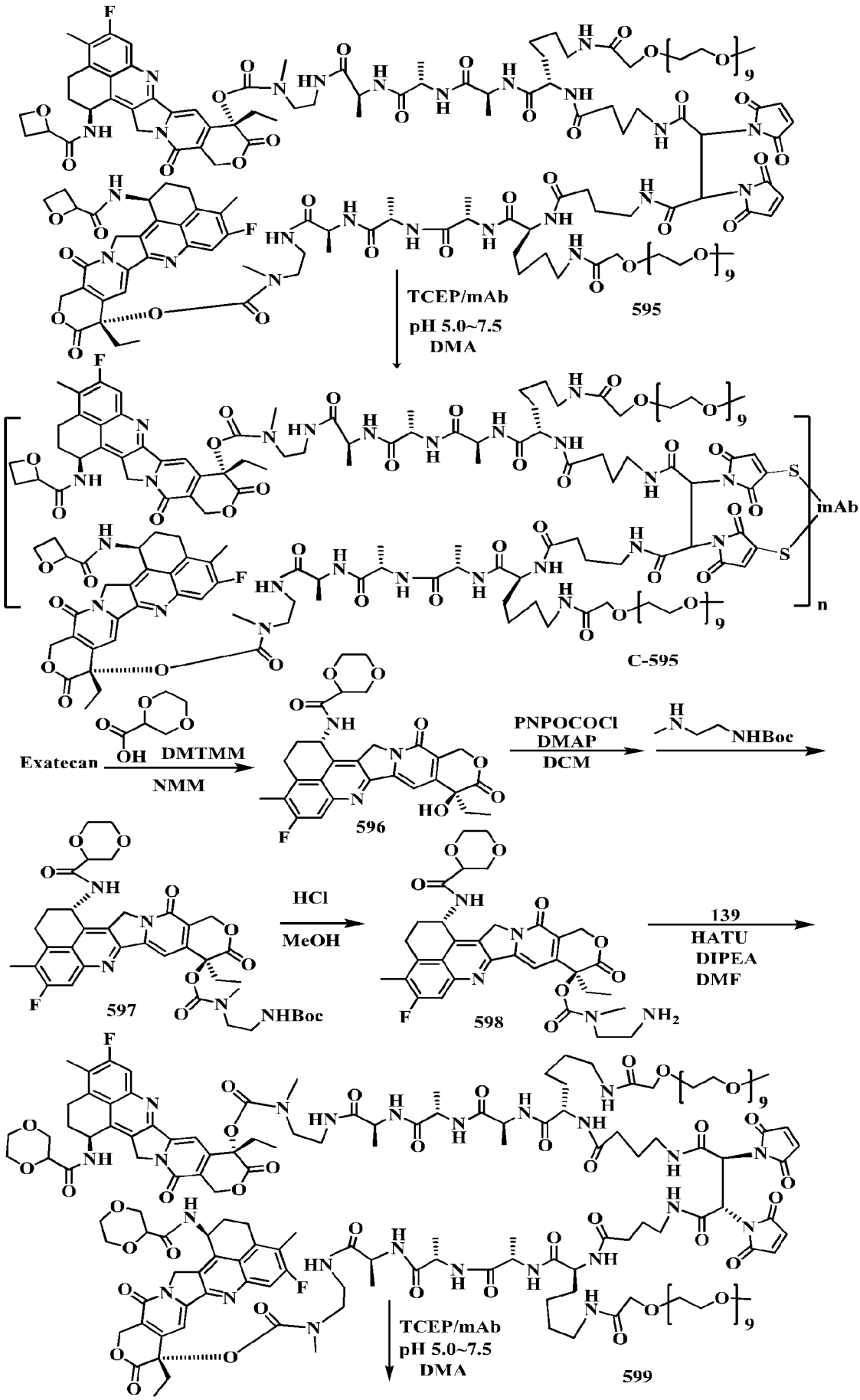

595
TCEP/mAb
pH 5.0~7.5
DMA
C-595
mAb
Exatecan
DMTMM
NMM
596
PNPOCOCl
DMAP
DCM
NHBoc
597
HCl
MeOH
598
139
HATU
DIPEA
DMF
599
TCEP/mAb
pH 5.0~7.5
DMA

Fig. 55

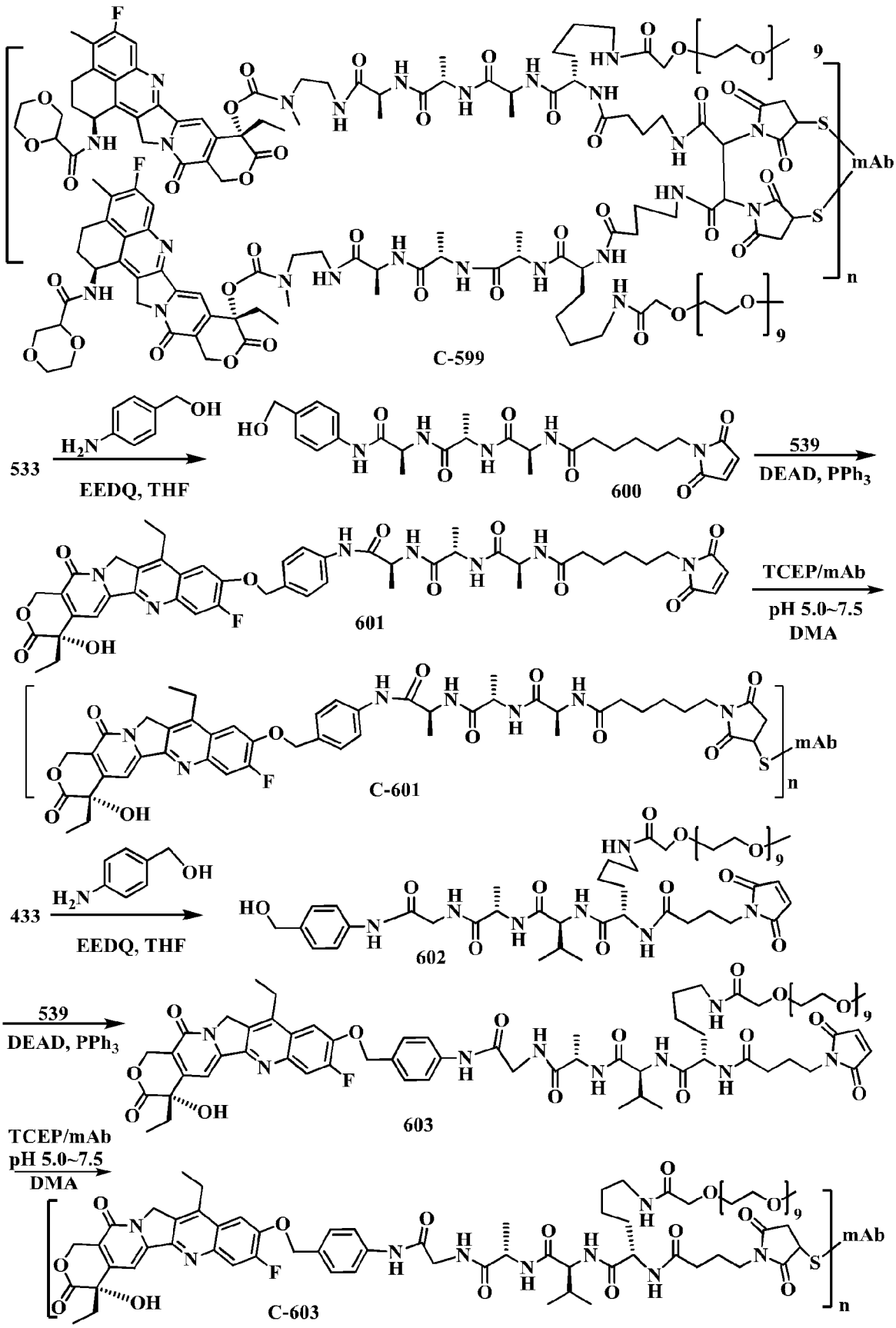

C-599
533
EEDQ, THF
600
539
DEAD, PPh3
601
TCEP/mAb
pH 5.0~7.5
DMA
C-601
433
EEDQ, THF
602
539
DEAD, PPh3
603
TCEP/mAb
pH 5.0~7.5
DMA
C-603

Fig. 56

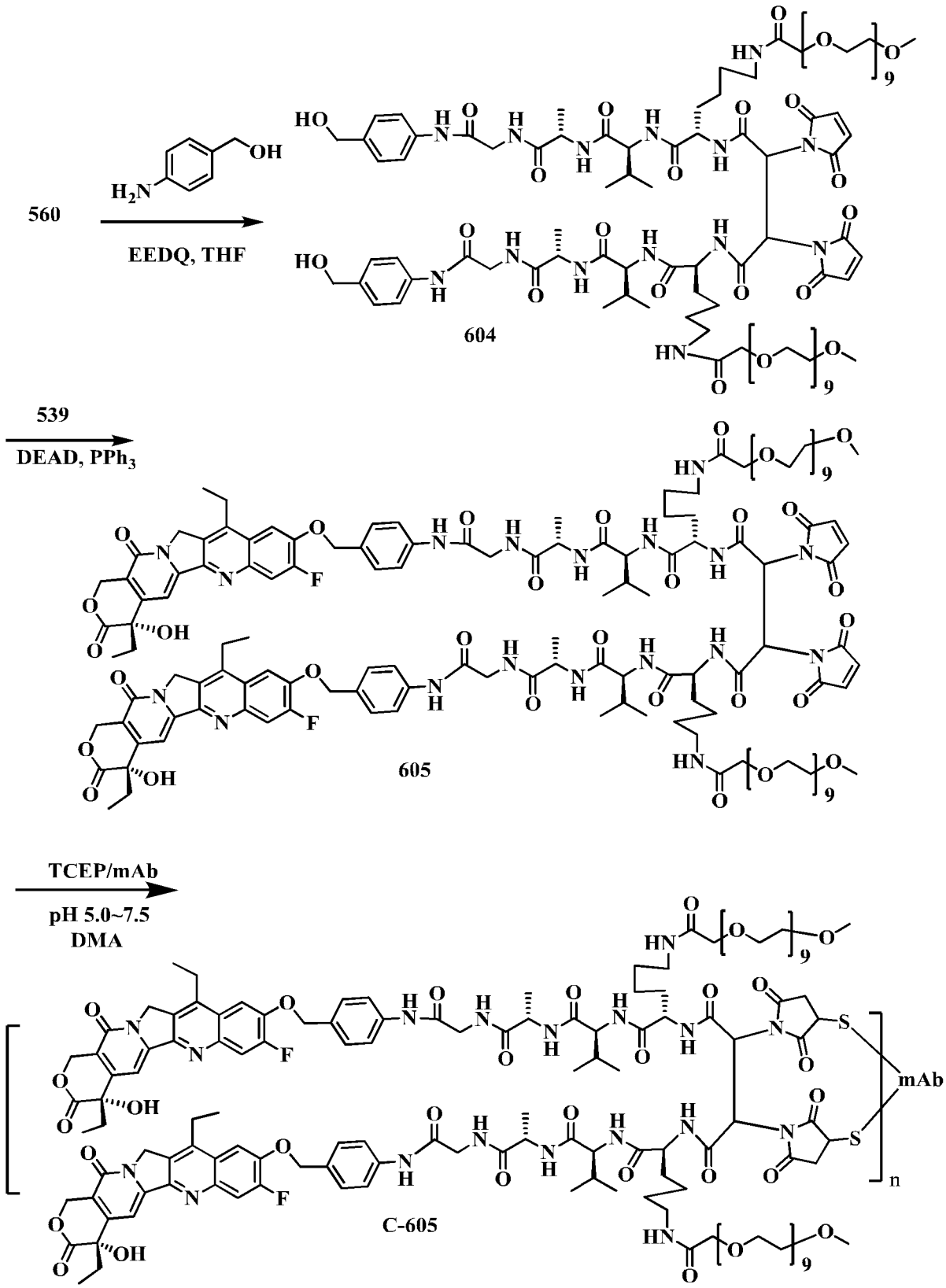
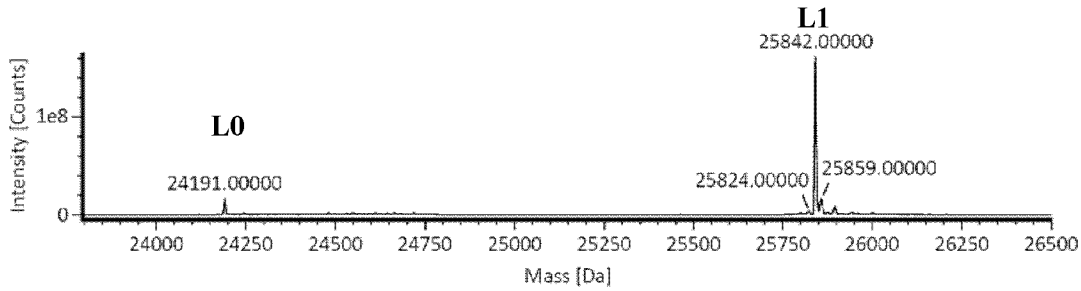

560
EEDQ, THF
604
539
DEAD, PPh3
605
TCEP/mAb
pH 5.0~7.5
DMA
C-605
mAb

Fig. 57

(a). A light chain of a BCMA-Tub ADC:



(b). A heavy chain of a BCMA-Tub ADC:

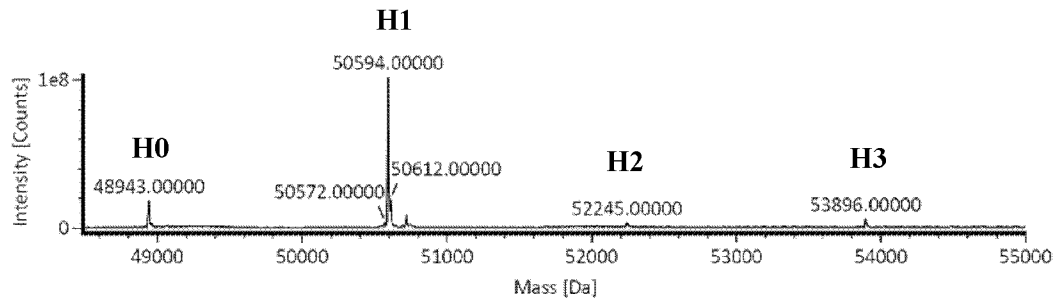


Fig. 58 (a), (b)

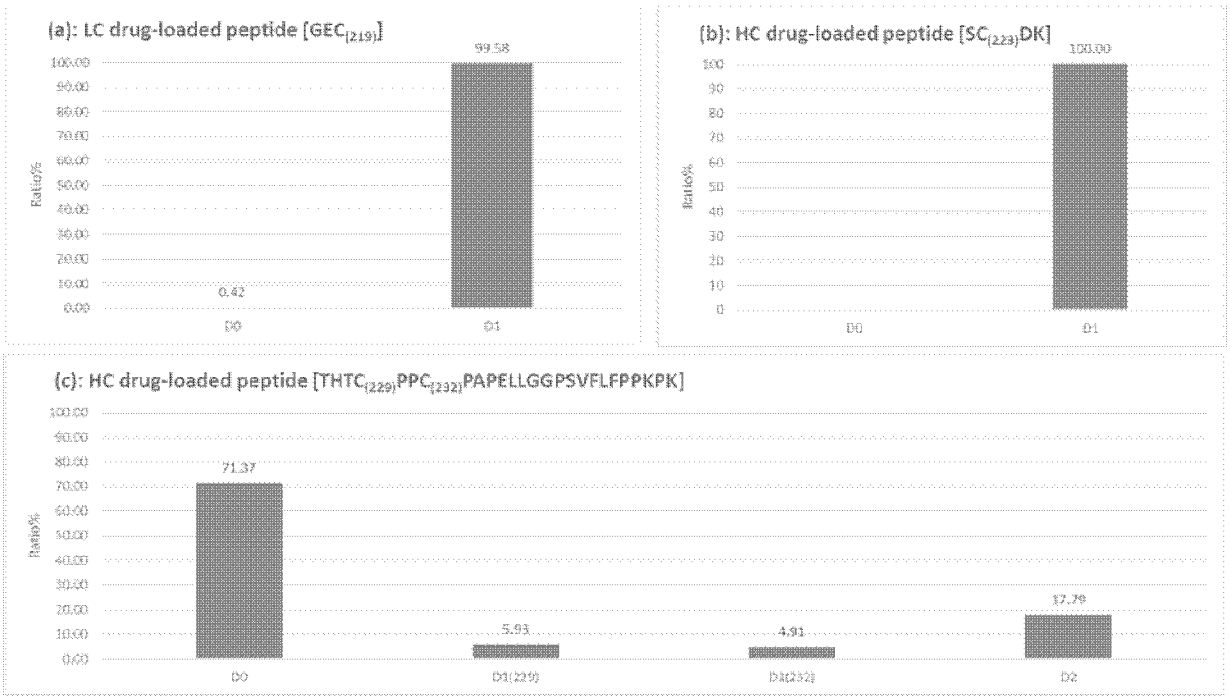


Fig. 59

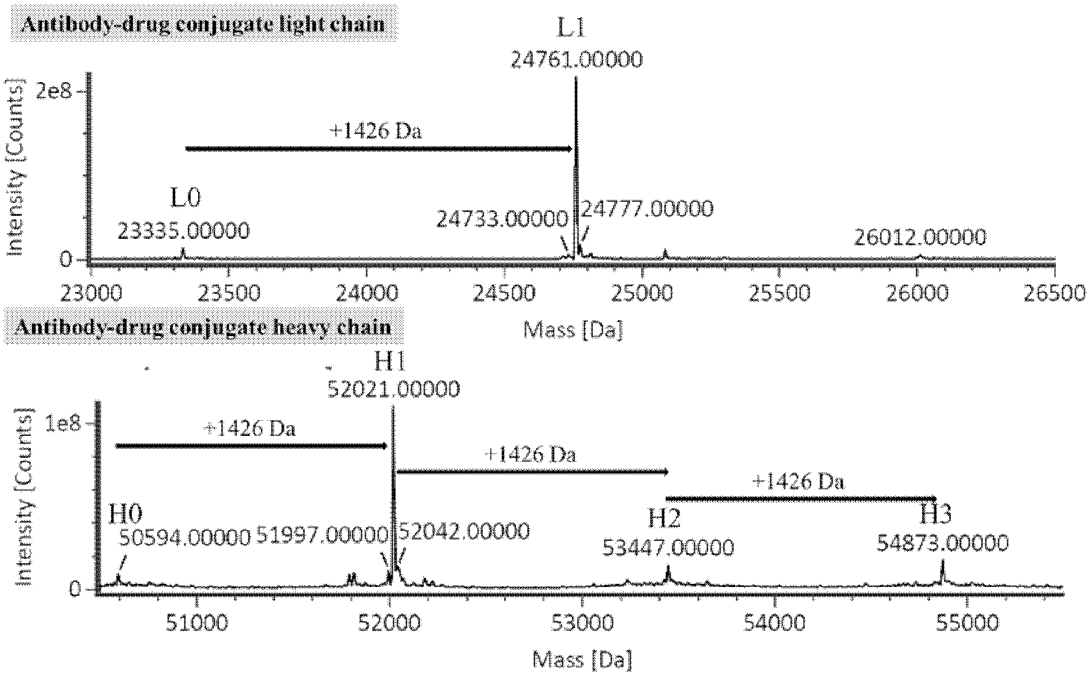

Antibody-drug conjugate light chain
L1
24761.00000
+1426 Da
L0
23335.00000
24733.00000
24777.00000
26012.00000
Intensity [Counts]
2e8
0
23000
23500
24000
24500
25000
25500
26000
26500
Mass [Da]
Antibody-drug conjugate heavy chain
H1
52021.00000
+1426 Da
+1426 Da
+1426 Da
H0
50594.00000
51997.00000
52042.00000
H2
53447.00000
H3
54873.00000
Intensity [Counts]
1e8
0
51000
52000
53000
54000
55000
Mass [Da]

Fig . 60

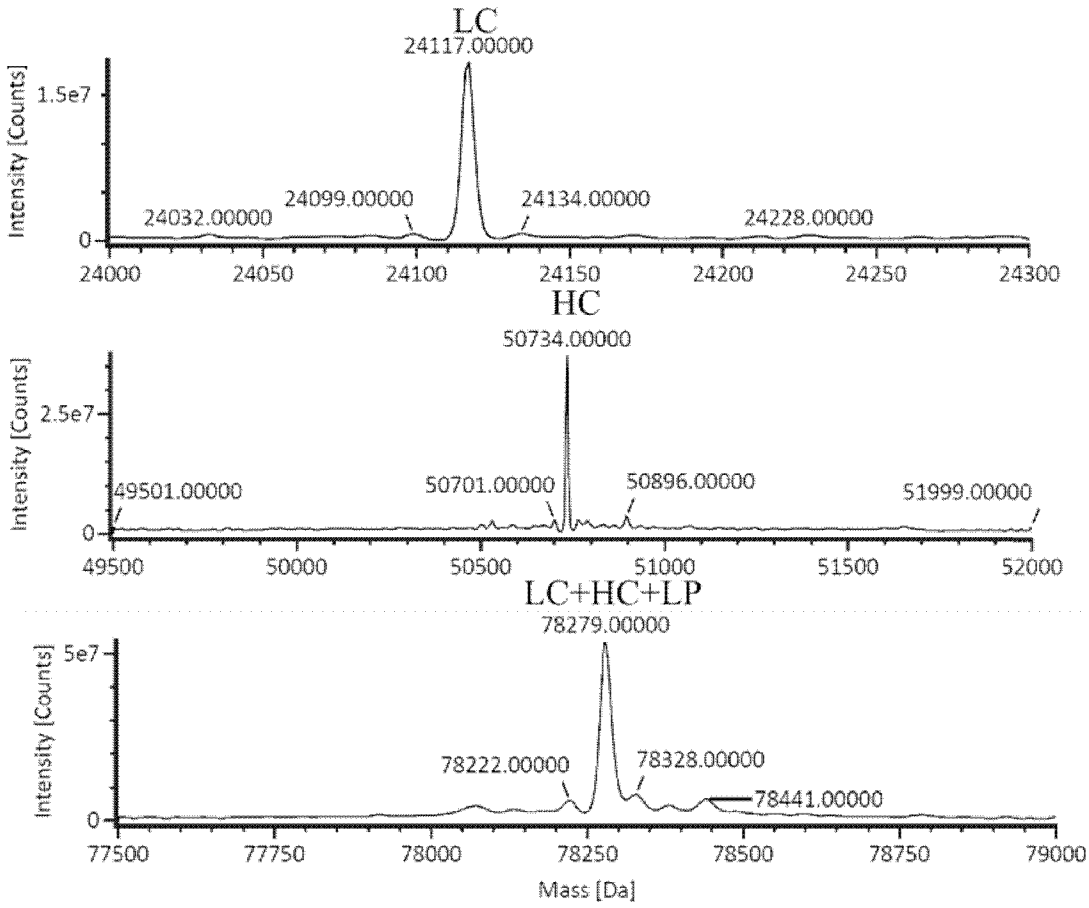

LC
24117.00000
Intensity [Counts]
1.5e7
0
24032.00000
24099.00000
24134.00000
24228.00000
24000
24050
24100
24150
24200
24250
24300
HC
50734.00000
Intensity [Counts]
2.5e7
0
49501.00000
50701.00000
50896.00000
51999.00000
49500
50000
50500
51000
51500
52000
LC+HC+LP
78279.00000
Intensity [Counts]
5e7
0
78222.00000
78328.00000
78441.00000
77500
77750
78000
78250
78500
78750
79000
Mass [Da]

Fig. 61

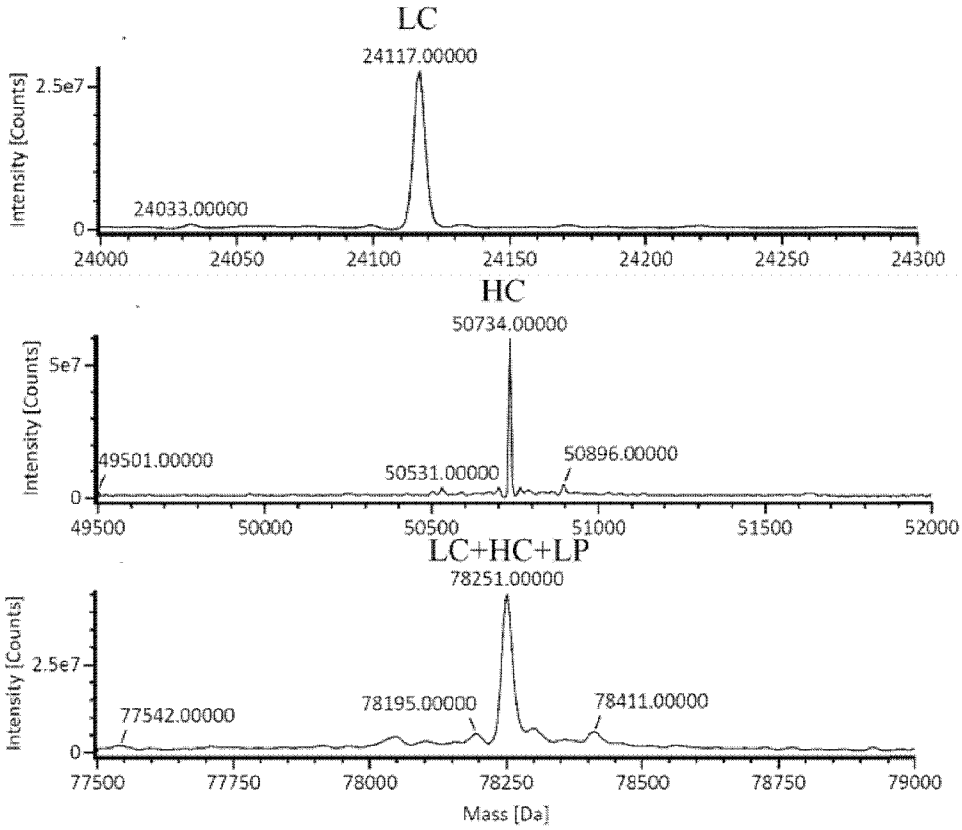

LC
24117.00000
24033.00000
Intensity [Counts]
2.5e7
0
24000
24050
24100
24150
24200
24250
24300
HC
50734.00000
49501.00000
50531.00000
50896.00000
5e7
49500
50000
50500
51000
51500
52000
LC+HC+LP
78251.00000
77542.00000
78195.00000
78411.00000
77500
77750
78000
78250
78500
78750
79000
Mass [Da]

Fig. 62

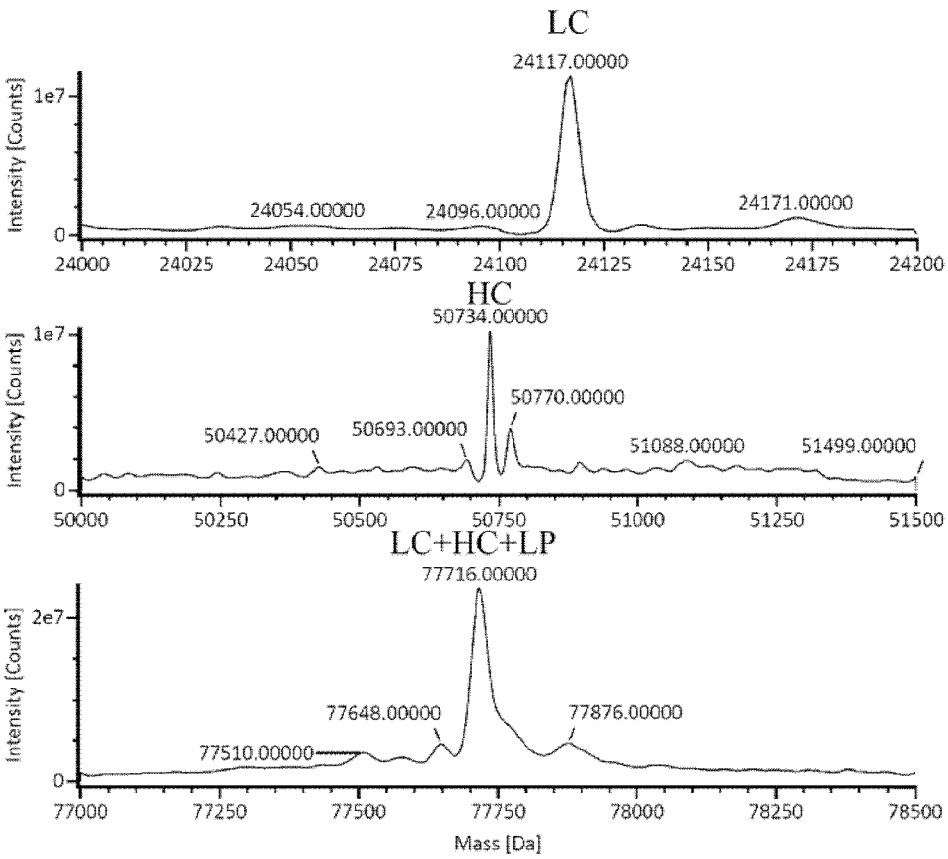

LC
24117.00000
24054.00000
24096.00000
24171.00000
Intensity [Counts]
1e7
0
24000
24025
24050
24075
24100
24125
24150
24175
24200
HC
50734.00000
50770.00000
50427.00000
50693.00000
51088.00000
51499.00000
50000
50250
50500
50750
51000
51250
51500
LC+HC+LP
77716.00000
77648.00000
77876.00000
77510.00000
2e7
77000
77250
77500
77750
78000
78250
78500
Mass [Da]

Fig. 63

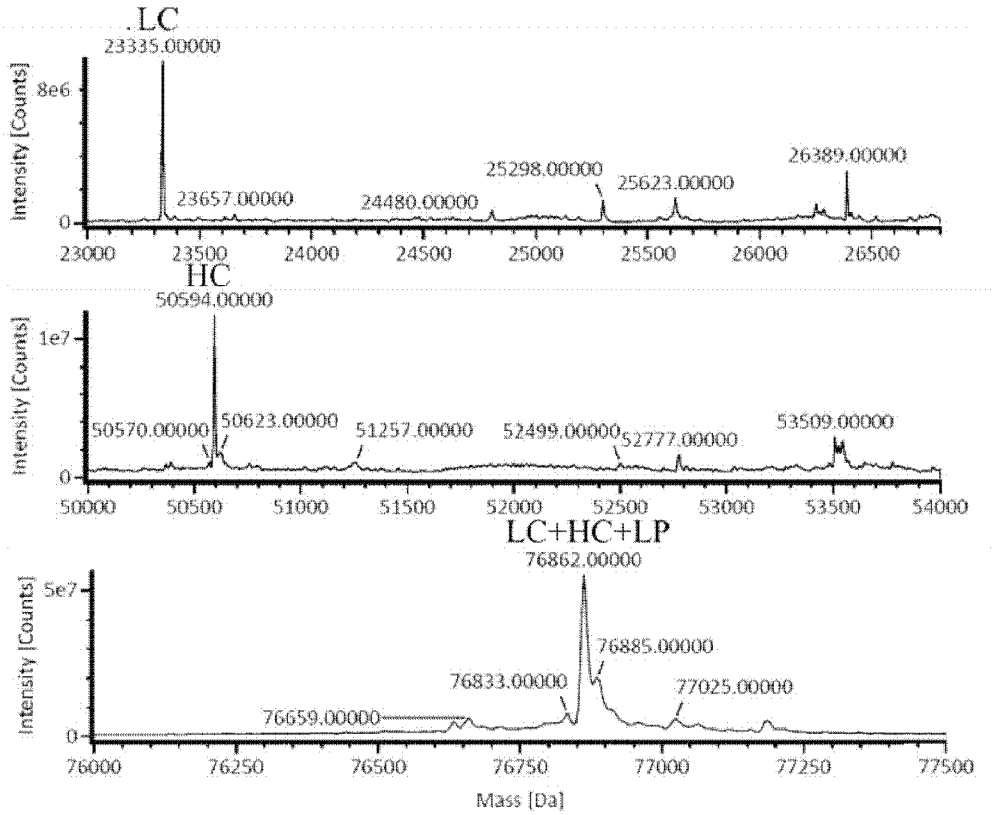

LC
23335.00000
23657.00000
24480.00000
25298.00000
25623.00000
26389.00000
Intensity [Counts]
8e6
0
23000
23500
24000
24500
25000
25500
26000
26500
HC
50594.00000
50570.00000
50623.00000
51257.00000
52499.00000
52777.00000
53509.00000
1e7
50000
50500
51000
51500
52000
52500
53000
53500
54000
LC+HC+LP
76862.00000
76885.00000
76833.00000
77025.00000
76659.00000
5e7
76000
76250
76500
76750
77000
77250
77500
Mass [Da]

Fig. 64

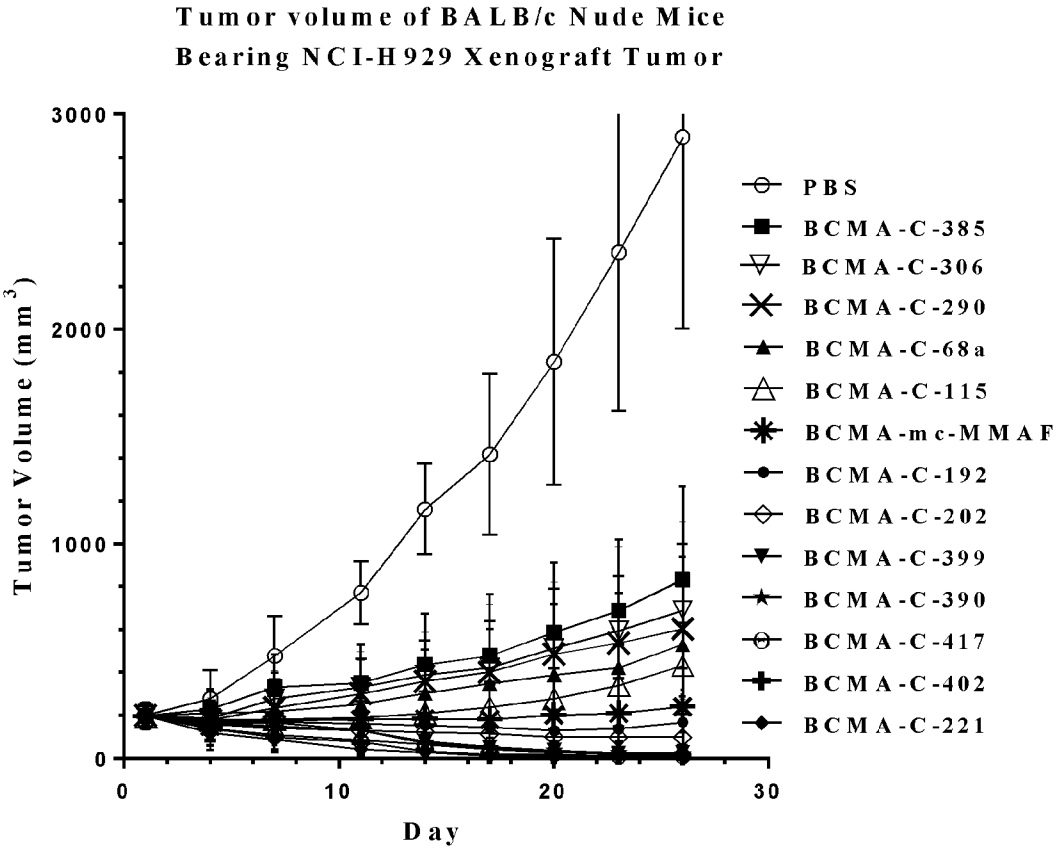

Tumor volume of BALB/c Nude Mice
Bearing NCI-H929 Xenograft Tumor
Tumor Volume (mm3)
3000
2000
1000
0
0
10
20
30
Day
PBS
BCMA-C-385
BCMA-C-306
BCMA-C-290
BCMA-C-68a
BCMA-C-115
BCMA-mc-MMAF
BCMA-C-192
BCMA-C-202
BCMA-C-399
BCMA-C-390
BCMA-C-417
BCMA-C-402
BCMA-C-221

Fig. 65

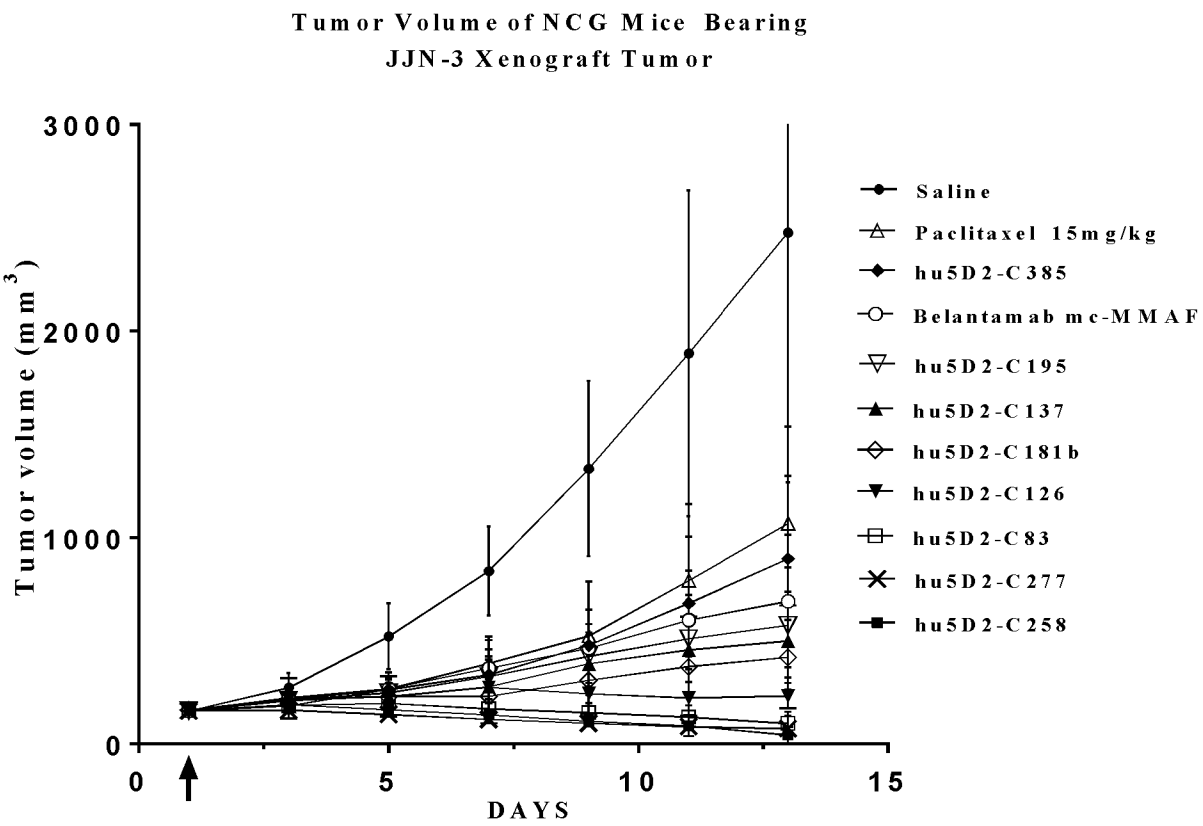

Tumor Volume of NCG Mice Bearing
JJN-3 Xenograft Tumor
Tumor volume (mm3)
3000
2000
1000
0
0
5
10
15
DAYS
Saline
Paclitaxel 15mg/kg
hu5D2-C385
Belantamab mc-MMAF
hu5D2-C195
hu5D2-C137
hu5D2-C181b
hu5D2-C126
hu5D2-C83
hu5D2-C277
hu5D2-C258

Fig.66

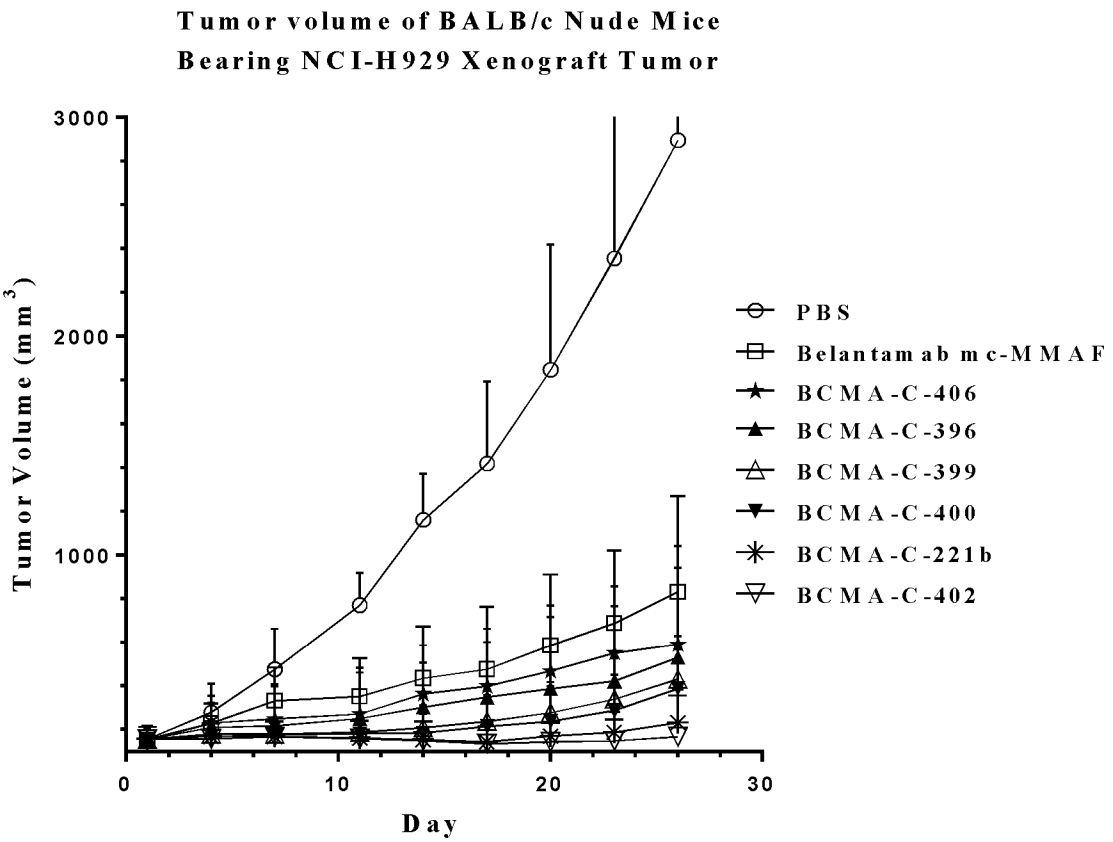

Tumor volume of BALB/c Nude Mice
Bearing NCI-H929 Xenograft Tumor
Tumor Volume (mm3)
3000
2000
1000
0
10
20
30
Day
PBS
Belantamab mc-MMAF
BCMA-C-406
BCMA-C-396
BCMA-C-399
BCMA-C-400
BCMA-C-221b
BCMA-C-402

Fig. 67

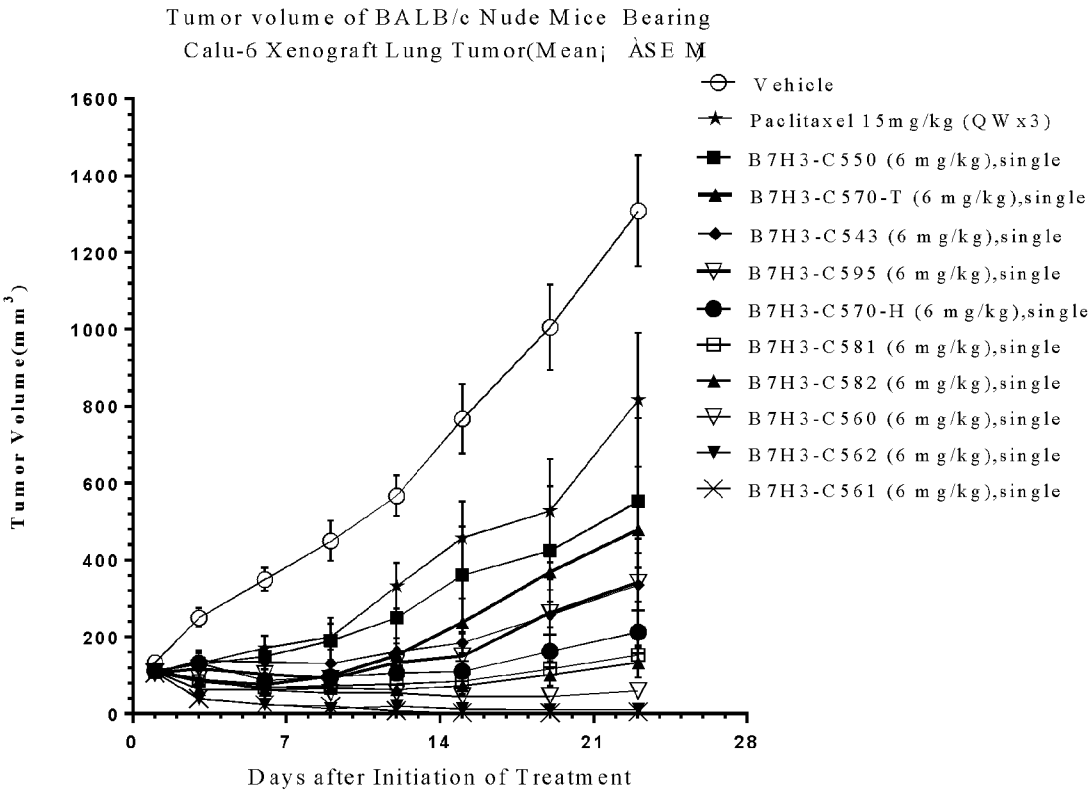

Tumor volume of BALB/c Nude Mice Bearing
Calu-6 Xenograft Lung Tumor(Mean¡ ÀSE M
Vehicle
Paclitaxel 15mg/kg (QW x3)
B7H3-C550 (6 mg/kg),single
B7H3-C570-T (6 mg/kg),single
B7H3-C543 (6 mg/kg),single
B7H3-C595 (6 mg/kg),single
B7H3-C570-H (6 mg/kg),single
B7H3-C581 (6 mg/kg),single
B7H3-C582 (6 mg/kg),single
B7H3-C560 (6 mg/kg),single
B7H3-C562 (6 mg/kg),single
B7H3-C561 (6 mg/kg),single
Tumor Volume(mm3)
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
Days after Initiation of Treatment

Fig. 68

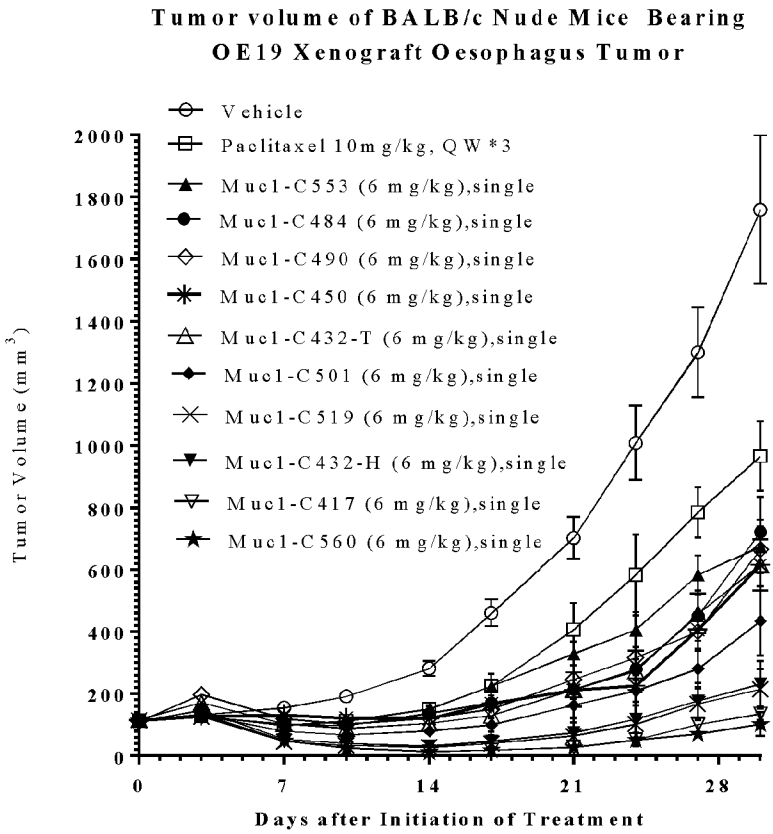

Tumor volume of BALB/c Nude Mice Bearing
OE19 Xenograft Oesophagus Tumor
Vehicle
Paclitaxel 10mg/kg, QW*3
Muc1-C553 (6 mg/kg),single
Muc1-C484 (6 mg/kg),single
Muc1-C490 (6 mg/kg),single
Muc1-C450 (6 mg/kg),single
Muc1-C432-T (6 mg/kg),single
Muc1-C501 (6 mg/kg),single
Muc1-C519 (6 mg/kg),single
Muc1-C432-H (6 mg/kg),single
Muc1-C417 (6 mg/kg),single
Muc1-C560 (6 mg/kg),single
Tumor Volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
Days after Initiation of Treatment

Fig. 69

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/129122

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/00(2006.01)i; C07K 16/28(2006.01)i; A61K 39/395(2006.01)i; A61K 47/68(2017.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, SIPOABS, WOTXT, EPTXT, USTXT, CNTXT, CATXT, GBTXT, JPTXT, KRABS, CNABS, CNKI,STNext,ISI Web of Science: homogenous; homogeneity; transition metal; conjugate; cation ammo chelate; Zn; zinc amino; linker; ADC; antibody drug ;structure search; ZHAO Robert; HANGZHOU DAC BIOTECH;

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020164561 A1 (WUXI BIOLOGICS SHANGHAI CO., LTD. et al.) 20 August 2020 (2020-08-20) claims 1-8, description paragraphs 0019 and 0030	1-3
Y	WO 2020164561 A1 (WUXI BIOLOGICS SHANGHAI CO., LTD. et al.) 20 August 2020 (2020-08-20) claims 1-8, description paragraphs 0019 and 0030	4-20
Y	WO 2019127607 A1 (HANGZHOU DAC BIOTECH CO., LTD.) 04 July 2019 (2019-07-04) claims 1-23	4-20
Y	WO 2018086139 A1 (HANGZHOU DAC BIOTECH CO., LTD. et al.) 17 May 2018 (2018-05-17) claims 1-43	4-20
Y	WO 2015155753 A2 (SUZHOU M CONJ BIOTECH CO., LTD. et al.) 15 October 2015 (2015-10-15) claims 1-33	4-20

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 December 2022	**22 December 2022**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088, China** Facsimile No. **(86-10)62019451**	Authorized officer **CUI,Yiwen** Telephone No. **(86-10)53961855**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016059622 A2 (SUZHOU M-CONJ BIOTECH CO., LTD. et al.) 21 April 2016 (2016-04-21) claims 1-38	4-20
Y	WO 2015151081 A2 (SUZHOU M CONJ BIOTECH CO., LTD. et al.) 08 October 2015 (2015-10-08) claims 1-29	4-20
A	CN 107922477 A (DAIICHI SANKYO CO., LTD.) 17 April 2018 (2018-04-17) the whole document	1-20
PX	WO 2022078524 A2 (HANGZHOU DAC BIOTECH CO., LTD) 21 April 2022 (2022-04-21) claims 1-21	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/129122

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **18-19**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 18-19 relate to a method for synergistical treatment or prevention of a cancer, an autoimmune disease, or an infectious disease. They do not meet the criteria set out in PCT Rules 39.1 (iv). The search has been made and based on the use of the pharmaceutical composition for the manufacturing of medicament for the treatment of a disease.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/129122

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2020164561	A1	20 August 2020	JP	2022520260	A	29 March 2022
				CN	113423730	A	21 September 2021
				TW	202045540	A	16 December 2020
				EP	3924378	A1	22 December 2021
				US	2022040321	A1	10 February 2022
				KR	20210125034	A	15 October 2021
WO	2019127607	A1	04 July 2019	IL	275274	A	30 July 2020
				KR	20200097295	A	18 August 2020
				CA	3085634	A1	04 July 2019
				US	2020276261	A1	03 September 2020
				AU	2022205269	A1	11 August 2022
				CN	111093707	A	01 May 2020
				BR	112020010405	A2	24 November 2020
				JP	2021507928	A	25 February 2021
				EP	3621652	A1	18 March 2020
				CL	2020001600	A1	19 March 2021
				EA	202091217	A1	18 September 2020
				SG	11202004801W	A	29 June 2020
				AU	2017445144	A1	18 June 2020
				MX	2020006192	A	20 August 2020
				PH	12020550657	A1	19 April 2021
WO	2018086139	A1	17 May 2018	US	2022313837	A1	06 October 2022
				AU	2016429272	A1	02 May 2019
				EP	3888691	A1	06 October 2021
				US	2022313838	A1	06 October 2022
				EP	3538080	A1	18 September 2019
				NZ	752394	A	30 July 2021
				JP	2019536772	A	19 December 2019
				CN	110099682	A	06 August 2019
				KR	20220147720	A	03 November 2022
				KR	20220150408	A	10 November 2022
				US	2021308277	A1	07 October 2021
				KR	20210126796	A	20 October 2021
				US	2022313836	A1	06 October 2022
				US	2022323602	A1	13 October 2022
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				KR	20220147721	A	03 November 2022
				KR	20220147719	A	03 November 2022
				CA	3042442	A1	17 May 2018
				KR	20210126795	A	20 October 2021
				KR	20190085532	A	18 July 2019
				AU	2021200562	A1	25 February 2021
				PH	12019501056	A1	09 September 2019
WO	2015155753	A2	15 October 2015	JP	2018532695	A	08 November 2018
				CA	2991975	A1	15 October 2015
				CN	108289964	A	17 July 2018
WO	2016059622	A2	21 April 2016	AU	2021266317	A1	09 December 2021
				US	2021346523	A1	11 November 2021
				PH	12018501638	A1	27 May 2019

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/129122

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				US	2020215206	A1	09 July 2020
				CA	3013412	A1	21 April 2016
				US	2021052747	A1	25 February 2021
				US	2021353777	A1	18 November 2021
				AU	2016202632	A1	23 August 2018
				NZ	744940	A	25 March 2022
				EP	3411074	A2	12 December 2018
				HK	1259487	A1	29 November 2019
				SG	11201806594Q	A	27 September 2018
WO	2015151081	A2	08 October 2015	AU	2015242213	A1	08 March 2018
				CN	108449940	A	24 August 2018
				CA	2991973	A1	08 October 2015
				JP	2018524377	A	30 August 2018
				EP	3319936	A2	16 May 2018
				AU	2021202467	A1	20 May 2021
				ZA	201800825	B	29 May 2019
				AU	2022206809	A1	18 August 2022
				PH	12018500285	A1	29 August 2018
				NZ	739830	A	24 December 2021
CN	107922477	A	17 April 2018	BR	112017027690	A2	09 October 2018
				IL	256558	A	28 February 2018
				US	2018147292	A1	31 May 2018
				CA	2990572	A1	05 January 2017
				HK	1247211	A1	21 September 2018
				TW	201705982	A	16 February 2017
				IL	290959	A	01 April 2022
				US	2021386865	A1	16 December 2021
				JP	2021020956	A	18 February 2021
				TW	202130368	A	16 August 2021
				KR	20180021723	A	05 March 2018
				AU	2016286898	A1	04 January 2018
				EP	3315512	A1	02 May 2018
				JP	WO2017002776	A1	31 May 2018
				WO	2017002776	A1	05 January 2017
WO	2022078524	A2	21 April 2022	WO	2022078524	A3	25 August 2022