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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
04 May 2023 (04.05.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/077126 A1

(51) International Patent Classification:
A61K 47/54 (2017.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2022/078991

(22) International Filing Date:
31 October 2022 (31.10.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/273,777 29 October 2021 (29.10.2021) US

(71) Applicant: **TAMBO, INC.** [US/US]; 665 Third Street, Suite 250, San Francisco, California 94107 (US).

(72) Inventors: **ONETO, Jose Manuel Mejia**; c/o Tambo, Inc., 665 Third Street, Suite 250, San Francisco, California 94107 (US). **ZAKHARIAN, Michael**; c/o Tambo, Inc., 665 Third Street, Suite 250, San Francisco, California 94107 (US). **MAHMOODI, Amir**; c/o Tambo, Inc., 665 Third Street, Suite 250, San Francisco, California 94107 (US). **MCFARLAND, Jesse M.**; c/o Tambo, Inc., 665 Third Street, Suite 250, San Francisco, California 94107 (US).

(74) Agent: **TANNER, Lorna L.** et al.; SHEPPARD MULLIN RICHTER & HAMPTON LLP, 650 Town Center Drive, 10th Floor, Costa Mesa, California 92626-1993 (US).

(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 applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: TRANS-CYCLOOCTENE CONJUGATES

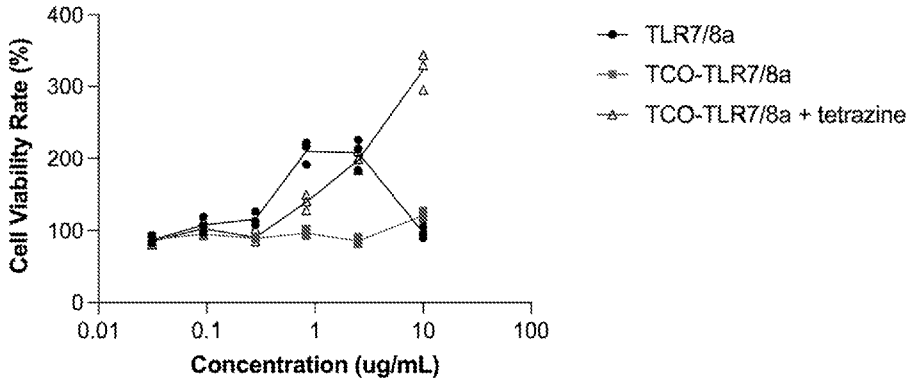


FIG. 1

(57) Abstract: The present disclosure relates generally trans-cyclooctene conjugates for bioorthogonal delivery of a payload to a targeted location in a subject. The compositions and methods have applications in the treatment of cancer, tumor growths, and immunotherapy.

WO 2023/077126 A1

TRANS-CYCLOOCTENE CONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) to U.S. Provisional Application Numbers 63/273,777, filed October 29, 2021, which is incorporated by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (2022-10-31_Sequence_Listing_63XT-342804-WO.xml; Size: 4,881 bytes; and Date of Creation: October 31, 2022) are herein incorporated by reference in their entireties.

FIELD

[0003] The present disclosure relates generally trans-cyclooctene conjugates for bioorthogonal delivery of a payload to a targeted location in a subject, which conjugates have applications, e.g., in the treatment of cancer, tumor growth, and immunotherapy.

BACKGROUND

[0004] Bioorthogonal conjugation or click reactions are selective and orthogonal (non-interacting with) functionalities found in biological systems, and have found use in various applications in the fields of chemistry, chemical biology, molecular diagnostics, and medicine, where they can be used to facilitate the selective manipulation of molecules, cells, particles and surfaces, and the tagging and tracking of biomolecules *in vitro* and *in vivo*. These reactions include the Staudinger ligation, the azide-cyclooctyne cycloaddition, and the inverse-electron-demand Diels-Alder reaction.

SUMMARY

[0005] Provided herein are conjugates for use in bioorthogonal reactions, which conjugates comprise a payload covalently bonded to one or more optionally substituted trans-cyclooctene moieties via a linker. In some embodiments, the payload is selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and a monoclonal antibody, or a derivative, or analog thereof.

[0006] In some embodiments, provided is a method for delivering an effective amount of a payload (i.e., an inhibitor of poly (ADP-ribose) polymerase (PARP inhibitor), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and a monoclonal antibody, or a derivative, or analog thereof) to a target location in a subject, the method comprising administering to the subject at the target location a therapeutic support composition as described herein, and administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein.

[0007] In some embodiments, provided is a method for treating cancer, comprising administering to a subject in need thereof, a therapeutic support composition as described herein to a target location, and

administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein.

[0008] In some embodiments, the cancer is metastatic. In some embodiments the cancer is melanoma, renal cancer, prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, kaposi's sarcoma, Non-Hodgkins lymphoma, Hodgkins lymphoma Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, or cutaneous T-cell lymphoma.

[0009] In some embodiments, the cancer is a melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is a lymphoma or leukemia. In some embodiments, the cancer is a hematolical malignancy.

BRIEF DESCRIPTION OF THE FIGURES

[0010] Fig. 1 and Fig. 2 show effect of the trans-cyclooctene conjugate of Example 20 in the absence or presence of tetrazine activator versus unmodified Gardiquimod on proliferation of fresh murine splenocytes. In Fig. 1, concentrations up to 10 μg/mL are tested. In Fig. 2, up to 50 μg/mL of conjugate with or without tetrazine is tested.

DETAILED DESCRIPTION

[0011] The following description sets forth exemplary embodiments of the present technology. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

1. Definitions

[0012] It is appreciated that certain features, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace subject matter that are, for example, compounds that are stable compounds (i.e., compounds that can be made, isolated, characterized, and tested for biological activity). In addition, all sub-combinations of the various embodiments and elements thereof (e.g., elements of the chemical groups listed in the embodiments describing such variables) are also specifically embraced and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

A. Definitions

[0013] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0014] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "an" and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0015] The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). The modifier "about" should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression "from about 2 to about 4" also discloses the range "from 2 to 4." The term "about" may refer to plus or minus 10% of the indicated number. For example, "about 10%" may indicate a range of 9% to 11%, and "about 1" may mean from 0.9-1.1. Other meanings of "about" may be apparent from the context, such as rounding off, so, for example "about 1" may also mean from 0.5 to 1.4.

[0016] The conjunctive term "or" includes any and all combinations of one or more listed elements associated by the conjunctive term. For example, the phrase "an apparatus comprising A or B" may refer to an apparatus including A where B is not present, an apparatus including B where A is not present, or an apparatus where both A and B are present. The phrases "at least one of A, B, . . . and N" or "at least one of A, B, . . . N, or combinations thereof" are defined in the broadest sense to mean one or more elements selected from the group comprising A, B, . . . and N, that is to say, any combination of one or more of the elements A, B, . . . or N including any one element alone or in combination with one or more of the other elements which may also include, in combination, additional elements not listed.

[0017] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic*

Chemistry, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987; the entire contents of each of which are incorporated herein by reference.

[0018] The term "alkoxy" as used herein, refers to an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, and tert-butoxy.

[0019] The term "alkyl" as used herein, means a straight or branched, saturated hydrocarbon chain containing from 1 to 30 carbon atoms. The term "lower alkyl" or "C_1-C_6-alkyl" means a straight or branched chain hydrocarbon containing from 1 to 6 carbon atoms. The term "C_1-C_3- alkyl" means a straight or branched chain hydrocarbon containing from 1 to 3 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *n*-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, *n*-heptyl, *n*-octyl, *n*-nonyl, and *n*-decyl.

[0020] The term "alkenyl" as used herein, means a hydrocarbon chain containing from 2 to 30 carbon atoms with at least one carbon-carbon double bond. The alkenyl group may be substituted or unsubstituted. For example, the alkenyl group may be substituted with an aryl group, such as a phenyl.

[0021] The term "alkynyl," as used herein, refers to straight or branched monovalent hydrocarbyl groups having from 2 to 30 carbon atoms, such as 2 to 20, or 2 to 10 carbon atoms and having at least 1 site of triple bond unsaturation. The term "alkyne" also includes non-aromatic cycloalkyl groups of from 5 to 20 carbon atoms, such as from 5 to 10 carbon atoms, having single or multiple rings and having at least one triple bond. Examples of such alkynyl groups include, but are not limited to acetylenyl (-C≡CH), and propargyl (-CH_2C≡CH), and cycloalkynyl moieties, such as, but not limited to, substituted or unsubstituted cyclooctyne moieties.

[0022] The term "alkoxyalkyl" as used herein, refers to an alkoxy group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein.

[0023] The term "alkylene" as used herein, refers to a divalent group derived from a straight or branched chain hydrocarbon of 1 to 30 carbon atoms, for example, of 2 to 10 carbon atoms. Representative examples of alkylene include, but are not limited to, -CH_2-, -$CH(CH_3)$-, -$C(CH_3)_2$-, -CH_2CH_2-, -$CH(CH_3)CH_2$-, -$C(CH_3)_2CH_2$-, -$CH_2CH_2CH_2$-, -$CH(CH_3)CH_2CH_2$-, -$C(CH_3)_2CH_2CH_2$-, -$CH_2C(CH_3)_2CH_2$-, -$CH_2CH_2CH_2CH_2$-, and –$CH_2CH_2CH_2CH_2CH_2$-.

[0024] The term "amino acid" refers to both natural and unnatural amino acids, protected natural and unnatural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids include 20 common amino

acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine) and pyrrolidine and selenocysteine. Amino acid analogs refer to compounds having the same basic chemical structure as a naturally occurring amino acid, i.e., by way of example only, an α-carbon attached to a hydrogen, carboxyl group, amino group, and R group. Such analogs can have a modified R group (e.g., norleucine as an example) or retain a modified peptide backbone while retaining the same basic chemical structure as a natural amino acid. Non-limiting examples of amino acid analogs include citrulline, homoserine, norleucine, methionine sulfoxide, methionine methylsulfonium, homophenylalanine, ornithine, formyl glycine, phenyl glycine, para-azidophenyl glycine, para-azidophenylalanine, para-acetophenylalanine, 4-(3-methyl-(1,2,4,5-tetrazine))-phenylglyine, and 4-(3-methyl-(1,2,4,5-tetrazine))-phenylalanine.

[0025] The term "aryl" as used herein, refers to a phenyl group, or bicyclic aryl or tricyclic aryl fused ring systems. Bicyclic fused ring systems are exemplified by a phenyl group appended to the parent molecular moiety and fused to a phenyl group. Tricyclic fused ring systems are exemplified by a phenyl group appended to the parent molecular moiety and fused to two other phenyl groups. Representative examples of bicyclic aryls include, but are not limited to, naphthyl. Representative examples of tricyclic aryls include, but are not limited to, anthracenyl. The monocyclic, bicyclic, and tricyclic aryls are connected to the parent molecular moiety through any carbon atom contained within the rings, and can be unsubstituted or substituted.

[0026] The term "azide" as used herein, refers to the functional group $-N_3$.

[0027] The term "cycloalkyl" as used herein, refers to a carbocyclic ring system containing three to ten carbon atoms, zero heteroatoms and zero double bonds. Representative examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl and cyclodecyl. "Cycloalkyl" also includes carbocyclic ring systems in which a cycloalkyl group is appended to the parent molecular moiety and is fused to an aryl group as defined herein, a heteroaryl group as defined herein, or a heterocycle as defined herein.

[0028] The term "cycloalkenyl" as used herein, means a non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and preferably having from 5-10 carbon atoms per ring. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl or cycloheptenyl.

[0029] The term "cyclooctene" as used herein, refers to a substituted or unsubstituted non-aromatic cyclic alkyl group of 8 carbon atoms, having a single ring with a double bond. Examples of such cyclooctene groups include, but are not limited to, substituted or unsubstituted trans-cyclooctene (TCO).

[0030] The term "fluoroalkyl" as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by fluorine. Representative

examples of fluoroalkyl include, but are not limited to, 2-fluoroethyl, 2,2,2-trifluoroethyl, trifluoromethyl, difluoromethyl, pentafluoroethyl, and trifluoropropyl such as 3,3,3-trifluoropropyl.

[0031] The term "alkoxyfluoroalkyl" as used herein, refers to an alkoxy group, as defined herein, appended to the parent molecular moiety through a fluoroalkyl group, as defined herein.

[0032] The term "fluoroalkoxy" as used herein, means at least one fluoroalkyl group, as defined herein, is appended to the parent molecular moiety through an oxygen atom. Representative examples of fluoroalkyloxy include, but are not limited to, difluoromethoxy, trifluoromethoxy and 2,2,2-trifluoroethoxy.

[0033] The term "halogen" or "halo" as used herein, means Cl, Br, I, or F.

[0034] The term "haloalkyl" as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by a halogen.

[0035] The term "haloalkoxy" as used herein, means at least one haloalkyl group, as defined herein, is appended to the parent molecular moiety through an oxygen atom.

[0036] The term "heteroalkyl" as used herein, means an alkyl group, as defined herein, in which one or more of the carbon atoms has been replaced by a heteroatom selected from S, Si, O, P and N. The heteroatom may be oxidized. Representative examples of heteroalkyls include, but are not limited to, alkyl ethers, secondary and tertiary alkyl amines, and alkyl sulfides.

[0037] The term "heteroaryl" as used herein, refers to an aromatic monocyclic ring or an aromatic bicyclic ring system or an aromatic tricyclic ring system. The aromatic monocyclic rings are five or six membered rings containing at least one heteroatom independently selected from the group consisting of N, O and S (e.g. 1, 2, 3, or 4 heteroatoms independently selected from O, S, and N). The five membered aromatic monocyclic rings have two double bonds and the six membered six membered aromatic monocyclic rings have three double bonds. The bicyclic heteroaryl groups are exemplified by a monocyclic heteroaryl ring appended to the parent molecular moiety and fused to a monocyclic cycloalkyl group, as defined herein, a monocyclic aryl group, as defined herein, a monocyclic heteroaryl group, as defined herein, or a monocyclic heterocycle, as defined herein. The tricyclic heteroaryl groups are exemplified by a monocyclic heteroaryl ring appended to the parent molecular moiety and fused to two of a monocyclic cycloalkyl group, as defined herein, a monocyclic aryl group, as defined herein, a monocyclic heteroaryl group, as defined herein, or a monocyclic heterocycle, as defined herein. Representative examples of monocyclic heteroaryl include, but are not limited to, pyridinyl (including pyridin-2-yl, pyridin-3-yl, pyridin-4-yl), pyrimidinyl, pyrazinyl, thienyl, furyl, thiazolyl, thiadiazolyl, isoxazolyl, pyrazolyl, and 2-oxo-1,2-dihydropyridinyl. Representative examples of bicyclic heteroaryl include, but are not limited to, chromenyl, benzothienyl, benzodioxolyl, benzotriazolyl, quinolinyl, thienopyrrolyl, thienothienyl, imidazothiazolyl, benzothiazolyl, benzofuranyl, indolyl, quinolinyl, imidazopyridine, benzooxadiazolyl, and benzopyrazolyl. Representative examples of tricyclic heteroaryl

include, but are not limited to, dibenzofuranyl and dibenzothienyl. The monocyclic, bicyclic, and tricyclic heteroaryls are connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the rings, and can be unsubstituted or substituted.

[0038] The term "heterocycle" or "heterocyclic" as used herein, means a monocyclic heterocycle, a bicyclic heterocycle, or a tricyclic heterocycle. The monocyclic heterocycle is a three-, four-, five-, six-, seven-, or eight-membered ring containing at least one heteroatom independently selected from the group consisting of O, N, and S. The three- or four-membered ring contains zero or one double bond, and one heteroatom selected from the group consisting of O, N, and S. The five-membered ring contains zero or one double bond and one, two or three heteroatoms selected from the group consisting of O, N and S. The six-membered ring contains zero, one or two double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. The seven- and eight-membered rings contains zero, one, two, or three double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. Representative examples of monocyclic heterocycles include, but are not limited to, azetidinyl, azepanyl, aziridinyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, 1,3-dithiolanyl, 1,3-dithianyl, 1,3-dimethylpyrimidine-2,4(1H,3H)-dione, imidazolinyl, imidazolidinyl, isothiazolinyl, isothiazolidinyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolinyl, oxadiazolidinyl, oxazolinyl, oxazolidinyl, oxetanyl, piperazinyl, piperidinyl, pyranyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydrothienyl, thiadiazolinyl, thiadiazolidinyl, 1,2-thiazinanyl, 1,3-thiazinanyl, thiazolinyl, thiazolidinyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl (thiomorpholine sulfone), thiopyranyl, and trithianyl. The bicyclic heterocycle is a monocyclic heterocycle fused to a phenyl group, or a monocyclic heterocycle fused to a monocyclic cycloalkyl, or a monocyclic heterocycle fused to a monocyclic cycloalkenyl, or a monocyclic heterocycle fused to a monocyclic heterocycle, or a spiro heterocycle group, or a bridged monocyclic heterocycle ring system in which two non-adjacent atoms of the ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Representative examples of bicyclic heterocycles include, but are not limited to, benzopyranyl, benzothiopyranyl, chromanyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzothienyl, 2,3-dihydroisoquinoline, 2-azaspiro[3.3]heptan-2-yl, azabicyclo[2.2.1]heptyl (including 2-azabicyclo[2.2.1]hept-2-yl), 2,3-dihydro-1*H*-indolyl, isoindolinyl, octahydrocyclopenta[*c*]pyrrolyl, octahydropyrrolopyridinyl, and tetrahydroisoquinolinyl. Tricyclic heterocycles are exemplified by a bicyclic heterocycle fused to a phenyl group, or a bicyclic heterocycle fused to a monocyclic cycloalkyl, or a bicyclic heterocycle fused to a monocyclic cycloalkenyl, or a bicyclic heterocycle fused to a monocyclic heterocycle, or a bicyclic heterocycle in which two non-adjacent atoms of the bicyclic ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Examples of tricyclic heterocycles include, but are not limited to, octahydro-2,5-epoxypentalene, hexahydro-2*H*-2,5-methanocyclopenta[*b*]furan, hexahydro-1*H*-1,4-methanocyclopenta[*c*]furan, aza-adamantane (1-azatricyclo[3.3.1.$1^{3,7}$]decane), and oxa-adamantane (2-oxatricyclo[3.3.1.$1^{3,7}$]decane). The monocyclic, bicyclic, and tricyclic heterocycles

are connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the rings, and can be unsubstituted or substituted.

[0039] The term "hydroxyl" as used herein, means an –OH group.

[0040] The term "hydroxyalkyl" as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by a hydroxyl group.

[0041] In some instances, the number of carbon atoms in a hydrocarbyl substituent (e.g., alkyl or cycloalkyl) is indicated by the prefix "C_x-C_y-" or "C_{x-y}," wherein x is the minimum and y is the maximum number of carbon atoms in the substituent. Thus, for example, "C_1-C_3-alkyl" and "C_{1-3}alkyl" refer to an alkyl substituent containing from 1 to 3 carbon atoms. The two conventions "C_x-C_y-" and "C_{x-y}" are used interchangeably and have the same meaning.

[0042] The term "substituted" refers to a group that may be further substituted with one or more non-hydrogen substituent groups. Substituent groups include, but are not limited to, halogen, =O, =S, cyano, nitro, fluoroalkyl, alkoxyfluoroalkyl, fluoroalkoxy, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, heteroalkyl, cycloalkyl, cycloalkenyl, aryl, heteroaryl, heterocycle, cycloalkylalkyl, heteroarylalkyl, arylalkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, alkylene, aryloxy, phenoxy, benzyloxy, amino, alkylamino, acylamino, aminoalkyl, arylamino, sulfonylamino, sulfinylamino, sulfonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, sulfinyl, -COOH, ketone, amide, carbamate, and acyl.

[0043] The term "tetrazine" refers to a substituted or unsubstituted aromatic cyclic group of 2 carbon atoms and 4 nitrogen atoms, having a single ring with three double bonds. Examples of tetrazine groups include 1,2,3,4-tetrazine and 1,2,4,5-tetrazine. As used herein, 1,2,4,5-tetrazine is referred to as a "Tz" group.

[0044] The term "selectively delivering" refers to delivering an agent (e.g., a payload) to an organ or tissue (or portion thereof) in need of treatment or diagnosis, without significant binding to other non-target organs or tissues (or portions thereof).

[0045] The term "payload" refers to an agent for delivery to a target site in a subject. Payloads include therapeutic agents.

[0046] The term "therapeutic agent" refers to an agent capable of treating and/or ameliorating a condition or disease, or one or more symptoms thereof, in a subject. Therapeutic agents of the present disclosure also include prodrug forms of therapeutic agents.

[0047] The term "diagnostic agent" refers to agents that assist in diagnosing conditions or diseases. Representative diagnostic agents include imaging agents such as paramagnetic agents, optical probes, radionuclides, and the like. Paramagnetic agents are imaging agents that are magnetic under an externally applied field. Examples of paramagnetic agents include, but are not limited to, iron particles including iron nanoparticles and iron microparticles. Optical probes are fluorescent compounds that can

be detected by excitation at one wavelength of radiation and detection at a second, different, wavelength of radiation. Optical probes of the present disclosure include, but are not limited to, Cy5.5, Alexa 680, Cy5, DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate) and DiR (1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide). Other optical probes include quantum dots. Radionuclides are elements that undergo detectable radioactive decay. Radionuclides useful in embodiments of the present disclosure include, but are not limited to, ^{3}H, ^{11}C, ^{13}N, ^{18}F, ^{19}F, ^{60}Co, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{82}Rb, ^{89}Zr, ^{90}Sr, ^{90}Y, ^{99}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{129}I, ^{131}I, ^{137}Cs, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, Rn, Ra, Th, U, Pu, and ^{241}Am.

[0048] The term "targeting agent" refers to a chemical or biological agent that specifically binds to a target (e.g., a targeted organ or tissue), thereby forming a stable association between the targeting agent and the specific target. By "stably associated" or "stable association" is meant that a moiety is bound to or otherwise associated with another moiety or structure under standard physiological conditions. Bonds may include covalent bonds and non-covalent interactions, such as, but not limited to, ionic bonds, hydrophobic interactions, hydrogen bonds, van der Waals forces (e.g., London dispersion forces), dipole-dipole interactions, and the like. A targeting agent may be a member of a specific binding pair, such as, but are not limited to: a member of a receptor/ligand pair; a ligand-binding portion of a receptor; a member of an antibody/antigen pair; an antigen-binding fragment of an antibody; a hapten; a member of a lectin/carbohydrate pair; a member of an enzyme/substrate pair; biotin/avidin; biotin/streptavidin; digoxin/antidigoxin; a member of a DNA or RNA aptamer binding pair; a member of a peptide aptamer binding pair; and the like. Targeting agents include ligands that specifically bind (or substantially specifically bind) a particular clinically-relevant target receptor or cell surface target. The ligand can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or other molecule with a specific affinity for a target receptor or cell surface target. Examples of receptors and cell surface targets include, but are not limited to, PD-1, CTLA-4, HER2/neu, HER1/EGFR, VEGFR, 4-1BB, GITR, LT4 - human mAb directed against the inhibitory immune checkpoint receptor immunoglobulin-like transcript 4 (ILT4; leukocyte immunoglobulin-like receptor subfamily B member 2, LILRB2, lymphocyte immunoglobulin-like receptor 2, LIR2, monocyte/macrophage immunoglobulin-like receptor 10, MIR-10, CD85d, or other cellular receptors or cell surface targets. Additional examples are included in various embodiments disclosed herein.

[0049] The term "targeted organ or tissue" refers to an organ or tissue that is being targeted for delivery of the payload. Representative organs and tissues for targeting include those that can be targeted by chemical or biological targeting agents, as well as those organs and tissues that cannot be targeted by chemical or biological targeting agents.

[0050] The term "implanting" refers to surgical implantation into a subject's body.

[0051] The term "contacting" or "contact" refers to the process of bringing into contact at least two distinct species such that they can interact with each other, such as in a non-covalent or covalent binding

interaction or binding reaction. It should be appreciated, however, the resulting complex or reaction product can be produced directly from an interaction or a reaction between the added reagents or from an intermediate from one or more of the added reagents or moieties, which can be produced in the contacting mixture.

[0052] The term "binding agent" refers to an agent having a functional group capable of forming a covalent bond to a complementary functional group of another binding agent in a biological environment. Binding between binding agents in a biological environment may also be referred to as bioconjugation. Binding agents include bioorthogonal binding agents, which are binding agents having bioorthogonal functional groups. Bioorthogonal functional groups of bioorthogonal binding agents selectively react with a complementary bioorthogonal functional group of another bioorthogonal binding partner. Selective reaction between bioorthogonal binding partners can minimize side reactions with other binding agents, biological compounds, or other non-complementary bioorthogonal binding agents or non-complementary bioorthogonal functional groups. Bioorthogonal moieties or functional groups of bioorthogonal binding agents include, but are not limited to, an azide and alkyne for formation of a triazole via Click-chemistry reactions, trans-cyclooctene (TCO) and tetrazine (Tz) (e.g., 1,2,4,5-tetrazine), and others. The binding agents useful in the present disclosure may have a high reactivity with the corresponding binding agent so that the reaction is rapid.

[0053] The term "functionalized" refers to a moiety having a functional group attached to the moiety, such as for example a moiety having a binding agent functional group (e.g., a bioorthogonal functional group) attached thereto.

[0054] The term "administering" refers to any suitable route of administration to a subject, such as, but not limited to, oral administration, administration as a suppository, topical contact, parenteral, intravenous, intraperitoneal, intramuscular, intralesional, intranasal or subcutaneous administration, intrathecal administration, or the implantation of a slow-release device e.g., a mini-osmotic pump, to the subject.

[0055] The term "parenterally," as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0056] The term "leaving group" refers to an atom (or a group of atoms) with electron withdrawing ability that can be displaced as a stable species, taking with it the bonding electrons. Examples of suitable leaving groups include halides (e.g., Br, Cl, I), sulfonate esters (e.g., triflate, mesylate, tosylate, and brosylate), and nitrophenols.

[0057] The term "pharmaceutically effective amount" and "therapeutically effective amount" refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent or reduce the risk of the occurrence or reoccurrence of the disease or disorder or

symptom(s) thereof. In reference to tumorigenic proliferative disorders, a pharmaceutically or therapeutically effective amount comprises an amount sufficient to, among other things, cause the tumor to shrink or decrease the growth rate of the tumor.

[0058] As used herein, the term "subject," "patient," or "organism" includes humans and mammals (e.g., mice, rats, pigs, cats, dogs, and horses). Typical subjects to which an agent(s) of the present disclosure may be administered may include mammals, particularly primates, especially humans. For veterinary applications, suitable subjects may include, for example, livestock such as cattle, sheep, goats, cows, swine, and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, suitable subjects may include mammals, such as rodents (e.g., mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like.

[0059] The term "treating" or "treatment" as used herein means the treating or treatment of a disease or medical condition or symptom(s) thereof in a patient, such as a mammal (particularly a human) that includes: (a) ameliorating the disease or medical condition or symptom(s) thereof, such as, eliminating or causing regression of the disease or medical condition or symptom(s) thereof in a patient; (b) suppressing the disease or medical condition or symptom(s) thereof, for example by, slowing or arresting the development of the disease or medical condition or symptom(s) thereof in a patient; or (c) alleviating a symptom of the disease or medical condition or symptom(s) thereof in a patient.

[0060] The term "physiological conditions" is meant to encompass those conditions compatible with living cells, *e.g.*, predominantly aqueous conditions of a temperature, pH, salinity, *etc.* that are compatible with living cells.

[0061] For compounds described herein, groups and substituents thereof may be selected in accordance with permitted valence of the atoms and the substituents, such that the selections and substitutions result in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0062] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0063] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7

and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

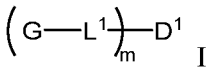
[0064] The compounds may exist as stereoisomers wherein asymmetric or chiral centers are present. The stereoisomers are "*R*" or "*S*" depending on the configuration of substituents around the chiral carbon atom. The terms "*R*" and "*S*" used herein are configurations as defined in IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, in Pure Appl. Chem., 1976, 45: 13-30. The disclosure contemplates various stereoisomers and mixtures thereof, and these are specifically included within the scope of this invention. Stereoisomers include enantiomers and diastereomers and mixtures of enantiomers or diastereomers. Individual stereoisomers of the compounds may be prepared synthetically from commercially available starting materials, which contain asymmetric or chiral centers or by preparation of racemic mixtures followed by methods of resolution well-known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography, and optional liberation of the optically pure product from the auxiliary as described in Furniss, Hannaford, Smith, and Tatchell, "Vogel's Textbook of Practical Organic Chemistry," 5th edition (1989), Longman Scientific & Technical, Essex CM20 2JE, England, or (2) direct separation of the mixture of optical enantiomers on chiral chromatographic columns, or (3) fractional recrystallization methods.

[0065] It should be understood that the compounds may possess tautomeric forms as well as geometric isomers, and that these also constitute an aspect of the invention.

[0066] The present disclosure also includes isotopically-labeled compounds, which are identical to those recited herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in the compounds of the invention are hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, and chlorine, such as, but not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. Substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements, and, hence, may be preferred in some circumstances. The compound may incorporate positron-emitting isotopes for medical imaging and positron-emitting tomography (PET) studies for determining the distribution of receptors. Suitable positron-emitting isotopes that can be incorporated are ^{11}C, ^{13}N, ^{15}O, and ^{18}F. Isotopically-labeled compounds disclosed herein can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using appropriate isotopically-labeled reagent in place of non-isotopically-labeled reagent.

B. Conjugates

[0067] Provided herein are conjugates for use in bioorthogonal reactions. In some embodiments, provided is a conjugate of Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein:

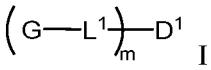
m is an integer from 1-150;

G, at each occurrence, is independently an optionally substituted trans-cyclooctene moiety;

D^1 is a payload selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, a monoclonal antibody, a topoisomerase inhibitor, lurbinectedin, MSA-2, gardiquimod, ciprofloxacin, mitomycin C, etoposide, and exatecan, or a derivative, or analog thereof;

L^1, at each occurrence, is independently a linker.

[0068] Provided herein are conjugates for use in bioorthogonal reactions. In some embodiments, provided is a conjugate of Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

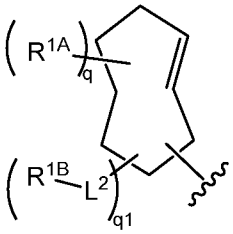
m is an integer from 1-150;

G, at each occurrence, is independently an optionally substituted trans-cyclooctene moiety;

D^1 is a payload selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and a monoclonal antibody, or a derivative, or analog thereof;

L^1, at each occurrence, is independently a linker.

[0069] In some embodiments of the conjugates described herein, each trans-cyclooctene moiety is independently:



wherein:

R^{1A}, at each occurrence, is independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, and C_{1-4}alkoxy;

q is 0, 1, or 2;

q1 is 0 or 1;

R^{1B}, at each occurrence, is independently selected from the group consisting of G^1, -OH, $–NR^{1c}–C_{1-4}alkylene–G^1$, $–NR^{1c}–C_{1-4}alkylene–N(R^{1d})_2$, $–NR^{1c}–C_{1-6}alkylene–N(C_{1-4}alkyl)_3^+$, $–N(R^{1c})CHR^{1e}CO_2H$, $–N(R^{1c})–C_{1-6}alkylene–CO_2H$, $–N(R^{1f})–C_{2-4}alkylene–(N(C_{1-4}alkylene–CO_2H)–C_{2-4}alkylene)_n–N(C_{1-4}alkylene–CO_2H)_2$, $–N(R^{1c})CHR^{1e}C(O)OC_{1-6}alkyl$, $–N(R^{1c})–C_{1-6}alkylene–C(O)OC_{1-6}alkyl$, $–N(R^{1f})–C_{2-4}alkylene–(N(C_{1-4}alkylene–C(O)OC_{1-6}alkyl)–C_{2-4}alkylene)_n–N(C_{1-4}alkylene–C(O)OC_{1-6}alkyl)_2$, $–N(R^{1c})–C_{1-6}alkylene–SO_3H$, $–N(R^{1c})–(CH_2CH_2O)_{1-3}–CH_2CH_2N((CH_2CH_2O)_{1-3}–C_{1-6}alkylene–CO_2H)_2$, and $–N(R^{1c})–CH(CH_2O–(CH_2CH_2O)_{0-2}–C_{1-6}alkylene–CO_2H)_2$;

R^{1c} and R^{1d}, at each occurrence, are independently hydrogen or C_{1-4}alkyl;

R^{1e}, at each occurrence, is independently $–C_{1-4}alkylene–CO_2H$, $–C_{1-4}alkylene–CONH_2$, or $–C_{1-4}alkylene–OH$;

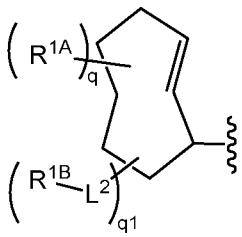
R^{1f}, at each occurrence, is independently hydrogen, C_{1-6}alkyl, or $C_{1-4}alkylene–CO_2H$;

n, at each occurrence, is independently 0, 1, 2, or 3;

L^2, at each occurrence, is independently selected from the group consisting of –C(O)– and C_{1-3}alkylene; and

G^1, at each occurrence, is independently an optionally substituted heterocyclyl.

[0070] In some embodiments of the conjugates described herein, each trans-cyclooctene moiety (G) is independently:



wherein:

R^{1A}, at each occurrence, is independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, and C_{1-4}alkoxy;

q is 0, 1, or 2;

q1 is 0 or 1;

R^{1B}, at each occurrence, is independently selected from the group consisting of G^1, -OH, $-NR^{1c}-C_{1-4}$alkylene$-G^1$, $-NR^{1c}-C_{1-4}$alkylene$-N(R^{1d})_2$, $-NR^{1c}-C_{1-6}$alkylene$-N(C_{1-4}alkyl)_3^+$, $-N(R^{1c})CHR^{1e}CO_2H$, $-N(R^{1c})-C_{1-6}$alkylene$-CO_2H$, $-N(R^{1f})-C_{2-4}$alkylene$-(N(C_{1-4}$alkylene$-CO_2H)-C_{2-4}$alkylene$)_n-N(C_{1-4}$alkylene$-CO_2H)_2$, $-N(R^{1c})CHR^{1e}C(O)OC_{1-6}$alkyl, $-N(R^{1c})-C_{1-6}$alkylene$-C(O)OC_{1-6}$alkyl, $-N(R^{1f})-C_{2-4}$alkylene$-(N(C_{1-4}$alkylene$-C(O)OC_{1-6}$alkyl$)-C_{2-4}$alkylene$)_n-N(C_{1-4}$alkylene$-C(O)OC_{1-6}alkyl)_2$, $-N(R^{1c})-C_{1-6}$alkylene$-SO_3H$, $-N(R^{1c})-(CH_2CH_2O)_{1-3}-CH_2CH_2N((CH_2CH_2O)_{1-3}-C_{1-6}$alkylene$-CO_2H)_2$, and $-N(R^{1c})-CH(CH_2O-(CH_2CH_2O)_{0-2}-C_{1-6}$alkylene$-CO_2H)_2$;

R^{1c} and R^{1d}, at each occurrence, are independently hydrogen or C_{1-4}alkyl;

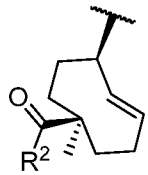
R^{1e}, at each occurrence, is independently $-C_{1-4}$alkylene$-CO_2H$, $-C_{1-4}$alkylene$-CONH_2$, or $-C_{1-4}$alkylene$-OH$;

R^{1f}, at each occurrence, is independently hydrogen, C_{1-6}alkyl, or C_{1-4}alkylene$-CO_2H$;

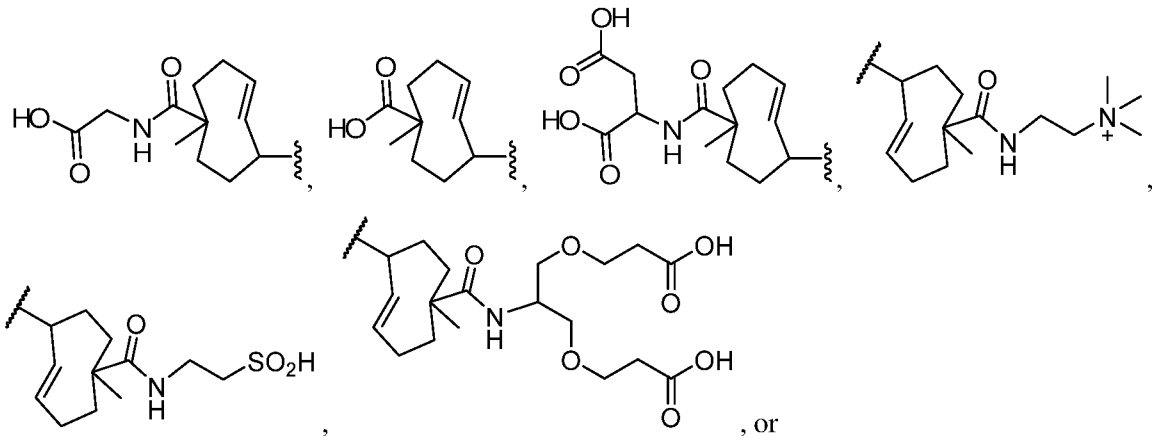
n, at each occurrence, is independently 0, 1, 2, or 3;

L^2, at each occurrence, is independently selected from the group consisting of –C(O)– and C_{1-3}alkylene; and

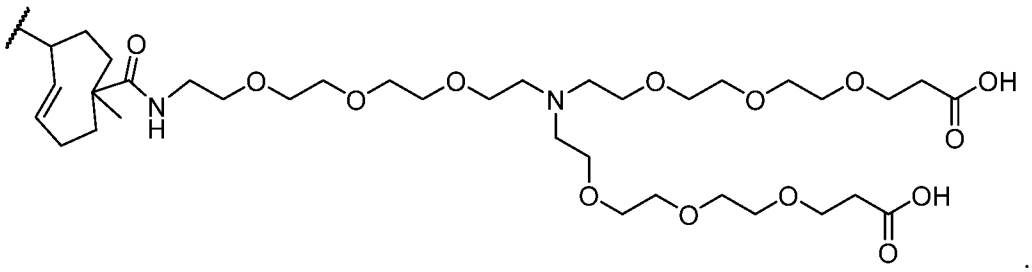
G^1, at each occurrence, is independently an optionally substituted heterocyclyl.

[0071] In some embodiments, the trans-cyclooctene moiety (G) is  , and R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

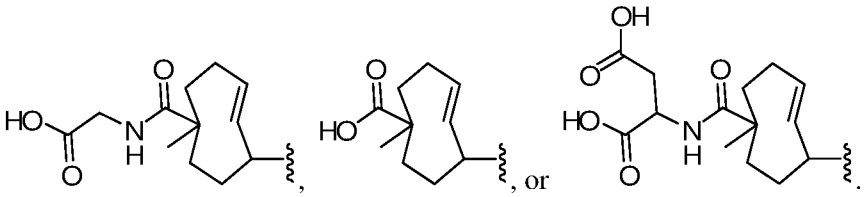
[0072] In some embodiments, the trans-cyclooctene moiety (G) is:

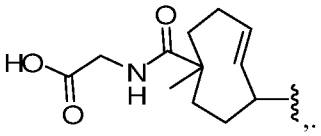


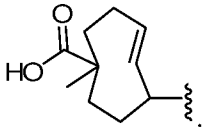
, or

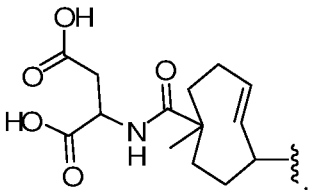


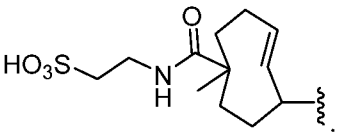
[0073] In some embodiments, the trans-cyclooctene moiety is:

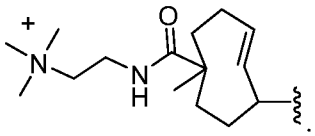


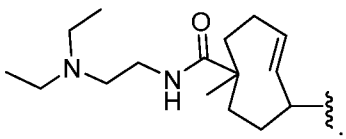
[0074] In some embodiments, the trans-cyclooctene moiety is  .

[0075] In some embodiments, the trans-cyclooctene moiety is  .

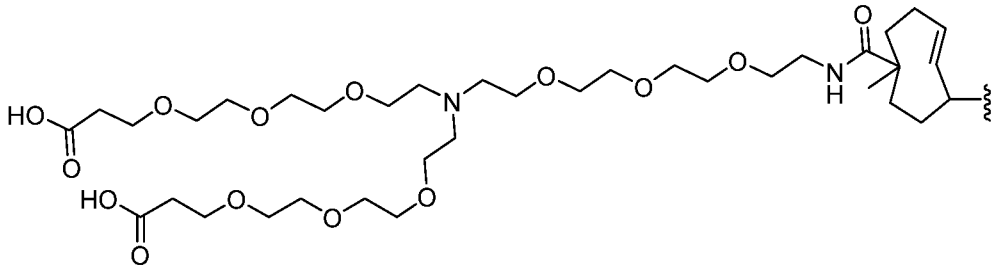
[0076] In some embodiments, the trans-cyclooctene moiety is  .

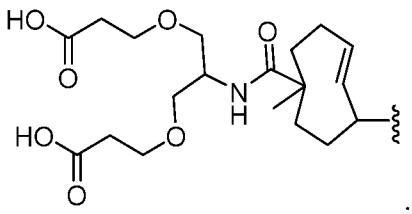
[0077] In some embodiments, the trans-cyclooctene moiety is  .

[0078] In some embodiments, the trans-cyclooctene moiety is  .

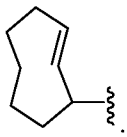
[0079] In some embodiments, the trans-cyclooctene moiety is  .

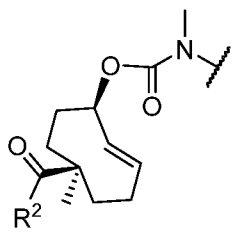
[0080] In some embodiments, the trans-cyclooctene moiety is





[0081] In some embodiments, the trans-cyclooctene moiety is .

[0082] In some embodiments, the trans-cyclooctene moiety is  .

[0083] In some embodiments, G–L^1, at each occurrence, is independently  , and R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

[0084] In some embodiments, m is 1-20. In some embodiments, m is 1-10. In some embodiments, m is 1-5. In some embodiments, m is 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1. In some embodiments, m is 1.

[0085] Also provided is a pharmaceutical composition comprising the conjugate, or a pharmaceutically acceptable salt thereof, as disclosed herein and a pharmaceutically acceptable carrier.

Payloads

[0086] The term "payload" as used herein is intended to refer to an inhibitor of poly (ADP-ribose) polymerase (PARP inhibitor), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and a monoclonal antibody, or a derivative, or analog thereof.

[0087] In certain embodiments, the terms "derivative" or "analog" or "derived from" as used in reference to a payload, means that one or more atoms, including hydrogen or non-hydrogen atoms, of the original, unmodified payload is replaced by a covalent bond to one or more linker L^1. The D^1 payloads are derived from the known payload and are modified to be covalently bonded to at least one optionally substituted trans-cyclooctene via a linker L^1. The D^1 payloads, even after modification to arrive at the

compounds described herein, maintain biological activity which is comparable to that observed in the original, unmodified payload. In certain embodiments, the D^1 payloads exhibit a binding activity or inhibition which is at least about 98%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, or about 50% of that observed in the original, unmodified payload.

[0088] In certain embodiments, a hydrogen atom bound to a heteroatom (e.g., N, O, or S) of the original, unmodified payload is replaced by a covalent bond to a linker L^1. In certain embodiments, a halogen atom on a payload is replaced for attachment to the remainder of the compound. In certain embodiments, a hydrogen atom on a payload is replaced for attachment to the remainder of the compound. In certain embodiments, the hydrogen atom is on a heteroatom. In certain embodiments, the hydrogen atom is on a nitrogen. In certain embodiments, the hydrogen atom is on an oxygen. In certain embodiments, the hydrogen atom is on a carbon.

[0089] In some embodiments, at least one payload is selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, an anti-CD3 (αCD3) monoclonal antibody, lurbinectedin, MSA-2, gardiquimod, ciprofloxacin, paclitaxel, gemcitabine, mitomycin C, etoposide, exatecan, and MMAE, or a derivative, or analog thereof.

[0090] In some embodiments, at least one payload is selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and an anti-CD3 (αCD3) monoclonal antibody, or a derivative, or analog thereof.

[0091] In some embodiments, at least one payload is selected from lurbinectedin, MSA-2, gardiquimod, ciprofloxacin, paclitaxel, gemcitabine, mitomycin C, etoposide, exatecan, Seco-Duocarmycin SA, and MMAE, or a derivative, or analog thereof.

[0092] A monoclonal antibody for use herein as a payload can be an entire monoclonal antibody, or a fragment thereof (e.g., antigen-binding fragment (Fab)). In some embodiments, the antibody is an immune cell engager, and as such would induce or elicit an immune response. In some embodiments, the antibody, or fragment thereof, targets one or more of CD3 (NCBI Gene ID 916), CD28 (NCBI Gene ID 940), CD137 (4-1BB) (NCBI Gene ID 3604), CD16 (NCBI Gene ID 2214), NKG2D (NCBI Gene ID 22914), CD64 (NCBI Gene ID 2209), GITR/TNFRSF18 (NCBI Gene ID 8487), CD25 (NCBI Gene ID 3559), CD40 (NCBI Gene ID 958), CD4 (NCBI Gene ID 920), CXCR4 (NCBI Gene ID 7852), G-CSFR (NCBI Gene ID 1441), GM-CSFR (NCBI Gene ID 1438), CD122 (NCBI Gene ID 3560), PD1 (NCBI Gene ID 5133), CTLA4 (NCBI Gene ID 1493), LAG3 (NCBI Gene ID 3902), TIGIT (NCBI Gene ID 201633), NCR1 (NCBI Gene ID 9437), TIM3 (NCBI Gene ID 84868), VISTA (NCBI Gene ID 64115), CD134 (NCBI Gene ID 7293), CD27 (NCBI Gene ID 939), CD40L (NCBI Gene ID 959), ICOS (NCBI Gene ID 29851), BAFFR (NCBI Gene ID 115650), LFA-1 (NCBI Gene ID 3689), or BTLA (NCBI Gene ID 151888).

[0093] In certain embodiments, the payload is an antibody or antibody fragment which targets CD3, such as OKT3, SP34, UCHT1, teplizumab, otelixizumab, visilizumab, or foralumab, or an antibody fragment derived therefrom.

[0094] In certain embodiments, the payload is an antibody or antibody fragment which targets CD28, such as theralizumab, TGN1412, or FR104, or an antibody fragment derived therefrom.

[0095] In certain embodiments, the payload is an antibody or antibody fragment which targets CD137 (4-1BB), such as utomilumab, urelumab, LVGN6051, or AGEN2373, or an antibody fragment derived therefrom.

[0096] In certain embodiments, the payload is an antibody or antibody fragment which targets CD16, such as AFM13, or an antibody fragment derived therefrom.

[0097] In certain embodiments, the payload is an antibody or antibody fragment which targets NKG2D, such as NNC0152-0002 or JNJ-64304500, or an antibody fragment derived therefrom.

[0098] In certain embodiments, the payload is an antibody or antibody fragment which targets CD64, such as H22, or an antibody fragment derived therefrom.

[0099] In certain embodiments, the payload is an antibody or antibody fragment which targets GITR/TNFRSF18, such as MK-4166, TRX518, MS-986156, AMG-228, or INCAGN01876, or an antibody fragment derived therefrom.

[0100] In certain embodiments, the payload is an antibody or antibody fragment which targets CD25, such as daclizumab, RG6292, basiliximab, or HuMax-TAC, or an antibody fragment derived therefrom.

[0101] In certain embodiments, the payload is an antibody or antibody fragment which targets CD40, such as iscalimab, ABBV-323, bleselumab (ASKP-1240), BI-655064, FFP-104, BMS986090, dacetuzumab, or lucatumumab, or an antibody fragment derived therefrom.

[0102] In certain embodiments, the payload is an antibody or antibody fragment which targets CD4, such as MAX.16H5, IT1208, zanolimumab (HuMax-CD4), UB-421, or MTRX1011A, or an antibody fragment derived therefrom.

[0103] In certain embodiments, the payload is an antibody or antibody fragment which targets CXCR4, such as F50067, or an antibody fragment derived therefrom.

[0104] In certain embodiments, the payload is an antibody or antibody fragment which targets G-CSFR, such as CSL324, or an antibody fragment derived therefrom.

[0105] In certain embodiments, the payload is an antibody or antibody fragment which targets GM-CSFR, such as mavrilimumab, or an antibody fragment derived therefrom.

[0106] In certain embodiments, the payload is an antibody or antibody fragment which targets CD122, such as Hu-Mik(beta)1, or an antibody fragment derived therefrom.

[0107] In certain embodiments, the payload is an antibody or antibody fragment which targets PD-1, such as CC-90006, cemiplimab, camrelizumab, or TSR-042, or an antibody fragment derived therefrom.

[0108] In certain embodiments, the payload is an antibody or antibody fragment which targets CTLA4, such as tremelimumab or ipilimumab, or an antibody fragment derived therefrom.

[0109] In certain embodiments, the payload is an antibody or antibody fragment which targets LAG3, such as relatlimab (BMS-986016), GSK2831781, cemiplimab (REGN3767), favezelimab, ieramilimab, or mavezelimab, or an antibody fragment derived therefrom.

[0110] In certain embodiments, the payload is an antibody or antibody fragment which targets TIGIT, such as BMS-986207, tiragolumab, vibostolimab, etigilimab, domvanalimab, ASP-8374, IBI939, BGB-A1217, COM902, or M6223, or an antibody fragment derived therefrom.

[0111] In certain embodiments, the payload is an antibody or antibody fragment which targets NCR1, such as hNKp46.02, or an antibody fragment derived therefrom.

[0112] In certain embodiments, the payload is an antibody or antibody fragment which targets TIM3, such as cobolimab, Sym023, LY3321367, BMS-986258, SHR-1702, dabatolimab, or INCAGN02390, or an antibody fragment derived therefrom.

[0113] In certain embodiments, the payload is an antibody or antibody fragment which targets VISTA, such as SG7, K01401-020, CI-8993, or JNJ-61610588, or an antibody fragment derived therefrom.

[0114] In certain embodiments, the payload is an antibody or antibody fragment which targets CD134, such as KHK4083 or ISB830, or an antibody fragment derived therefrom.

[0115] In certain embodiments, the payload is an antibody or antibody fragment which targets CD27, such as varlilumab, MK-5890, or CDX-527, or an antibody fragment derived therefrom.

[0116] In certain embodiments, the payload is an antibody or antibody fragment which targets CD40L, such as dapirolizumab, or an antibody fragment derived therefrom.

[0117] In certain embodiments, the payload is an antibody or antibody fragment which targets ICOS, such as MEDI-570, KY1044, JTX-2011, or GSK3359609, or an antibody fragment derived therefrom.

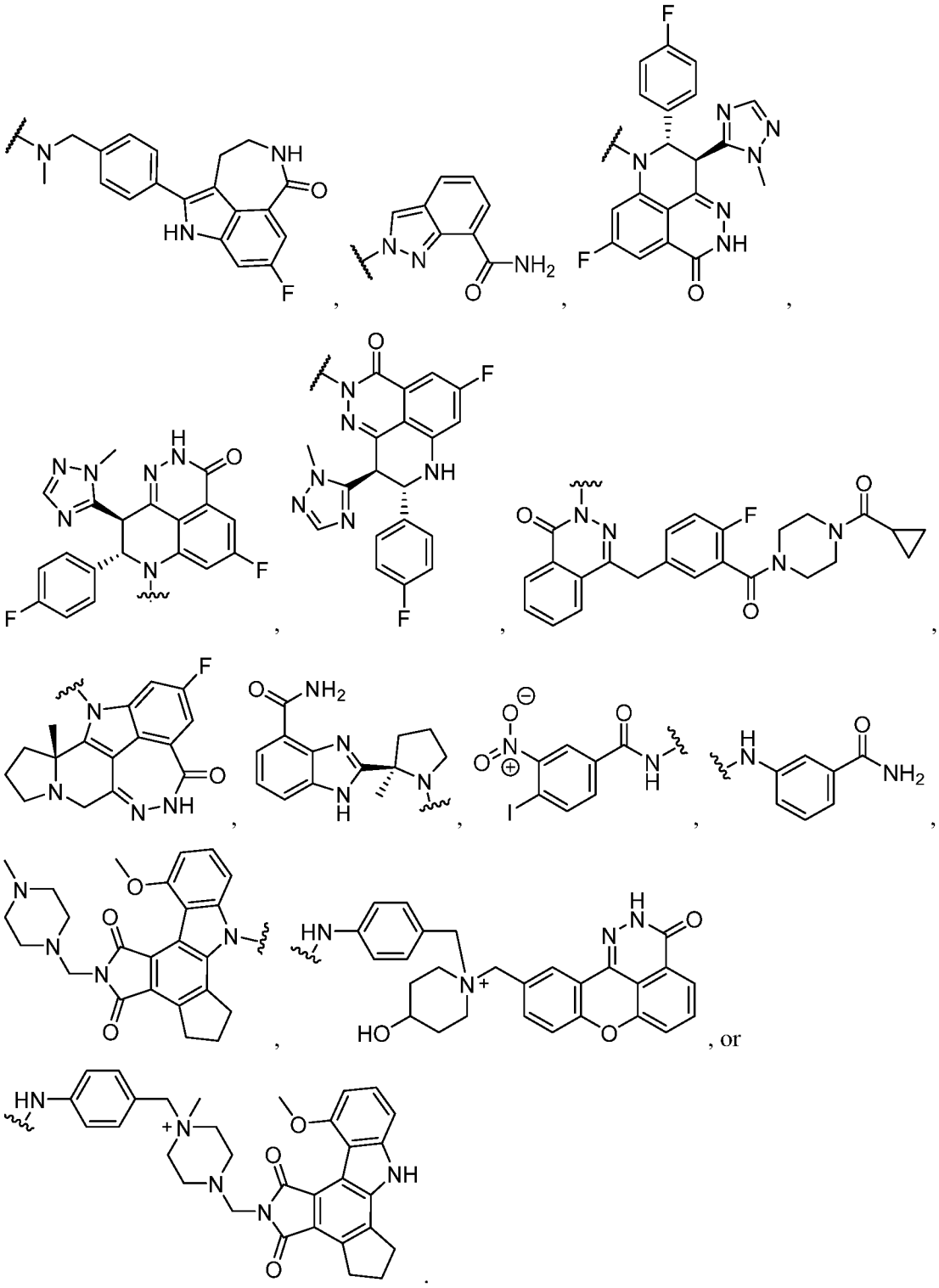
[0118] In certain embodiments, the payload is an antibody or antibody fragment which targets BAFFR, such as ianalumab, or an antibody fragment derived therefrom.

[0119] In certain embodiments, the payload is an antibody or antibody fragment which targets LFA-1, such as efalizumab, or an antibody fragment therefrom.

[0120] In certain embodiments, the payload is an antibody or antibody fragment which targets BTLA, such as icatolimab, or an antibody fragment derived therefrom.

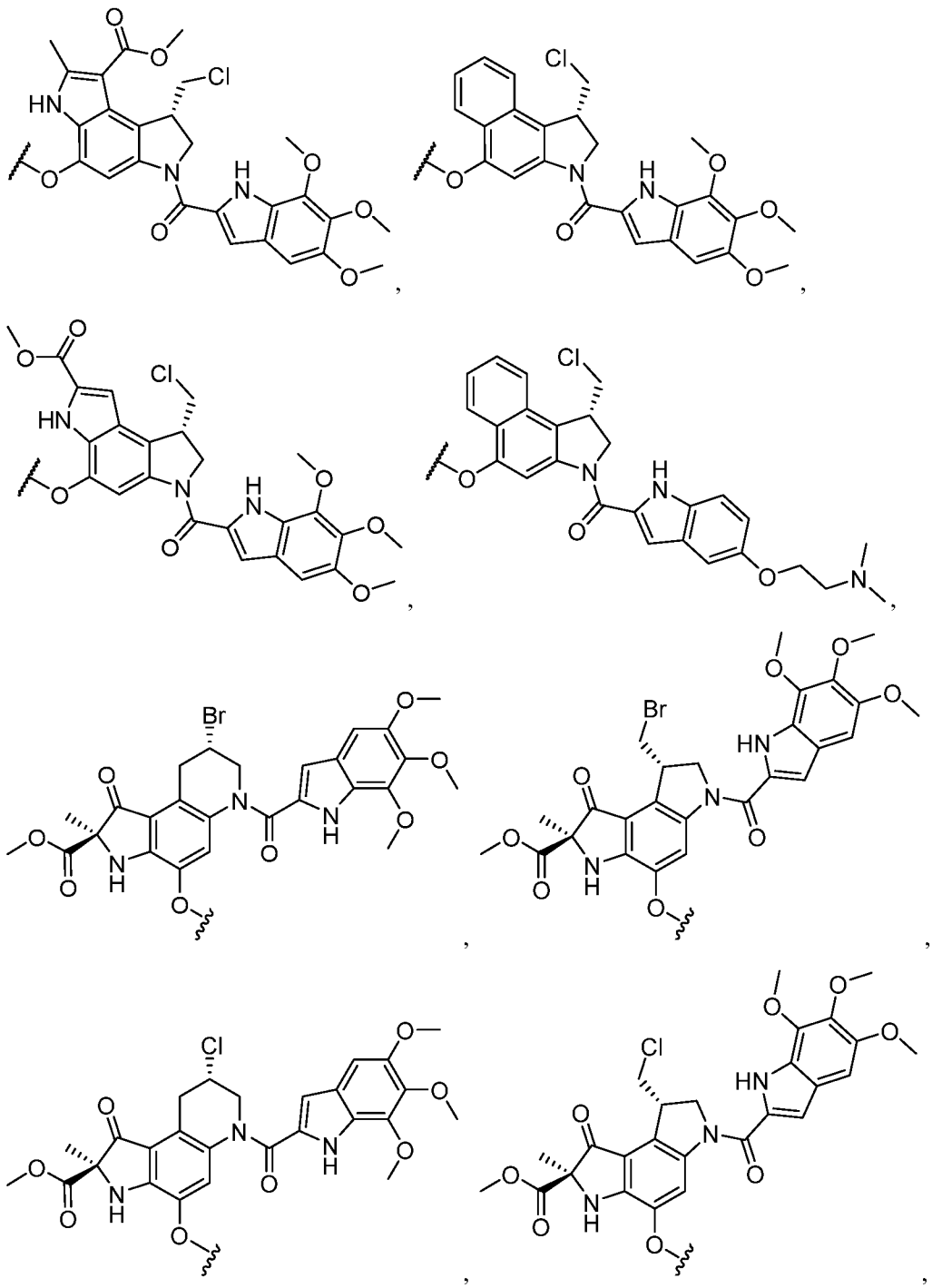
[0121] In some embodiments, a payload is an inhibitor of poly (ADP-ribose) polymerase (PARP), or a derivative, or analog thereof. In some embodiments, the inhibitor of poly (ADP-ribose) polymerase (PARP inhibitor) is niraparib, talazoparib, olaparib, pamiparib, rucaparib, veliparib, iniparib, 3-aminobenzamide, CEP-9722, E7016, or a derivative, or analog thereof.

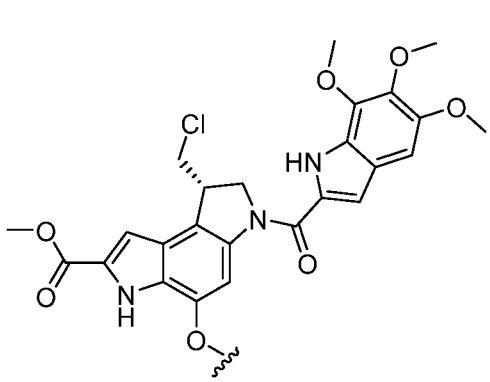
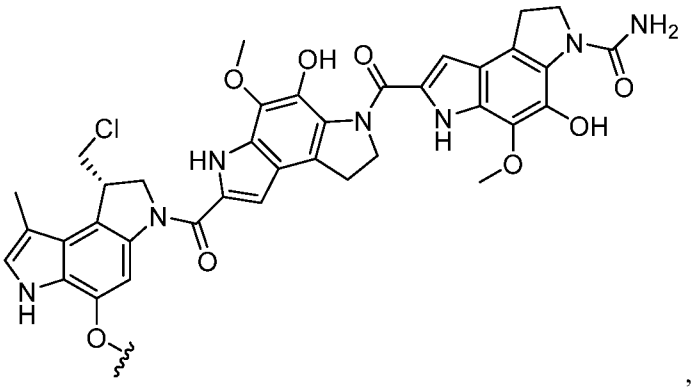
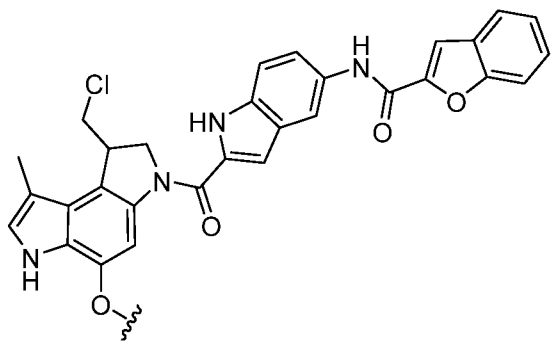
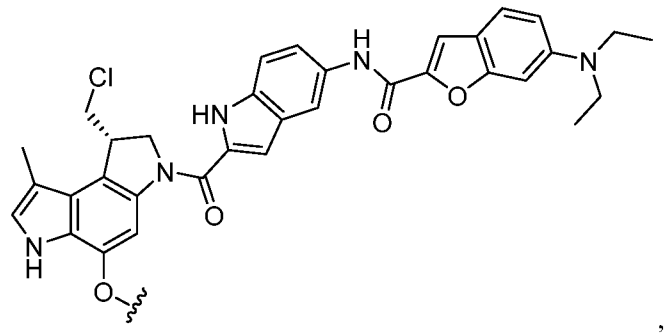
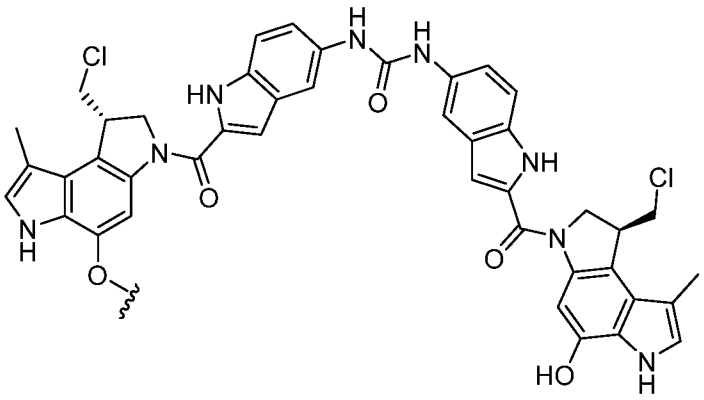
[0122] In some embodiments, D^1 is:



[0123] In some embodiments, a payload is a duocarmycin, or a derivative, or analog thereof. In some embodiments, the duocarmycin is duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, carzelesin, bizelesin, or a derivative, or analog thereof.

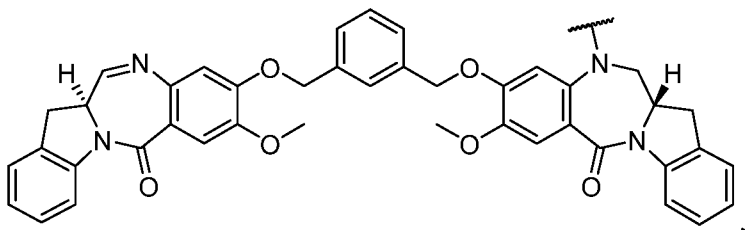
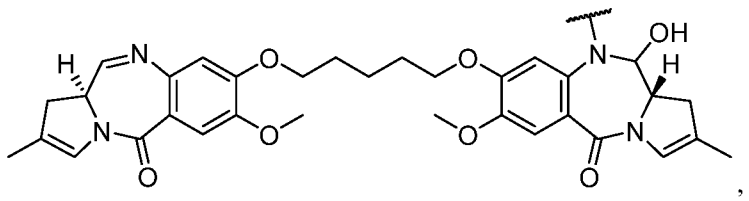
[0124] In some embodiments, D^1 is:

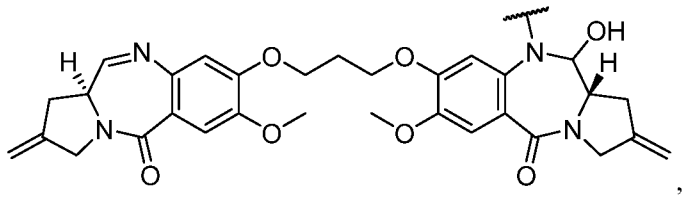



,

,

,

,

or .

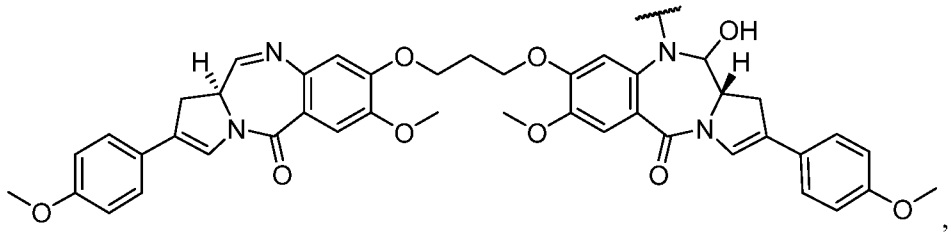
[0125] In some embodiments, a payload is a pyrrolobenzodiazepine (PBD), or a derivative, or analog thereof. In some embodiments, the pyrrolobenzodiazepine (PBD) is [1,2]diazepino[3,4-e]indole, or a derivative, or analog thereof.

[0126] In some embodiments, D^1 is:

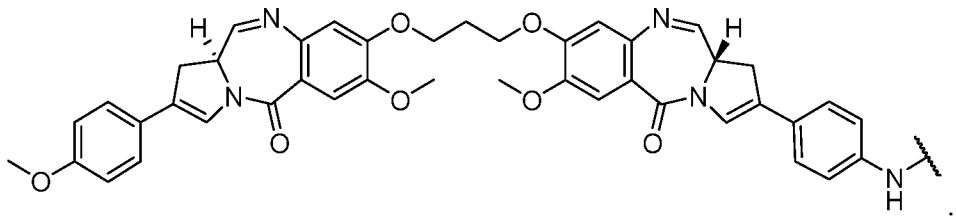

,

,



,



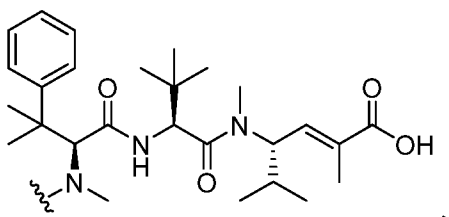
, or



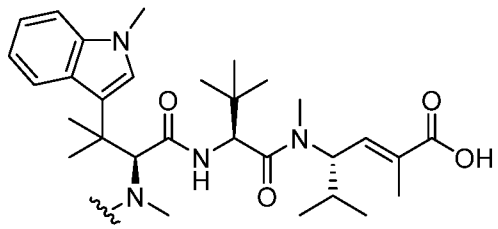
.

[0127] In some embodiments, a payload is an inhibitor of tubulin polymerization. In some embodiments, a payload is hemiasterlin, HTI-286, or a derivative, or analog thereof.

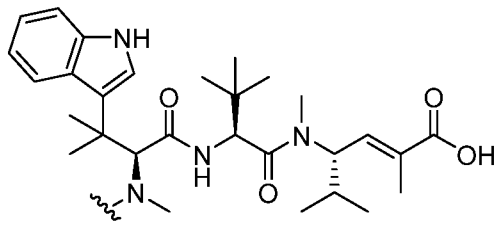
[0128] In some embodiments, D^1 is derived from:



,

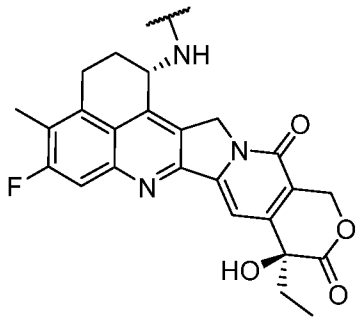
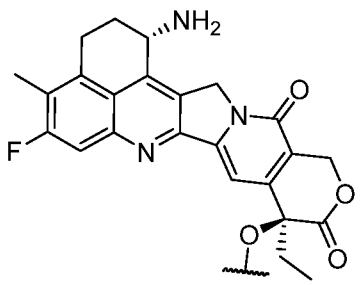


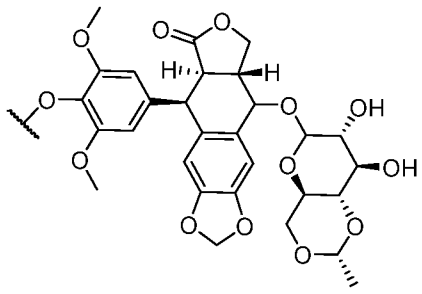
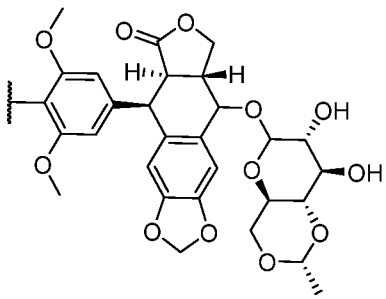
, or

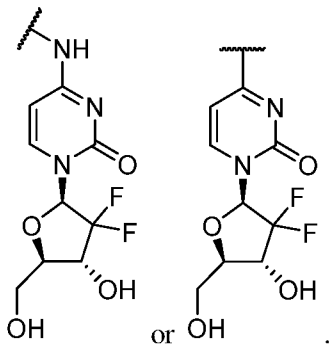


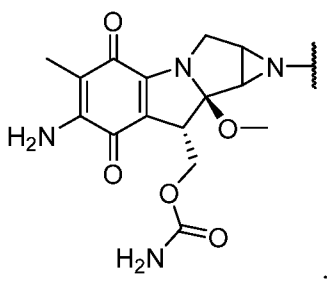
.

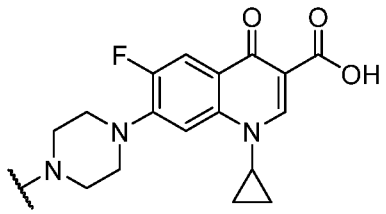
[0129] In some embodiments, D^1 is a topoisomerase inhibitor. In some embodiments, D^1 is camptothecin, or a derivative, or analog thereof. In some embodiments, D^1 is topotecan, irinotecan, silatecan, cositecan, exatecan, lurtotecan, gimatecan, belotecan, or rubitecan.

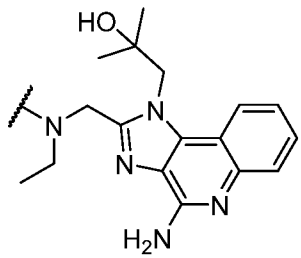
[0130] In some embodiments, D^1 is  or  .

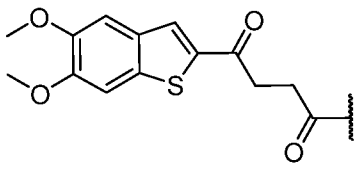
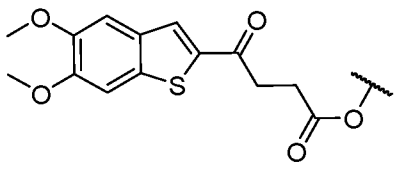
[0131] In some embodiments, D^1 is  or  .

[0132] In some embodiments, D^1 is  or .

[0133] In some embodiments, D^1 is  .

[0134] In some embodiments, D^1 is  .

[0135] In some embodiments, D^1 is  .

[0136] In some embodiments, D^1 is  or  .

[0137] In some embodiments, the payload comprises a polypeptide. In some embodiments, the polypeptide comprises one or more lysine, serine, threonine, or tyrosine residues. In some embodiments, the linker L^1 is covalently bonded to a lysine, serine, threonine, or tyrosine residue present on the payload. In some embodiments, the polypeptide comprises one or more lysine residues. In some embodiments, the linker L^1 is covalently bonded to a lysine residue present on the payload.

[0138] In some embodiments, the payload comprises an N-terminal amino acid, wherein the linker L^1 is covalently bonded to a N-terminal amino acid.

[0139] In some embodiments, m is 1-20.

[0140] In some embodiments of the conjugates described herein, linker L^1 may be a bond.

[0141] In some embodiments of the conjugates described herein, linker L^1 may have 1 to 100 linking atoms, and may include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms, or from 1 to 40 linking atoms, or from 1 to 30 linking atoms, or from 1 to 20 linking atoms, or from 1 to 10 linking atoms, or from 1 to 5 linking atoms, or from 5 to 30 linking atoms, or from 10 to 30 linking atoms, or from 5 to 40 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms.

[0142] In some embodiments of the conjugates described herein, the linker L^1 in Formula I may comprise one or more (e.g., 1-10 or 1-5) chain heteroatoms (e.g., O, N, S) and one or more (e.g., 1-10 or 1-5) alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene moieties; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or

heterocycloalkylene moiety, may be independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl.

[0143] In some embodiments of the conjugates described herein, the linker L^1 may be of the formula:

$$-Y^{10}-(CH_2)_{n'}-Y^{20}-(CH_2)_{m''}-Y^{30}-$$

wherein:

each of Y^{10}, Y^{20}, and Y^{30} are independently a bond, $-NR^{110}-$, -O-, $-S(O)_{0-2}-$, $-NR^{110}C(O)-$, $-C(O)NR^{110}-$, $-NR^{110}S(O)_2-$, $-S(O)_2NR^{110}-$, $-CR^{120}=N-NR^{110}-$, $-NR^{110}-N=CR^{120}-$, -C(O)-, -OC(O)-, -OC(O)O-, alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene is independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl;

each R^{110} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

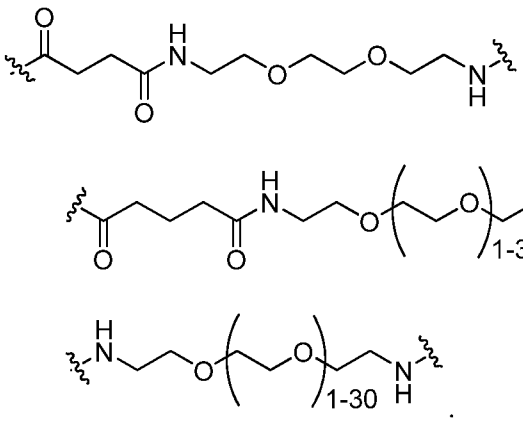
each R^{120} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl; and

n' and m" are each independently 0, 1, 2, 3, 4, 5, 6, 7, or 8.

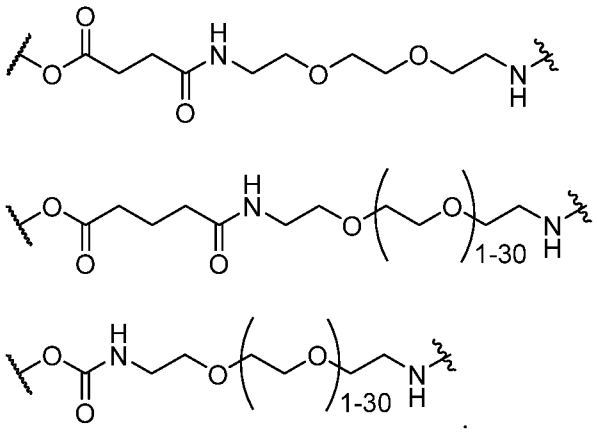
[0144] In certain embodiments, the linker L^1 is not a bond. In some embodiments, L^1 is a cleavable linker. In some embodiments, L^1 is a non-cleavable linker.

[0145] In certain embodiments, each R^{110} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl; and each R^{120} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl.

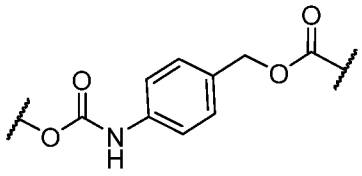
[0146] Representative linkers include, but are not limited to, those shown below:



[0147] Representative linkers include, but are not limited to, those shown below:



[0148] In some embodiments of the conjugates described herein, the linker in Formula I may comprise one or more of polyethylene glycol (e.g., PEG having an average molecular weight of from 300 g/mol to 10,000 g/mol), ethylene-1,2-diylbis(methylcarbamate, an arylene (e.e., phenylene), ethylene-oxy, amine, ester, amide, carbamate, ketone (i.e., formyl), or carbonate. The linker in Formula I may comprise



[0149] In some embodiments of the conjugates described herein, the linker L^1 may comprise one or more natural or unnatural amino acids, which may be referred to as a peptide linker. Where the drug (D^1) comprises an amino moiety, the linker may be bound thereto using a peptide linker made up of a carboxylic acyl unit, and one or more amino acids making up a protein or peptide sequence. The linker may also contain a self-immolating spacer which spaces the drug and the protein peptide sequence.

[0150] In some embodiments of the conjugates described herein, the linker L^1 may be a peptide linker represented by "A—Y—Z—X—W" in which "A" is the carboxylic acyl unit, "Y" and "Z" are each one or more natural or unnatural amino acids and together form a peptide sequence, and "X" and "W" are optional additional linkers having from 1 to 50 linking atoms, or from 5 to 10 linking atoms, or from 1 to 10 linking atoms which spaces the peptide and the drug, D^1, or the bioorthogonal moiety. In certain embodiments, one or more of the amino acids in the peptide linker is N-methylated.

[0151] In some embodiments, Y may be at least one amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan and proline. In some embodiments, Y may be at least one amino acid selected from the group consisting of phenylalanine, alanine, and valine.

[0152] In some embodiments, Z may be at least one amino acid selected from the group consisting of alanine, lysine, lysine protected with acetyl or formyl, arginine, arginine protected with tosyl or nitro

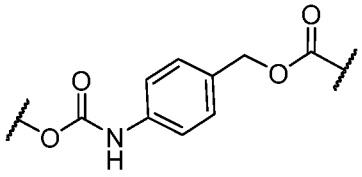
groups, histidine, ornithine, ornithine protected with acetyl or formyl, and citrulline. In some embodiments, Z may be at least one amino acid selected from the group consisting of alanine, lysine and citrulline.

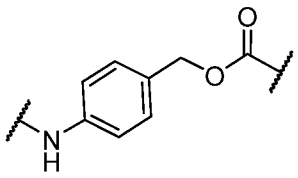
[0153] In some embodiments, Y-Z combinations include Valine-Citrulline; Valine-Alanine; and Alanine-Alanine.

[0154] In certain embodiments, A is -OC(O)-.

[0155] In certain embodiments, X is -OC(O)-.

[0156] In certain embodiments, W is -OC(O)-. In certain embodiments, X is absent and W is -OC(O)-.

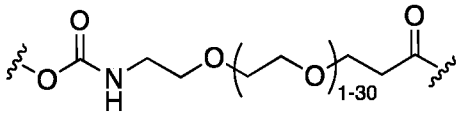
[0157] In certain embodiments, —X—W is  .

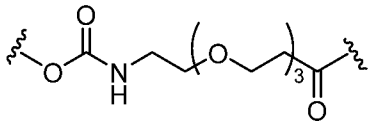
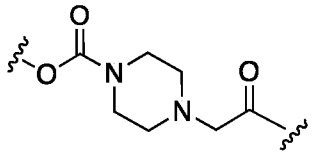
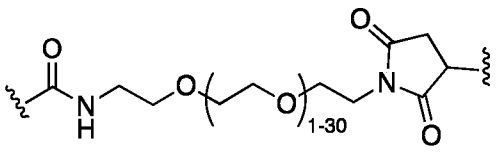
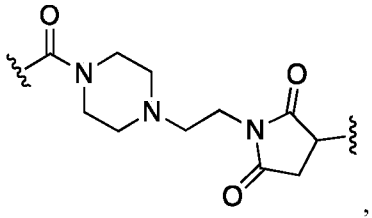
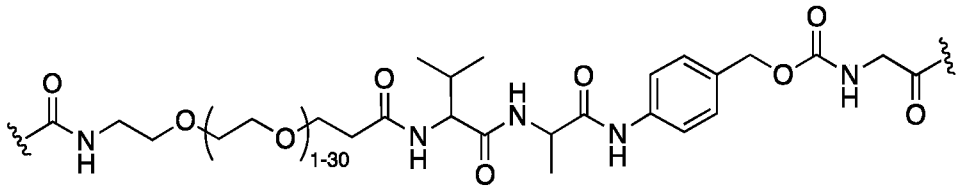
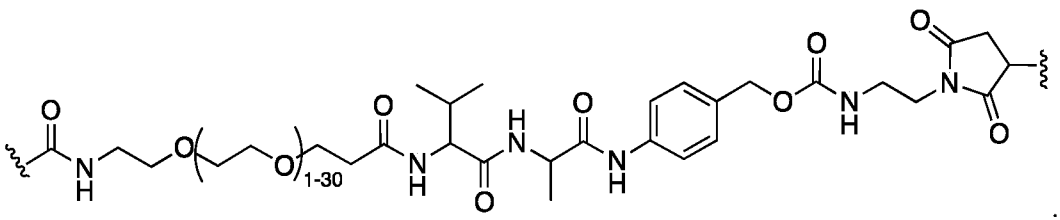
[0158] In certain embodiments, —X—W is  .

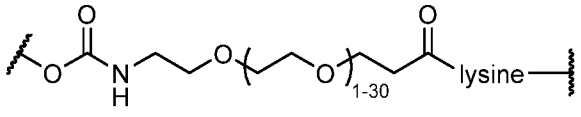
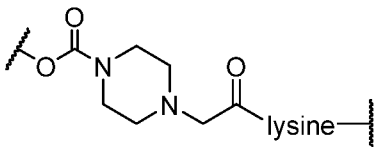
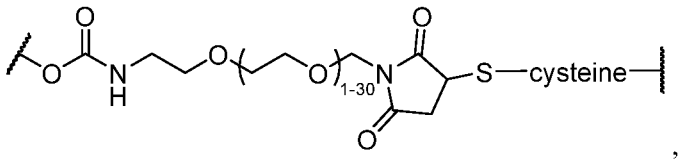
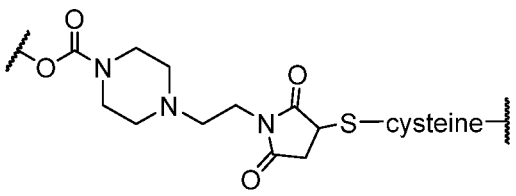
[0159] In certain embodiments, the peptide linker is specifically tailored so that it will be selectively cleaved (e.g., enzymatically cleaved) releasing the drug, such as by one or more of the tumor-associated proteases.

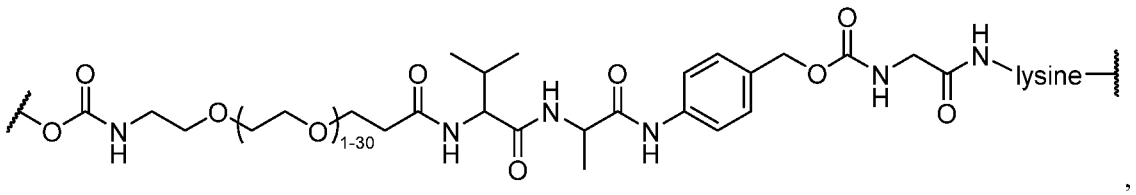
[0160] In certain embodiments, the peptide linker has a chain length of two to four amino acid residues (i.e., a di-, tri-, or tetra-peptide). It will be understood, however, that peptide linkers up to five, six, seven, or eight amino acid residues may also suitably be employed.

[0161] In certain embodiments, the peptide linker is Phe-Lys, Val-Lys, Val-Ala, Ala-Ala, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Ala-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Gly-Phe-Leu-Gly [SEQ ID NO: 1], Ala-Leu-Ala-Leu [SEQ ID NO:2], Phe-N^9-tosyl-Arg, or Phe-N^9-Nitro-Arg. In certain embodiments, the peptide linker is Phe-Lys, Val-Lys, Val-Ala, Ala-Ala, Val-Val, Val-Cit, or D-Phe-L-Phe-Lys. In certain embodiments, the peptide linker is Val-Cit, Val-Ala, or Ala-Ala.

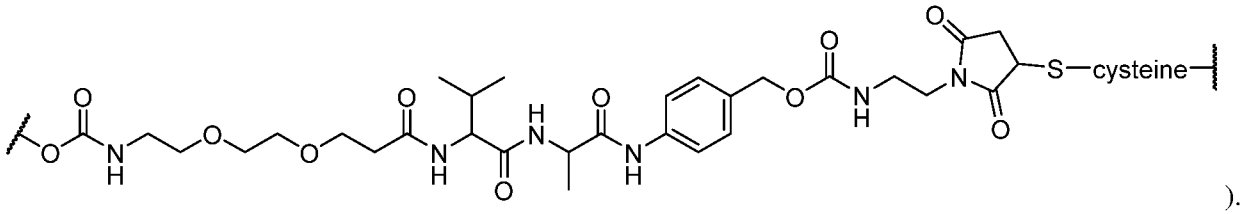
[0162] In certain embodiments, the linker L^1 in Formula I is: 

(e.g., ),  ,  ,  ,  , or  .

[0163] The foregoing linkers may attach on the right-hand side to amino acid side chains of D^1 such lysine or cysteine (e.g.,  ,  ,  ,  .



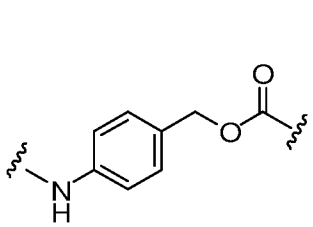
,



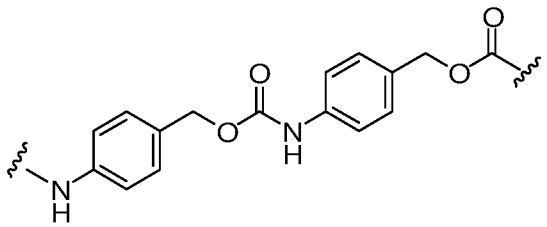
).

[0164] In some embodiments, L^1 is $–OC(O)L^4–$ or $–OC_{1-6}alkyleneC(O)L^4–$;

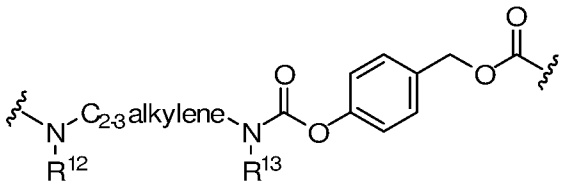
L^4 is a bond, $–N(R^{12})–C_{2-3}alkylene–N(R^{13})C(O)–$, $–CH(NHC(O)R^{14})C_{1-4}alkylene–S–S–C_{1-4}alkylene–OC(O)–$, $–NHNHC(O)CH(NHC(O)R^{15})CH_2C(O)–$, $–C_{1-6}alkylene–CH(G^x)OC(O)–$,



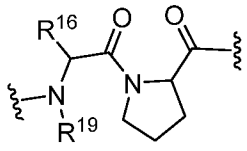
,



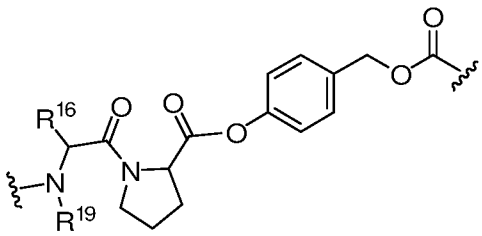
,



,



, or

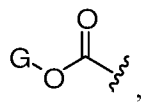


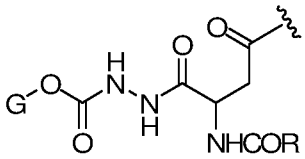
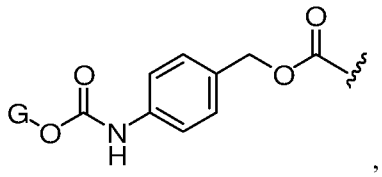
;

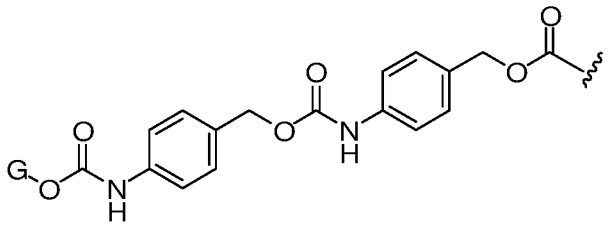
R^{12}, R^{13}, R^{14}, R^{15}, and R^{19} are each independently hydrogen or $C_{1-4}alkyl$;

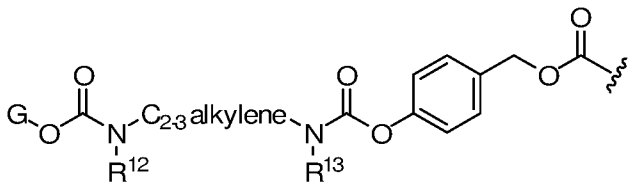
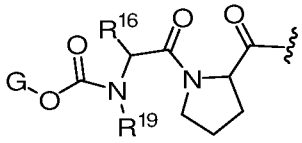
R^{16} is hydrogen, $C_{1-4}alkyl$, $–C_{1-4}alkylene–OH$, $–C_{1-4}alkylene–OC_{1-4}alkyl$, $–C_{1-4}alkylene–CO_2H$, or $–C_{1-4}alkylene–CONH_2$; and

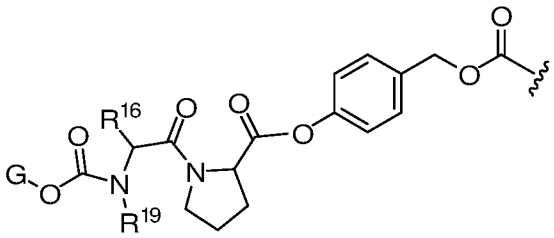
G^x is phenyl optionally substituted with 1-5 substituents independently selected from the group consisting of halogen, $C_{1-4}alkyl$, $C_{1-4}haloalkyl$, $C_{1-4}alkoxy$, cyano, and nitro.

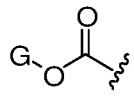
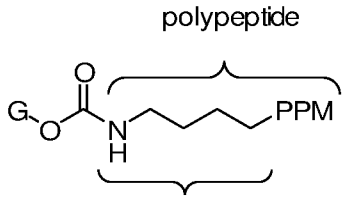
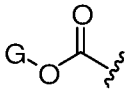
[0165] In some embodiments, G–L[1], at each occurrence, is independently  ,

 ,  ,  ,

 ,

 ,  , or

 .

[0166] In some embodiments, G–L[1], at each occurrence, is independently  . When attached to a lysine residue, the conjugate may have formula  , wherein PPM is a polypeptide moiety having the lysine residue and lysine side chain and the PPM may also have additional lysines, or other amino acid side chains conjugated to the group  .

[0167] In some embodiments, R^{1B} is selected from the group consisting of G^1, OH, $–NR^{1c}–C_{1\text{-}4}alkylene–G^1$, $–NR^{1c}–C_{1\text{-}4}alkylene–N(R^{1d})_2$, $–N(R^{1c})CHR^{1e}CO_2H$, $–N(R^{1c})CH_2CO_2H$, and $–N(R^{1f})–CH_2CH_2–(N(CH_2CO_2H)CH_2CH_2)_n–N(CH_2CO_2H)_2$;

R^{1e} is $–CH_2CO_2H$, $–CH_2CH_2CO_2H$, $–CH_2CONH_2$, $–CH_2CH_2CONH_2$, $–CH_2OH$, or $–CH(CH_3)OH$; and

R^{1f} is hydrogen or CH_2CO_2H.

[0168] In some embodiments, R^{1B} is selected from the group consisting of $–NR^{1c}–C_{2\text{-}4}$alkylene–$N(C_{1\text{-}4}alkyl)_3^+$, $–N(R^{1c})–C_{1\text{-}6}$alkylene–$SO_3H$, $–N(R^{1c})–(CH_2CH_2O)_{1\text{-}3}–CH_2CH_2N((CH_2CH_2O)_{1\text{-}3}–C_{1\text{-}6}alkylene–CO_2H)_2$, and $–N(R^{1c})–CH(CH_2O–(CH_2CH_2O)_{0\text{-}2}–C_{1\text{-}6}alkylene–CO_2H)_2$.

[0169] In some embodiments, R^{1B} is selected from the group consisting of $–NR^{1c}–CH_2CH_2–N(CH_3)_3^+$, $–N(R^{1c})–CH_2CH_2–SO_3H$, $–N(R^{1c})–(CH_2CH_2O)_3–CH_2CH_2N((CH_2CH_2O)_3–CH_2CH_2–CO_2H)_2$, and $–N(R^{1c})–CH(CH_2O–CH_2CH_2–CO_2H)_2$.

[0170] In some embodiments, R^{1A} is $C_{1\text{-}4}$alkyl.

[0171] In some embodiments, R^{1A} is CH_3.

[0172] In some embodiments, R^{1c} is hydrogen.

[0173] In some embodiments, R^{1A} is $C_{1\text{-}4}$alkyl;

R^{1B} is selected from the group consisting of G^1, OH, $-NR^{1c}-C_{1\text{-}4}$alkylene-G^1, $–NR^{1c}–C_{1\text{-}4}$alkylene–$N(R^{1d})_2$, $-N(R^{1c})CHR^{1e}CO_2H$, $-N(R^{1c})CH_2CO_2H$, and $-N(R^{1f})–CH_2CH_2-(N(CH_2CO_2H)CH_2CH_2)_n-N(CH_2CO_2H)_2$;

R^{1e} is $–C_{1\text{-}4}$alkylene–CO_2H;

R^{1f} is hydrogen or $C_{1\text{-}4}$alkylene–CO_2H;

G^1 is a 4- to 8-membered monocyclic heterocyclyl containing a first nitrogen and optionally one additional heteroatom selected from nitrogen, oxygen, and sulfur, G^1 being attached at the first nitrogen and optionally substituted with 1-4 substituents independently selected from the group consisting of
$C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$haloalkyl, halo, cyano, OH, $–OC_{1\text{-}4}$alkyl, and oxo; and

n is 0, 1, or 2.

[0174] In some embodiments, R^{1A} is CH_3;

R^{1e} is $–CH_2CO_2H$;

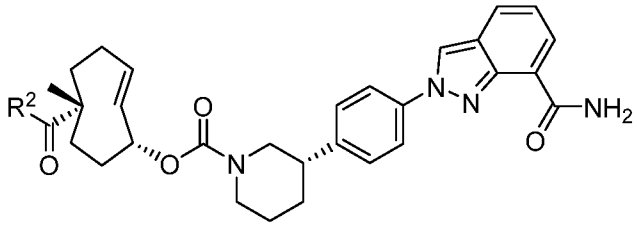
R^{1f} is hydrogen or CH_2CO_2H; and

G^1 is a piperazinyl, morpholinyl, piperidinyl, azepanyl, or pyrrolidinyl, attached through a ring nitrogen atom and optionally substituted with 1-4 substituents independently selected from the group consisting of $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$haloalkyl, halo, cyano, OH, $–OC_{1\text{-}4}$alkyl, and oxo.

[0175] In some embodiments, L^2 is –C(O)–.

[0176] In some embodiments, R^{1B} is selected from the group consisting of OH, $N(H)CH_2CO_2H$, $–N(H)CHR^{1e}CO_2H$, $–N(H)–CH_2CH_2–(N(CH_2CO_2H)CH_2CH_2)_n–N(CH_2CO_2H)_2$, and $–N(CH_2CO_2H)–CH_2CH_2–N(CH_2CO_2H)_2$; and R^{1e} is $–CH_2CO_2H$.

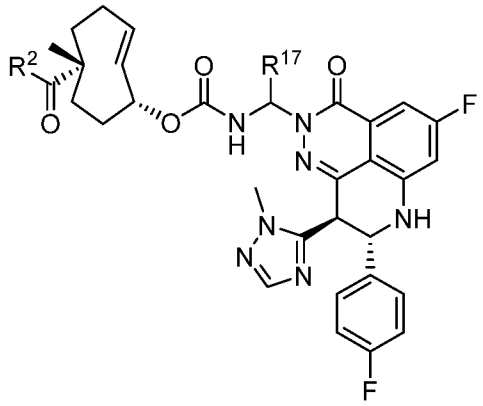
[0177] In certain embodiments, provided is a conjugate of Formula IIA:



IIA

or a pharmaceutically acceptable salt thereof, wherein R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

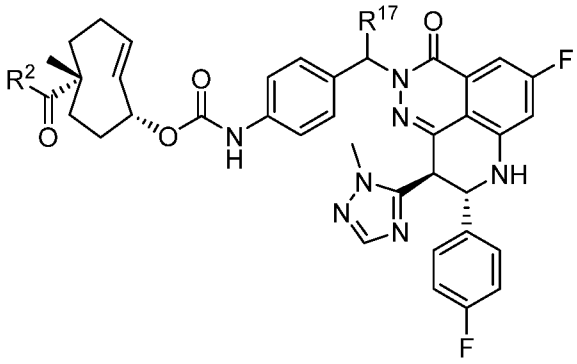
[0178] In certain embodiments, provided is a conjugate of Formula IIB:



IIB

or a pharmaceutically acceptable salt thereof, wherein R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether; and R^{17} is hydrogen, optionally substituted alkyl, or optionally substituted aryl.

[0179] In certain embodiments, provided is a conjugate of Formula IIC:



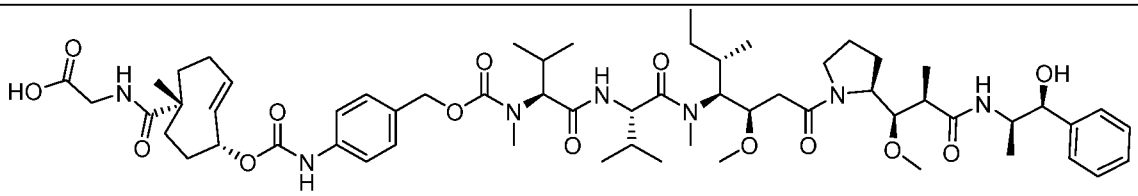
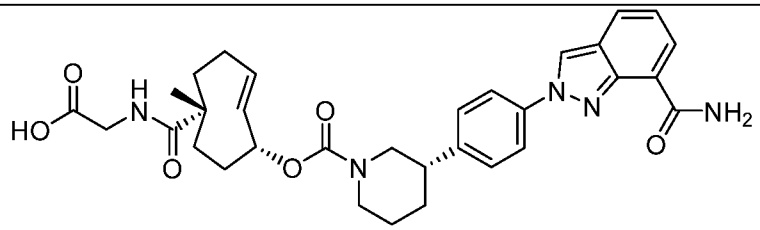
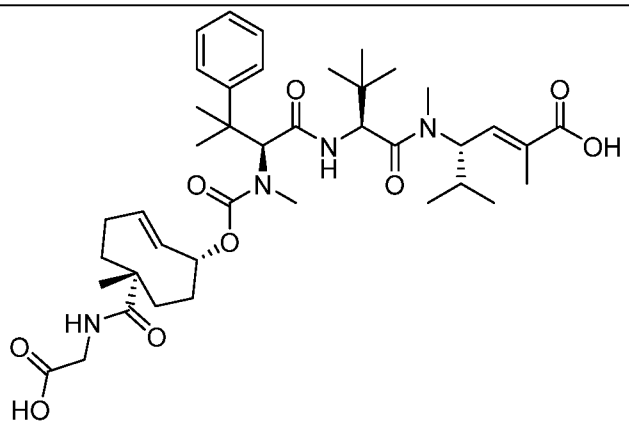
IIC

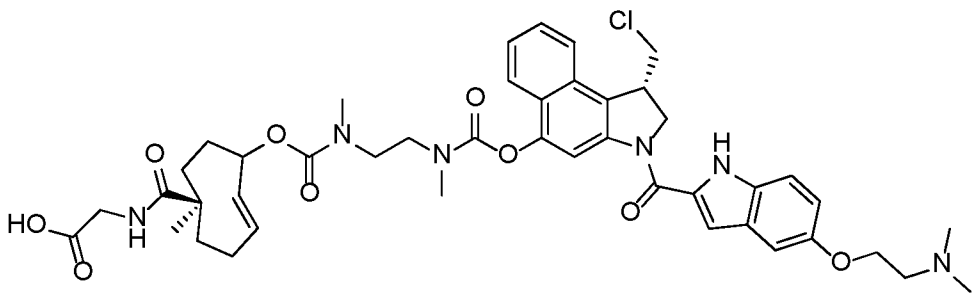
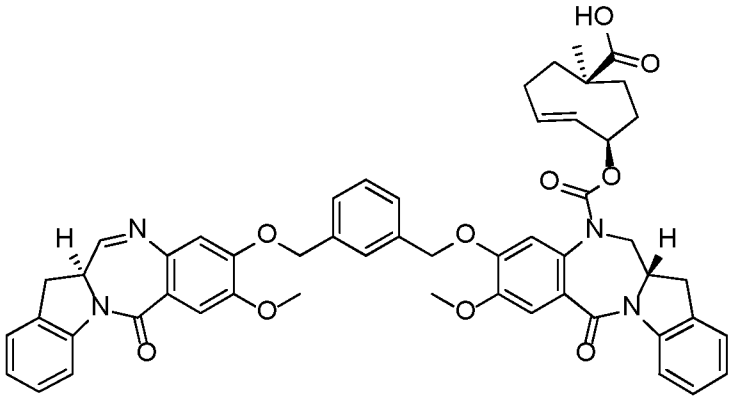
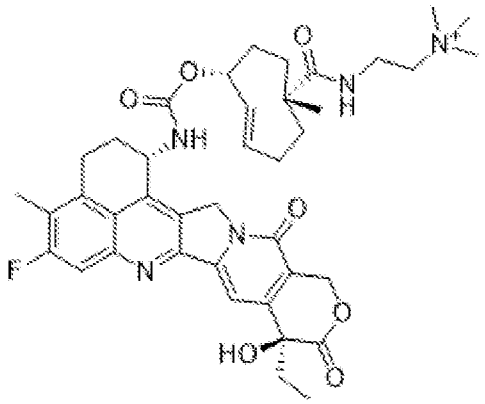
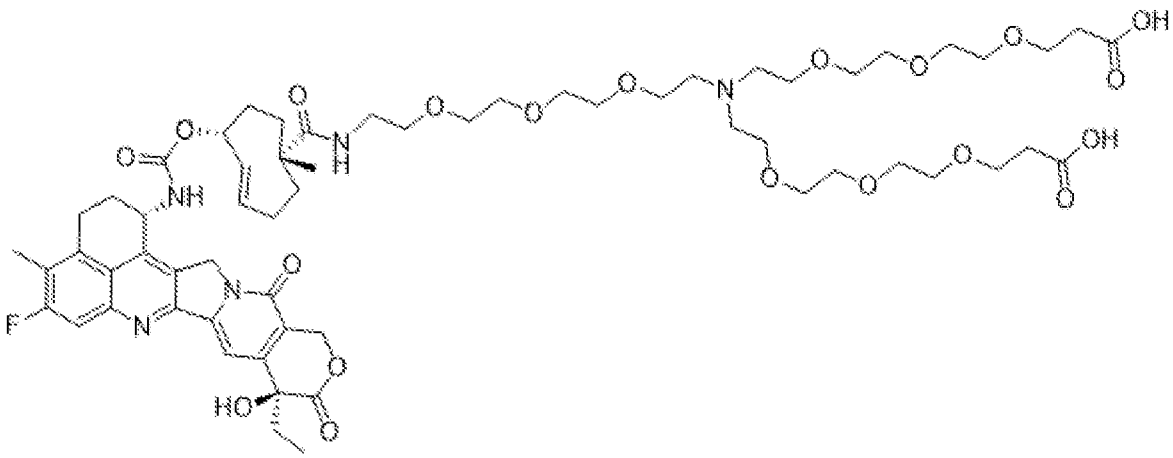
or a pharmaceutically acceptable salt thereof, wherein R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether; and R^{17} is hydrogen, optionally substituted alkyl, or optionally substituted aryl.

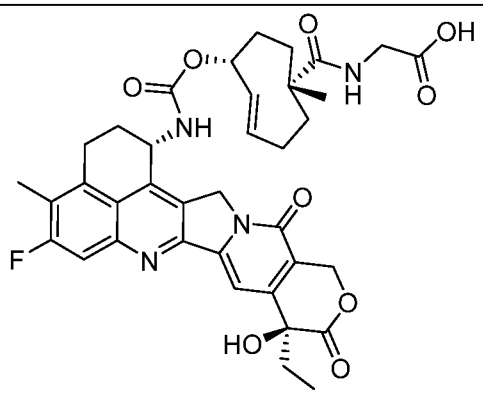
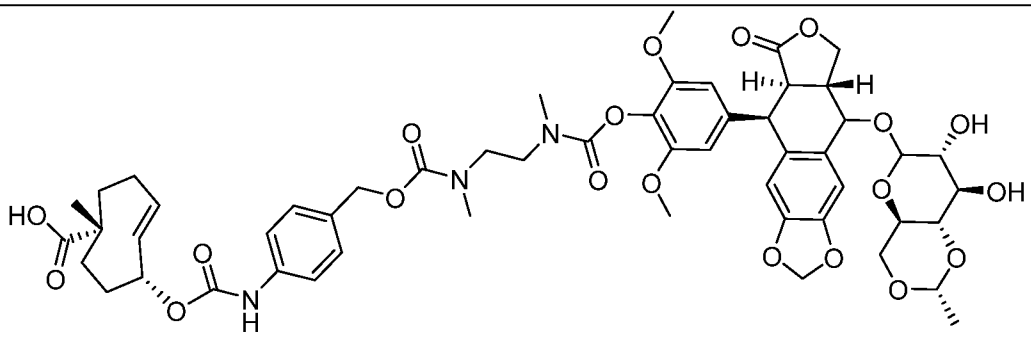
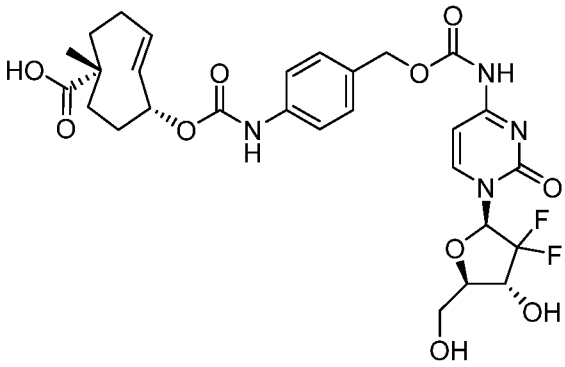
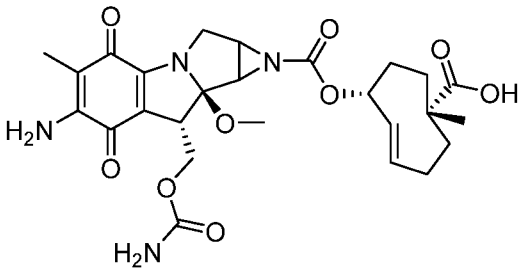
[0180] In some embodiments, R^{17} is hydrogen, alkyl, or aryl; wherein the alkyl, or aryl is optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, halo, cyano, OH, $–OC_{1-4}$alkyl, and oxo. In some embodiments, R^{17} is hydrogen, alkyl, or aryl; wherein the alkyl, or aryl is optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, halo, cyano, OH, $–OC_{1-4}$alkyl, and oxo.

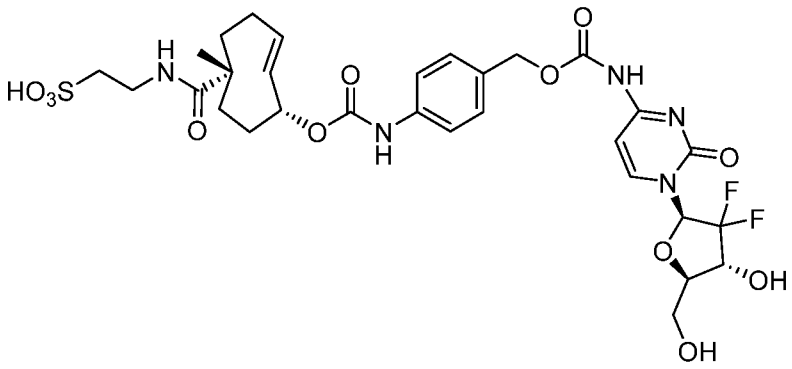
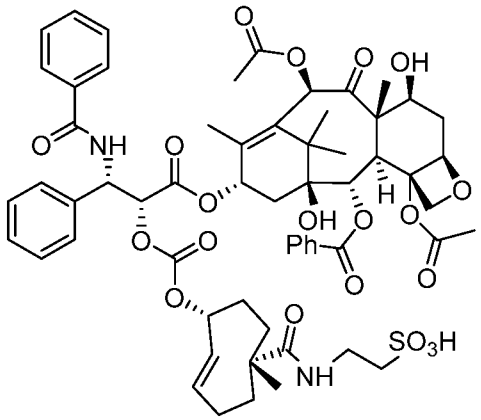
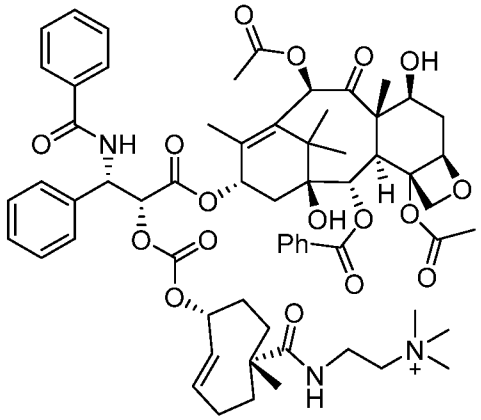
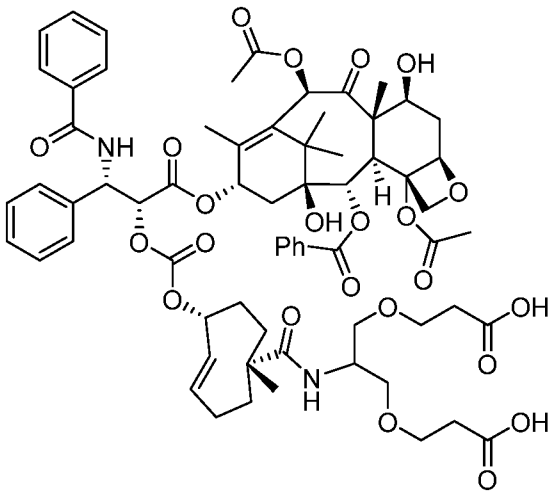
[0181] In certain embodiments, provided is a conjugate, or a pharmaceutically acceptable salt thereof, where the conjugate is selected from Table 1.

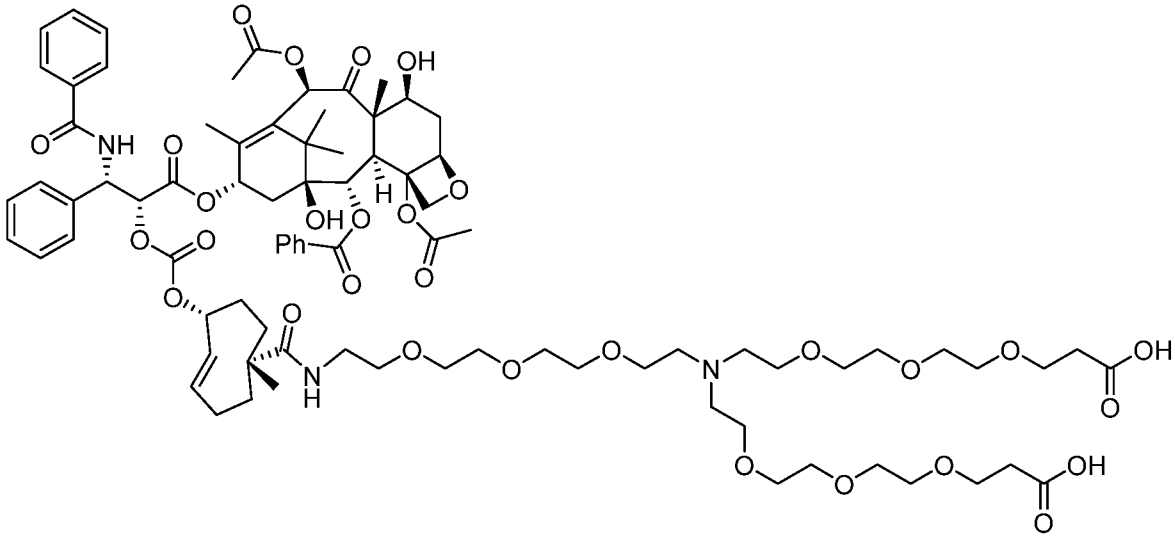
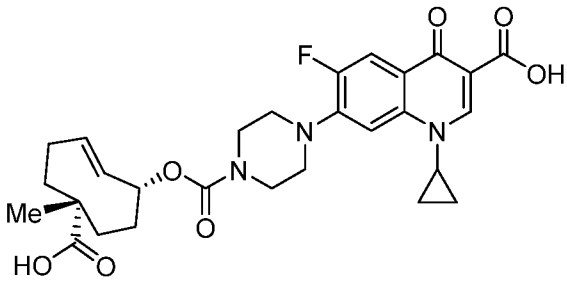
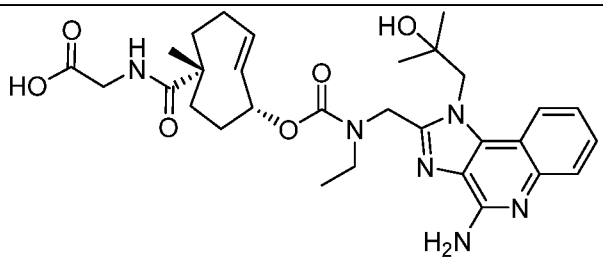
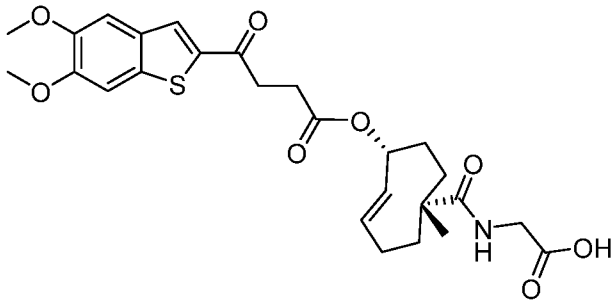
Table 1

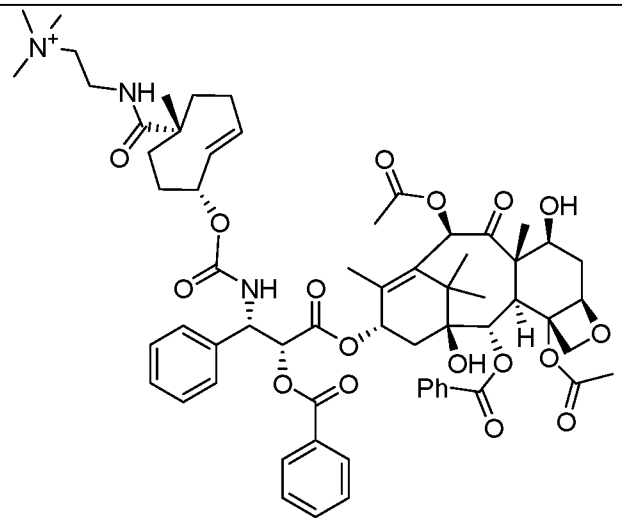
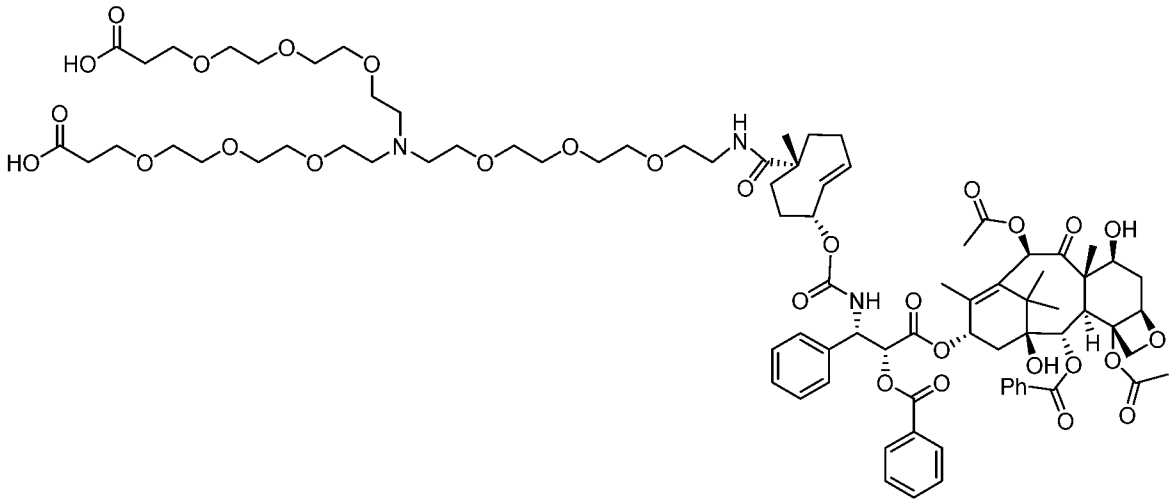
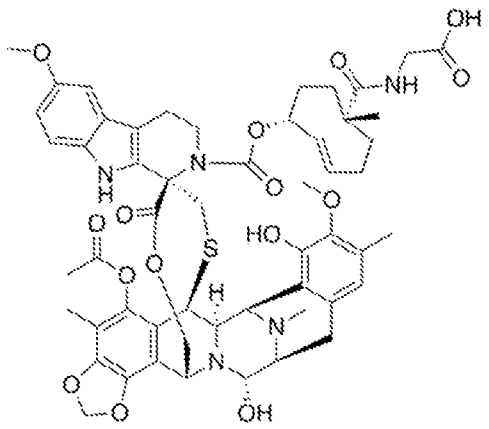
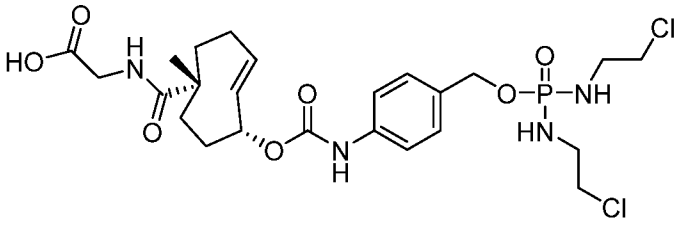
1	
2	
3	

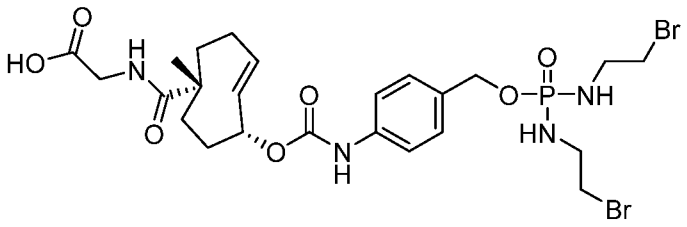
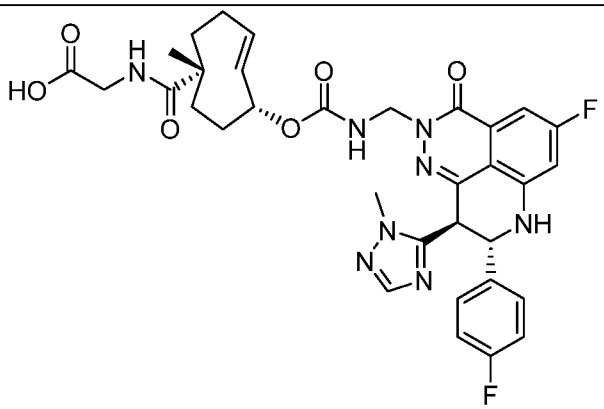
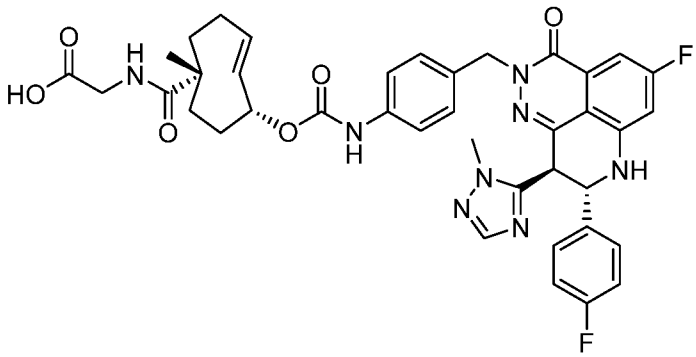
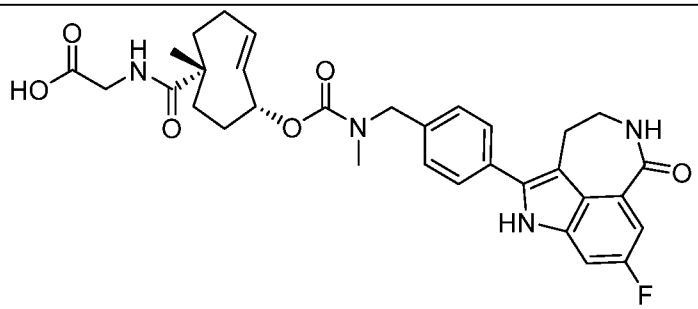
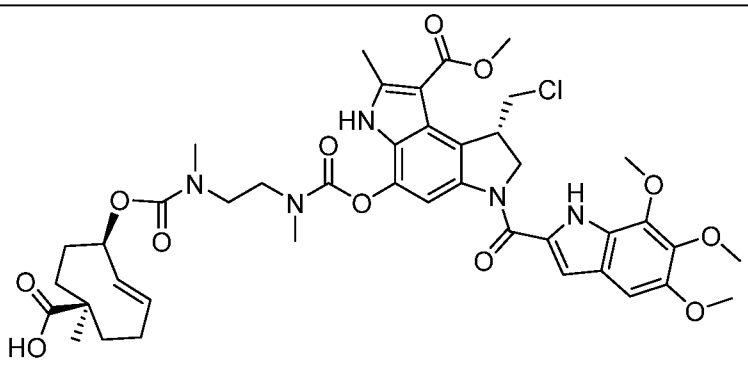
4	
5	
6	
7	

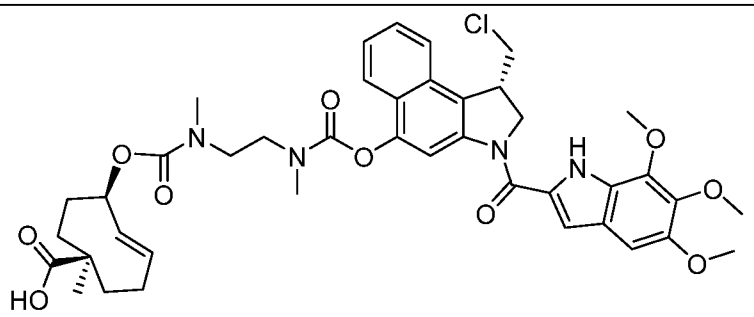
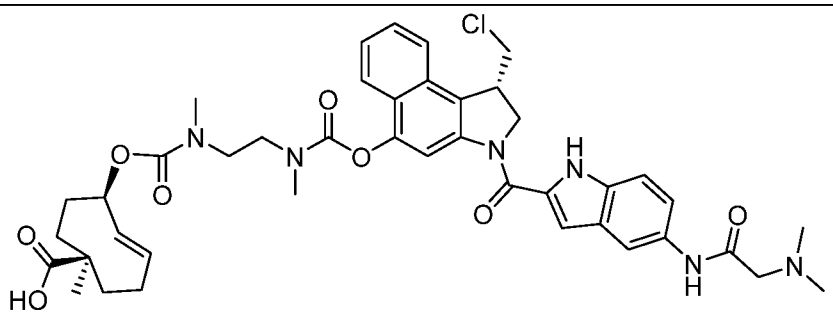
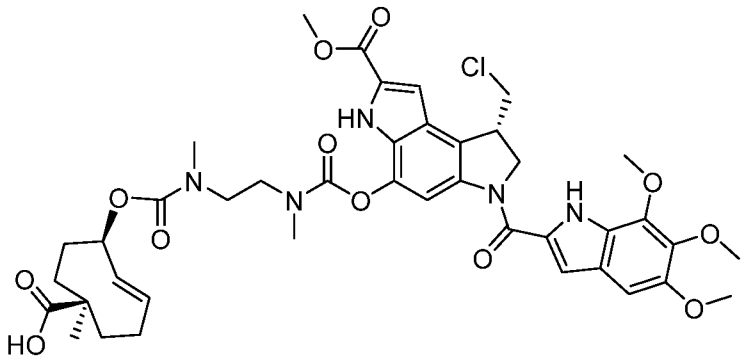
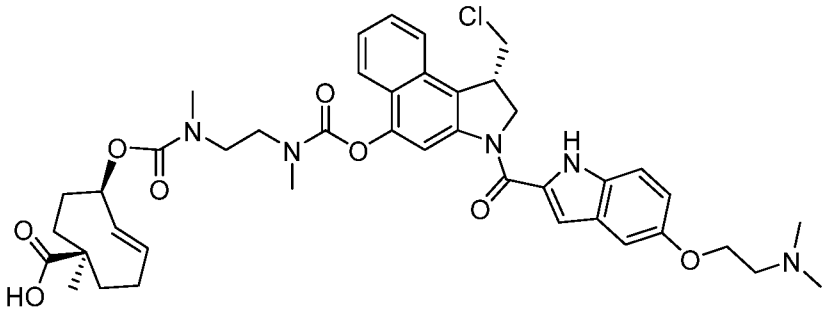
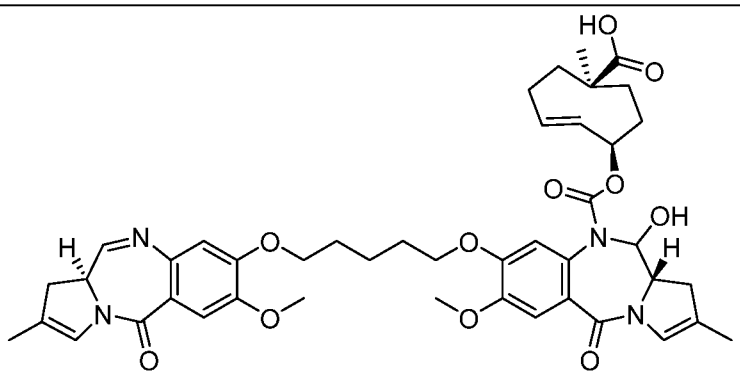
8	
9	
10	
11	

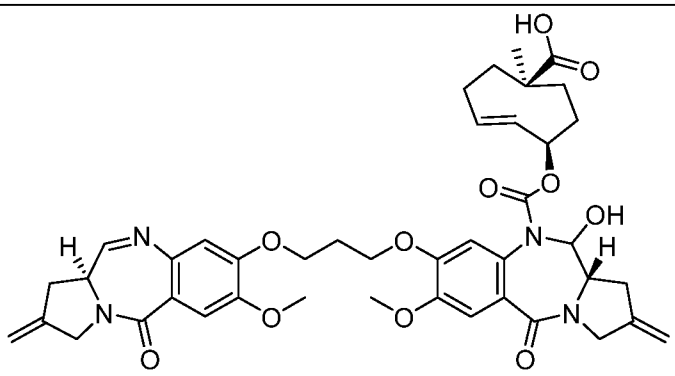
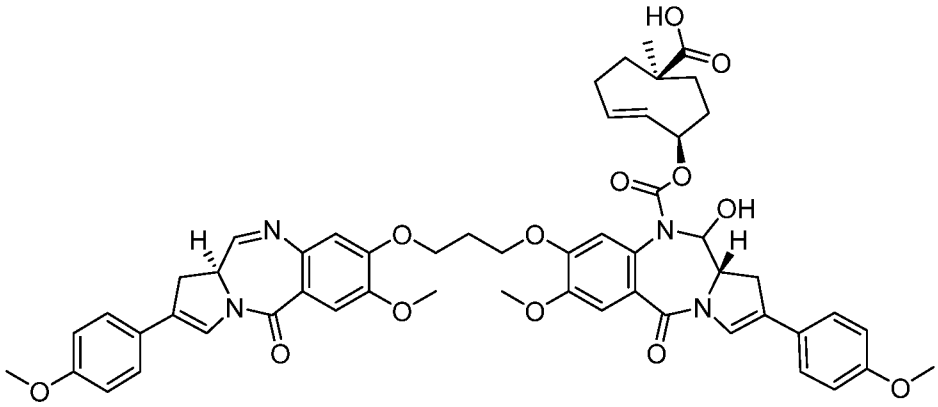
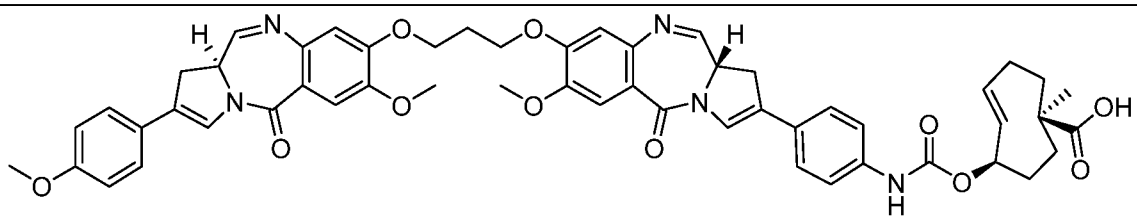
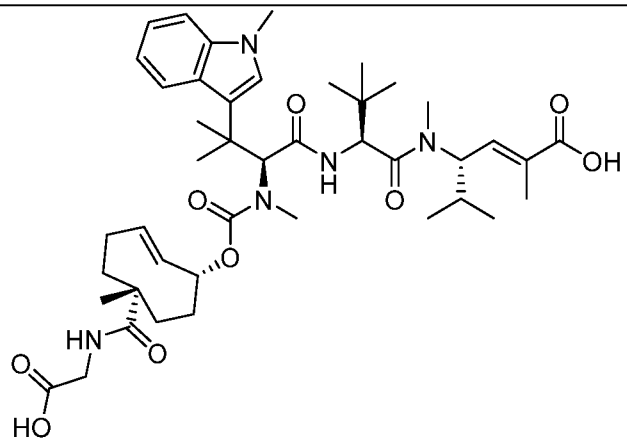
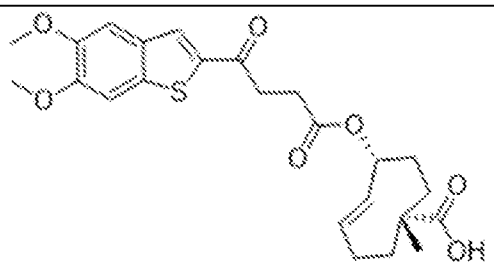
12	
13	
14	
15	

16	
17	
18	
19	

20	
21	
22	
23	

24	
25	
26	
27	
28	

29	
30	
31	
32	
33	

34	
35	
36	
37	
38	

[0182] In some embodiments, provided is a method for delivering an effective amount of a payload (i.e., an inhibitor of poly (ADP-ribose) polymerase (PARP inhibitor), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, and a monoclonal antibody, or a derivative, or analog thereof) to a target location in a subject, the method comprising administering to the subject at the target location a therapeutic support composition as described herein, and administering to the subject a conjugate, or the pharmaceutically acceptable salt or composition thereof, as described herein.

C. Therapeutic Support Compositions

[0183] The therapeutic support composition comprises a support. Supports may be biocompatible supports compositions, i.e., compatible with the subject's body. In some instances, a support is non-toxic to the subject and does not substantially react with tissue or biological compounds in the subject. For example, the support can be a hydrogel, among others. A support is capable of implantation into a subject's body and supporting binding agents (e.g., tetrazine-containing group), as well as payloads after the binding agents conjugate. Representative supports include, but are not limited to polymers, viscous or non-viscous liquid materials, gels, hydrogels, polysaccharide hydrogels, a cross-linked polymer matrix, a metal, a ceramic, a plastic, a bone graft material, alginate, cellulose, chitosan, hyaluronic acid, chondroitin sulfate, heparin, and the like. Supports also include particles, such as nanoparticles, microparticles, and the like.

[0184] Hydrogels may be polysaccharide hydrogels, alginate, cellulose, hyaluronic acid, chitosan, chitosin, chitin, hyaluronic acid, chondroitin sulfate, heparin, and the like. Other suitable sugar-based biomaterials include those described in *Polymer Advanced Technology*, 2014, 25, 448-460. Polymers that may be used as the support can include, but are not limited to, polyphosphazenes, polyanhydrides, polyacetals, poly(ortho esters), polyphosphoesters, polycaprolactones, polyurethanes, polylactides, polycarbonates, polyamides, and polyethers, and blends/composites/co-polymers thereof. Representative polyethers include, but are not limited to, poly(ethylene glycol) (PEG), polypropylene glycol) (PPG), triblock Pluronic ($[PEG]_n$-$[PPG]_m$-[PEG]n), PEG diacrylate (PEGDA), and PEG dimethacrylate (PEGDMA). The support can also include proteins and other poly(amino acids), such as collagen, gelatin, elastin and elastin-like polypeptides, albumin, fibrin, poly(gamma-glutamic acid), poly(L-lysine), poly(L-glutamic acid), poly(aspartic acid), and the like.

[0185] In some embodiments, the support is a hydrogel. In some embodiments, the support is an alginate. In some embodiments, the support is chitin. In some embodiments, the support is a hyaluronic acid (e.g., a non-hydrogel hyaluronic acid substantially without crosslinks). In some embodiments, the support is chitosin.

[0186] In certain embodiments, the support is a particle. Particles of the present disclosure can have a diameter that is 2 cm or less, such as 1.5 cm or less, or 1 cm or less, or 0.5 cm or less. For example, the particles can be nanoparticles or microparticles. Nanoparticles include particles having average

dimensions in the nanometer scale (e.g., 1000 nm or less). Microparticles are particles having average dimensions in the micrometer scale (e.g., 1000 μm or less). By "average" is meant the arithmetic mean. In some embodiments, the nanoparticles have a diameter ranging from 1 nm to 1 μm, such as from 10 nm to 1 μm, or 25 nm to 1 μm, or 50 nm to 1 μm, or 75 nm to 1 μm, or 100 nm to 1 μm, or 150 nm to 1 μm, or 200 nm to 1 μm, or 250 nm to 1 μm, or 300 nm to 1 μm, or 350 nm to 1 μm, or 400 nm to 1 μm, or 450 nm to 1 μm, or 500 nm to 1 μm. In other embodiments, the microparticles have a diameter ranging from 1 μm to 1 mm, such as from 10 μm to 1 mm, or 25 μm to 1 mm, or 50 μm to 1 mm, or 75 μm to 1 mm, or 100 μm to 1 mm, or 150 μm to 1 mm, or 200 μm to 1 mm, or 250 μm to 1 mm, or 300 μm to 1 mm, or 350 μm to 1 mm, or 400 μm to 1 mm, or 450 μm to 1 mm, or 500 μm to 1 mm. In further embodiments, small particles on the order of 10-100 nm in diameter may be assembled to form larger complexes, such as clusters or assemblies on the order of 1-10 μm. Particles of the present disclosure may be substantially spherical, such that the particles have a substantially circular cross-section. Other particle shapes may also be used, such as, but not limited to, ellipsoid, cubic, cylindrical, conical, needle, or other irregular shapes.

[0187] A "particle" may take the form of any fabricated material, a molecule, cryptophan, a virus, a phage, etc. The particle may be composed of a material, such as, but not limited to, a metal, a ceramic, a plastic, a glass, a composite, a polymer, a hydrogel, and the like. For example, the particles may be made of an inert material, such as alginate or iron oxide. In some examples, the particles may be magnetic and can be formed from a paramagnetic, super-paramagnetic or ferromagnetic material, or other material that responds to a magnetic field. Further, a particle may be of any shape, for example, spheres, rods, non-symmetrical shapes, etc. The particles, or a group of several particles in a complex, may be functionalized with a receptor that has a specific affinity to bind to or interact with a clinically relevant substrate. The receptor may be inherent to the particle itself. For example, the particle itself may be a virus or a phage with an inherent affinity for certain substrates. Additionally or alternatively, the particles can be functionalized by covalently or otherwise attaching or associating a receptor that specifically binds or otherwise recognizes a particular clinically relevant substrate. The functionalized receptor can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or any other molecule with a defined affinity for a target substrate. Examples of material that may be used for the "particles" and/or "carrier" include polylactic acid, polyglycolic acid, PLGA polymers, alginates and alginate derivatives, gelatin, collagen, fibrin, hyaluronic acid, laminin rich gels, agarose, natural and synthetic polysaccharides, polyamino acids, polypeptides, polyesters, poly anhydrides, polyphosphazines, poly(vinyl alcohols), poly(alkylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers, pluronic polyols, polyoxamers, poly(uronic acids), poly(vinylpyrrolidone) and copolymers or graft copolymers of any of the above. These examples do not limit their concentration, their cross-linking with different agents, their method of administration, their tailored degradation profiles and other characteristics known to those skilled in the art.

[0188] The particles, or a group of several particles in a complex, may be functionalized with a targeting agent (e.g., a ligand or antibody) that specifically binds (or substantially specifically binds) to a target (e.g., a target receptor or a cell surface target, such as a clinically relevant receptor or cell surface target (e.g., antigen)). The targeting agent may be attached directly to the particle itself. The targeting agent can be an antibody, peptide, nucleic acid, phage, bacteria, virus, or any other molecule with a specific affinity for a target receptor or cell surface target. In some instances, the receptor or cell surface target is PD-1, CTLA-4, HER2/neu, HER1/EGFR, VEGFR, 4-1BB, GITR, or other cellular receptors or cell surface targets.

[0189] In some embodiments, the targeting agent is a monoclonal antibody. A monoclonal antibody can be an entire monoclonal antibody, or a fragment thereof (e.g., antigen-binding fragment (Fab)). In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets one or more of CD25 (NCBI Gene ID 3559), CEA (NCBI Gene ID 634), CEACAM5 (NCBI Gene ID 1048), ASPH (NCBI Gene ID 444), EGFR (NCBI Gene ID 1956), EPCAM (NCBI Gene ID 4072), VEGFR (NCBI Gene ID 3791), PDGFR (NCBI Gene ID 5159), TROP2 (NCBI Gene ID 4070), Nectin4 (NCBI Gene ID 81607), PSMA (NCBI Gene ID 2346), BCMA (NCBI Gene ID 608), CD22 (NCBI Gene ID 933), CD20 (NCBI Gene ID 920), CD19 (NCBI Gene ID 930), CD79b (NCBI Gene ID 974), CD38 (NCBI Gene ID 952), CD45 (NCBI Gene ID 5788), Endoglin (NCBI Gene ID 2022), FGFR2 (NCBI Gene ID 14183), C4.4A (NCBI Gene ID 27076), Claudin-18.2 (NCBI Gene ID 51208), MMP9 (NCBI Gene ID 4318), Folate receptor (NCBI Gene ID 2348), DLL3 (NCBI Gene ID 10683), CD138 (NCBI Gene ID 6382), CD56 (NCBI Gene ID 4684), CD37 (NCBI Gene ID 951), CD74 (NCBI Gene ID 972), mesothelin (NCBI Gene ID 10232), IL-6R (NCBI Gene ID 3570), SLAMF7 (NCBI Gene ID 57823), BAFF (NCBI Gene ID 10673), MUC1 (NCBI Gene ID 4582), GPC3 (NCBI Gene ID 2719), HER2 (NCBI Gene ID 2064), HER3 (NCBI Gene ID 2065), CD30 (NCBI Gene ID 943), CD33 (NCBI Gene ID 945), CD123 (NCBI Gene ID 3563), GPNMB (NCBI Gene ID 10457), cMET (NCBI Gene ID 4233), CD142 (NCBI Gene ID 2152), NaPi2B (NCBI Gene ID 10568), GCC (NCBI Gene ID 2984), STEAP1 (NCBI Gene ID 26872), MUC16 (NCBI Gene ID 94025), CD70 (NCBI Gene ID 970), CD44 (NCBI Gene ID 960), (NCBI Gene ID), Antibody fragments (NCBI Gene ID), vWF (NCBI Gene ID 7450), TNF (NCBI Gene ID 7124), IL-6R (NCBI Gene ID 3570), BCMA (NCBI Gene ID 608), ADAMTS5 (NCBI Gene ID 11096), CX3CR1 (NCBI Gene ID 1524), CXCR4 (NCBI Gene ID 7852), TfR1 (NCBI Gene ID 7037), VEGFR (NCBI Gene ID 3791), or PSMA (NCBI Gene ID 2346).

[0190] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD25, such as Daclizumab, RG6292, basiliximab, or HuMax-TAC, or an antibody fragment derived therefrom.

[0191] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CEA, such as Labetuzumab, 15-1-32, PR1A3, or cT84.66, or an antibody fragment derived therefrom.

[0192] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CEACAM5, such as Tusamitiamab or CC4, or an antibody fragment derived therefrom.

[0193] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets ASPH, such as PAN-622, or an antibody fragment derived therefrom.

[0194] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets EGFR, such as Cetuximab, necitumumab, nimotuzumab, matuzumab, AMG595, depatuxizumab, dapatuxizumab, duligotuzumab, futuximab, GC1118, imgatuzumab, panitumumab, alutumumab, tomuzotuximab, or laprituximab, or an antibody fragment derived therefrom.

[0195] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets EPCAM, such as oportuzumab, citatuzumab, tucotuzumab, catumaxomab, edrecolomab, or adecatumumab, or an antibody fragment derived therefrom.

[0196] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets VEGFR, such as ramucizumab, ramucirumab, or vulinacimab, or an antibody fragment derived therefrom.

[0197] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PDGFR, such as olaratumab or ramucirumab, or an antibody fragment derived therefrom.

[0198] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TROP2, such as sacituzumab or Pr1E11, or an antibody fragment derived therefrom.

[0199] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets Nectin4, such as enfortumab, or an antibody fragment derived therefrom.

[0200] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PSMA, such as J591 or MLN591, or an antibody fragment derived therefrom.

[0201] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BCMA, such as belantamab, or an antibody fragment derived therefrom.

[0202] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD22, such as moxetumomab, inotuzumab, epratuzumab, or pinatuzumab, or an antibody fragment derived therefrom.

[0203] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD20, such as ublituximab, ofatumumab, rituximab, obinutuzumab, tositumomab, or ibritumomab, or an antibody fragment derived therefrom.

[0204] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD19, such as loncastuximab, XMAB-5574, MOR208, coltuximab, denintuzumab, taplitumomab, or MDX-1342, or an antibody fragment derived therefrom.

[0205] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD79b, such as polatuzumab, or an antibody fragment derived therefrom.

[0206] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD38, such as isatuximab, daratumumab, MOR202, or TAK-079, or an antibody fragment derived therefrom.

[0207] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD45, such as I-131-BC8, or Iomab-B, or an antibody fragment derived therefrom.

[0208] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets endoglin, such as carotuximab, or an antibody fragment derived therefrom.

[0209] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets FGFR2, such as bemarituzumab or aprutumab, or an antibody fragment derived therefrom.

[0210] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets C4.4A, such as lupartumab, or an antibody fragment derived therefrom.

[0211] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets Claudin-18.2, such as zolbetuximab, or claudiximab, or an antibody fragment derived therefrom.

[0212] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MMP9, such as andecaliximab, or an antibody fragment derived therefrom.

[0213] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets folate receptor, such as mirvetuximab, farletuzumab, MORAb-202, MORAb-003, or SP8166, or an antibody fragment derived therefrom.

[0214] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets DLL3, such as rovalpituzumab, or an antibody fragment derived therefrom.

[0215] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD138, such as indatuximab, or an antibody fragment derived therefrom.

[0216] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD56, such as lorvotuzumab, promiximab, or an antibody fragment derived therefrom.

[0217] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD37, such as BI 836826, otlertuzumab, or naratuximab, or an antibody fragment derived therefrom.

[0218] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD74, such as milatuzumab, or an antibody fragment derived therefrom.

[0219] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets mesothelin, such as anetumab, amatuximab, or MMOT-0530A, or an antibody fragment derived therefrom.

[0220] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets IL-6R, such as tocilizumab or sarilumab, or an antibody fragment derived therefrom.

[0221] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets SLAMF7, such as elotuzumab, or an antibody fragment derived therefrom.

[0222] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BAFF, such as belimumab, or an antibody fragment therefrom.

[0223] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MUC1, such as KL-6, MY.1E12, hMUC1-1H7, TAB004, huC242, clivatuzumab, 8HuDS6, gatipotuzumab, AR20.5, or cantuzumab, or an antibody fragment derived therefrom.

[0224] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GPC3, such as codrituzumab, ECT204, or MDX-1414, or an antibody fragment derived therefrom.

[0225] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets HER2, such as pertuzumab, trastuzumab, or margetuximab, or an antibody fragment derived therefrom.

[0226] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets HER3, such as patritumab, seribantumab, lumretuzumab, elgemtumab, AV-203, CDX-3379, or GSK284933, or an antibody fragment derived therefrom.

[0227] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD30, such as brentuximab, or an antibody fragment derived therefrom.

[0228] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD33, such as gemtuzumab, BI 835858, vadastuximab, or lintuzumab, or an antibody fragment derived therefrom.

[0229] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD123, such as KHK2823, taclotuzumab, or G4723A, or an antibody fragment derived therefrom.

[0230] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GPNMB, such as glembatumumab, or an antibody fragment derived therefrom.

[0231] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets cMET, such as telisotuzumab, onartuzumab, or SAIT301, or an antibody fragment derived therefrom.

[0232] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD142, such as tisotumab, or an antibody fragment derived therefrom.

[0233] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets NaPi2B, such as lifastuzumab, or an antibody fragment derived therefrom.

[0234] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets GCC, such as indusatumab, or an antibody fragment derived therefrom.

[0235] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets STEAP1, such as vandortuzumab, or an antibody fragment derived therefrom.

[0236] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets MUC16, such as sofituzumab, or an antibody fragment derived therefrom.

[0237] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD70, such as vorsetuzumab, or an antibody fragment derived therefrom.

[0238] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CD44, such as bivatuzumab, or an antibody fragment derived therefrom.

[0239] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets vWF, such as caplacizumab, or an antibody fragment derived therefrom.

[0240] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TNF, such as ozoralizumab, V565, or PF-05230905, or an antibody fragment derived therefrom.

[0241] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets IL-6R, such as vobarilizumab, or an antibody fragment derived therefrom.

[0242] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets BCMA, such as LCAR-B38M, or an antibody fragment derived therefrom.

[0243] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets ADAMTS5, such as M6495, or an antibody fragment derived therefrom.

[0244] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CX3CR1, such as BI 655088, or an antibody fragment derived therefrom.

[0245] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets CXCR4, such as AD-214 or ALX-0651, or an antibody fragment derived therefrom.

[0246] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets TfR1, such as TXB4, or an antibody fragment derived therefrom.

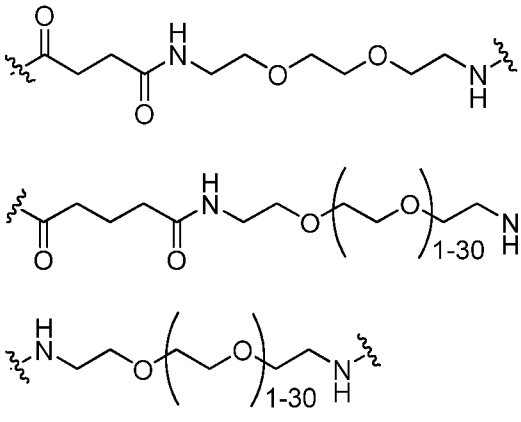
[0247] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets VEGFR, such as CDP791, or an antibody fragment derived therefrom.

[0248] In certain embodiments, the targeting agent is an antibody, or antibody fragment, that targets PSMA, such as GY1, or an antibody fragment derived therefrom.

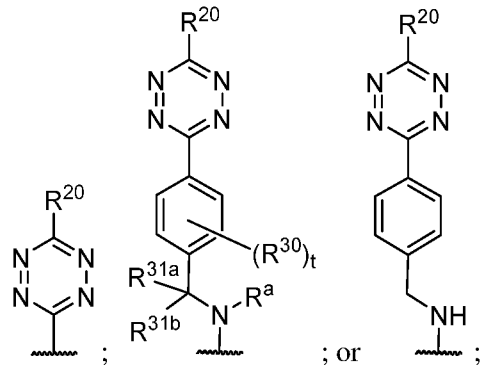
[0249] Other compounds or molecules, such as fluorophores or autofluorescent or luminescent markers, which may assist in detecting the particles (e.g., *in vivo* detection), may also be attached to the particles. The ligands and/or detectable labels may be attached directly to the particle or attached to the particle through bioorthogonal functional groups as described herein.

[0250] In certain embodiments, the support is a bone graft material, such as a bone graft substitute material. A bone graft substitute material is a material structurally similar to bone. In some instances, a bone graft substitute material is bioresorbable such that the bone graft substitute material can dissolve or be absorbed in the body over time. A bone graft substitute material can be osteoconductive, such that it facilitates blood vessel and new bone formation into the bone graft substitute material. In some instances, the bone graft substitute material is osteoinductive, such that it facilitates the formation of new bone through active recruitment of mesenchymal stem cells from the surrounding tissue. For example, growth factors, such as bone morphogenetic proteins, may be included in the bone graft substitute material. Bone graft substitute materials include, but are not limited to, hydroxyapatite, tricalcium phosphate, demineralized bone matrix, bovine collagen, calcium sulfate, calcium phosphate, cancellous bone chips, and the like, and combinations thereof.

[0251] Therapeutic support compositions of the present disclosure include a support and a first binding agent covalently linked to the support. The binding agent may be attached to the support on a surface of the support, such as a solvent-accessible surface of the support (e.g., a surface of the support that is in contact with the surrounding solvent). In some cases, the binding agent is attached directly to the support. For example, the binding agent may be covalently attached to the surface of the support, e.g., through a covalent bond, such as an amide, amine, ester, carbamate, urea, thioether, thiocarbamate, thiocarbonate, thiourea, etc. In some instances, the binding agent is covalently attached to the support through an amide bond. In other instances, the binding agent may be linked to the support via a linker. Any suitable linker can be used to link the binding agent to the support. Representative linkers can have from 1 to 100 linking atoms, and can include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms. Representative linkers include, but are not limited to, those shown below:

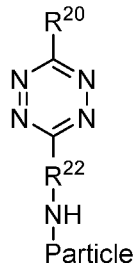


[0252] In certain embodiments, the therapeutic support compositions comprise a support and a tetrazine-containing group of formula:



wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R"', SC(=O)R"', OC(=S)R"', SC(=S)R"', S(=O)R', $S(=O)_2$R"', $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R"', SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R"; R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl; and R"' at each occurrence is independently selected from aryl and alkyl; R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl; R^a, R^{31a} and R^{31b} are each independently hydrogen, C_1-C_6-alkyl, or C_1-C_6-haloalkyl; and t is 0, 1, 2, 3, or 4.

[0253] In certain embodiments, the therapeutic support compositions have formula:

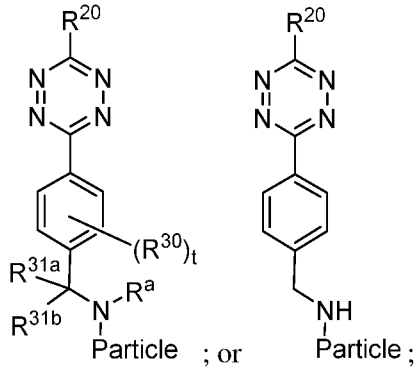


wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R"', SC(=O)R"', OC(=S)R"', SC(=S)R"', S(=O)R', $S(=O)_2$R"', $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R"', SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R"; R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl; R"' at each occurrence is

independently selected from aryl and alkyl; and R^{22} is a linker of 1 to 100 linking atoms, and can include ethylene-oxy groups, amines, esters, amides, carbamates, carbonates, and ketone functional groups. For example, linkers may have from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms.

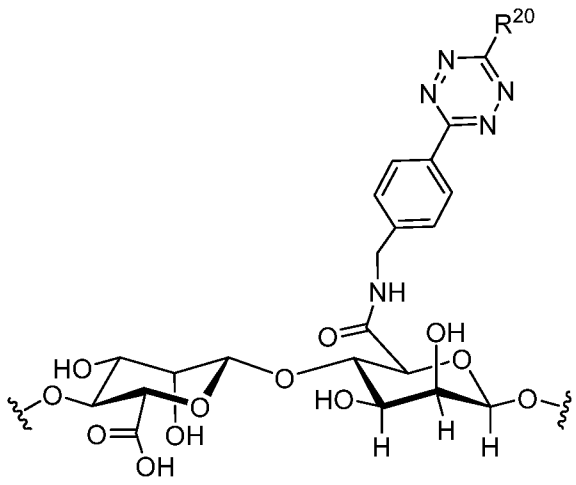
[0254] In certain embodiments, the therapeutic support compositions have formula:



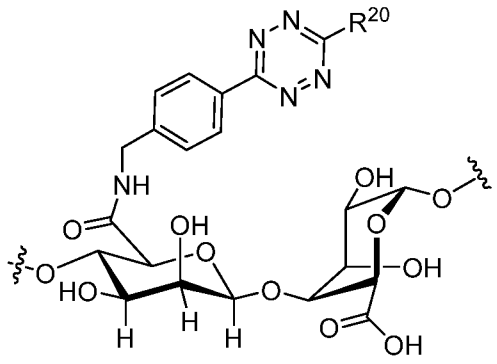
wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R'", SC(=O)R'", OC(=S)R'", SC(=S)R'", S(=O)R', $S(=O)_2$R'", $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R'", SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R"; R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl; R'" at each occurrence is independently selected from aryl and alkyl; R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl; R^a, R^{31a} and R^{31b} are each independently hydrogen, C_1-C_6-alkyl, or C_1-C_6-haloalkyl; and t is 0, 1, 2, 3, or 4.

[0255] In certain embodiments, the therapeutic support compositions comprise substituted alginate having units of formula:



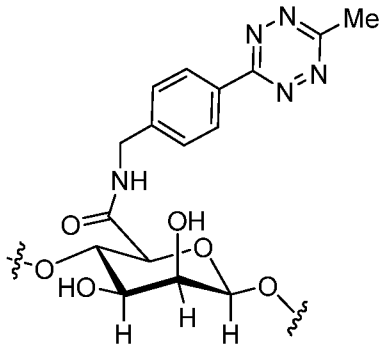
and/or



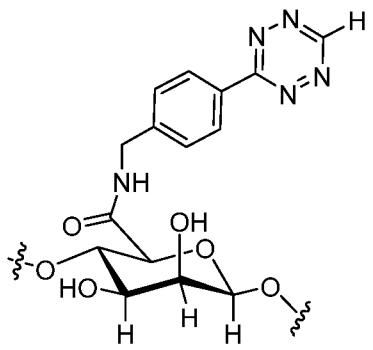
, or a salt thereof,

wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R"', SC(=O)R"', OC(=S)R"', SC(=S)R"', S(=O)R', $S(=O)_2$R"', $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R"', SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R"; R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl; and R"' at each occurrence is independently selected from aryl and alkyl.

[0256] In certain embodiments, the therapeutic support composition comprises units of formula:

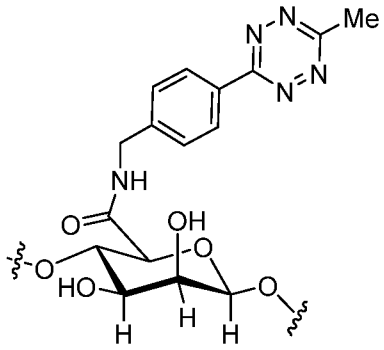


or



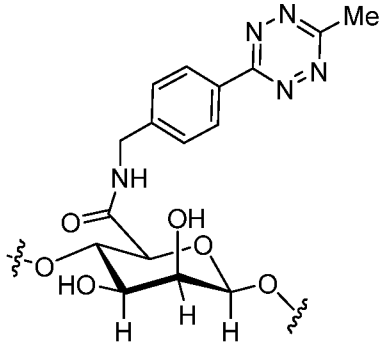
.

[0257] In some embodiments, the therapeutic support compositions comprise units of formula:

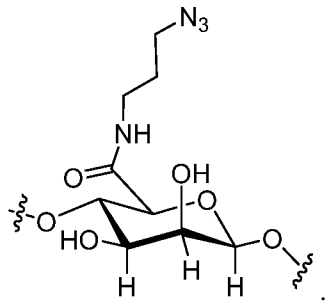


.

[0258] In some embodiments, the therapeutic support compositions comprise units of formula:

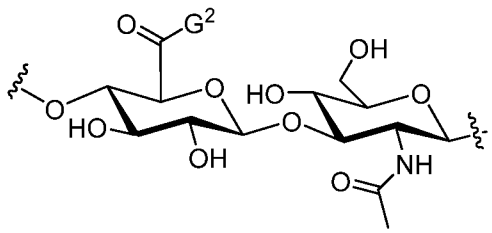


and

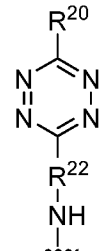


.

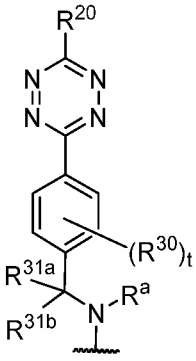
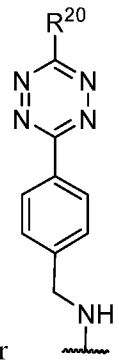
[0259] In some embodiments, the therapeutic support compositions comprise substituted hyaluronic acid having units of formula (II):

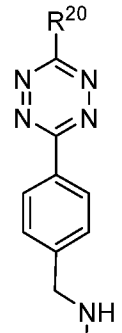


(II)



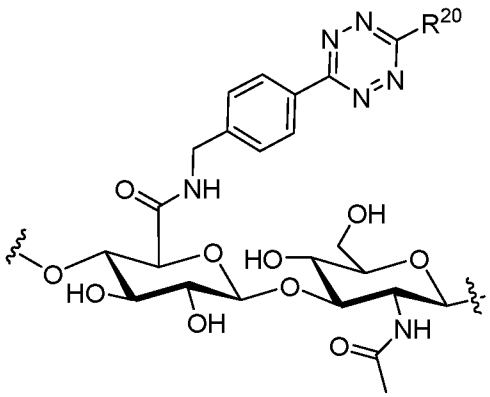
wherein G^2 is ; R^{22} is a linker of 1 to 100 linking atoms; and R^{20} is as defined herein.

[0260] In further embodiments, G^2 is  or  .



[0261] In still further embodiments, G^2 is ; and R^{20} is hydrogen or $C_{1\text{-}4}$alkyl.

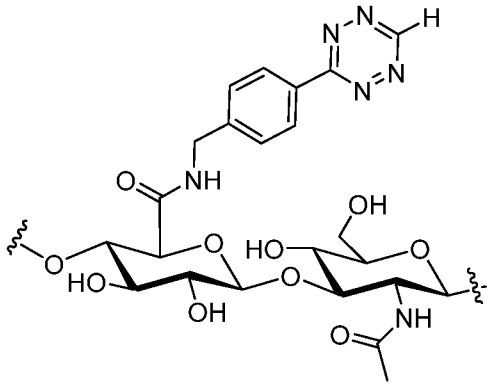
[0262] Compounds of formula (II) include compounds of formula (II-A):

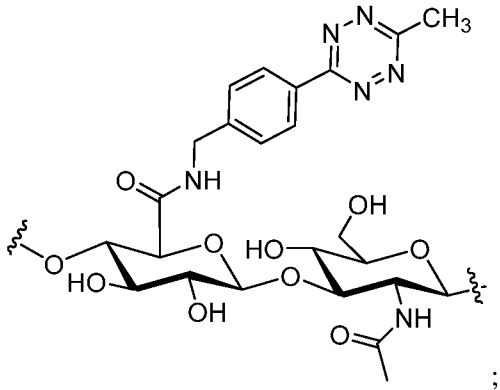


(II-A)

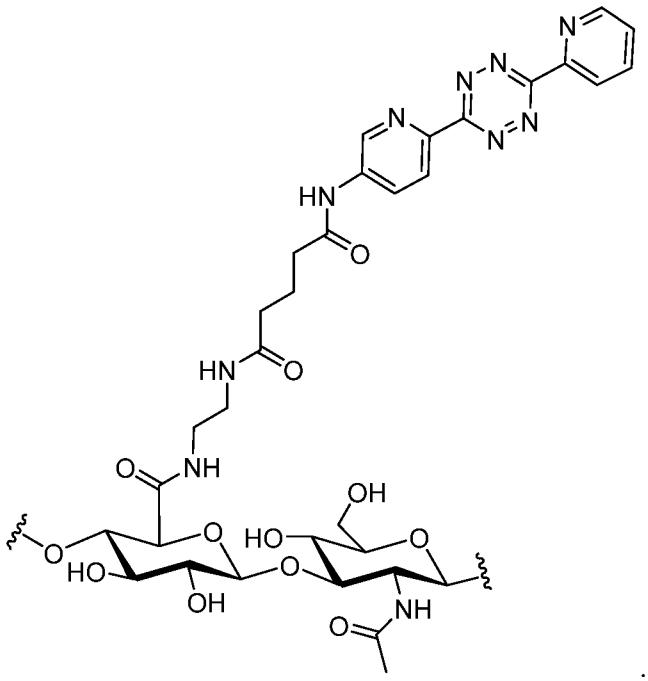
wherein R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R"', SC(=O)R"', OC(=S)R"', SC(=S)R"', S(=O)R', $S(=O)_2$R"', $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R"', SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R"; R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl; and R"' at each occurrence is independently selected from aryl and alkyl. In further embodiments according to formula (II-A), R^{20} is hydrogen or $C_{1\text{-}4}$alkyl.

[0263] In some embodiments, the therapeutic support compositions comprise units of formula:



; 

; ; or



.

[0264] Additional therapeutic support compositions are exemplified in WO2017/044983, WO/2015/139025A1, and WO/2014/205126A1, the entire contents of each of which is incorporated herein by reference in their entirety.

[0265] The hyaluronic acid derivative includes a hyaluronic acid having a plurality of glucuronic acid units and a tetrazine-containing group linked or directly bonded to a glucuronic acid unit of the hyaluronic acid. The hyaluronic acid may also have a plurality of N-acetylglucosamine units. In certain embodiments, the N-acetylglucosamine units of the hyaluronic acid are not linked or conjugated to the tetrazine-containing group.

[0266] The tetrazine-containing group can be linked or directly bonded through a carboxylic acid of a glucuronic acid unit. The tetrazine-containing group can be incorporated into the hyaluronic acid from about 0.1% to about 80% as measured by the % of carboxylic acids being linked or conjugated to the tetrazine-containing group, such as about 1% to about 75%, about 5% to about 75%, about 10% to about

50%, or about 40% to about 75% as measured by the % of carboxylic acids being linked or conjugated to the tetrazine-containing group.

D. Methods of Treatment

[0267] Aspects of the present disclosure include methods for delivering a payload to a target location in a subject. In certain embodiments, the method includes selectively delivering a payload to the target location in a subject. Selective delivery of the payload includes delivering the payload to the target location (e.g., an organ or tissue, or portion thereof), without targeting other locations in the subject (e.g., other organs or tissues, or portions thereof) that do not need administration of the payload. Selective delivery of the payload may be achieved through use of the support compositions and the functionalized payloads described herein.

[0268] In some instances, a support composition of the present disclosure may be localized to a desired target location in a subject. For example, methods of the present disclosure may include administering to a subject a support composition as described herein. The support composition may be administered to the subject at a desired target location in the subject. In some instances, the support composition may be implanted into the subject at the desired target location in the subject. In some embodiments, the support composition may be attached to a targeting agent as described herein, and the method may include administering the support composition to the subject (e.g., administered systemically). In these embodiments, the support composition that is attached to a targeting agent may localize at a desired target location in the subject through specific binding of the targeting agent to its target (e.g., antibody-antigen interaction, and the like), or may localize on the surface of a desired target (e.g., a cell surface) through specific binding of the targeting agent to its target (e.g., antibody-antigen interaction, and the like).

[0269] As described herein, selective binding between bioorthogonal binding partners (e.g., between a tetrazine binding agent of the support composition and its complementary trans-cyclooctene binding agent of a functionalized payload) may occur. Due to the localized administration of the support composition to a desired location in the subject as described above, the selective binding between the binding agent of the support composition and its complementary binding agent of the functionalized payload will localize the payload to the desired target location. Accordingly, in certain embodiments, the method includes administering to the subject a functionalized payload such that the functionalized payload binds to the support composition to form a support complex. For example, the functionalized payload may be administered systemically to the subject. Upon administration of the functionalized payload to the subject, contact between the binding agent of the support composition and the complementary binding agent of the functionalized payload may occur, such that the binding agent and its complementary binding agent bind to one another to form a support complex, thereby selectively delivering the payload to the target location in the subject. In some embodiments, selective delivery of

the functionalized payload results in a concentration of the payload at the target location that is greater than the concentration of the payload elsewhere in the subject (e.g., at non-targeted areas in the subject).

[0270] Provided herein is a method of treating cancer comprising administering to a subject in need thereof, a therapeutically effective amount of a conjugate as described herein, or a pharmaceutically acceptable salt thereof, and a therapeutic support composition.

[0271] In some embodiments, the cancer is metastatic. In some embodiments the cancer is melanoma, renal cancer, prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, Kaposi's sarcoma, Non-Hodgkin lymphoma, Hodgkin's lymphoma Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, or cutaneous T-cell lymphoma.

[0272] In certain embodiments, the approach can be used for the treatment and/or diagnosis of hematological malignancies such as myelodysplastic syndromes, acute myeloid leukemia, myeldysplastic syndromes, chronic myelogenous leukemia, chronic myelomonocytic leukemia, primary myelofibrosis, diffuse large B-cell lymphoma, chronic lymphocytic leukemia, monoclonal gammopathy, plasma cell myeloma, follicular lymphoma, marginal zone lymphoma, classical Hodgkin lymphoma, monoclonal B-cell lymphocytosis, lymphoproliferative disorder NOS, T-cell lymphoma, precursor B-lymphoblastic leukemia, mantle cell lymphoma, plasmacytoma, Burkitt lymphoma, T-cell leukemia, hairy-cell leukemia, precursor T-lymphoblastic leukemia, nodular lymphocyte predominant Hodgkin lymphoma, as well as others.

[0273] In some embodiments, the cancer is a melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer.

[0274] In some embodiments, the cancer is a solid tumor.

[0275] In some embodiments, the cancer is a soft tissue sarcoma.

[0276] In some embodiments, the soft tissue sarcoma is a fibrosarcoma, rhabdomyosarcoma, or Ewing's sarcoma.

[0277] In some embodiments, the method also comprises enhancing or eliciting an immune response. In some embodiments the immune response is an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

[0278] In some embodiments, the method further comprising administering a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an

immunomodulatory agent, or a trans-cyclooctene prodrug thereof. Anticancer agents, immunomodulatory agents, and their trans-cyclooctene prodrugs are known in the art.

[0279] Indications for this approach include cancer, both hematological and solid cancers. In certain embodiments, the approach can be used for the treatment and/or diagnosis of soft tissue sarcomas: rhabdomyosarcoma, fibrosarcoma, Ewing's sarcoma, and all the different subtypes of soft tissue sarcoma as well as osteosarcoma. The compositions can be for the treatment and/or diagnosis of pigmented vilonodular synovitis.

[0280] The compositions of the present disclosure find use in treatment and/or diagnosis of a condition or disease in a subject that is amenable to treatment or diagnosis by administration of the payload (e.g., the parent drug (i.e., the drug prior to conjugation to the composition)). By "treatment" is meant that at least an amelioration of the symptoms associated with the condition afflicting the subject is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g., symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g., terminated, such that the subject no longer suffers from the condition, or at least the symptoms that characterize the condition. Treatment may include inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease. Treatment may include relief, that is, causing the regression of clinical symptoms. For example, in the context of cancer, the term "treating" includes any or all of: reducing growth of a solid tumor, inhibiting replication of cancer cells, reducing overall tumor burden, prolonged survival and ameliorating one or more symptoms associated with a cancer.

[0281] The subject to be treated can be one that is in need of therapy, where the subject to be treated is one amenable to treatment using the parent drug. Accordingly, a variety of subjects may be amenable to treatment using the compositions disclosed herein. Generally, such subjects are "mammals," with humans being of interest. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees, and monkeys).

[0282] In certain embodiments, the functionalized payloads, therapeutic support compositions, additional therapeutic agents, and methods can be used for the treatment, prevention, and/or diagnosis of solid tumors, including but not limited to, melanoma (e.g., unresectable, metastatic melanoma), renal cancer (e.g., renal cell carcinoma), prostate cancer (e.g., metastatic castration resistant prostate cancer), ovarian cancer (e.g., epithelial ovarian cancer, such as metastatic epithelial ovarian cancer), endometrial carcinoma, breast cancer (e.g., triple negative breast cancer), glioblastoma (e.g., glioblastoma multiforme), and lung cancer (e.g., non-small cell lung cancer), soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular

carcinoma, Kaposi's sarcoma, Non-Hodgkin lymphoma, Hodgkin lymphoma Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, prostatic adenocarcinoma, nasopharyngeal carcinoma, cutaneous T-cell lymphoma, among others. The disclosed approach lends itself well as an adjuvant / neoadjuvant system. For example, particles as disclosed herein could be placed during the biopsy, once the results from the study come back, the practitioner could deliver the appropriate cocktail to the desired site in the body. This would minimize the size of the tumor particularly in the context of a surgically resectable tumor. Then at the end of the surgery, the surgeon could place more particles around the surgical cavity and treat the patient with further doses of treatment (e.g. chemotherapy through the disclosed approach) to minimize the risk of any cancer cells that may have been missed in the surgical margins..

[0283] In certain embodiments, the disclosed methods provide the ability to place particles as disclosed herein at the time of the biopsy. When the results return, the practitioner can deliver through to the biopsy site immunomodulatory agents such as TLR agonists, STING agonists, chemokines (agents that attract cancerous cells and/or immune cells) and adjuvants to enhance the immune system with fewer side effects as well as the chemotherapeutics agents combined with immunotherapy agents. This combination approach would be beneficial to patients. The chemotherapy agent would treat the solid tumor or specific location, while the enhanced response of the immunotherapy would help with distant metastatic sites. For example, in certain embodiments, the disclosed compositions and methods could employ or be used with anthracyclines, taxanes, gemcitabine and other agents to enhance the efficacy of one or more immunomodulatory agents such as ipilimumab, nivolumab, pembrolizumab, avelumab (also known as MSB0010718C; Pfizer).

Cancer

[0284] The disclosed methods may be used to treat or prevent cancer, including metastatic cancer. Cancer is a group of related diseases that may include sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enablement of replicative immortality, induction of angiogenesis, and the activation of invasion and metastasis. The disclosed methods may enhance or elicits an immune response against a cancer in the subject. The immune response may lead to an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

[0285] Cancer that may be treated by the disclosed methods, includes, but is not limited to, astrocytoma, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain cancer, brain stem cancer, brain stem glioma, breast cancer, cervical cancer, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, diffuse intrinsic pontine glioma, ductal cancer, endometrial cancer, ependymoma, Ewing's sarcoma, esophageal cancer, eye cancer, fibrosarcoma, gallbladder cancer, gastric cancer, gastrointestinal cancer, germ cell tumor, glioma, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, macroglobulinemia,

melanoma, mesothelioma, mouth cancer, multiple myeloma, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, skin cancer, small cell lung cancer, small intestine cancer, soft tissue carcinoma, soft tissue sarcoma, solid tumor, squamous cell carcinoma, stomach cancer, T-cell lymphoma, testicular cancer, throat cancer, thymoma, thyroid cancer, trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor.

[0286] In some embodiments, the cancer that may be treated by the disclosed methods is melanoma, renal cancer, prostate cancer, ovarian cancer, breast cancer, glioma, lung cancer, soft tissue carcinoma, soft tissue sarcoma, osteosarcoma, or pancreatic cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is a soft tissue carcinoma. In some embodiments, the cancer is afibrosarcoma. In some embodiments, the cancer is diffuse intrinsic pontine glioma. In some embodiments, the cancer is a metastatic cancer.

[0287] Without being bound by a particular theory, local release of certain anti-cancer agents using the compounds and methods of the invention may produce or contribute to immunogenic cell death (ICD). For example, certain anti-cancer agents (e.g., anthracyclines, cyclophosphamide, oxaliplatin) have been reported to induce ICD. Kroemer et al. Annu. Rev. Immunol. 2013 (31), 51-72. Immunogenic apoptosis of cancer cells can induce an effective antitumor immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. ICD is characterized by secretion of damage-associated molecular patterns (DAMPs). Three important DAMPs which are exposed to the cell surface during ICD. Calreticulin (CRT), one of the DAMP molecules, which is normally in the lumen of endoplasmic reticulum (ER), is translocated after the induction of immunogenic apoptosis to the surface of dying cell where it functions as an "eat me" signal for professional phagocytes. Other important surface exposed DAMPs are heat-shock proteins (HSPs), namely HSP70 and HSP90, which are under stress condition also translocated to the plasma membrane. On the cell surface they have an immunostimulatory effect, based on their interaction with number of antigen-presenting cell (APC) surface receptors like CD91 and CD40 and also facilitate crosspresentation of antigens derived from tumor cells on MHC class I molecule, which than leads to the CD8+ T cell response. Other important DAMPs, characteristic for ICD are secreted amphoterin (HMGB1) and ATP. HMGB1 is considered to be late apoptotic marker and its release to the extracellular space seems to be required for the optimal release and presentation of tumor antigens to dendritic cells. It binds to several pattern recognition receptors (PRRs) such as Toll-like receptor (TLR) 2 and 4, which are expressed on APCs. The most recently found DAMP released during immunogenic cell death is ATP, which functions as a ”find-me“ signal for monocytes when secreted and induces their attraction to the site of apoptosis. Kroemer et. al. Curr. Op. Immunol. 2008 (20), 504-511.

[0288] Thus, local release of ICD inducers using the compounds and methods of the invention may be beneficially combined with one or more immunomodulatory agents.

[0289] In certain embodiments, the functionalized payloads, therapeutic support compositions, and methods can be used for the treatment, prevention, and/or diagnosis of solid tumors, including but not limited to, melanoma (e.g. , unresectable, metastatic melanoma), renal cancer (e.g., renal cell carcinoma), prostate cancer (e.g., metastatic castration resistant prostate cancer), ovarian cancer (e.g., epithelial ovarian cancer, such as metastatic epithelial ovarian cancer), breast cancer (e.g., triple negative breast cancer), glioblastoma (e.g., glioblastoma multiforme), and lung cancer (e.g., non-small cell lung cancer), soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, among others.

[0290] The disclosed approach lends itself well as an adjuvant / neoadjuvant system. For example, therapeutic support compositions as disclosed herein could be placed during the biopsy, once the results from the study come back, the practitioner could administer the appropriate cocktail to deliver treatment to the desired site in the body (compound of Formula I and optional additional therapeutic agent(s)). The results of the biopsy may indicate the amount and type of treatment to deliver to the site of a tumor. For example, chemokines (agents that attract cancerous cells and/or immune cells) and adjuvants to enhance the immune system with fewer side effects as well as the chemotherapeutics agents could be delivered and combined with immunotherapy agents.

[0291] The disclosed compounds and compositions may be administered prior to surgical resection. The disclosed methods may minimize the size of the tumor prior to surgical resection. This would minimize the size of the tumor particularly in the context of a surgically resectable tumor. The disclosed conjugates, compounds and compositions may be administered during surgical resection. The disclosed conjugates, compounds and compositions may be administered after surgical resection. Therapeutic support composition may be placed around the surgical cavity at the end of surgical resection and the subject may then be treated with further doses of a treatment to minimize the risk of any cancer cells that may have been missed in the surgical margins.

[0292] The disclosed methods may include multiple systemic doses of functionalized payload that focus at one location. The disclosed methods may be used to deliver a second payload. The disclosed methods may be used to administer a second functionalized payload if the tumor is resistant to the first payload. A second payload may be a TCO-labeled payload of gemcitabine or docetaxel. The TCO-labeled payload of gemcitabine or docetaxel may be administered in combination with doxorubicin. The second functionalized payload may be activated by the therapeutic support composition used for the first prodrug.

[0293] The functionalized payloads disclosed herein may function as adjuvants. This combination approach would be beneficial to patients. The chemotherapy agent would treat the solid tumor or specific location and may enhance or elicit an immune response, while the enhanced response of the

immunotherapy of the functionalized payload and/or separate agent may help with distant metastatic sites. For example, in certain embodiments, the disclosed compositions and methods could employ or be used with anthracyclines, auristatins, vinca alkaloids, taxanes, gemcitabine, campothecin analogues and other agents to enhance the efficacy of ipilimumab, nivolumab, pembrolizumab, avelumab (also known as MSB0010718C; Pfizer).

[0294] The disclosed methods may be used to treat diffuse intrinsic pontine gliomas. Diffuse intrinsic pontine gliomas (DIPG) are pediatric brainstem tumors that may be highly malignant and may be difficult to treat. There is no known curative treatment for DIPG, and survival odds have remained dismal over the past four decades. DIPG patients have a median overall survival of just 11 months, with a two-year survival rate below 10%. DIPG account for 75–80% of brainstem tumors in children, affecting an estimated 200–300 children in the U.S. each year. The rarity of this devastating disease and previous lack of experimental model systems has impeded research, and over the past four decades survival odds have remained the same. Diagnosis of DIPG may begin with clinical symptoms and may be confirmed by MRI. The disease may begin with several months of generalized symptoms, including behavioral changes and difficulties in school, double vision, abnormal or limited eye movements, an asymmetric smile, loss of balance, and weakness. Alternately, severe neurologic deterioration may happen more quickly, with symptoms present for less than a month prior to diagnosis. Clinical examination may reveal the triad of multiple cranial neuropathies, long tract signs such as hyperreflexia and clonus, as well as ataxia. Expansion of the pons section of the brainstem may cause obstructive hydrocephalus and increased intracranial pressure.

[0295] Nuclei critical for life-sustaining function such as breathing and heartbeat in are located in the pons and without treatment, breathing and heartbeat may be damaged by DIPG.

[0296] The disclosed methods may be used to deliver molecular payloads to the site of a DIPG . The disclosed methods may include delivering drugs systemically that are only activated at the tumor site. The disclosed methods may be used as a neoadjuvant or adjuvant therapy. The biomaterial may be placed during a biopsy. The results of the biopsy may indicate the amount and type of treatment to deliver to the site of a tumor. The disclosed compounds and compositions may be administered prior to surgical resection. The disclosed methods may minimize the size of the tumor prior to surgical resection. The disclosed compounds and compositions may be administered during surgical resection. The disclosed compounds and compositions may be administered after surgical resection. Biomaterial may be placed around the surgical cavity at the end of surgical resection and the subject may then be treated with further doses of a treatment. The disclosed biodegradable gel may be implanted at the time of biopsy or surgery. The disclosed methods may not require an additional invasive procedure to deliver additional doses of the disclosed compounds and compositions.

[0297] The disclosed methods may include multiple systemic doses of functionalized payload that focus at one location. The disclosed methods may be used to deliver a second payload. The disclosed methods

may be used to administer a second functionalized payload if the tumor is resistant to the first payload. A second payload may be a TCO-labeled payload of paclitaxel, docetaxel, anthracyclines, auristatins, vinca alkaloids, taxanes, gemcitabine, campothecin analogues, or other agents . The TCO-labeled payload of gemcitabine, paclitaxel, or docetaxel may be administered in combination with doxorubicin. The second functionalized payload may be activated by the therapeutic support composition used for the first prodrug.

Modes of Administration

[0298] Methods of treatment may include any number of modes of administering a disclosed conjugate, compound or composition. Modes of administration may include tablets, pills, dragees, hard and soft gel capsules, granules, pellets, skin patches, skin creams, skin gels, aqueous, lipid, oily or other solutions, emulsions such as oil-in-water emulsions, liposomes, aqueous or oily suspensions, syrups, elixirs, solid emulsions, solid dispersions or dispersible powders. In the pharmaceutical composition, the conjugate, compound or compositions disclosed herein may also be dispersed in a microparticle, e.g. a nanoparticulate composition.

[0299] For parenteral administration, the conjugates, compounds or compositions disclosed herein may be dissolved or suspended in a physiologically acceptable diluent, such as water, buffer, oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. Suitable oils may include, for example, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil. For parenteral administration, the conjugates, compounds or compositions disclosed herein may be administered in the form of an aqueous, lipid, oily or other kind of solution or suspension, or even administered in the form of liposomes or nano-suspensions.

[0300] The term "parenterally," as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0301] Therapeutic support compositions are preferably administered locally at the site of a tumor, such as by injection or implantation. Functionalized payloads, such as conjugates of Formula I or (III), may be administered by any convenient route, in view of a subject's condition and judgment of medical professionals. Parenteral administration is a suitable means of administering conjugates of Formula I.

[0302] The amount of composition administered to a subject can be initially determined based on guidance of a dose and/or dosage regimen of the parent drug. In general, the compositions can provide for targeted delivery and/or enhanced serum half-life of the bound drug, thus providing for at least one of reduced dose or reduced administrations in a dosage regimen. Thus, the compositions can provide for reduced dose and/or reduced administration in a dosage regimen relative to the parent drug prior to being conjugated in a composition of the present disclosure.

[0303] The pharmaceutical formulation may be provided in unit dosage form. In such form the pharmaceutical formulation may be subdivided into unit doses containing appropriate quantities of the compositions of the present disclosure. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, such as packeted tablets, capsules, and powders in pouches, vials or ampoules.

[0304] In some embodiments, provided is a kit comprising a conjugate, or a pharmaceutically acceptable salt thereof, as described herein, or the pharmaceutical composition comprising the same, and instructions for use thereof.

[0305] In some embodiments, the kit further comprising the therapeutic support composition.

[0306] Compositions of the present disclosure can be present in any suitable amount, and can depend on various factors including, but not limited to, weight and age of the subject, state of the disease, etc. Suitable dosage ranges for the composition of the present disclosure include from 0.1 mg to 10,000 mg, or 1 mg to 1000 mg, or 10 mg to 750 mg, or 25 mg to 500 mg, or 50 mg to 250 mg. For instance, suitable dosages for the composition of the present disclosure include 1 mg, 5 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg or 1000 mg.

[0307] In some embodiments, multiple doses of a composition are administered. The frequency of administration of a composition can vary depending on any of a variety of factors, e.g., severity of the symptoms, condition of the subject, etc. For example, in some embodiments, a composition is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

[0308] The compositions of the present disclosure can be administered at any suitable frequency, interval and duration. For example, the composition of the present disclosure can be administered once an hour, or two, three or more times an hour, once a day, or two, three, or more times per day, or once every 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days, so as to provide the desired dosage level to the subject. When the composition of the present disclosure is administered more than once a day, representative intervals include 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 minutes, as well as 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr, 20 hr, and 24 hours. The composition of the present disclosure can be administered once, twice, or three or more times, for an hour, for 1 to 6 hours, for 1 to 12 hours, for 1 to 24 hours, for 6 to 12 hours, for 12 to 24 hours, for a single day, for 1 to 7 days, for a single week, for 1 to 4 weeks, for a month, for 1 to 12 months, for a year or more, or even indefinitely.

[0309] The compositions of the present disclosure can be co-administered with another active agent. Co-administration includes administering the composition of the present disclosure and active agent

within 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr, 20 hr, or 24 hours of each other. Co-administration also includes administering the composition of the present disclosure and active agent simultaneously or approximately simultaneously (e.g., within about 1 min, 5 min, 10 min, 15 min, 20 min, or 30 minutes of each other), or sequentially in any order. In addition, the composition of the present disclosure and the active agent can each be administered once a day, or two, three, or more times per day so as to provide the desired dosage level per day.

[0310] Co-administration can be accomplished by coimplantation or coinjection.

[0311] In some embodiments, co-administration can be accomplished by co-formulation, e.g., preparing a single pharmaceutical formulation including both the composition of the present disclosure and the active agent. In other embodiments, the composition of the present disclosure and the active agent can be formulated separately and co-administered to the subject.

[0312] The composition of the present disclosure and the active agent can be present in a formulation in any suitable weight ratio, such as from 1:100 to 100:1 (w/w), or 1:50 to 50:1, or 1:25 to 25:1, or 1:10 to 10:1, or 1:5 to 5:1 (w/w). The composition of the present disclosure and the other active agent can be present in any suitable weight ratio, such as 1:100 (w/w), 1:75, 1:50, 1:25, 1:10, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 25:1, 50:1, 75:1, or 100:1 (w/w). Other dosages and dosage ratios of the composition of the present disclosure and the active agent are suitable in the formulations and methods described herein.

Combination Therapies

[0313] In one aspect, the invention provides a method of treating cancer or enhancing or eliciting an immune response comprising administering to a subject in need thereof: a therapeutically effective amount of a conjugate of the invention (e.g., Formula I), or a pharmaceutically acceptable salt or composition thereof; a therapeutic support composition, as described herein; and a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof.

[0314] The invention also provides a pharmaceutical combination comprising a conjugate described herein, or a pharmaceutically acceptable salt, or composition thereof; a therapeutic support composition, as described herein; and an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof, for use in the treatment or prevention of a cancer or for use in enhancing or eliciting an immune response.

[0315] The invention also provides the use of a pharmaceutical combination comprising a conjugate described herein, or a pharmaceutically acceptable salt, or composition thereof; a therapeutic support composition; and a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug

thereof for the treatment or prevention of a cancer or for use in enhancing or eliciting an immune response.

[0316] In the methods and uses described herein, the components of the pharmaceutical combinations may be administered/used simultaneously, separately, or sequentially, and in any order, and the components may be administered separately or as a fixed combination. For example, the delay of progression or treatment of diseases according to the invention may comprise administration of the first active ingredient in free or pharmaceutically acceptable salt form and administration of the second active ingredient in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts or effective amounts, e.g. in daily dosages corresponding to the amounts described herein. The individual active ingredients of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single dosage forms. The present disclosure is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly. Thus, a pharmaceutical combination, as used herein, defines either a fixed combination in one dosage unit form or separate dosages forms for the combined administration where the combined administration may be independently at the same time or at different times. As a further example, the therapeutic support composition and conjugate may be administered/used simultaneously (e.g., through coinjection or coimplantation), separately, or sequentially, followed by administration of the additional therapeutic agent selected from the group consisting of an anticancer agent, an immunomodulatory agent, or a trans-cyclooctene prodrug thereof.

[0317] The methods and uses in treating cancer include administering/localizing the therapeutic support composition at a tumor. In the methods and uses disclosed herein, the administration of the conjugate, or a pharmaceutically acceptable salt, or composition thereof; the therapeutic support composition; and the additional therapeutic agent may inhibit the growth of the tumor.

[0318] Additional therapeutic agent(s) may be administered simultaneously or sequentially with the disclosed conjugates and compositions. Sequential administration includes administration before or after the disclosed conjugates and compositions. An additional therapeutic agent may be administered before the disclosed conjugates and compositions. An additional therapeutic agent may be administered after the disclosed conjugates and compositions. An additional therapeutic agent may be administered at the same time as the disclosed conjugates and compositions. In some embodiments, the additional therapeutic agent or agents may be administered in the same composition as the disclosed conjugates. In other embodiments, there may be an interval of time between administration of the additional therapeutic agent and the disclosed conjugates or compositions. In some embodiments, administration of an additional therapeutic agent with a disclosed conjugate or composition may allow lower doses of the other therapeutic agents and/or administration at less frequent intervals. When used in combination with one or more other active ingredients, the conjugates or compositions of the present invention and the other

active ingredients may be used in lower doses than when each is used singly. Accordingly, the pharmaceutical compositions of the present invention include those that contain one or more other active ingredients, in addition to a conjugates of the present disclosure.

Anticancer agents

[0319] Exemplary anti-cancer agents include, but are not limited to, Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin- stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil), Afatinib Dimaleate, Afinitor (Everolimus), Aldara (Imiquimod), Aldesleukin, Alemtuzumab, Alimta (Pemetrexed Disodium), Aloxi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi, Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and I 131 Iodine Tositumomab), Bicalutamide, Bleomycin, Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Busulfan, Busulfex (Busulfan), Cabazitaxel, Cabozantinib- S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carboplatin, Carboplatin-Taxol, Carfilzomib, Casodex (Bicalutamide), CeeNU (Lomustine), Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, Chlorambucil, Chlorambucil-Prednisone, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cometriq (Cabozantinib-S-Malate), COPP, COPP-ABV, Cosmegen (Dactinomycin), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytarabine, Cytarabine liposomal, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Efudex (Fluorouracil), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysanthemi), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, Fareston (Toremifene), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil), Fluorouracil, Folex (Methotrexate), Folex PFS (Methotrexate), Folfiri, Folfiri- Bevacizumab, Folfiri-Cetuximab, Folfirinox, Folfox (Leucovorin, Fluorouracil, Oxaliplatin), Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gazyva (Obinutuzumab), Gefitinib,

Gemcitabine Hydrochloride, Gemcitabine-Cisplatin, Gemcitabine-Oxaliplatin, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hyper-CVAD, Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Ifex (Ifosfamide), Ifosf amide, Ifosfamidum (Ifosfamide), Imatinib Mesylate, Imbruvica (Ibrutinib), Imiquimod, Inlyta (Axitinib), Intron A (Recombinant Interferon Alfa- 2b), Iodine 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Istodax (Romidepsin), Ixabepilone, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Kadcyla (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Kyprolis (Carfilzomib), Lapatinib Ditosylate, Lenalidomide, Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Lomustine, Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron Depot- 3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megace (Megestrol Acetate), Megestrol Acetate, Mekinist (Trametinib), Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mitomycin C, Mitozytrex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin- stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Nelarabine, Neosar (Cyclophosphamide), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilotinib, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Ofatumumab, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Ontak (Denileukin Diftitox), OEPA, OPPA, Oxaliplatin, Paclitaxel, Paclitaxel Albumin- stabilized Nanoparticle Formulation, Palifermin, Palonosetron Hydrochloride, Pamidronate Disodium, Panitumumab, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, Pegaspargase, Peginterferon Alfa-2b, PEG-Intron (Peginterferon Alfa-2b), Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Provenge (Sipuleucel-T), Purinethol (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Rasburicase, R-CHOP, R-CVP, Recombinant HPV Bivalent Vaccine, Recombinant HPV Quadrivalent Vaccine, Recombinant Interferon Alfa- 2b, Regorafenib, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Rituxan (Rituximab), Rituximab, Romidepsin, Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc), Sipuleucel-T, Sorafenib Tosylate, Sprycel (Dasatinib), Stanford V, Sterile Talc Powder

(Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa- 2b), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), Tafinlar (Dabrafenib), Talc, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Tositumomab and 1 131 Iodine Tositumomab, Totect (Dexrazoxane Hydrochloride), Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride), Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Vandetanib, VAMP, Vectibix (Panitumumab), VelP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, Vismodegib, Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), Xelox, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Zaltrap (Ziv-Aflibercept), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), and Zytiga (Abiraterone Acetate).

[0320] The anticancer agent may be a PBD dimer, calicheamicin, speromycin, tubulysin B, rhizoxin, dolastatin, didemnin B, camptothecin, CBI, temsirolimus, actinomycin D, epothilone B, taxol, cryptophycin, SN38, velcade, bruceantin, DAVLBH, DM1, Phyllanthoside, Alimta, T2 Toxin, MMC, vantalanib, vinorelbine, brefeldin, sunitinib, daunomycin, semaxanib, tarceva, iressa, irinotecan, LY-541503, geldanomycin, gemcitabine, methotrexate, gleevec, topotecan, bleomycin, doxorubicin, cisplatin, N-mustards, etoposide, or 5-FU.

[0321] In certain embodiments, an anticancer agent is an anthracycline. In certain embodiments, anticancer agent is a taxane. In certain embodiments, anticancer agent is gemcitabine. In certain embodiments, anticancer agent is doxorubicin. In certain embodiments, anticancer agent is docetaxel. In certain embodiments, anticancer agent is SN38. In certain embodiments, anticancer agent is monomethyl auristatin E. In certain embodiments, an anticancer agent is an alkylating agent, antimetabolite (folate antagonist, purine antagonist, pyrimidine antagonist), antibiotic, taxane, vinca alkaloid, or campothecin analogue.

7. Synthesis of the Compounds

[0322] The conjugates may be prepared using the methods disclosed herein and routine modifications thereof, which will be apparent given the disclosure herein and methods well known in the art. Conventional and well-known synthetic methods may be used in addition to the teachings herein. The synthesis of typical compounds described herein may be accomplished as described in the following

examples. If available, reagents and starting materials may be purchased commercially, e.g., from Sigma Aldrich or other chemical suppliers.

[0323] It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[0324] Additionally, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art. For example, numerous protecting groups are described in Wuts, P. G. M., Greene, T. W., & Greene, T. W. (2006). Greene's protective groups in organic synthesis. Hoboken, N.J., Wiley-Interscience, and references cited therein.

[0325] Furthermore, the conjugates of this disclosure may contain one or more chiral centers. Accordingly, if desired, such conjugates can be prepared or isolated as pure stereoisomers, i.e., as individual enantiomers or diastereomers or as stereoisomer-enriched mixtures. All such stereoisomers (and enriched mixtures) are included within the scope of this disclosure, unless otherwise indicated. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well-known in the art. Alternatively, racemic mixtures of such conjugates can be separated using, for example, chiral column chromatography, chiral resolving agents, and the like.

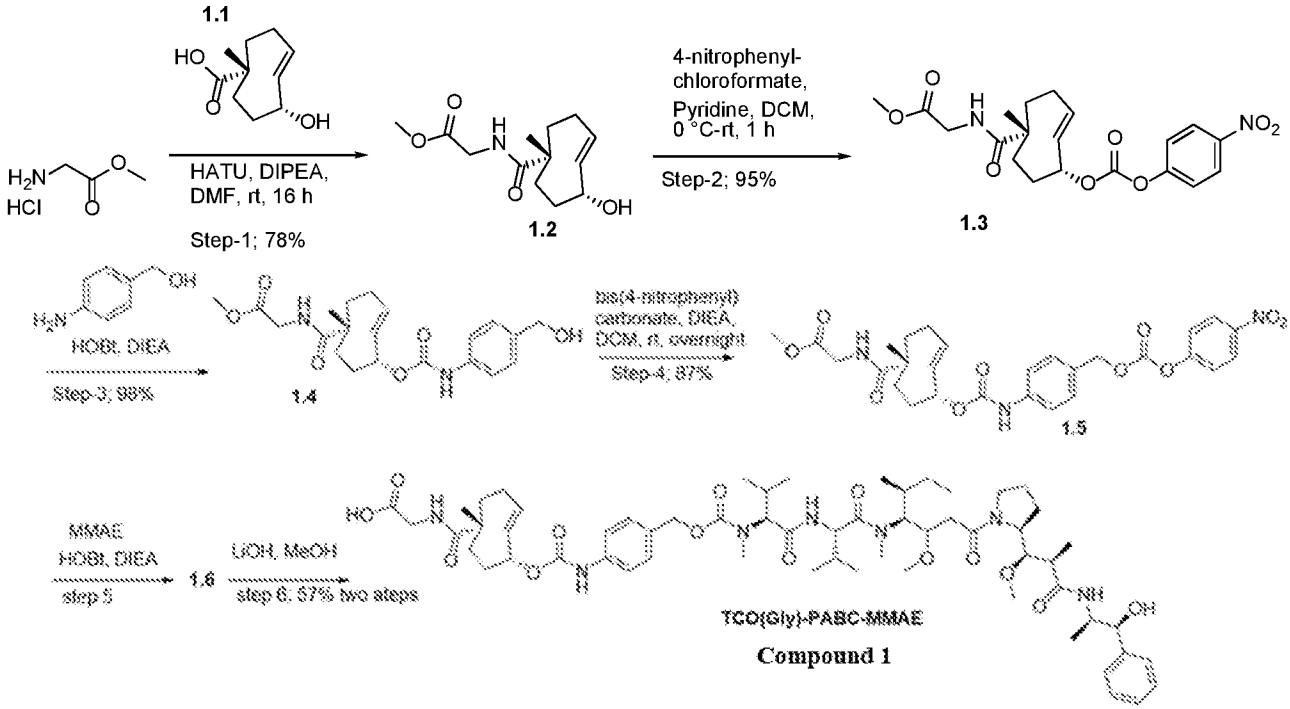
[0326] The starting materials for the following reactions are generally known compounds or can be prepared by known procedures or obvious modifications thereof. For example, many of the starting materials are available from commercial suppliers such as Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemce or Sigma (St. Louis, Missouri, USA). Others may be prepared by procedures or obvious modifications thereof, described in standard reference texts such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-15 (John Wiley, and Sons, 1991), Rodd's Chemistry of Carbon Compounds, Volumes 1-5, and Supplementals (Elsevier Science Publishers, 1989) organic Reactions, Volumes 1-40 (John Wiley, and Sons, 1991), March's Advanced Organic Chemistry, (John Wiley, and Sons, 5th Edition, 2001), and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989).

EXAMPLES

[0327] The following examples are included to demonstrate specific embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques to function well in the practice of the disclosure, and thus can be considered

to constitute specific modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1: Synthesis of TCO(Gly)-PABC-MMAE (Compound 1)



[0328] Methyl ((1R,6R,E)-6-hydroxy-1-methylcyclooct-4-ene-1-carbonyl)glycinate (1.2). Step-1: To a mixture of methyl glycinate (5.1 g, 32.6 mmol, 2.0 eq.) in DMF (100.0 mL) and DIPEA (14.9 mL, 85.5 mmol, 5.3 eq.) were added (1R,6R,E)-6-hydroxy-1-methylcyclooct-4-ene-1-carboxylic acid (**1.1**), (3.0 g, 16.3 mmol, 1.0 eq), and HATU (12.4 g, 32.6 mmol, 2.0 eq.) sequentially. The mixture was stirred at rt overnight, diluted with EtOAc (400 mL) and water (400 mL). The aqueous layer was extracted with EtOAc (400 mL) once. The combined organic layer was dried with Na_2SO_4 and filtered. The filtrate was concentrated and purified by flash chromatography (220 g, ISCO column) eluting with a gradient of EtOAc in hexanes (0-100%) and isocratic at 100% EtOAc in hexane to afford 3.25 g (78% yield) of methyl ((1R,6R,E)-6-hydroxy-1-methylcyclooct-4-ene-1-carbonyl)glycinate.

[0329] Methyl ((1R,6R,E)-1-methyl-6-(((4-nitrophenoxy)carbonyl)oxy)cyclooct-4-ene-1-carbonyl)glycinate (1.3). Step-2: To a solution of compound **1.2** (1.0 g, 4.0 mmol, 1.0 eq.) in anhydrous DCM (30 mL) was added pyridine (0.9 g, 12 mmol, 3.0 eq.). The mixture was cooled in an ice bath. To this mixture was added a solution of p-nitrophenyl chloroformate (1.0 g, 5 mmol, 1.3 eq.) in DCM (5 mL) over two minutes. The mixture was stirred at rt for 1 h and partitioned with EtOAc and water. The organic phase was washed with aq. sodium bicarbonate solution, water and then dried with sodium sulfate, filtered, and concentrated. The resulting residue was dissolved in a minimal amount of DCM and purified by flash chromatography on a silica gel column (40 g, ISCO) with a stepwise gradient

of EtOAc in DCM (0-20%) as eluent to afford 1.6 g (95%) of methyl ((1R,6R,E)-1-methyl-6-(((4-nitrophenoxy)carbonyl)oxy)cyclooct-4-ene-1-carbonyl)glycinate (+ESI)$[M+H]^+$= 421.1

[0330] Methyl ((1R,6R,E)-6-(((4-(hydroxymethyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycinate (1.4). Step-3: To a solution of compound-**1.3** (138 mg, 0.33 mmol, 1.0 eq.), (4-aminophenyl)methanol (40.4 mg, 0.33 mmol, 1.0 eq.), and HOBt (111 mg. 0.66, 2.0 eq) in DMF (2.0 mL) was added DIEA (85 mg, 0.66 mmol, 2.0 eq.) sequentially. The mixture was stirred at rt overnight and monitoring by LCMS. Upon the completion of the reaction, the mixture was loaded directly in C18 flash chromatography (25 g, Agela) with a stepwise gradient of acetonitrile in water (0-60% for 16 min; product eluted out at ~40% acetonitrile in water). The fractions were collected and diluted with EtOAc (~200 mL). The aqueous layer was extracted two more time with EtOAc (100 mL each). The combined organic layer was dried with Na_2SO_4, filtered, and concentrated to afford 130 mg (98%) of methyl ((1R,6R,E)-6-(((4-(hydroxymethyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycinate. (+ESI)$[M+Na]^+$ = 427.3.

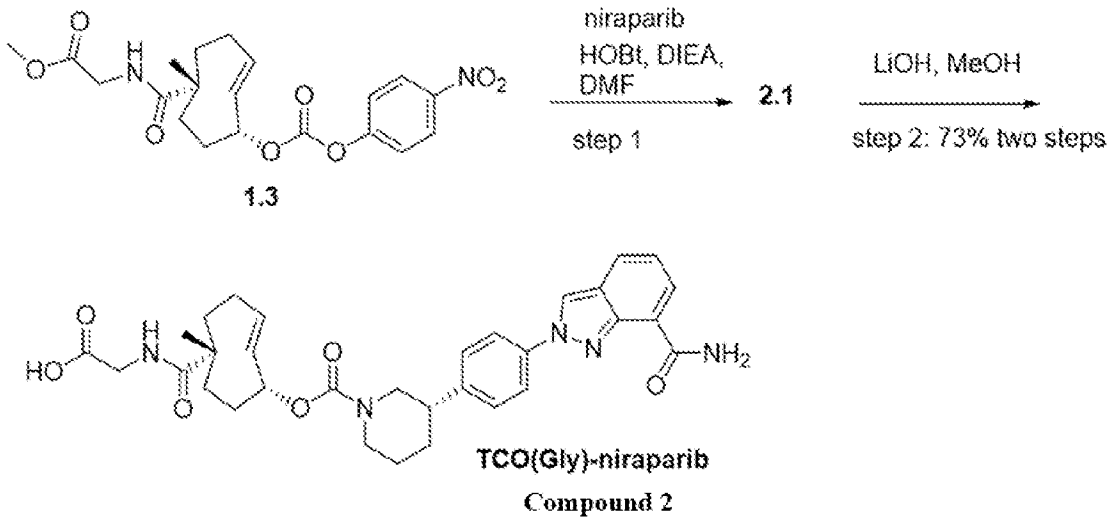
[0331] Methyl ((1R,6R,E)-1-methyl-6-(((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy) cyclooct-4-ene-1-carbonyl)glycinate (1.5). Step-4: To a solution of compound 1.4 (130 mg, 0.32 mmol, 1.0 eq.) and bis(4-nitrophenyl) carbonate (108 mg, 0.35 mmol, 1.1 eq.) in dried DCM (8 mL) was added DIEA (83 mg, 0.64 mmol, 2.0 eq.). The mixture was stirred at rt overnight, diluted with DCM (30 mL), washed with $NaHCO_3$ (20 mL) twice, dried with Na_2SO_4, filtered, and concentrated under reduce pressure. The crude was purified by flash chromatography using a gradient of EtOAc and hexane (0-80%; product eluted around 70%) as eluent to afford 160 mg (87%) of methyl ((1R,6R,E)-1-methyl-6-(((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)cyclooct-4-ene-1-carbonyl)glycinate. (+esi)$[M+H]^+$=570.2 (minor ion). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.32 (d, J = 9.1 Hz, 2H), 7.79 (t, J = 5.8 Hz, 1H), 7.57 (d, J = 9.2 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 5.91 (ddd, J = 14.3, 9.9, 3.9 Hz, 1H), 5.79 – 5.67 (m, 1H), 5.24 (s, 2H), 5.15 (s, 1H), 3.80 – 3.65 (m, 2H), 3.60 (s, 3H), 2.30-2.03 (m, 3H), 2.02-1.93 (m, 1H), 1.92-1.78 (m, 2H), 1.70 (d, J = 13.4 Hz, 1H), 1.58 (dd, J = 14.4, 6.2 Hz, 1H), 1.05 (s, 3H).

[0332] Methyl ((1R,6R,E)-6-(((4-(((((2S)-1-(((2S)-1-(((4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycinate (1.6). Step 5: To a solution of compound 1.5 (40 mg, 0.07 mmol, 1.0 eq.) and (2S)-N-((4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamide (50 mg, 0.07 mmol, 1.0 eq), and HOBt (27 mg, 0.14 mmol, 2.0 eq.; 80% pure) in DMF (1.0 mL) was added DIPEA (14 mg, 0.14 mmol, 2.0 eq.). The mixture was stirred

at rt overnight and monitored by LCMS. Upon the consuming of compound 1.5, the mixture was loaded directly on a C18 cartridge (25 g, Agela) and purified with a step gradient with acetonitrile and water (0-100%; compound eluted out around 65%). The fractions were collected and partially concentrated to remove most of the acetonitrile and take it to the next step directly without drying all the way. (+esi)$[M+H]^+$=1248.6.

[0333] ((1R,6R,E)-6-(((4-(((((2S)-1-(((2S)-1-(((4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycine (TCO(Gly)-PABC-MMAE). Step 6: To compound 1.6 in water from the above step was diluted with MeOH (3.0 mL). To the mixture was added LiOH (7 mg, 0.28 mmol, 4 eq.). The mixture was stirred at rt for 1 h and monitoring by LCMS. Upon the completion of the reaction, the mixture was partially concentrated to remove most of the MeOH and acidified to pH 3 with HCl (1 N) to observed a sticky solid. To the aqueous solution was extracted with EtOAc (10 mL) four times. The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The product was transferred to a 40 mL vial and re-dissolved in a mixture of acetonitrile and water (1:1; 8 mL) and lyophilized to afford 45 mg (57% over two steps) of ((1R,6R,E)-6-(((4-(((((2S)-1-(((2S)-1-(((4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycine. (+esi)$[M+H]^+$= 1134.8. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.98-7.71 (m, 2H), 7.57-7.44 (m, 2H), 7.44 – 7.27 (m, 4H), 7.26-7.16 (m, 1H), 6.10-5.97 (m, 1 H), 5.74 (d, J = 16.7 Hz, 1H), 5.26-5.12 (m, 3 H), 5.06 (d, J = 13.2 Hz, 1H), 4.80 – 4.50 (m, 5H), 4.45 – 4.15 (m, 4H), 4.13-4.02 (m, 1H), 3.91 – 3.65 (m, 5H), 3.61-3.39 (m, 5H), 3.36 (s, 3H), 3.25-3.08 (m, 2H), 3.02 – 2.85 (m, 2H), 2.60 – 2.38 (m, 2H), 2.36-2.13 (m, 4H), 2.13 – 1.77 (m, 6H), 1.77 – 1.53 (m, 3H), 1.51-1.26 (m, 5H), 1.26 – 1.09 (m, 8H), 1.09 – 0.69 (m, 15H).

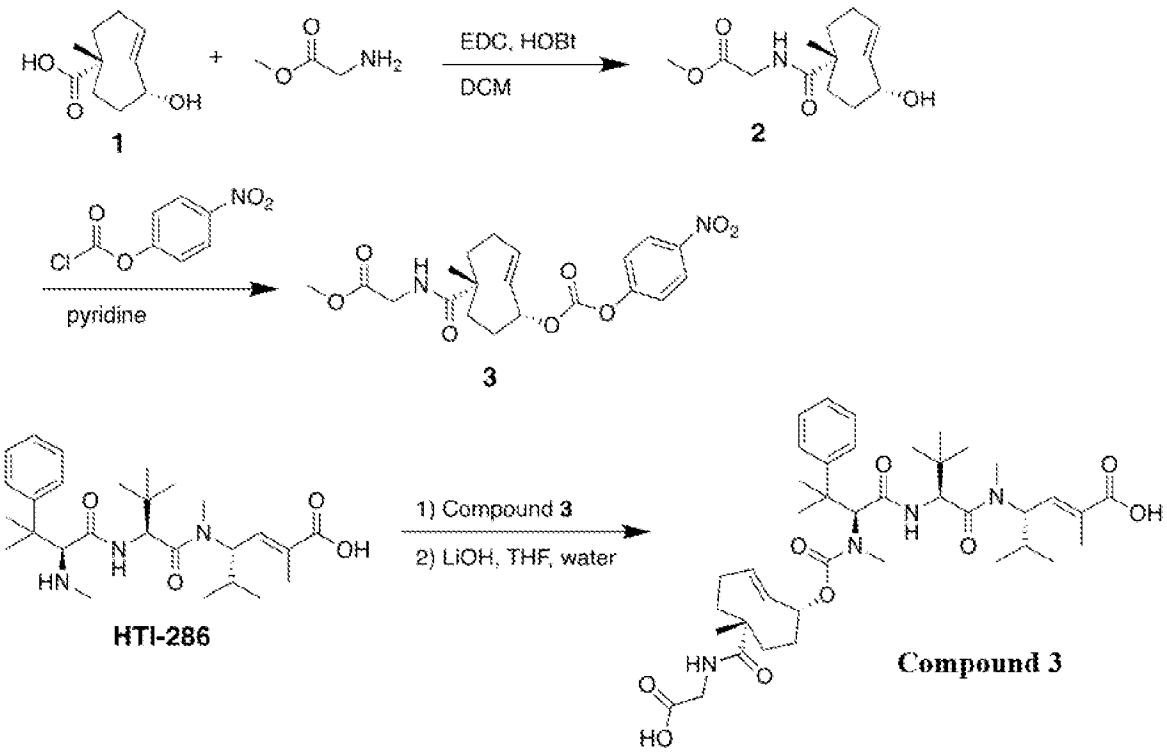
Example 2: Synthesis of TCO(gly)-niraparib (Compound 2)



[0334] (1R,6R,E)-6-((2-methoxy-2-oxoethyl)carbamoyl)-6-methylcyclooct-2-en-1-yl (S)-3-(4-(7-carbamoyl-2H-indazol-2-yl)phenyl)piperidine-1-carboxylateglycinate (2.1). Step-1: To a solution of compound-1.3 (39 mg, 0.09 mmol, 1.0 eq.), niraparib (30 mg, 0.09 mmol, 1.0 eq.), and HOBt (111 mg. 0.66, 2.0 eq) in DMF (1.0 mL) was added DIEA (12 mg, 0.19 mmol, 2.0 eq.) sequentially. The mixture was stirred at rt overnight and monitoring by LCMS. Upon the completion of the reaction, the mixture was loaded directly in C18 flash chromatography (12 g, ISCO) with a stepwise gradient of acetonitrile in water (0-100% for 12 min; product eluted out at ~70% acetonitrile in water). The fractions were collected and partially concentrated to remove most of the acetonitrile and take it to the next step directly without drying all the way. (+ESI)$[M+H]^+$=602.6.

[0335] ((1R,6R,E)-6-(((S)-3-(4-(7-carbamoyl-2H-indazol-2-yl)phenyl)piperidine-1-carbonyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycine (TCO(Gly)-Niraparib). Step 2: To compound 2.1 in water from the above step was diluted with MeOH (3.0 mL). To the mixture was added LiOH (9 mg, 0.37 mmol, 4 eq.). The mixture was stirred at rt for 1 h and monitoring by LCMS. Upon the completion of the reaction, the mixture was partially concentrated to remove most of the MeOH and acidified to pH 3 with HCl (1 N). The precipitate was collected and dried to afford 40 mg (73% over two steps) of ((1R,6R,E)-6-(((4-(((((2S)-1-(((2S)-1-(((4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)oxy)-1-methylcyclooct-4-ene-1-carbonyl)glycine as a white powder. (+esi)$[M+H]^+$= 588.7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.03 (s, 1H), 8.19 (d, J = 7.0 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.75-7.62 (m, 1H), 7.57 (s, 2H), 7.29 (t, J = 7.7 Hz, 1H), 5.92 (s, 1H), 5.75 (d, J = 16.7 Hz, 1H), 5.22 (s, 1H), 4.43 – 4.08 (m, 3H), 3.81 (d, J = 15.3 Hz, 2H), 3.23 – 2.79 (m, 4H), 2.33 (s, 2H), 2.25 – 1.80 (m, 6H), 1.80-1.53 (m, 2H), 1.18 (s, 3H).

Example 3: Gly-TCO-HTI-286 conjugate (Compound 3)



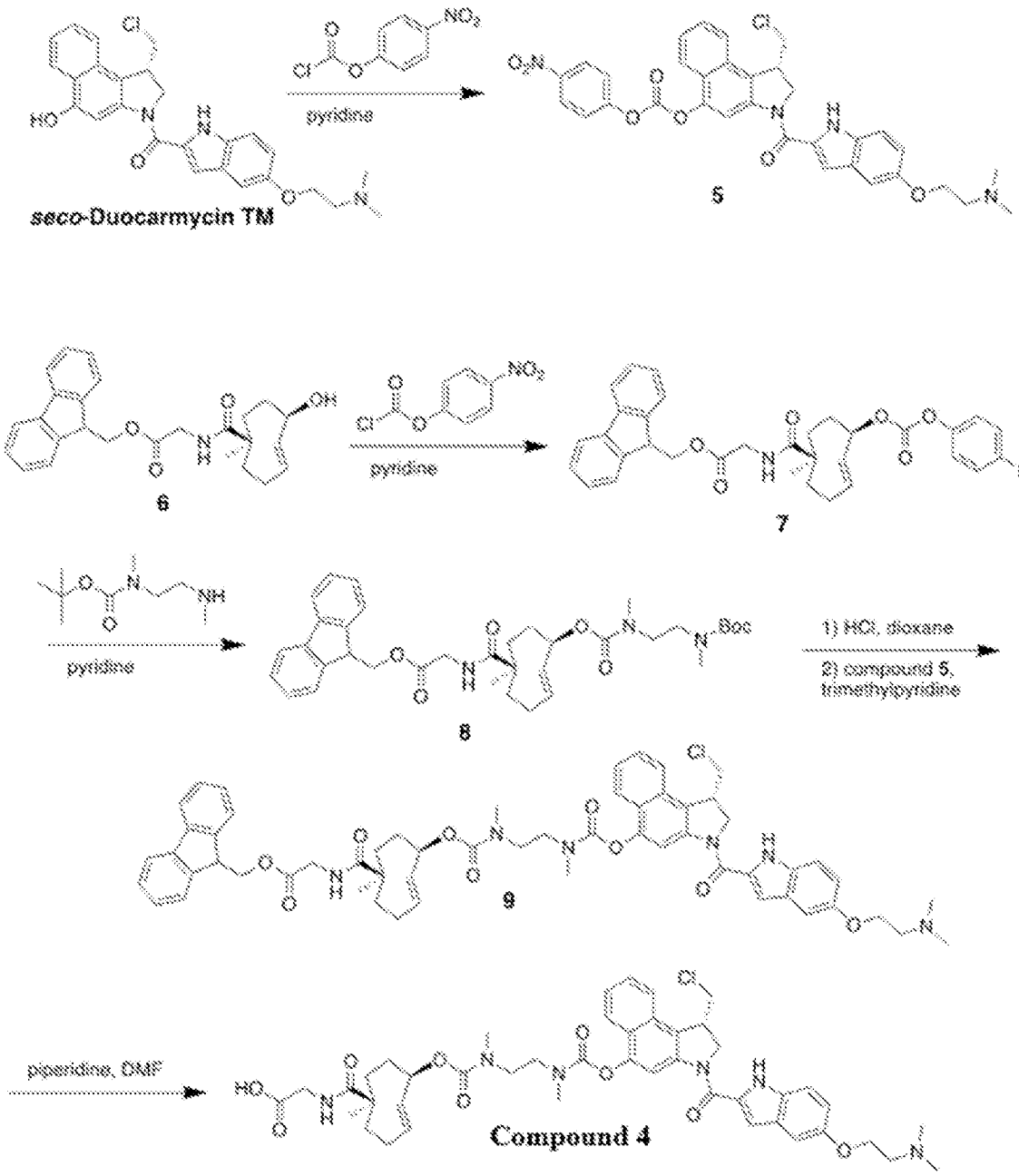
[0336] Gly(OMe)-TCO (**2**). A solution of trans-cyclooctene **1**, glycine methyl ester, DIPEA, and HOBt in DCM is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added EDC and the resulting mixture is stirred for 1 h at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is partitioned between ethyl acetate and citrate buffer (pH 4.5). The organic layer is then washed with citrate buffer (2x) followed by sodium bicarbonate (2x), and brine. The organic layer is dried over sodium sulfate, filtered, and the filtrate is concentrated under reduced pressure. The resulting residue is purified by flash chromatography to yield intermediate **2**.

[0337] Gly(OMe)-TCO-para-nitrophenyl carbonate (**3**). A solution of intermediate **2** and pyridine in DCM is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added para-nitrophenyl chloroformate and the resulting mixture is stirred at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is partitioned between ethyl acetate and citrate buffer (pH 4.5). The organic layer is then washed with citrate buffer (2x) followed by sodium bicarbonate (3x), and brine. The organic layer is dried over sodium sulfate, filtered, and the filtrate is concentrated under reduced pressure. The resulting residue is purified by flash chromatography to yield carbonate **3**.

[0338] Gly-TCO-HTI-286 (**4**). To a solution of **HTI-286** and pyridine in DMF in a round bottom flask fitted with a magnetic stir bar is added carbonate **3**. The reaction mixture is stirred at ambient temperature until the starting material is consumed as monitored by HPLC. To the reaction mixture is added lithium hydroxide (solution in water) and THF. The mixture is stirred for an additional period at ambient temperature. The reaction mixture is then concentrated under reduced pressure and the resulting

residue is purified by reverse phase chromatography (10-100% MeCN/water with 0.1% formic acid) to yield the desired product **Compound 3**.

Example 4: Gly-TCO-spacelink-DuoTM conjugate (Compound 4)



[0339] Seco-Duocarmycin TM – PNP carbonate (**5**). To a solution of seco-Duocarmycin TM in DCM is added pyridine and para-nitrophenylchloroformate. The reaction mixture is stirred at ambient temperature until the starting material is consumed, as monitored by HPLC. The reaction mixture is then partitioned between ethyl acetate and citrate buffer. The organic layer is washed with citrate buffer (2x), followed by sodium bicarbonate (3x) and brine. The organic layer is then dried over sodium sulfate, filtered, and the filtrate is concentrated under reduced pressure to yield carbonate **5**, which is used without further purification.

[0340] Gly(OFm)-TCO-para-nitrophenyl carbonate (**7**). A solution of alcohol **6** and pyridine in DCM is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added para-nitrophenyl chloroformate and the resulting mixture is stirred at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is then partitioned between ethyl acetate and buffer (pH 4.5). The organic layer is washed with citrate buffer (2x) followed by sodium bicarbonate (3x), and brine. The organic layer is dried over sodium sulfate, filtered, and the filtrate is concentrated under reduced pressure. The resulting residue is purified by flash chromatography to yield carbonate **7**.

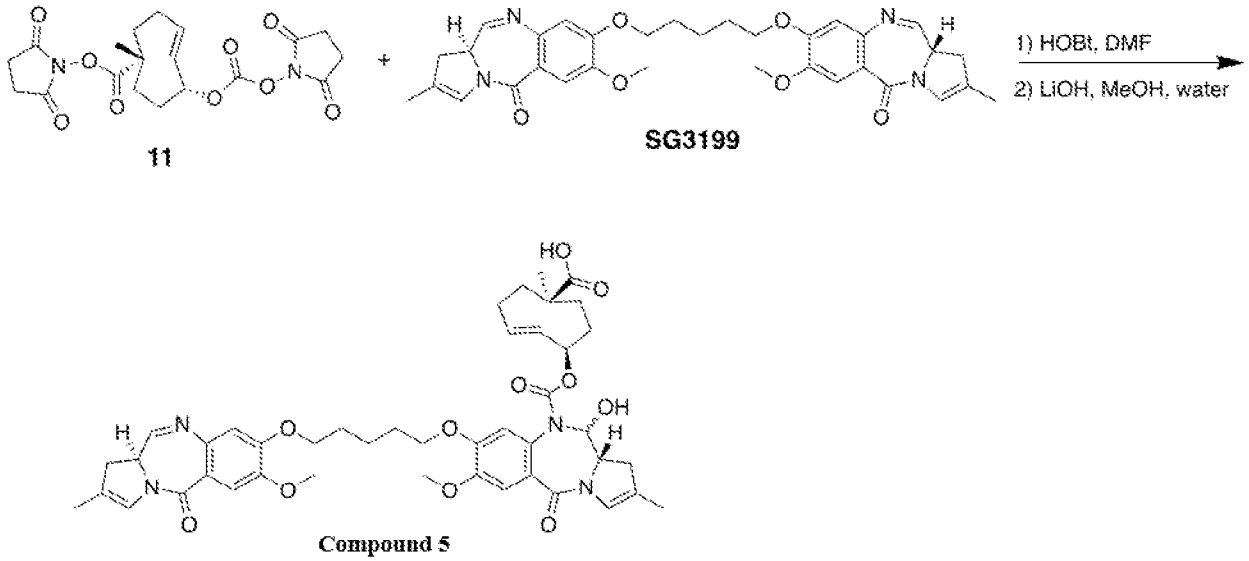
[0341] Gly(OFm)-TCO-N-Boc-spacelink carbamate (**8**). A solution of carbonate **7** and pyridine in DCM is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added N-Boc-N,N'-dimethyl-1,2-diaminoethane and the resulting mixture is stirred at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is then partitioned between ethyl acetate and citrate buffer (pH 4.5). The organic layer is washed with citrate buffer (2x) followed by sodium bicarbonate (3x), and brine. The organic layer is dried over sodium sulfate, filtered, and the filtrate is concentrated under reduced pressure. The resulting residue is purified by flash chromatography to yield carbamate **8**.

[0342] Gly(OFm)-TCO-spacelink-DuoTM (**9**). A solution of carbamate **8** in dioxane is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added HCl (4 M in dioxane) and the resulting mixture is stirred at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is then concentrated under reduced pressure and the resulting residue is used without further purification.

[0343] The residue is resuspended in DCM and carbonate **5** is added to the solution. To the mixture is added 2,6-lutidine and the resulting reaction mixture is stirred at ambient temperature until the carbonate is consumed. The reaction mixture is then concentrated under reduced pressure and the resulting residue is purified by flash chromatography to yield carbamate **9**.

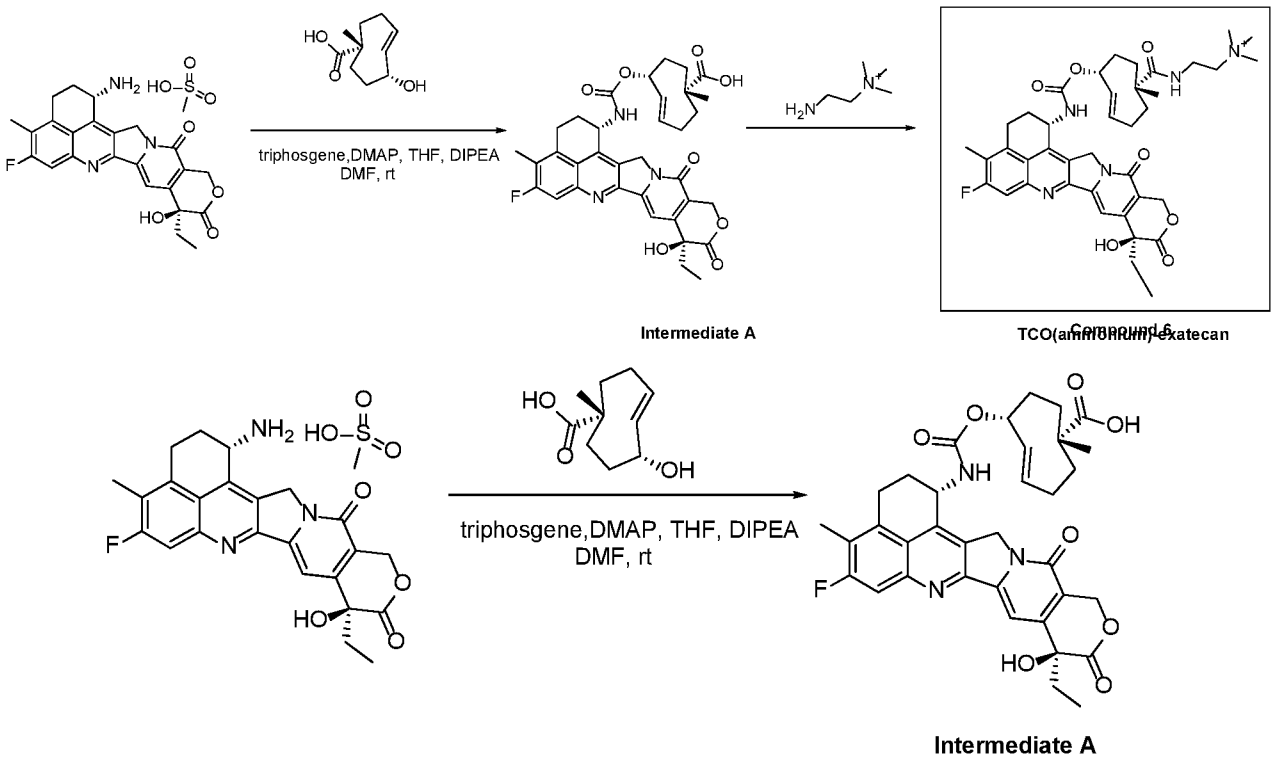
[0344] Gly-TCO-spacelink-DuoTM (**10**). A solution of carbamate **9** in DMF is charged to a round bottom flask fitted with a magnetic stir bar. To the solution is added piperidine and the resulting mixture is stirred at ambient temperature. The starting material is observed to be consumed by HPLC. The reaction mixture is then concentrated under reduced pressure and the resulting residue is purified by reverse phase chromatography (10-100% MeCN/water with 0.1% formic acid) to yield the desired product **Compound 4**.

Example 5: TCO-PBD conjugate (Compound 5)

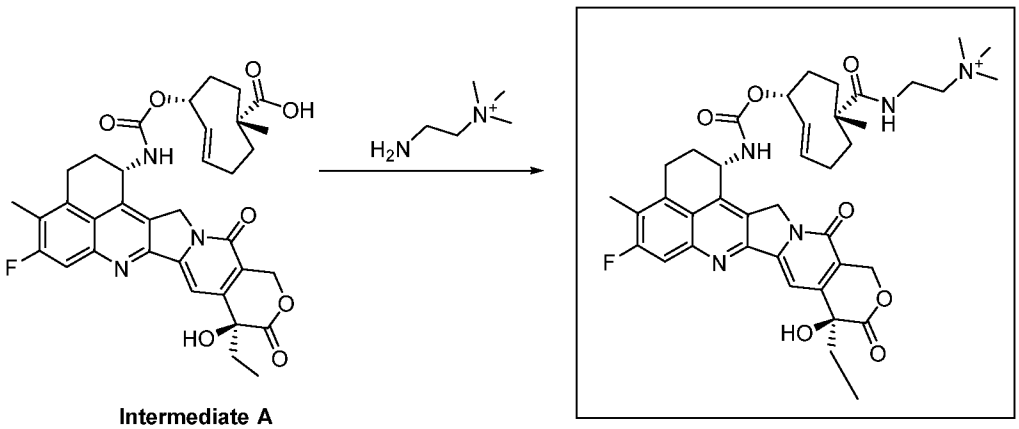


[0345] TCO-PBD (**12**). To a solution of **SG3199** in DMF is added bis-NHS-TCO **11** and HOBt. The reaction mixture is stirred at ambient temperature protected from light until the starting material is consumed. To the reaction mixture is added lithium hydroxide (solution in water) and methanol and the resulting solution is stirred for additional time at ambient temperature. The reaction mixture is then concentrated under reduced pressure and the resulting residue is purified by reverse phase chromatography (10-100% MeCN/water with 0.1% ammonium formate) to yield the desired product **Compound 5**.

Example 6: TCO(ammonium)-exatecan conjugate (Compound 6)



[0346] To a mixture of triphosgene (177 mg, 0.6 mmol) in THF (10 mL) was added (1R,6R,E)-6-hydroxy-1-methylcyclooct-4-ene-1-carboxylic acid (220 mg, 1.20 mmol) and DMAP (292 mg, 2.40 mmol). The mixture was stirred at room temperature for 30 min. The mixture was added to a mixture of (1S,9S)-1-amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl -1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione methanesulfonate (700 mg, 1.32 mmol) and DIPEA (510 mg, 3.96 mmol) in DMF (10 mL). The resulting mixture was stirred at room temperature for 12 hr. The mixture was concentrated and purified by Prep-HPLC (CH_3CN/H_2O(FA)) 0% to 70%) to give **Intermediate A** (110 mg, yield 14%). LCMS: (m/z, $C_{35}H_{36}FN_3O_8$) = 646.3 $[M+H]^+$

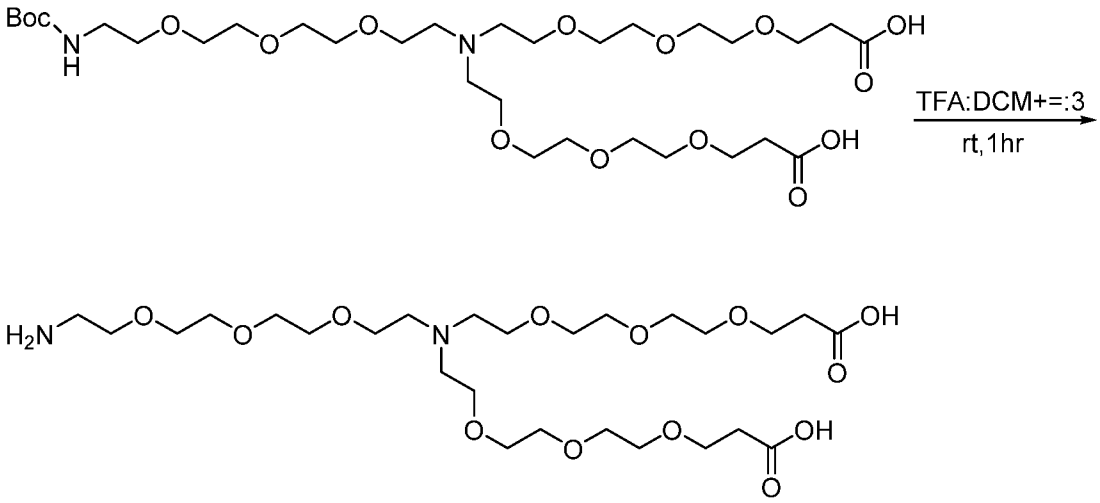


Intermediate A

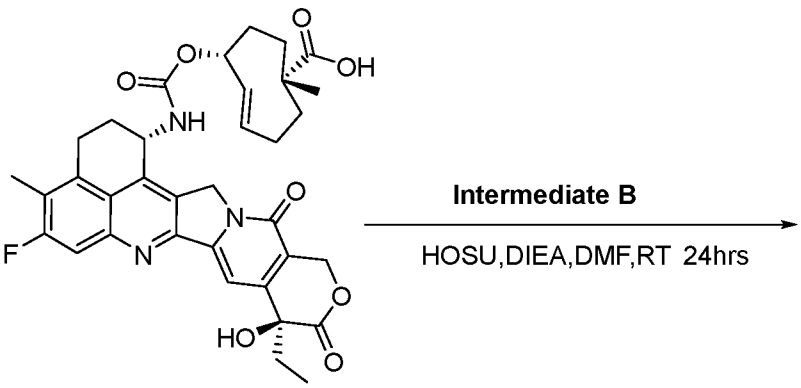
Compound 6
TCO(ammonium)-exatecan

[0347] To a solution of **Intermediate A** (65 mg, 100 μmol) and HOSU (18 mg, 150 μmol) in DMF (5 mL) was added DIEA (38 mg, 300 μmol). The mixture was stirred at room temperature for 30 min and then 2-amino-N,N,N-trimethylethan-1-aminium (21 mg, 110 μmol) was added. The mixture was stirred at 25 °C for additional 24 hr. The mixture was concentrated and purified by Prep-HPLC (CH_3CN/H_2O with 0.01% formic acid) 0% to 70%) to give **Compound 6** (21 mg, 29%).

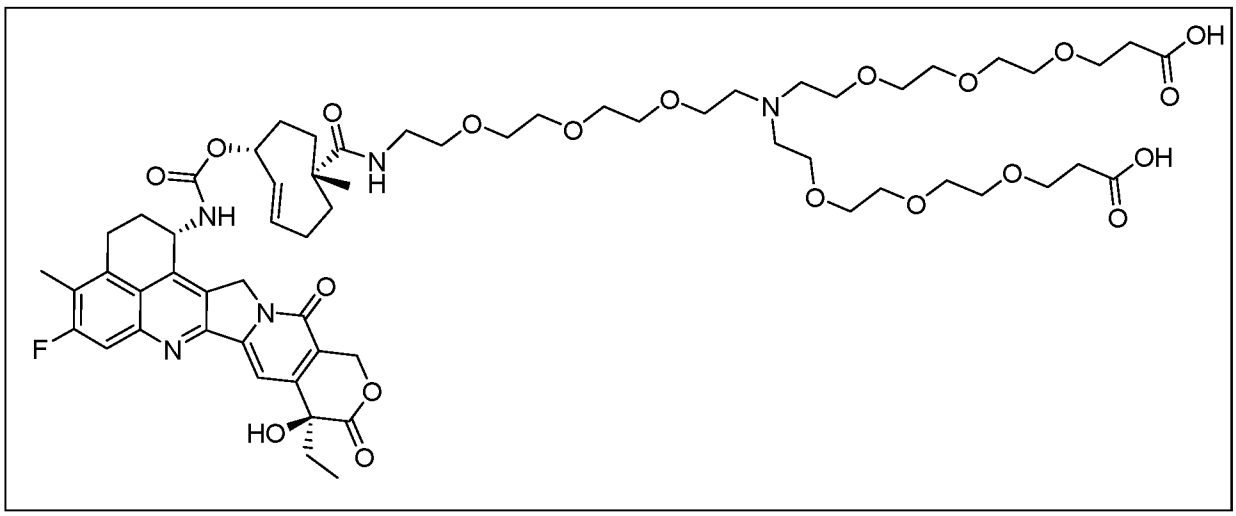
Example 7: TCO(PEG)-exatecan conjugate (Compound 7)



Intermediate B



Intermediate A

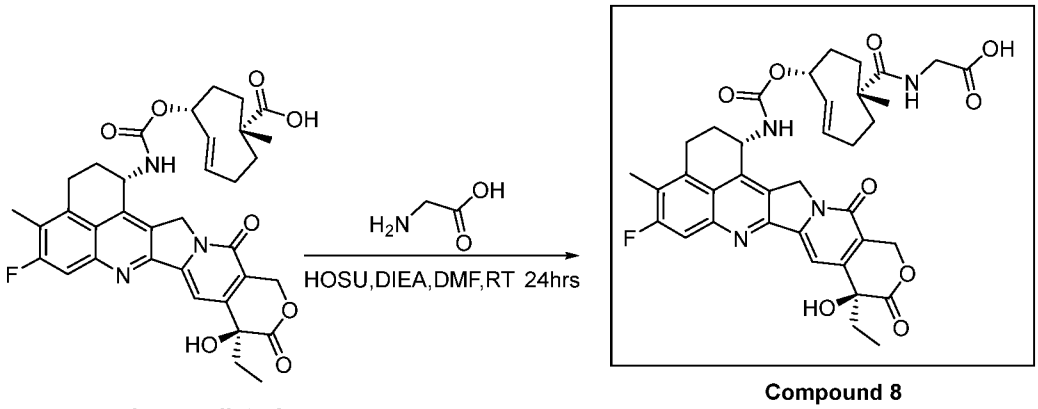


Compound 7
TCO(PEG)-exatecan

[0348] A solution of 13-(2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecan-16-yl) -4,7,10,16,19,22-hexaoxa-13-azapentacosanedioic acid (42 mg, 60 µmol) in TEA:DCM (1:5) (5 mL) was

stirred at 0 °C for 1 hr. The mixture was concentrated to give the crude **Intermediate B.** Then to a solution of intermediate A (65 mg, 100 μmol) and HOSU (18 mg, 150 μmol) in DMF (5 mL) was added DIEA (38 mg, 300 μmol). The mixture was stirred at 25 °C for 30 min. Then the above crude intermediate B (21 mg, 110 μmol) was added. The mixture was stirred at 25 °C for 24 hr. The mixture was concentrated and purified by Prep-HPLC (CH_3CN/H_2O with 0.05% TFA) 0% to 70%) to give **Compound 7** (20 mg, 27.2%).

Example 8: TCO(gly)-exatecan conjugate (Compound 8)

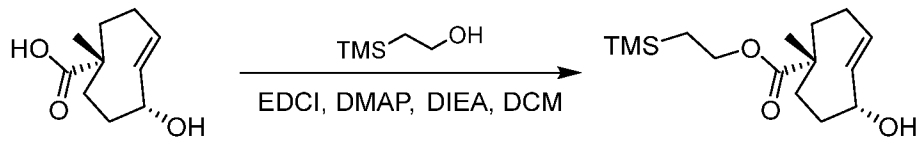


Intermediate A

Compound 8
TCO(gly)-exatecan

[0349] To a solution of Intermediate A (65 mg, 100 μmol) and HOSU (18 mg, 150 μmol) in DMF (5 mL) was added DIEA (38 mg, 300 μmol). The mixture was stirred at 25 °C for 30 min. Then glycine (16 mg, 200 μmol) and $NaHCO_3$ (17 mg, 200 μmol) was added. The mixture was stirred at 25 °C for 5 hr. The mixture was concentrated and purified by Prep-HPLC (CH_3CN/H_2O with 0.01% formic acid) 0% to 70%) to give **Compound 8** (32 mg, 45.2%).

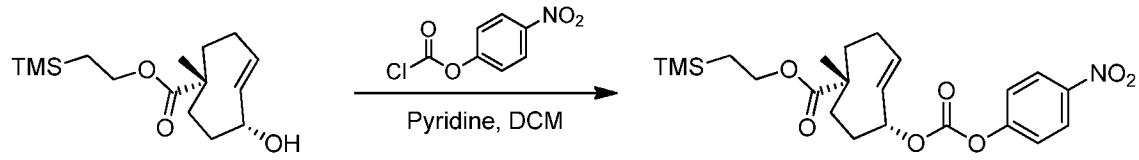
Example 9: TCO-PABC-spacelink-Etoposide (Compound 9)



1 2

[0350] To a solution of **1** (1.70 g, 9.23 mmol) in DCM (20.0 mL) was added 2-trimethylsilylethanol (1.64 g, 13.8 mmol) and DMAP (1.69 g, 13.8 mmol), EDCI (2.65 g, 13.8 mmol), DIEA (1.79 g, 13.8 mmol) at 0 °C. The mixture was stirred at 25 °C for 16 hrs. TLC (petroleum ether: ethyl acetate = 1 : 1, R_f = 0.4) indicated compound **1** was consumed completely. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 5/1). Intermediate **2** (1.80 g, 68.6% yield) was obtained.

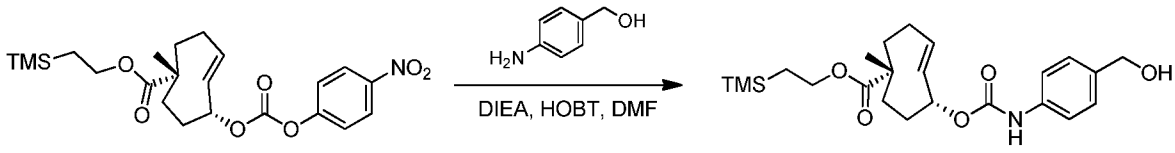
[0351] ^1H NMR: (400 MHz, $CDCl_3$) δ 6.15 - 6.00 (m, 1H), 5.63 (dd, J = 2.0, 16.4 Hz, 1H), 4.48 (s, 1H), 4.13 - 4.08 (m, 2H), 2.31 - 2.15 (m, 3H), 1.94 - 1.81 (m, 4H), 1.58 - 1.56 (m, 2H), 1.09 (s, 3H), 0.98 - 0.94 (m, 2H), 0.03 (s, 9H).



2 3

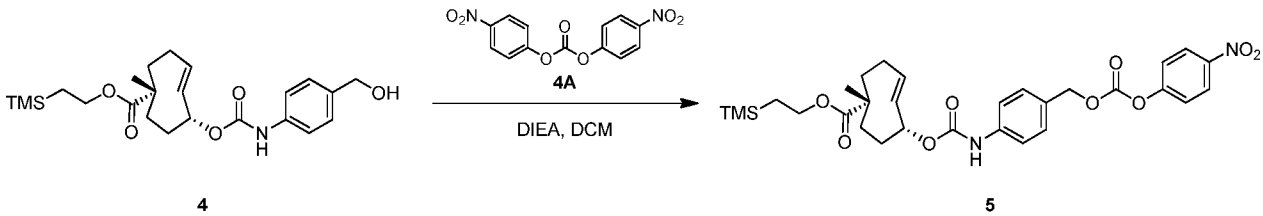
[0352] To a solution of intermediate **2** (1.80 g, 6.33 mmol) in DCM (40.0 mL) was added (4-nitrophenyl) carbonochloridate (5.10 g, 25.3 mmol) and pyridine (2.50 g, 31.6 mmol). The mixture was stirred at 25 °C for 12 hrs. TLC (petroleum ether : ethyl acetate = 3 : 1, R_f = 0.4) indicated intermediate **2** was consumed completely and a major new spot was formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 3/1). Intermediate **3** (1.80 g, 63.3% yield) was obtained.

[0353] ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.29 (dd, J = 9.2, 3.2 Hz, 2H), 7.40 (dd, J = 9.2, 2.8 Hz, 2H), 6.11- 6.03 (m, 1H), 5.64 (dd, J = 2.4, 16.4 Hz, 1H), 5.29 (s, 1H), 4.15 - 4.10 (m, 2H), 2.40 - 2.17 (m, 4H), 1.98 - 1.92 (m, 3H), 1.71 - 1.65 (m, 1H), 1.14 (s, 3H), 1.03 - 0.95 (m, 2H), 0.09 (s, 9H).



3 4

[0354] To a solution of (4-aminophenyl)methanol (2.47 g, 20.0 mmol) in DMF (15.0 mL) was added HOBt (811 mg, 6.01 mmol), DIEA (2.59 g, 20.0 mmol) and intermediate **3** (1.80 g, 4.00 mmol). The mixture was stirred at 20 °C for 12 hrs. TLC (petroleum ether : ethyl acetate = 3 : 1, R_f = 0.2) indicated intermediate **3** was consumed completely and one major new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with H_2O (100 mL) and extracted with EtOAc (30.0 mL * 4), the combined organic phase washed with brine (30.0 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 5/1 to 3/1). Intermediate **4** (1.60 g, 92.2% yield) was obtained.

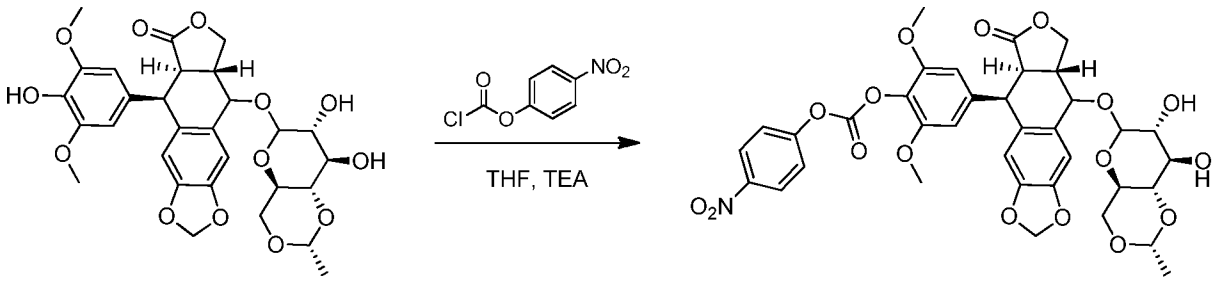


4 5

[0355] To a solution of intermediate **4** (1.40 g, 3.23 mmol) in DCM (20.0 mL) was added DIEA (1.25 g, 9.69 mmol) and **4A** (1.96 g, 6.46 mmol). The mixture was stirred at 25 °C for 2 hrs. LCMS (ES20203-16-P1C, product: RT = 1.214 mins) showed a main peak with desired mass was detected. The mixture

was dilued with DCM (100 mL), washed with 1 M HCl (30.0 mL * 2) and brine (20.0 mL * 2), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO_2, petroleum ether/ethyl acetate = 10/1 to 5/1). Intermediate **5** (1.40 g, 67.2% yield) was obtained.

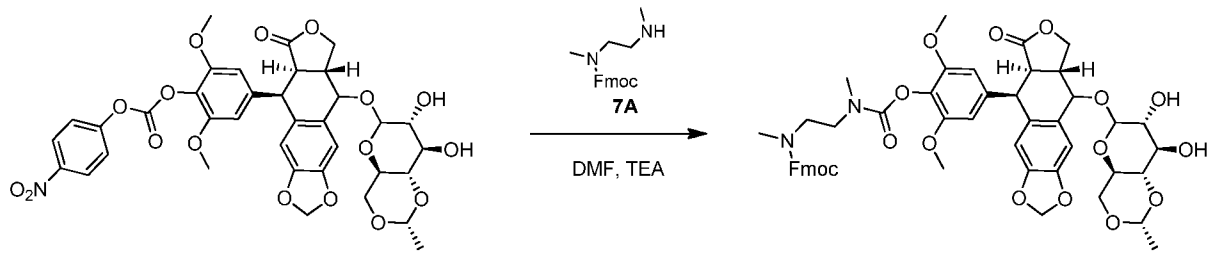
[0356] ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.30- 8.26 (m, 2H), 7.48 - 7.36 (m, 6H), 6.79 (s, 1H), 6.01 - 5.93 (m, 1H), 5.66 (dd, J = 2.8, 16.8Hz, 1H), 5.30 (s, 1H), 5.26 (s, 2H), 4.15 - 4.11 (m, 2H), 2.32 - 2.21 (m, 2H), 2.21 - 2.13 (m, 2H), 1.93 - 1.81 (m, 3H), 1.70 - 1.61 (m, 1H), 1.14 (s, 3H), 1.03 - 0.96 (m, 2H), 0.05 (s, 9H).



6 **7**

[0357] To a solution of intermediate **6** (2.00 g, 3.40 mmol) in THF (70.0 mL) was added TEA (5.16 g, 51.0 mmol) and (4-nitrophenyl) carbonochloridate (1.03 g, 5.10 mmol) THF (30.0 mL). The mixture was stirred at 25 °C for 2 hrs. LCMS (ES19514-19-p2a2, product: RT = 0.866 min) showed a main peak with desired mass was detected. The mixture was treated with 200 mL isopropyl ether and then the mixture was filtered and the filter cake was collected to give the product. The crude product was used into the next step without further purification. Intermediate **7** (2.00 g, crude) was obtained.

[0358] LCMS: $[M+H]^+$ 754.0

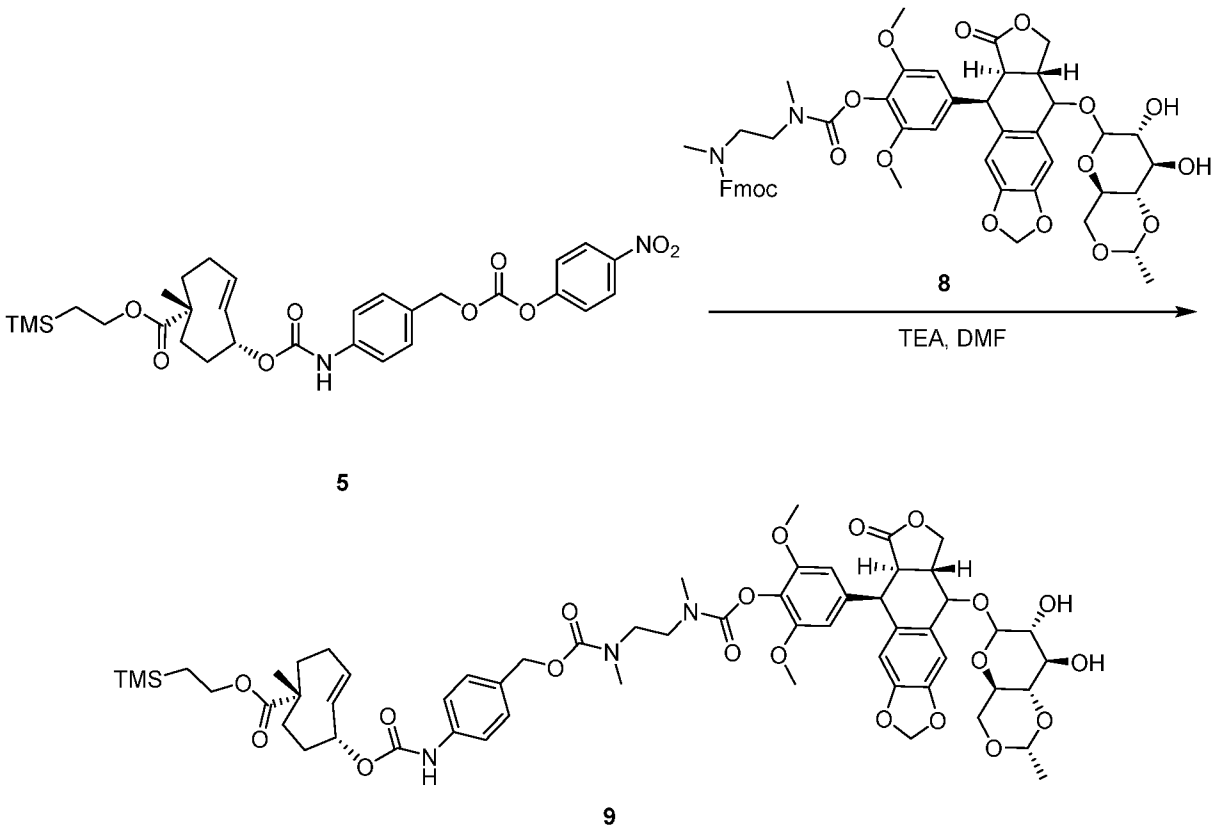


7 **8**

[0359] To a solution of intermediate **7** (1.00 g, 930 μmol) and **7A** (432 mg, 1.39 mmol) in DMF (10.0 mL) was added TEA (188 mg, 1.86 mmol). The mixture was stirred at 25 °C for 0.5 hr. LCMS (ES20203-20-P1B, product: RT = 1.005 mins) showed a main peak with desired mass was detected. The mixture was poured into water (100 mL), the solid separated out and then the mixture was filtered and the solid washed with water (20.0 mL * 2) and dissolved with EtOAc (60.0 mL) and the organic phase dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was

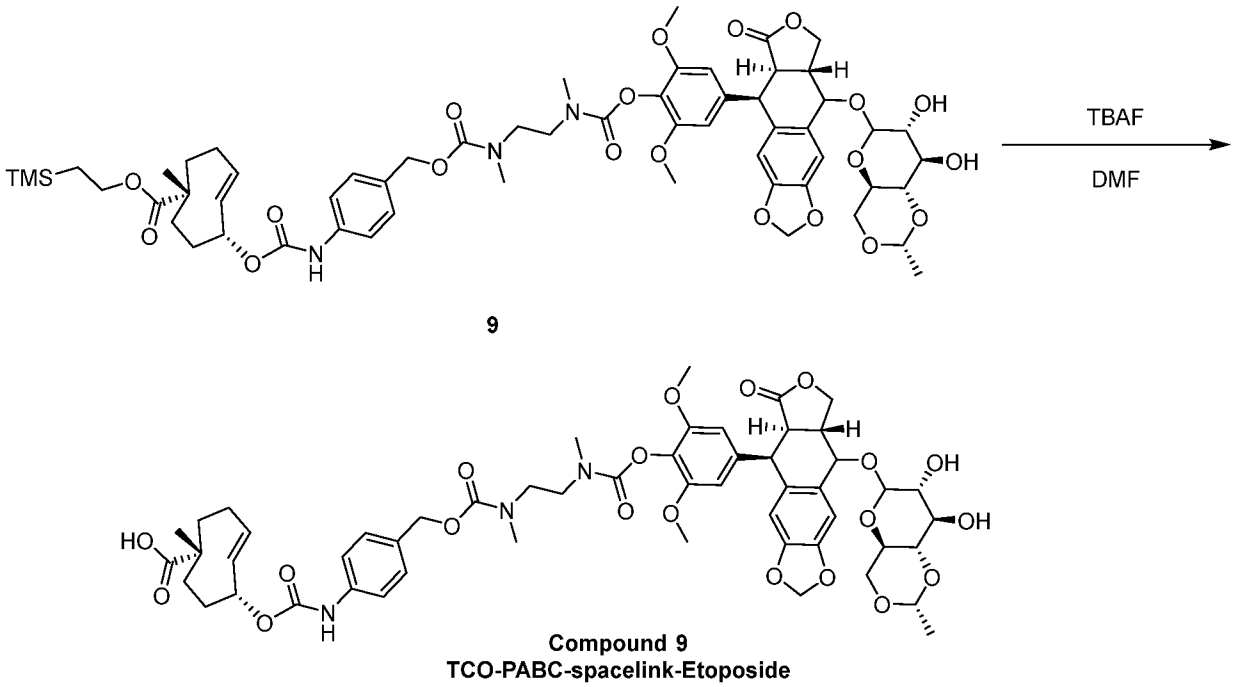
purified by silica gel column chromatography (SiO_2, petroleum ether/ethyl acetate = 20/1 to 5/1). Intermediate **8** (600 mg, 69.8% yield) was obtained.

[0360] LCMS: $[M+H]^+$ 925.3



[0361] A mixture of intermediate **5** (170 mg, 280 μmol), intermediate **8** (300 mg, 260 μmol) in DMF (5.0 mL) was added TEA (363 mg, 3.59 mmol) at 20 °C. The mixture stirred at 20 °C for 12 hrs. LCMS (ES20203-21-P1A, product: RT = 1.093 mins) showed a main peak with desired mass was detected. The mixture was filtered and the filtrate was purified by prep-HPLC (Welch XB-C18 7 μm 110 Å 250*50 mm; mobile phase: [water (0.01 mol/L NH_3HCO_3 in H_2O)-ACN]; B%: 55%-75%, Retention time: 37 min, flow rate 60 mL/min). Intermediate **9** (180 mg, 59.7% yield) was obtained.

[0362] Note: Fmoc group of intermediate **8** was removed in situ and the resulting amine reacted with the nitrophenyl carbonate in one pot.

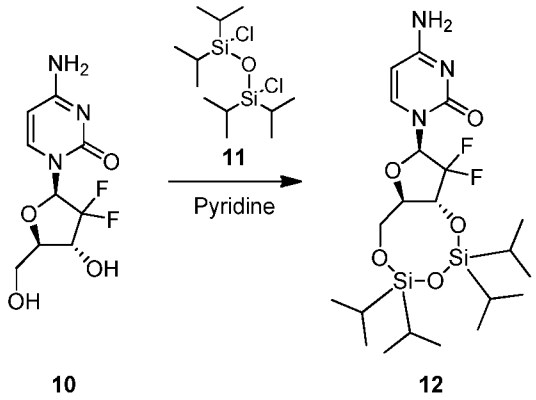


[0363] To a mixture of intermediate **9** (300 mg, 258 μmol) in DMF (10.0 mL) was added TBAF (1 M, 1.29 mmol) at 20 °C. The mixture stirred at 20 °C for 3 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and purified by prep-HPLC (Welch XB-C18 7 μm 110 Å 250*50 mm; mobile phase: [water(0.1% TFA in H_2O)-ACN]; B%: 38%-58%, Retention time: 20 min, 20 mL/min). **Compound 9** (120 mg, 41.8% yield) was obtained

[0364] LCMS: $[M+Na]^+$ 1084.3

[0365] ^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.80 - 9.61 (m, 1H), 7.52 - 7.38 (m, 2H), 7.34 - 7.19 (m, 3H), 6.74 (s, 1H), 6.55 (s, 2H), 6.00 (d, J = 5.6 Hz, 2H), 5.95 - 5.84 (m, 1H), 5.75 - 5.65 (m, 1H), 5.15 (s, 1H), 5.00 (s, 2H), 4.75 - 4.66 (m, 2H), 4.43 (d, J = 3.2 Hz, 1H), 4.38 (d, J = 7.2 Hz, 1H), 4.29 (t, J = 8.8 Hz, 1H), 4.14 (dd, J = 3.2, 9.6 Hz, 1H), 3.94 (d, J = 2.4 Hz, 1H), 3.77 - 3.73 (m, 1H), 3.68 (d, J = 4.4 Hz, 5H), 3.33 (s, 2H), 3.25 (s, 6H), 2.80 (s, 6H), 2.49 - 2.40 (m, 2H), 2.26 - 2.16 (m, 2H), 2.07 - 1.93 (m, 2H), 1.90 - 1.78 (m, 3H), 1.63 - 1.54 (m, 2H), 1.34 - 1.28 (m, 1H), 1.22 (d, J = 5.2 Hz, 3H), 1.02 (s, 3H), 0.94 (t, J = 7.2 Hz, 2H).

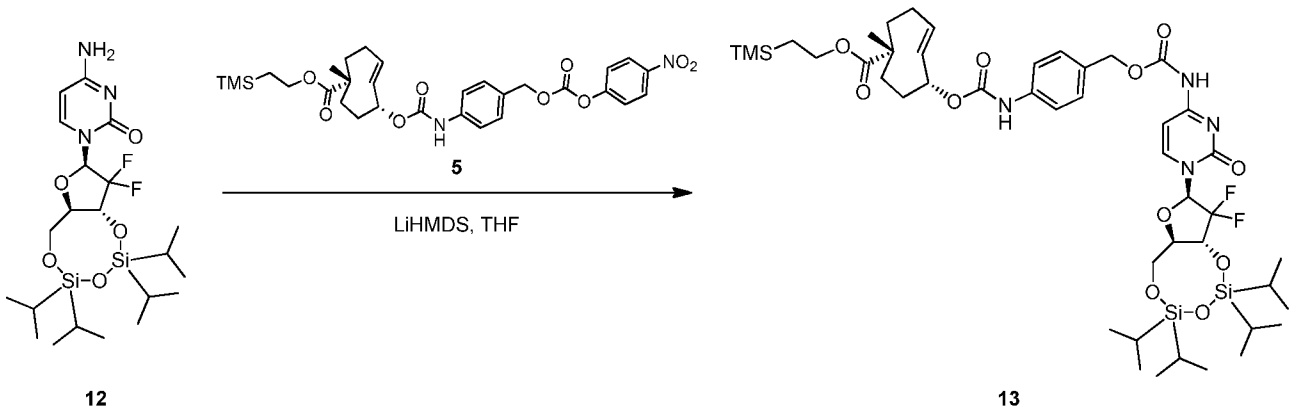
Example 10: TCO-PABC-Gemcitabine (Compound 10)



10 12

[0366] To a solution of intermediate **10** (500 mg, 1.90 mmol) in pyridine (30.0 mL) was added compound **11** (629 mg, 1.99 mmol) at 0 °C. The mixture was stirred at 25 °C for 12 hrs. LCMS indicated compound **10** was consumed completely and a main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove the solvent. The residue was purified by column chromatography (SiO_2, DCM/MeOH = 20/1). Intermediate **12** (800 mg, 83.3% yield) was obtained.

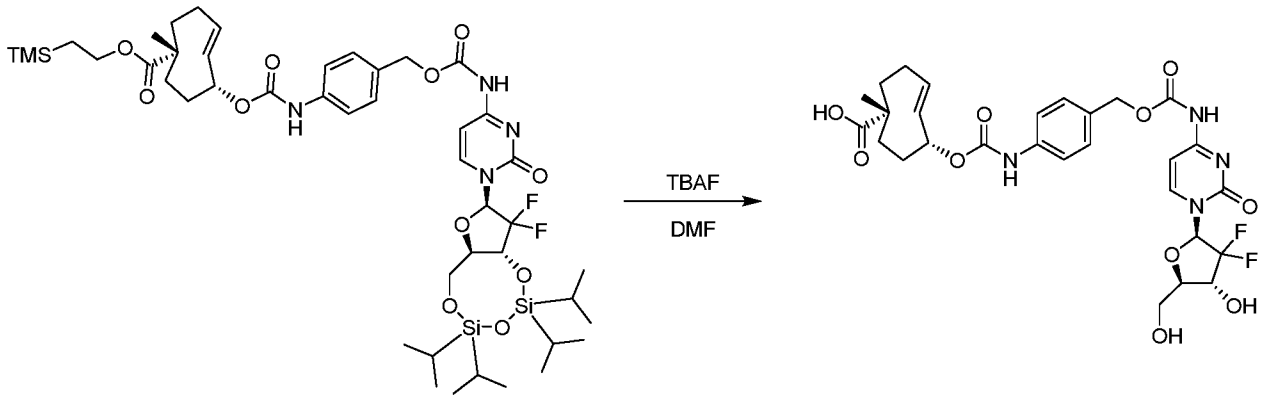
[0367] LCMS: $[M+H]^+$ 506.1



12 13

[0368] To a mixture of intermediate **12** (304 mg, 600 μmol) and compound **5** (300 mg, 501 μmol) in THF (20.0 mL) was added LiHMDS (1 M, 1.50 mmol) at -30 °C. The mixture stirred at -30 °C for 0.5 hr. And then a solution of compound **5** (300 mg, 501 μmol) in THF (20.0 mL) was added dropwise to the reaction and stirred at -30 °C for 1 hr. LCMS showed a main peak with desired mass was detected. The mixture was quenched by addition saturated ammonium chloride solution (100 mL) and diluted with H_2O (100 mL), the mixture was extracted with EtOAc (40.0 mL * 3), the combined organic phase washed with brine (20.0 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO_2, petroleum ether/ethyl acetate = 20/1 to 5/1). Intermediate **13** (260 mg, 53.8 % yield) was obtained.

[0369] LCMS: $[M+Na]^+$ 987.3



13

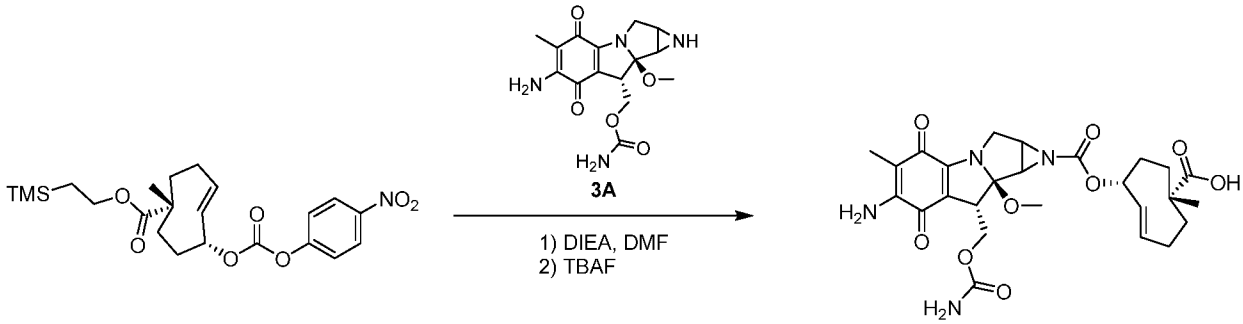
Compound 10
TCO-PABC-gemcitabine

[0370] To a mixture of intermediate **13** (260 mg, 270 µmol) in DMF (10.0 mL) was added TBAF (1 M, 2.15 mmol) at 20 °C. The mixture stirred at 20 °C for 2 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Welch Xtimate C18 100*40 mm*3 µm; mobile phase: [water(TFA)-ACN]; B%: 26%-66%,8min). **Compound 10** (105 mg, 62.5% yield) was obtained

[0371] LCMS: $[M+H]^+$ 623.2

[0372] HNMR: (400 MHz, DMSO-d_6) δ 12.63 - 11.69 (m, 1H), 11.19 - 10.72 (m, 1H), 9.91 - 9.66 (m, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 7.6 Hz, 1H), 6.44 - 6.24 (m, 1H), 6.20 - 6.10 (m, 1H), 5.99 - 5.82 (m, 1H), 5.76 - 5.65 (m, 1H), 5.16 (s, 1H), 5.12 (s, 2H), 4.29 - 4.10 (m, 1H), 3.92 - 3.86 (m, 1H), 3.81 (d, J = 13.2 Hz, 1H), 3.66 (dd, J = 3.6, 12.4 Hz, 1H), 2.22 (d, J = 6.0 Hz, 2H), 2.09 - 1.78 (m, 6H), 1.65 - 1.57 (m, 1H), 1.03 (s, 3H).

Example 11: TCO-Mitomycin C (Compound 11)



3

Compound 11
TCO-mitomycin C

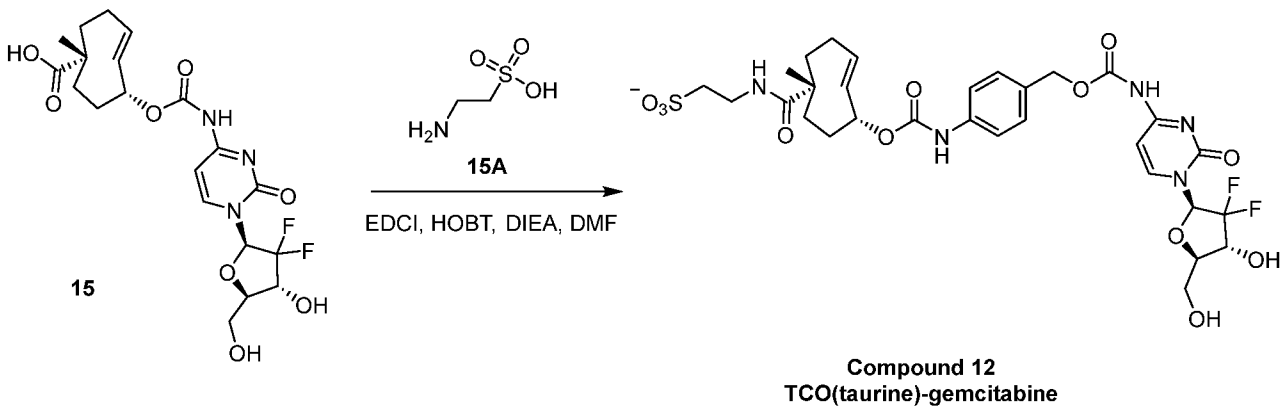
[0373] To a mixture of intermediate **3A** (350 mg, 1.05 mmol), DIEA (345 mg, 2.67 mmol) and DMAP (109 mg, 890 µmol) in DMF (5.0 mL) was added a solution of intermediate **3** (400 mg, 890 µmol) in DMF (5.0 mL) at 0 °C. The mixture stirred at 20 °C for 12 hrs. Then to the reaction mixture was added TBAF (1 M, 4.45 mmol) at 0 °C and stirred at 20 °C for 2 hrs. LCMS showed a main peak with desired mass was detected. The reaction mixture was diluted with ice water (100 mL), then extracted with DCM

(100 mL * 4) and organic layers were dried over filtered and concentrated under reduced pressure to give a residue. The aqueous phase was quenched by addition 1 M HCl 100 mL at 0 °C and NaClO solution 100 mL at 0 °C. The residue was purified by prep-HPLC (Welch XB-C18 7 µm 110 Å 250*50 mm; mobile phase: [water(0.01 mol/L NH_3HCO_3 in H_2O)-ACN]; B%: 10-30%-40 min. Retention time: 20 min, 20 mL/min). **Compound 11** (102 mg, 20.8% yield) was obtained.

[0374] LCMS: $[M+H]^+$ 545.2

[0375] ^{1}HNMR: (400 MHz, DMSO-d_6) δ 7.25 - 6.97 (m, 2H), 6.67 - 6.31 (m, 2H), 5.99 - 5.79 (m, 1H), 5.65 - 5.53 (m, 1H), 5.10 - 5.04 (m, 1H), 5.02 - 4.95 (m, 1H), 4.35 - 4.28 (m, 1H), 4.04 - 3.95 (m, 1H), 3.68 (d, J = 4.8 Hz, 1H), 3.53 (d, J = 6.4 Hz, 2H), 3.14 (s, 3H), 2.58 - 2.56 (m, 1H), 2.24 - 2.01 (m, 4H), 1.88 - 1.72 (m, 3H), 1.68 (s, 3H), 1.60 - 1.52 (m, 1H), 0.97 (s, 3H).

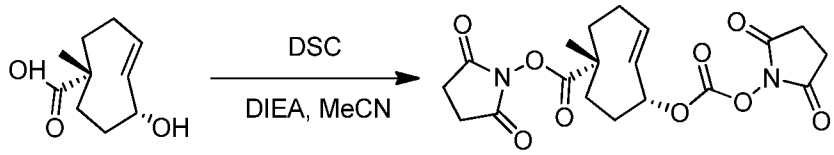
Example 12: TCO-(taurine)-Gemcitabine (Compound 12)



[0376] To a mixture of intermediate **15** (200 mg, 422 µmol) and intermediate **15A** (106 mg, 845 µmol) in DMF (2.0 mL) was added EDCI (162 mg, 845 µmol), HOBt (114 mg, 845 µmol) and DIEA (164 mg, 1.27 mmol) at 20 °C. The mixture stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Welch Xtimate C18 100*40 mm*3 µm; mobile phase: [water (TFA)-ACN]; B%: 10%-40%, 8 min). **Compound 12** (102 mg, 39.8% yield) was obtained.

[0377] ^{1}HNMR: (400 MHz, DMSO-d_6) δ 8.23 (d, J = 7.6 Hz, 1H), 7.51 (s, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.17 (t, J = 7.6 Hz, 1H), 5.99 - 5.86 (m, 1H), 5.68 (d, J = 16.4 Hz, 1H), 5.16 (s, 1H), 4.27 - 4.12 (m, 1H), 3.88 (d, J = 8.4 Hz, 1H), 3.82 (s, 1H), 3.66 (s, 3H), 3.30 - 3.26 (m, 2H), 2.55 (s, 1H), 2.26 - 2.14 (m, 2H), 2.11 - 1.79 (m, 5H), 1.66 (d, J = 12.8 Hz, 1H), 1.51 - 1.38 (m, 1H), 1.31 - 1.12 (m, 1H), 1.00 (s, 3H).

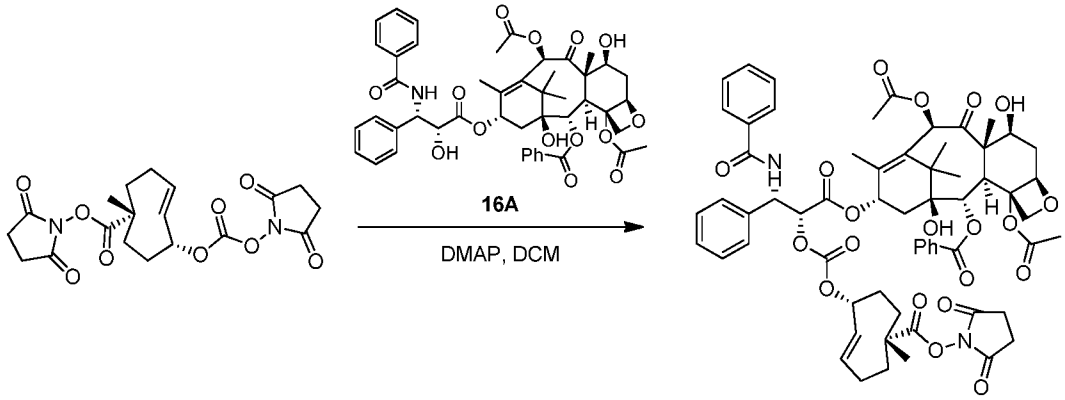
Example 13: TCO-(taurine)-Ptx (Compound 13)



1 16

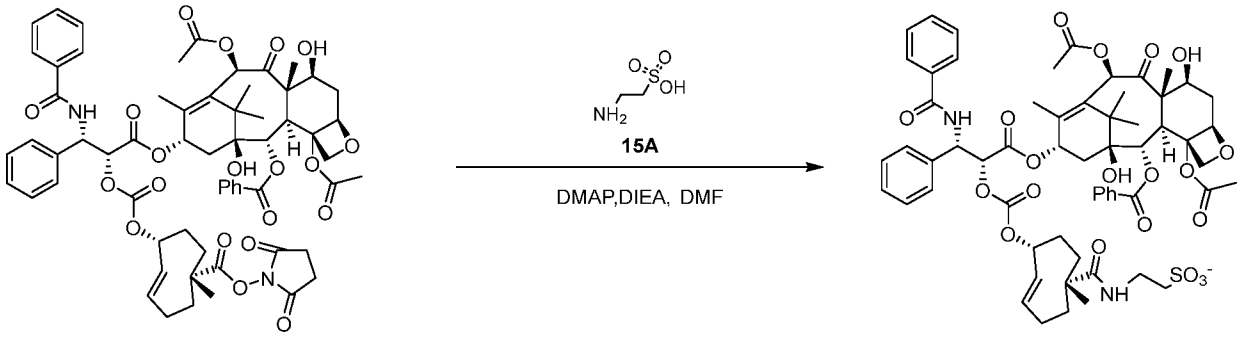
[0378] DIEA (491 mg, 3.80 mmol) was added to a stirred suspension of compound **1** (100 mg, 540 µmol) and DSC (598 mg, 2.33 mmol) in MeCN (2.0 mL) and the mixture was stirred at 20 °C for 12 hrs. TLC (petroleum ether : ethyl acetate = 1 : 1, R_f = 0.3) showed a major new spot was formed. The mixture was poured into water (50.0 mL), extracted with EA (30.0 mL * 3), the combined organic phase washed with brine (30.0 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1 to 1/1). Intermediate **16** (130 mg, 56.7% yield) was obtained.

[0379] ^{1}HNMR: (400 MHz, $CDCl_3$) δ 6.14 - 6.01 (m, 1H), 5.63 (dd, J = 2.4, 16.8 Hz, 1H), 5.29 (s, 1H), 2.87 - 2.81 (m, 8H), 2.51 - 2.26 (m, 4H), 2.20 - 1.94 (m, 4H), 1.28 (s, 3H).



16 17

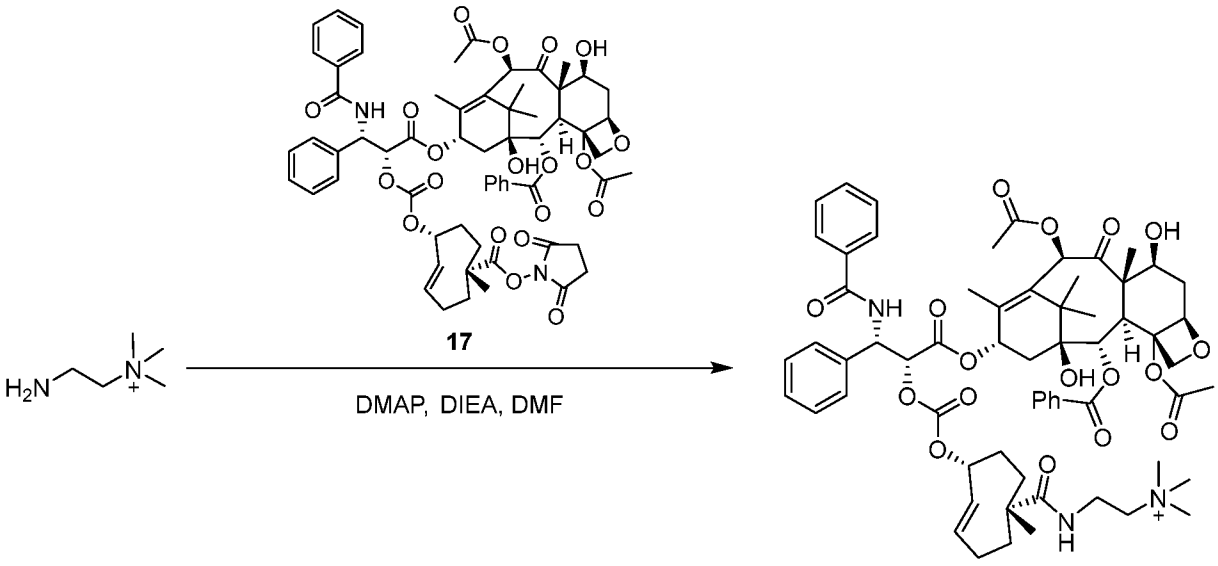
[0380] To a mixture of intermediate **16A** (106 mg, 124 µmol) and DMAP (34.0 mg, 275 µmol) in DCM (2.0 mL) was added intermediate **16** (58.0 mg, 137 µmol). The mixture stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was concentrated under reduced pressure. The residue was purified by prep-TLC (DCM/MeOH = 10/1, R_f = 0.4). Intermediate **17** (130 mg, 79.5% yield) was obtained. LCMS: $[M\text{-}CO_2\text{-}OSu]^{\bullet+}$ 1020.9.



17 Compound 13 TCO-taurine-Ptx

[0381] A mixture of intermediate **17** (50.0 mg, 43.1 µmol), intermediate **15A** (16.2 mg, 129 µmol), DMAP (10.5 mg, 86.1 µmol) and DIEA (33.4 mg, 258 µmol) in DMF (5.0 mL) was stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water(TFA)-ACN]; B%: 40%-70%, 10 min) and further purified by prep-HPLC (column: YMC-Actus Triart C18, 250*30 mm, 5 um, 120 Å; mobile phase: [water-ACN]; B%: 20-70% -40 min. Retention time: 22 min, 20 ml/min). **Compound 13** (6.80 mg, 12.0% yield) was obtained. LCMS: $[M+H]^+$ 1171.2

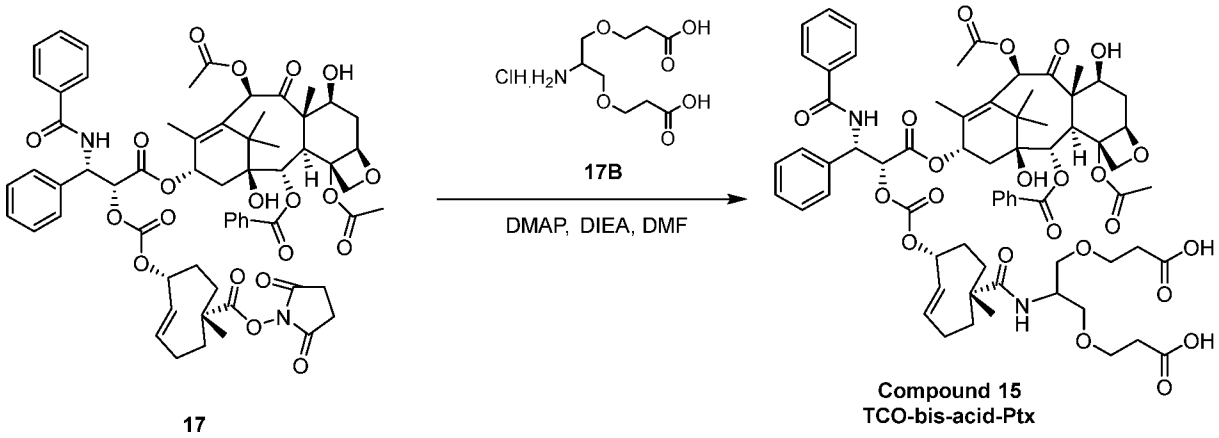
Example 14: TCO-ammonium-Ptx (Compound 14)



20 Compound 14 TCO-ammonium-Ptx

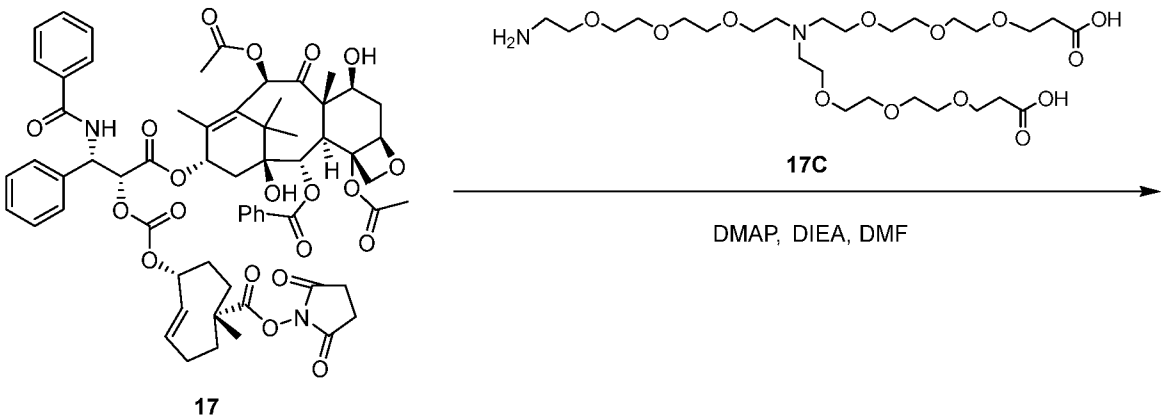
[0382] A mixture of intermediate **17** (50.0 mg, 43.1 µmol), intermediate **20** (18.0 mg, 129 µmol, HCl), DIEA (33.4 mg, 258 µmol) and DMAP (10.5 mg, 86.1 mmol) in DMF (1.0 mL) stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Welch Xtimate C18 150*25 mm*5 µm; mobile phase: [water (TFA)-ACN]; B%: 33%-63%, Retention time: 11 min). **Compound 14** (24.0 mg, 45.3% yield) was obtained. LCMS: $[M]^+$ 1148.4

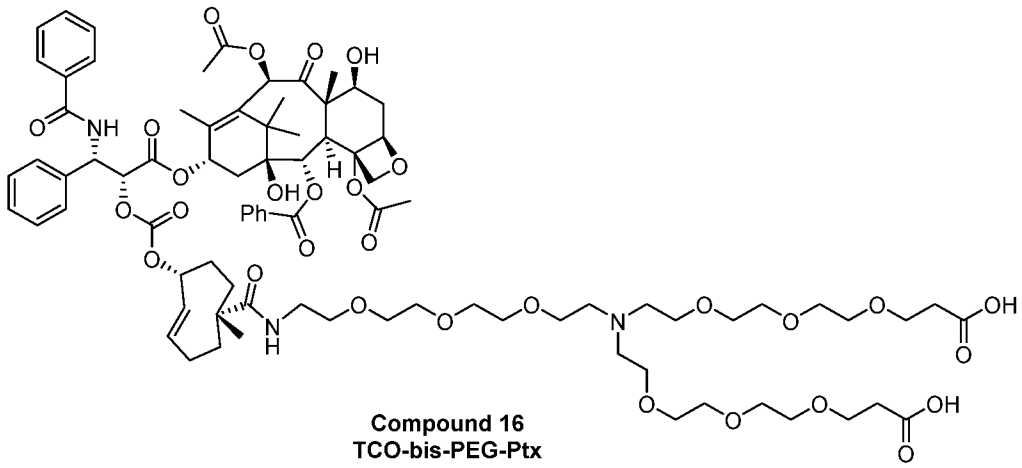
Example 15: TCO-bis-acid-Ptx (Compound 15)



[0383] A mixture of intermediate **17** (40.0 mg, 34.5 µmol), intermediate **17B** (37.4 mg, 138 µmol, HCl), DMAP (8.42 mg, 68.9 µmol) and DIEA (26.7 mg, 207 µmol) in DMF (1.0 mL) stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Welch Xtimate C18 100*40 mm*3 µm; mobile phase: [water (TFA)-ACN]; B%: 32%-62%, 8 min) and then the mixture was adjusted the pH=8~9 with $NH_3.H_2O$ before lyophilization. **Compound 15** (14 mg, 30.6% yield). LCMS: $[M+H]^+$ 1282.4

Example 16: TCO-bis-PEG-Ptx (Compound 16)

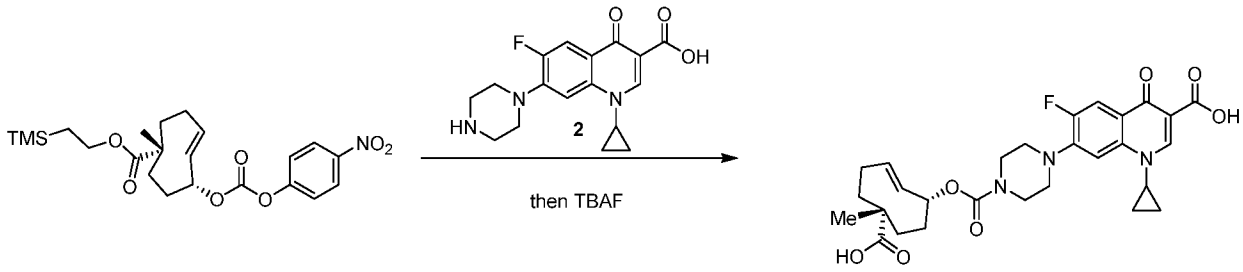




[0384] A mixture of intermediate **17** (40.0 mg, 34.5 µmol), intermediate **17C** (41.4 mg, 68.9 µmol), DMAP (8.42 mg, 68.9 µmol) and DIEA (4.45 mg, 34.5 µmol) in DMF (5.0 mL) stirred at 20 °C for 12 hrs. LCMS showed a main peak with desired mass was detected. The mixture was filtered and concentrated under reduced pressure. The mixture was purified by prep-HPLC (column: YMC-Actus Triart C18 150*30 mm*5 µm; mobile phase: [water(TFA)-ACN]; B%: 43%-63%, 10.5 min) and further purified by prep-HPLC (column: YMC-Actus Triart C18, 250*10 mm, 5 µm, 120 Å; mobile phase: [water-ACN]; B%: 30-60%-50 min. Retention time: 30min, 2 mL/min). **Compound 16** (10.8 mg, 14.3% yield) was obtained.

[0385] LCMS: $[M+H]^+$ 1282.4

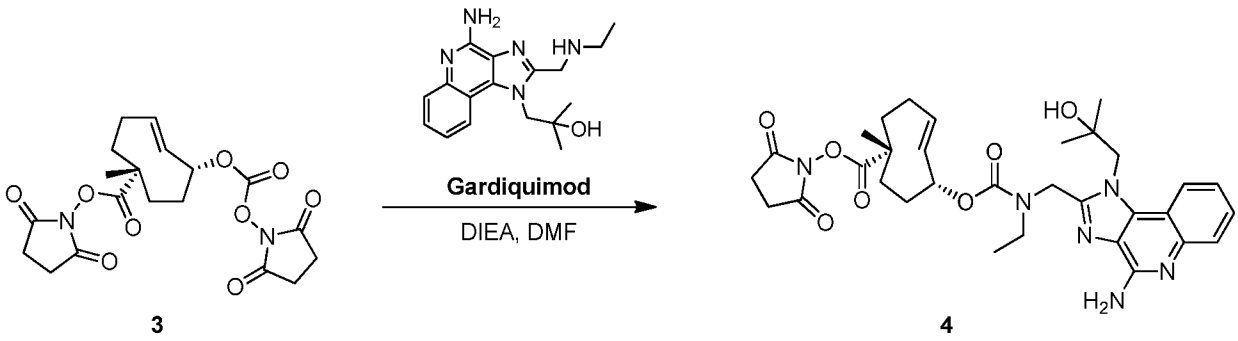
Example 17: TCO-Cipro (Compound 17)



[0386] To a solution of intermediate **2** (ciprofloxacin, 180 mg, 489 µmol) in DMF (0.3 mL) was added DIEA (172 mg, 232 µL) and the intermediate **1** (200 mg, 445 µmol) in DMF (0.6 mL), the mixture was stirred at 25 °C for 12 hrs , then HOBt (120 mg, 890 µmol) was in DMF (0.3 mL) was added to the solution stirred at 25 °C for 2 hrs, then TBAF (1 M, 3.5 mL) was added to the solution, the mixture was stirred at 25 °C for 10 hrs. LCMS showed the reactant was consumed and one main peak was desired product. The residue was purified by prep-HPLC (TFA condition) to give **Compound 17** (106 mg, 44.0% yield).

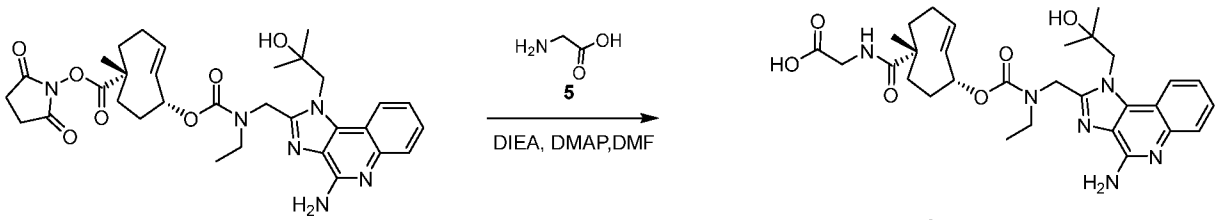
[0387] 1HNMR: (400 MHz, DMSO-d_6) δ ppm 15.20 (br s, 1 H), 12.02 (br s, 1 H), 8.68 (s, 1 H), 7.95 (d, J = 13.05 Hz, 1 H), 7.61 (d, J = 7.53 Hz, 1 H), 5.78-5.90 (m, 1 H), 5.66-5.74 (m, 1 H), 5.16 (br s, 1 H), 3.73-3.87 (m, 2 H), 3.60 (br s, 3 H), 3.36-3.40 (m, 4 H), 2.14-2.25 (m, 2 H), 2.03-2.12 (m, 1 H), 1.90-1.97 (m, 1 H), 1.72-1.88 (m, 3 H), 1.57-1.64 (m, 1 H), 1.31-1.36 (m, 2 H), 1.19 (br s, 2 H), 1.02 (s, 3 H). LCMS: $[M+H]^+$ 542.1

Example 18: TCO-TLR7/8a (Compound 18)



3 4

[0388] To a solution of Gardiquimod (100 mg, 319 μmol) in DMF (1 mL) was added DIEA (82.5 mg, 638 μmol) and intermediate **3** (202 mg, 479 μmol). The mixture was stirred at 25 °C for 4 hrs. LC-MS showed Gardiquimod was consumed completely and one main peak with desired mass was detected. The intermediate **4** (200 mg, crude) was used into the next step without further purification.

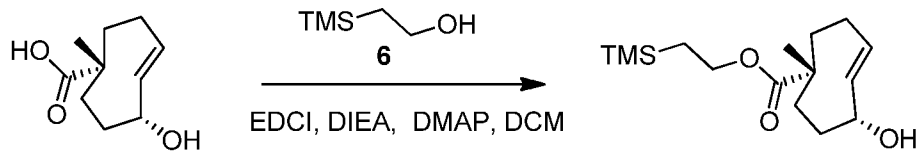


4 Compound 18 TCO-TLR7/8a

[0389] To a solution of intermediate **4** (200 mg, 322 μmol) in DMF (4 mL) was added intermediate **5** (242 mg, 3.22 mmol) and DIEA (250 mg, 1.93 mmol) and DMAP (236 mg, 1.93 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed intermediate **4** was consumed completely and one main peak with desired mass was detected. The product was purified by prep-HPLC (0.1% TFA conditions) to give **Compound 18** (200 mg, 53.4% yield).

[0390] **^{1}HNMR**: (400 MHz, MeOD) δ 1.02 - 2.31 (m, 20 H), 3.52 - 3.90 (m, 4 H), 4.90 - 5.27 (m, 5 H), 5.40 - 6.04 (m, 2 H), 7.29 - 7.40 (m, 1 H), 7.52 - 7.64 (m, 1 H), 7.67 - 7.82 (m, 2 H), 8.51 (br d, *J* = 8.38 Hz, 1 H). HRMS: $[M+H]^+$ 583.3102.

Example 19: TCO-STINGa (Compound 19)



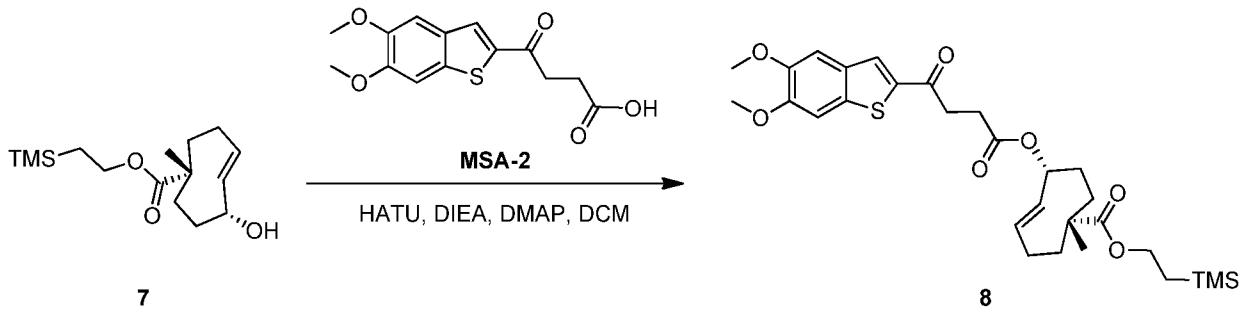
2 7

[0391] To a solution of intermediate **2** (2.0 g, 10.8 mmol) in DCM (10 mL) was added DIEA (4.21 g, 32.5 mmol) and EDCI (4.16 g, 21.7 mmol) and DMAP (2.65 g, 21.7 mmol) and intermediate **6** (1.54 g, 13.0 mmol). The mixture was stirred at 25 °C for 16 hrs. TLC indicated intermediate**2** was consumed

completely and one new spot formed. The reaction mixture was partitioned between DCM (10 mL) and H_2O (10 mL). The organic phase was separated, washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 20 / 1 to 10 / 1) to give intermediate **7** (800 mg, 25.9% yield).

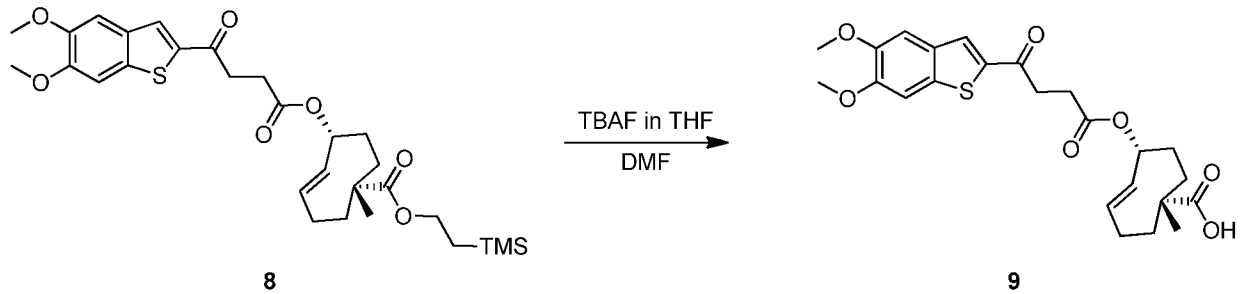
[0392] Note: After the compound **7** was stored at 0 °C for 12 hrs, TLC showed one new spot formed compare with the pure compound **7**.

[0393] **^{1}HNMR:** (400MHz, $CDCl_3$) δ 0.03 - 0.07 (m, 9 H), 0.94 - 1.00 (m, 2 H), 1.10 (s, 3 H), 1.56 (br dd, *J* = 15.57, 6.07 Hz, 2 H), 1.77 - 2.00 (m, 5 H), 2.14 - 2.35 (m, 3 H), 4.05 - 4.17 (m, 2 H), 4.48 (br s, 1 H), 5.64 (dd, *J* = 16.63, 2.38 Hz, 1 H), 5.98 - 6.14 (m, 1 H).



7 **8**

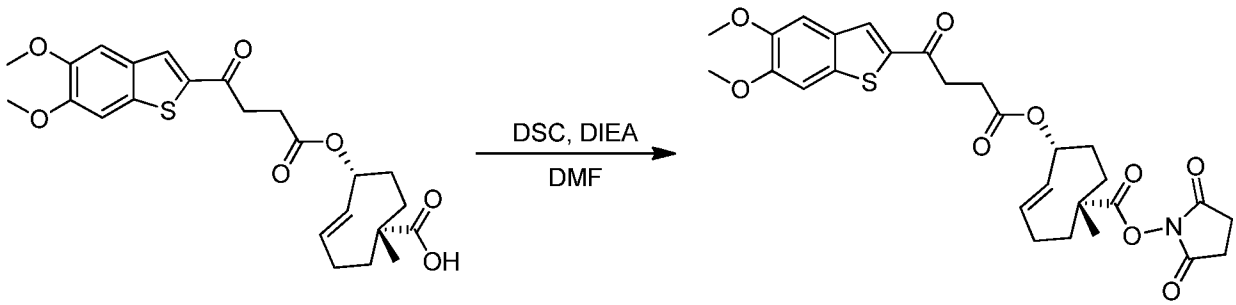
[0394] To a solution of **MSA-2** (250 mg, 849 µmol) in DCM (10 mL) was added intermediate **7** (725 mg, 2.55 mmol) and DIEA (329 mg, 2.55 mmol) and DMAP (156 mg, 1.27 mmol) and HATU (646 mg, 1.70 mmol). The mixture was stirred at 25 °C for 2 hrs. LC-MS showed **MSA-2** was consumed completely and one main peak with desired mass was detected. The residue was purified by prep-HPLC (0.1% TFA conditions) to give intermediate **8** (210 mg, 44.1% yield).



8 **9**

[0395] To a solution of intermediate **8** (200 mg, 357 µmol) in DMF (2 mL) was added TBAF (1 M, 1.43 mL, 1.43 mmol). The mixture was stirred at 25 °C for 2 hrs. LC-MS showed intermediate **8** was consumed completely and one main peak with desired mass was detected. The residue was purified by prep-HPLC (0.1% TFA conditions) to give intermediate **9** (88 mg, 53.6% yield).

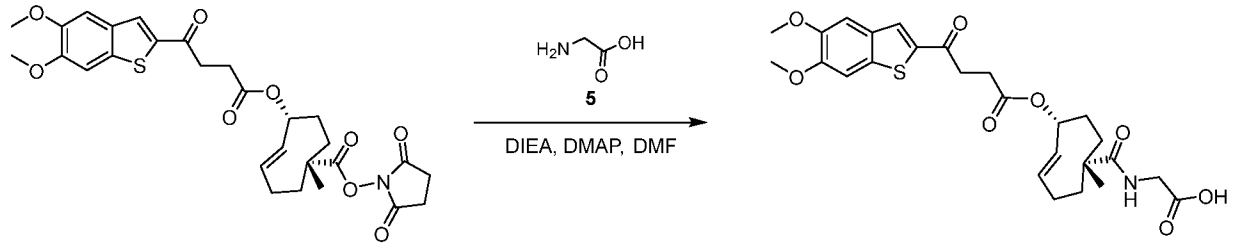
[0396] LCMS: $[M+Na]^+$ 483.0



9 10

[0397] To a solution of intermediate **9** (88 mg, 191 μmol) in DMF (0.2 mL) was added DSC (97.9 mg, 382 μmol) and DIEA (49.4 mg, 382 μmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed intermediate **9** was consumed completely and one main peak with desired mass was detected. The residue was purified by prep-HPLC (0.1% TFA) to give intermediate **10** (60 mg, 56.3% yield).

[0398] LCMS: $[M+H]^+$ 558.0



10

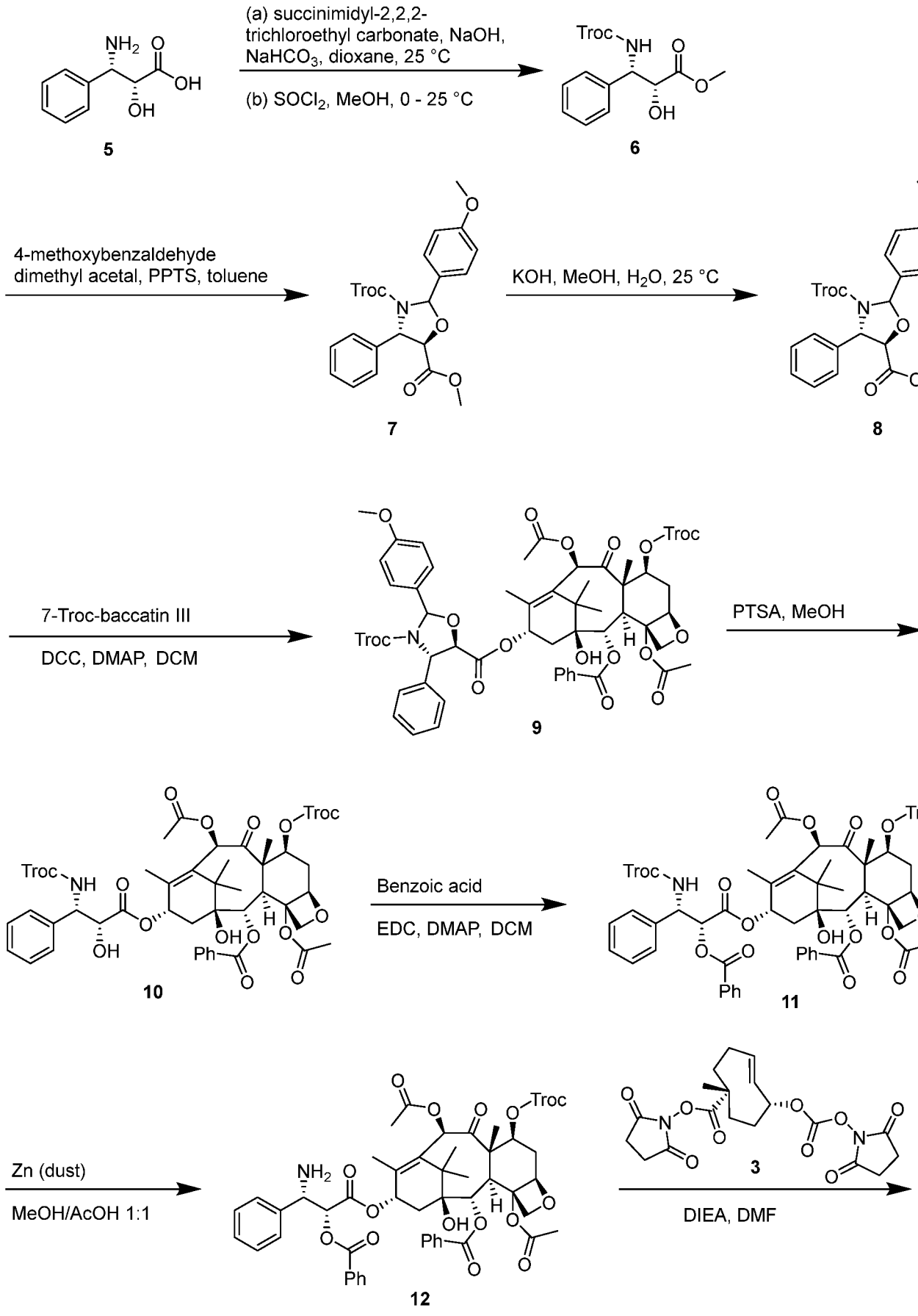
Compound 19
TCO-STINGa

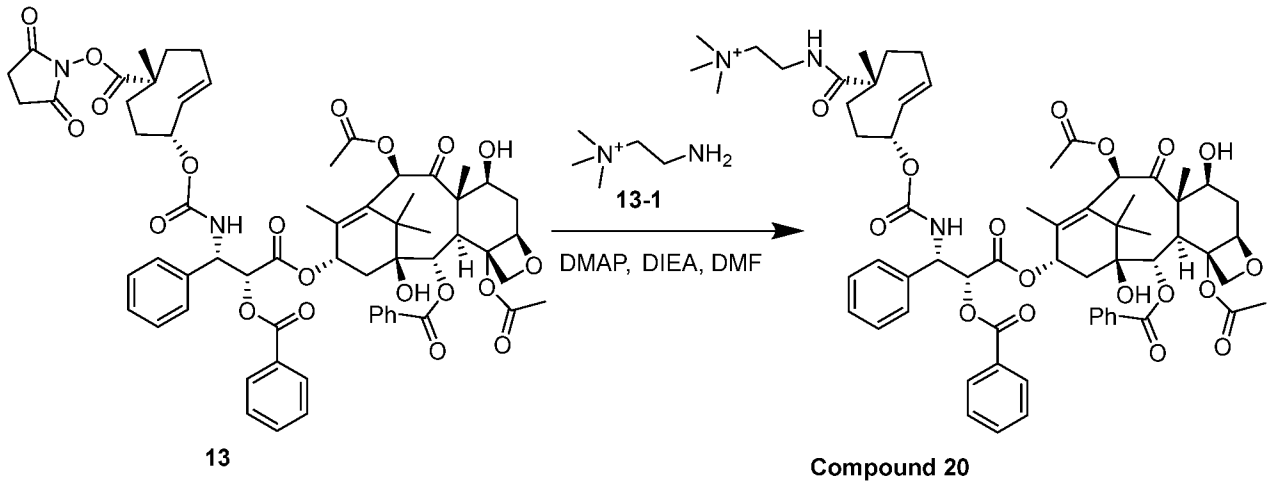
[0399] To a solution of intermediate **10** (60 mg, 107.60 μmol) in DMF (1 mL) was added intermediate **5** (80.7 mg, 1.08 mmol) and DIEA (139 mg, 1.08 mmol) and DMAP (131 mg, 1.08 mmol). The mixture was stirred at 25 °C for 24 hrs. LC-MS showed ~20% intermediate **10** was remained, then added intermediate **5** (80.7 mg, 1.08 mmol), the mixture was stirred at 25 °C for 24 hrs. LC-MS showed intermediate **10** consumed completely and one main peak with desired mass was detected. The crude was purified by prep-HPLC (0.1% TFA conditions) to give **Compound 19** (30 mg, 80% purity) (contained DIEA residue), then re-purified by prep-HPLC (0.1% TFA conditions) to give **Compound 19** (10 mg, 17.9% yield).

[0400] **^{1}HNMR:** (400MHz, $CDCl_3$) δ 1.14 (s, 3 H), 1.16 - 1.31 (m, 1 H), 1.58 - 1.65 (m, 1 H), 1.62 - 1.62 (m, 1 H), 1.81 - 1.89 (m, 3 H), 1.96 - 2.12 (m, 3 H), 2.19 - 2.25 (m, 1 H), 2.20 - 2.33 (m, 1 H), 2.88 (br t, J = 6.38 Hz, 2 H), 3.35 (br t, J = 6.32 Hz, 2 H), 3.46 - 3.69 (m, 11 H), 3.96 (s, 3 H), 3.98 (s, 3 H), 5.27 (br s, 1 H), 5.60 (dd, J = 16.45, 2.06 Hz, 1 H), 5.82 - 5.95 (m, 1 H), 6.16 (br s, 1 H), 7.93 (s, 1 H).

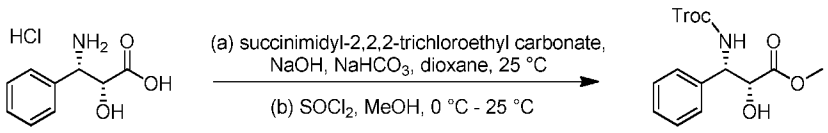
[0401] LCMS: $[M+Na]^+$ 540.1

Example 20: Synthesis of Compound 20





General procedure for preparation of intermediate 6

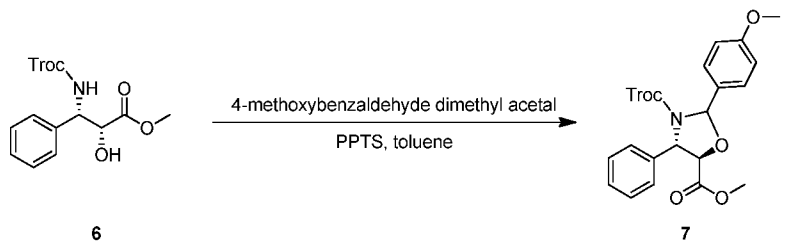


[0402] To a solution of intermediate **5** (150 g, 689 mmol, HCl) in NaOH (1 M, 1.38 L) and $NaHCO_3$ (1 M, 1.38 L) was added (2, 5-dioxopyrrolidin-1-yl) 2, 2, 2-trichloroethyl carbonate (210 g, 723 mmol) in dioxane (1 L). The mixture was stirred at 25 °C for 2 hrs. The reaction mixture was concentrated under reduced pressure to remove dioxane. The residue was extracted with MTBE (5 L), then the aqueous phase was adjusted pH~4 with *Sat.* $KHSO_4$ aq. and extracted with EtOAc (5 L). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. To a solution of above crude in MeOH (2 L) was added $SOCl_2$ (90.2 g, 758 mmol) and the mixture was stirred at 25 °C for 2 hrs. LC-MS showed reaction was completed and one main peak with desired mass was detected. The reaction mixture was adjusted pH~9-10 with *Sat.* $NaHCO_3$ aq., then extracted with EtOAc (5 L). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude. The crude was precipitated by PE (10 Vol) to give intermediate **6** (190 g, 74.4% yield).

[0403] **^{1}H NMR:** (400 MHz, $CDCl_3$): δ 3.25 (br s, 1 H) 3.85 (s, 3 H) 4.64 - 4.83 (m, 2 H) 5.30 (dd, *J* = 9.51, 1.13 Hz, 1 H) 5.92 (br d, *J* = 9.38 Hz, 1 H) 7.30 - 7.45 (m, 5 H).

[0404] **LCMS (m/z):** 391.9/393.9 $(M+H)^+$.

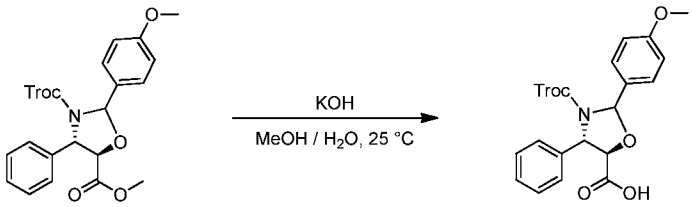
General procedure for preparation of intermediate 7



6 7

[0405] To a solution of intermediate **6** (185 g, 499 mmol) in toluene (1.9 L) was added 4-methylbenzenesulfonic acid pyridine (3.90 g, 15.4 mmol) and 4-methoxybenzaldehyde dimethyl acetal (121 g, 666 mmol). The mixture was stirred at 110 °C for 4 hrs. LC-MS showed one main peak with desired mass was detected. Then reaction mixture was allowed to cool to 25 °C, The reaction mixture was concentrated under reduced pressure to remove toluene. The residue was diluted with H_2O (500 mL), then extracted with EtOAc (500 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give intermediate **7** (285 g, crude) which was carried forward as is.

General procedure for preparation of intermediate 8



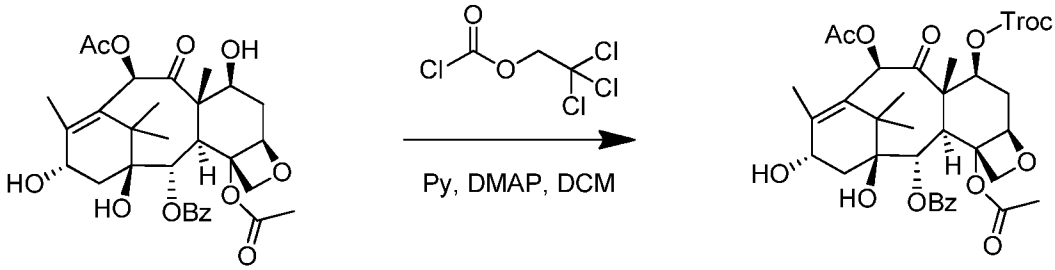
7 8

[0406] To a solution of intermediate **7** (285 g, crude) in MeOH (2000 mL) was added KOH (42.5 g, 758 mmol) in H_2O (1000 mL). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate **7** was consumed completely and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove MeOH. The residue was extracted with MTBE (5 L). The aqueous phase layers were diluted with *sat.* $KHSO_4$ (1L) aq. extracted with EtOAc (5 L), the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude. The crude was precipitated by PE (10 Vol) to give intermediate **8** (95.0 g, 34.3% yield).

[0407] ^{1}H NMR (400 MHz, MeOD): δ 3.82 (s, 3 H) 4.41 - 4.47 (m, 1 H) 4.50 - 4.56 (m, 1 H) 4.60 (d, *J* = 4.88 Hz, 1 H) 5.47 (d, *J* = 4.75 Hz, 1 H) 6.46 (s, 1 H) 6.86 - 6.94 (m, 2 H) 7.34 - 7.46 (m, 7 H).

[0408] LCMS (m/z): 495.9 $(M+Na)^+$.

General procedure for preparation of 7-Troc-baccatin III

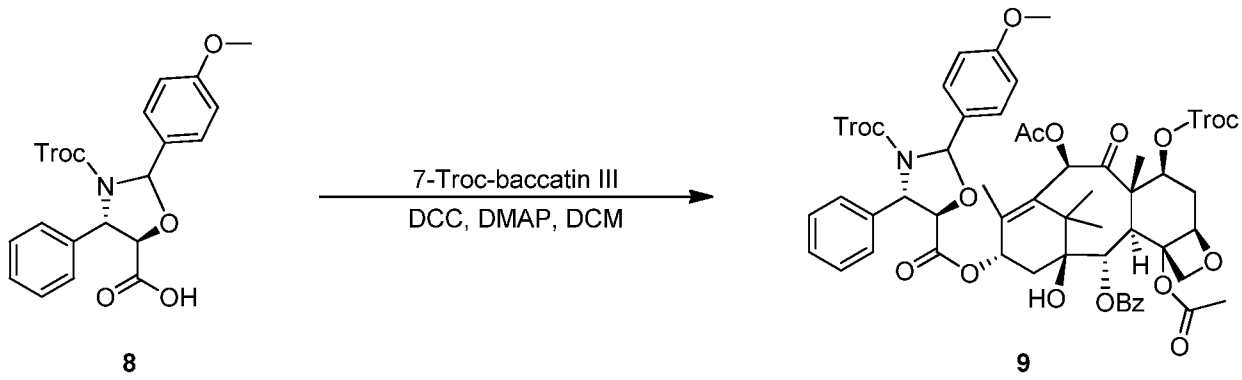


baccatin III **7-Troc-baccatin III**

[0409] To a solution of baccatin III (30.0 g, 51.1 mmol) in DCM (300 mL) was added DMAP (625 mg, 5.11 mmol) and pyridine (14.2 g, 179 mmol) and 2,2,2-trichloroethyl carbonochloridate (15.2 g, 71.6 mmol). The mixture was stirred at 25 °C for 0.5 hrs. LC-MS showed baccatin III was consumed completely and one main peak with desired mass was detected. The residue was diluted with water (300 mL) and extracted with DCM (300 mL) and washed with water (200 mL) and brine (200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **7-Troc-baccatin III** (45.0 g, 34.3% yield).

[0410] LCMS (m/z): 761.5/763.5 $(M+Na)^+$.

General procedure for preparation of intermediate 9

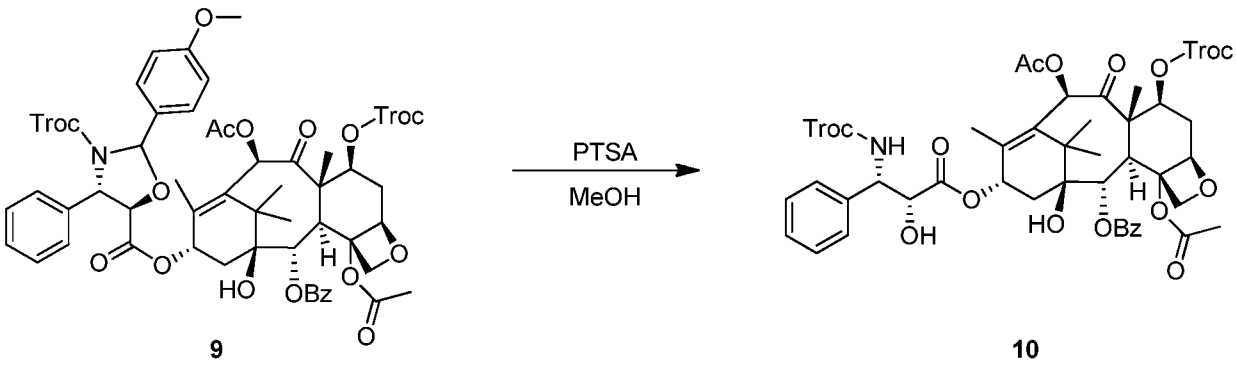


8 **9**

[0411] To a solution of 7-Troc-baccatin III (26.0 g, 34.1 mmol) and intermediate **8** (32.4 g, 68.2 mmol) in DCM (1000 mL) was added DMAP (4.20 g, 34.1 mmol) and DCC (21.1 g, 102 mmol). The mixture was stirred at 0 °C for 1 hrs. LC-MS showed intermediate **8** was consumed completely and one main peak with desired mass was detected. The reaction mixture filtered. The crude was washed by *sat.* NH_4Cl aq. (100 mL) and water (1000 mL) dried over Na_2SO_4, filtered and concentrated under reduced pressure to give intermediate **9** (35.0 g, crude).

[0412] LCMS (m/z): 1240.0/1242.0 $(M+Na)^+$.

General procedure for preparation of intermediate 10

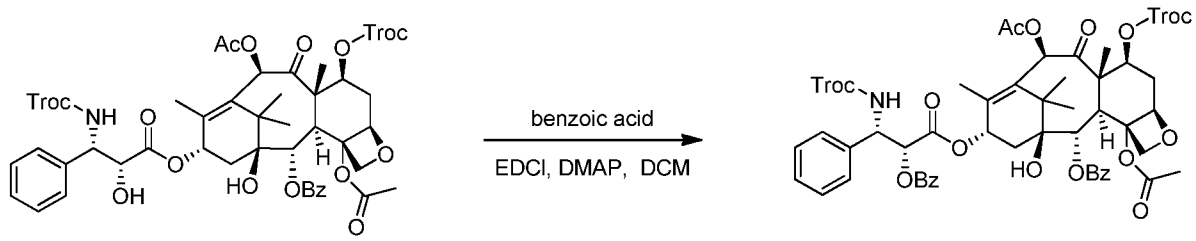


9 10

[0413] To a solution of intermediate **9** (80.0 g, 65.6 mmol) in MeOH (350 mL) was added 4-methylbenzenesulfonic acid; hydrate (24.9 g, 131 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed ~50% of intermediate **9** was remained and one main peak with desired mass was detected. The reaction mixture filtered, concentrated and the residue was purified by prep-HPLC (Water (0.1% TFA)-ACN). The elution was concentrated under reduced pressure to remove solvent, then, extracted with EtOAc (500 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give intermediate **10** (13.0 g, 17.9% yield).

[0414] LCMS (m/z): 1120.2 $(M+Na)^+$.

General procedure for preparation of intermediate 11

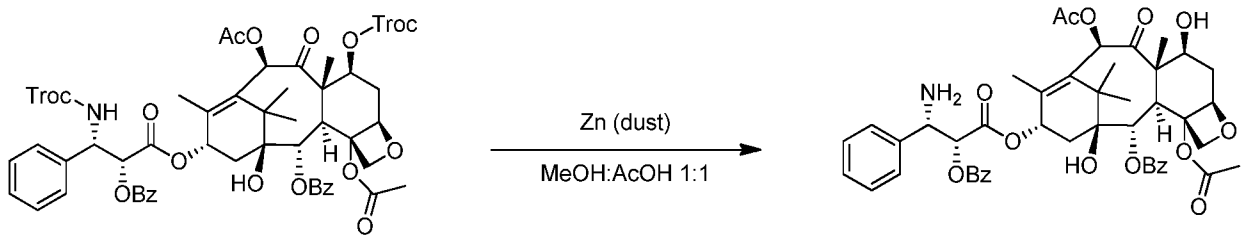


10 11

[0415] To a solution of intermediate **10** (13.0 g, 11.8 mmol) and DMAP (722 mg, 5.90 mmol) and EDCI (2.70 g, 14.2 mmol) and benzoic acid (1.70 g, 14.2 mmol) in DCM (260 mL). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate **10** was consumed completely and one main peak with desired mass was detected. The reaction mixture was washed with sat. citric acid aq. (100 mL), sat. NaHCO3 aq. (100 mL) and water (200 mL), dried over $NaSO_4$, filtered and concentrated under reduced pressure to give intermediate **11** (11.0 g, 77.3% yield).

[0416] LCMS (m/z): 1204.1 $(M+H)^+$.

General procedure for preparation of intermediate 12

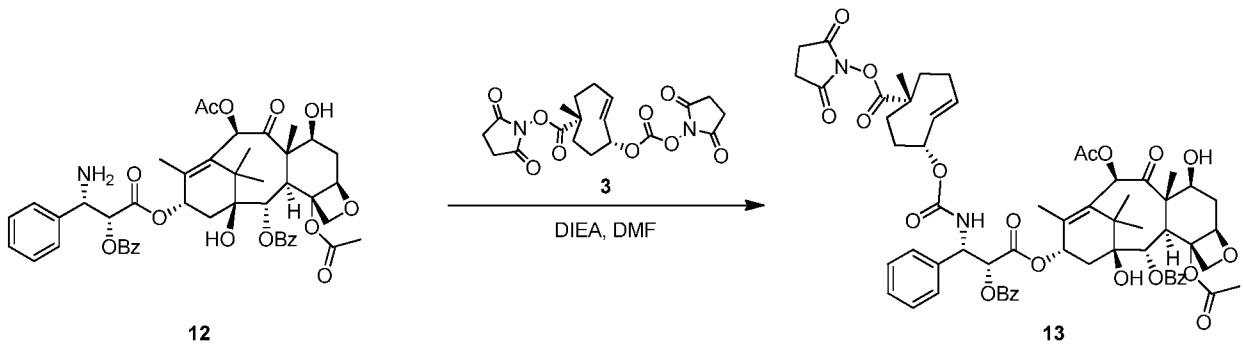


11 12

[0417] To a solution of intermediate **11** (20.0 g, 16.6 mmol) in MeOH (200 mL) and AcOH (200 mL) was added Zn dust (21.6 g, 331 mmol). The mixture was stirred at 25 °C for 1 hrs. LC-MS showed intermediate 11 was consumed completely and one main peak with desired mass was detected. The reaction mixture was filtered and diluted with H_2O (500 mL), then extracted with EtOAc (100 mL * 3). The combined organic layers were washed with *sat.* $NaHCO_3$ aq. (200 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give intermediate **12** (5.0 g, 21% yield).

[0418] LCMS (m/z): 854.3 $(M+H)^+$.

General procedure for preparation of intermediate 13

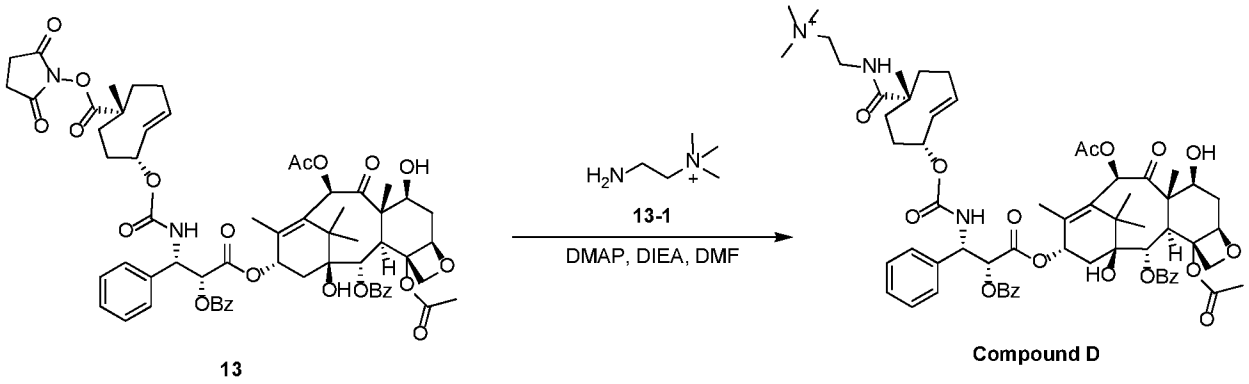


12 13

[0419] To a solution of intermediate **12** (5.00 g, 5.90 mmol), DIEA (1.50 g, 11.7 mmol) and **3** (3.90 g, 8.80 mmol) in DMF (50 mL). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed ~50% intermediate 12 was remained and one main peak with desired mass was detected. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give intermediate **13** (505 mg, 7.4% yield).

[0420] LCMS (m/z): 1161.4 $(M+H)^+$.

General procedure for preparation of Compound 20

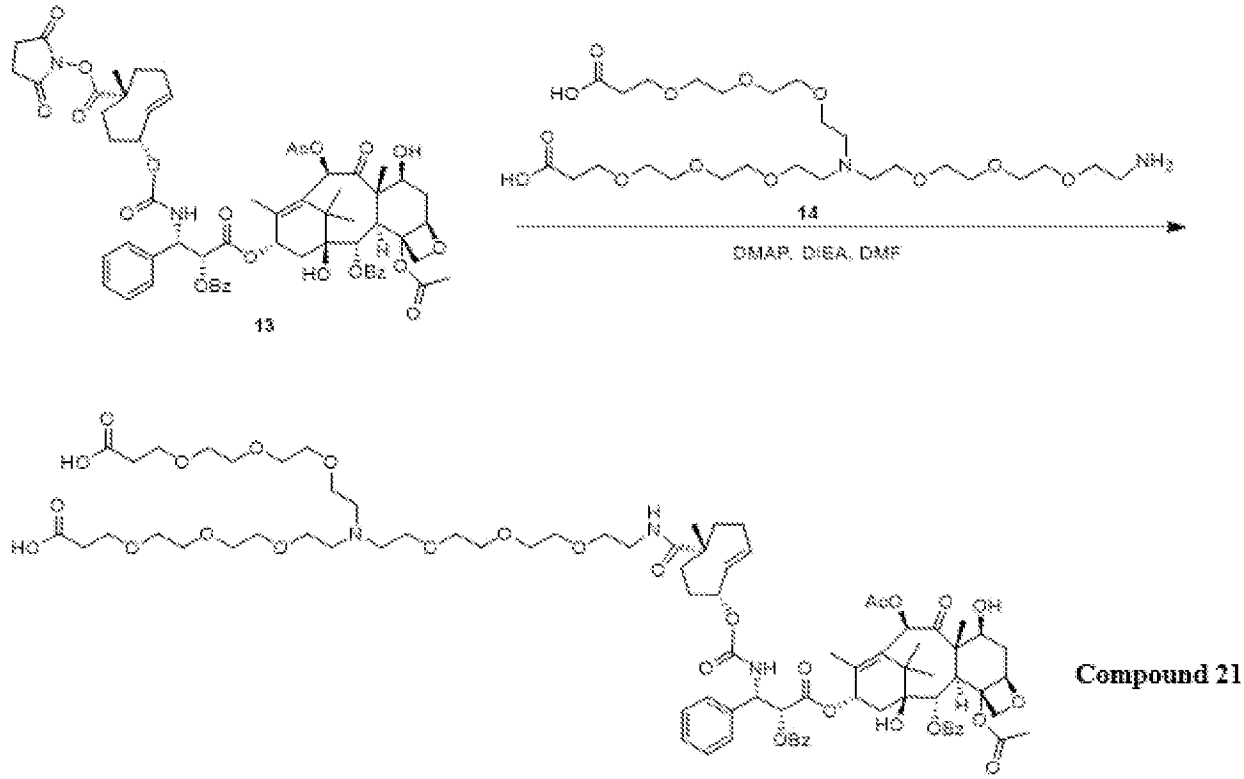


13 Compound D

[0421] To a solution of compound intermediate **13** (150 mg, 0.13 mmol) in DMF (1.50 mL) was added DMAP (94.7 mg, 0.78 mmol) and compound intermediate **13-1** (66.7 mg, 0.65 mmol) and DIEA (100 mg, 0.78 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed intermediate13 was consumed completely and one main peak with desired mass was detected. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give **Compound 20** (75.0 mg, 50.5% yield).

[0422] LCMS (m/z): 1148.5 $(M)^+$.

Example 21: General procedure for preparation of Compound 21



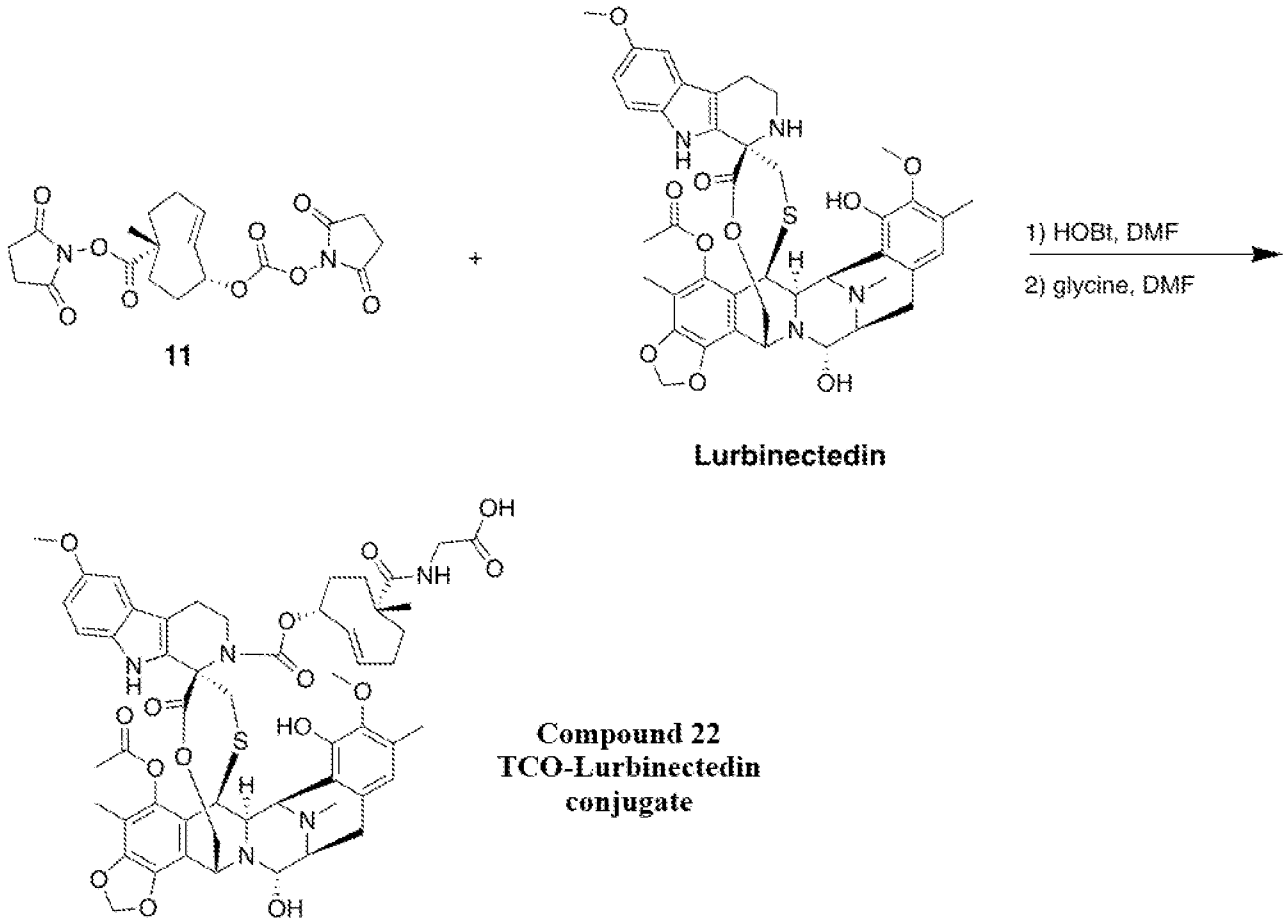
13 Compound 21

[0423] To a solution of intermediate **13** (350 mg, 0.30 μmol) and DMAP (221 mg, 1.81 mmol) and intermediate **14** (249 mg, 0.39 mmol, HCl) in DMF (0.3 mL). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed intermediate **13** was consumed completely and one main peak with desired mass

was detected. The residue was purified by prep-HPLC (Water (0.1% TFA)-ACN) to give **Compound 21** (205 mg, 41.3% yield).

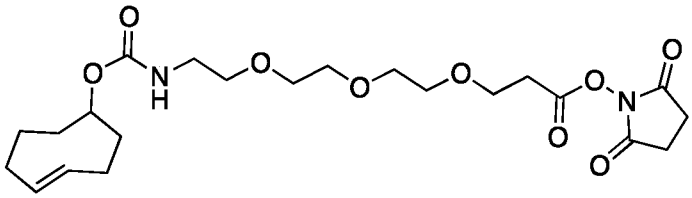
[0424] LCMS (m/z): 1646.5 $(M+H)^+$.

Example 22: TCO-Lurbinectedin conjugate (Compound 22)



[0425] TCO-Lurbinectedin (**Compound 22**). To a solution of Lurbinectedin in DMF is added bis-NHS-TCO **11** and HOBt. The reaction mixture is stirred at ambient temperature protected from light until the starting material is consumed. To the reaction mixture is added glycine and optionally a base and the resulting solution is stirred for additional time at ambient temperature. The reaction mixture is then concentrated under reduced pressure and the resulting residue is purified by reverse phase chromatography (10-100% MeCN/water with 0.1% ammonium formate) to yield the desired product **Compound 22**.

Example 23: Anti-CD3-Fab-TCO Therapeutic Conjugate

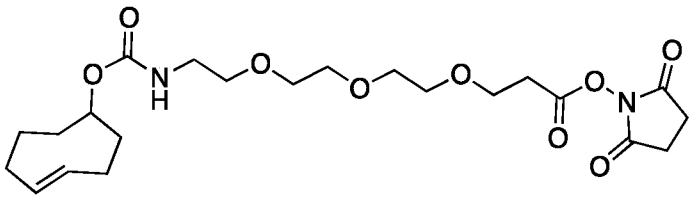


TCO-PEG3-NHS

[0426] Fab is prepared from OKT3 using a commercial kit (Pierce™ Fab Preparation Kit #44985) according to the manufacturers protocol and purified by protein G resin (BioVision #6511-25). To the purified Fab is added 10 mM TCO-PEG3-NHS is prepared in DMSO. The two components are reacted at 3:1 drug to protein molar ratio at 25 °C for 2 hours before it is dialyzed against PBS, pH 7.4 to remove excess TCO-PEG3-NHS compound from the protein component. The resulting solution of therapeutic targeting moiety is analyzed by SDS-Page and LCMS to confirm the formation of the therapeutic targeting moiety. It is contemplated that approximately 1-2 TCOs will be covalently bonded to each Fab, on average, as confirmed by LCMS.

Example 24: anti-CD3 Fab – PEG3-TCO Conjugate Preparation

[0427] Fab of the anti-CD3 antibody 2C11 was synthesized by plasmid construction, HEK293 cell expression and purification. The Fab-TCO conjugate was prepared by reacting TCO-PEG3-NHS (structure shown below, purchased from SiChem; catalog No. SC-8406) to primary amines on the Fab to form stable amide bonds.



TCO-PEG3-NHS

Synthesis of Fab of 2C11

[0428] Vector construction: Coding sequences (listed below) was synthesized and subcloned into expression vector. Constructed plasmids were transformed to E.coli for propagation. NucleoBond Xtra Maxi Plus EF kit was used for large scale plasmid generation. Purified plasmids were checked by agarose gel and confirmed by sequencing.

2C11-Fab HC sequence:

[0429] EVQLVESGGGLVQPGKSLKLSCEASGFTFSGYGMHWVRQAPGRGLESVAYITSSSINIKY ADAVKGRFTVSRDNAKNLLFLQMNILKSEDTAMYYCARFDWDKNYWGQGTMVTVSSAKTTA

PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKI (SEQ ID NO. 1).

2C11-Fab LC sequence:

[0430] DIQMTQSPSSLPASLGDRVTINCQASQDISNYLNWYQQKPGKAPKLLIYYTNKLADGVPS RFSGSGSGRDSSFTISSLESEDIGSYYCQQYYNYPWTFGPGTKLEIKRADAAPTVSIFPPSSEQLTS GGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHN SYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO. 2).

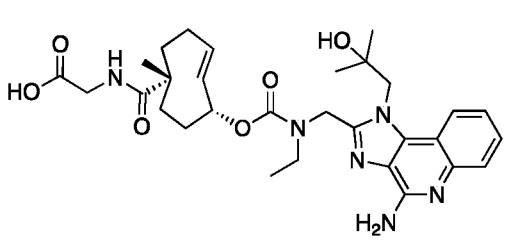
[0431] Protein expression: The constructs containing heavy chain and light chain of the Fab were co-transfected into HEK293 cells with PEI. The culture medium was harvested at 7 days post transfection.

[0432] Protein purification: Conditional medium expressing target Fab was harvested by centrifugation and filtration, then loaded onto CaptureSelect LC-kappa (murine) affinity column (Mabselect Prism). The loading buffer was PBS, pH 8.0, washed with PBS, pH 8.0, followed by PBS containing, 0.2% Triton X-100/114, pH 8.0, followed by PBS, pH 8.0. The protein was eluted with 50 mM Sodium-citrate buffer containing 150 mM NaCl, pH 3.0. The collected solution was neutralized with 1M Tris, 1M arginine buffer, pH 9.0. The affinity purified protein was further purified by gel filtration with Superdex S-200 column chromatography. Purified Fab was analyzed by SDS-PAGE, SEC-HPLC, and endotoxin measurement.

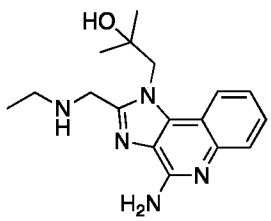
[0433] Conjugate preparation: Fab protein was dialyzed against PBS, pH 7.4 overnight with one buffer exchange at about 4 hours from the start. 10 mM TCOt-PEG3-NHS was prepared in DMSO. The two components were reacted at 3:1 drug to protein molar ratio at 25°C for 2 hours before it was dialyzed against PBS, pH 7.4 to remove excess TCO-PEG3-NHS compound from the protein component. LCMS analysis demonstrated the average loading of 1.9 TCO-PEG3 per Fab.

Biochemical Examples

Biochemical Example 1: TLR7/8a (Gardiquimod); effect on proliferation of fresh murine splenocytes



TCO-gly-gardiquimod



Gardiquimod

[0434] Lymphocytes were isolated from spleens of C57BL/6 mice. Spleens were grinded and cells strained through a 70 μm cell strainer using DBPS. Red blood cells were lysed, and cells were washed with DPBS. Isolated lymphocytes were suspended in culture medium. Cells were plated at 50,000

cells/well in a 96-well plate at 90 µL/well, then incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity overnight. The following day, compounds were prepared and 10 µL of compound-media was added to wells of the 96-well plate in triplicate. The following conditions were tested: unmodified gardiquimod; TCO-gly-gardiquimod; TCO-gly-gardiquimod plus tetrazine. The following concentrations of gardiquimod and TCO-gly-gardiquimod were used: 10 µg/mL, 2.5 µg/mL, 0.83 µg/mL, 0.28 µg/mL, 0.093 µg/mL, and 0.031 µg/mL. DMSO-medium was added to Blank and Control wells, leading to a final concentration of 0.1% DMSO. The plates were incubated for 48 hours. Plates were then analyzed by CellTiter-Glo Luminescent assay to assess cell viability, per manufacturer's instructions (Promega-G7573). The inhibition rate (IR) of the tested compounds was determined by the following formula: IR (%) = (1– (RLU compound – RLU blank) / (RLU control – RLU blank))*100%. The inhibitions of different doses of the tested compounds were calculated in Excel, and then used to plot inhibition curves and evaluate related parameters, such as Min (%), Max (%) and IC_{50}. The data were interpreted by GraphPad Prism.

[0435] As shown in Fig. 1 and Fig. 2, treatment with unmodified gardiquimod resulted in concentration-dependent proliferation/cell viability rate changes. Fig. 1 shows the results from an experiment with the highest concentration tested as 10 µg/mL. Fig. 2 shows a repeat of the experiment with TCO-gly-gardiquimod in the absence or presence of tetrazine up to 50 µg/mL concentrations. TCO-gly-gardiquimod had no to minimal effects on cell viability over the various concentrations. Even at the highest dose (50 µg/mL) its activity was minimal. This suggests an effective attenuation of activity. On the other hand, treatment with TCO-gly-gardiquimod in the presence of tetrazine led to a concentration-dependent increase in cell viability/proliferation rate. At concentrations over 5 µg/mL TCO-gly-gardiquimod in the presence of tetrazine displayed even greater activity on proliferation compared to unmodified gardiquimod, suggesting potentially superior activity compared to the unmodified drug.

[0436] Therapeutic support compositions as described herein can be prepared as described in WO2018/187740. Methods for testing and using the conjugates in combination with the support compositions can likewise be found in WO2018/187740.

[0437] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

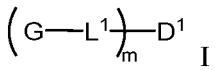
[0438] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[0439] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0440] It is to be understood that while the disclosure has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the disclosure. Other aspects, advantages and modifications within the scope of the disclosure will be apparent to those skilled in the art to which the disclosure pertains.

What is claimed is:

1. A conjugate of Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

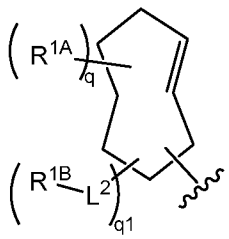
m is an integer from 1-150;

G, at each occurrence, is independently an optionally substituted trans-cyclooctene moiety;

D^1 is a payload selected from an inhibitor of poly (ADP-ribose) polymerase (PARP), a duocarmycin, a pyrrolobenzodiazepine (PBD), hemiasterlin, HTI-286, a monoclonal antibody, a topoisomerase inhibitor, lurbinectedin, MSA-2, gardiquimod, ciprofloxacin, mitomycin C, etoposide, and exatecan, or a derivative, or analog thereof;

L^1, at each occurrence, is independently a linker.

2. The conjugate of claim 1, or a pharmaceutically acceptable salt thereof, wherein each trans-cyclooctene moiety is independently:



wherein:

R^{1A}, at each occurrence, is independently selected from the group consisting of $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$haloalkyl, and $C_{1\text{-}4}$alkoxy;

q is 0, 1, or 2;

q1 is 0 or 1;

R^{1B}, at each occurrence, is independently selected from the group consisting of G^1, -OH, $–NR^{1c}–C_{1\text{-}4}$alkylene$–G^1$, $–NR^{1c}–C_{1\text{-}4}$alkylene$–N(R^{1d})_2$, $–NR^{1c}–C_{1\text{-}6}$alkylene$–N(C_{1\text{-}4}alkyl)_3^+$, $–N(R^{1c})CHR^{1e}CO_2H$, $–N(R^{1c})–C_{1\text{-}6}$alkylene$–CO_2H$, $–N(R^{1f})–C_{2\text{-}4}$alkylene$–(N(C_{1\text{-}4}$alkylene$–CO_2H)–C_{2\text{-}4}$alkylene$)_n–N(C_{1\text{-}4}$alkylene$–CO_2H)_2$, $–N(R^{1c})CHR^{1e}C(O)OC_{1\text{-}6}$alkyl, $–N(R^{1c})–C_{1\text{-}6}$alkylene$–C(O)OC_{1\text{-}6}$alkyl, $–N(R^{1f})–C_{2\text{-}4}$alkylene$–(N(C_{1\text{-}4}$alkylene$–C(O)OC_{1\text{-}6}$alkyl$)–C_{2\text{-}4}$alkylene$)_n–N(C_{1\text{-}4}$alkylene$–C(O)OC_{1\text{-}6}alkyl)_2$, $–N(R^{1c})–C_{1\text{-}6}$alkylene$–SO_3H$, $–N(R^{1c})–(CH_2CH_2O)_{1\text{-}3}–CH_2CH_2N((CH_2CH_2O)_{1\text{-}3}–C_{1\text{-}6}$alkylene$–CO_2H)_2$, and $–N(R^{1c})–CH(CH_2O–(CH_2CH_2O)_{0\text{-}2}–C_{1\text{-}6}$alkylene$–CO_2H)_2$;

R^{1c} and R^{1d}, at each occurrence, are independently hydrogen or $C_{1\text{-}4}$alkyl;

R^{1e}, at each occurrence, is independently $–C_{1-4}$alkylene–CO_2H, $–C_{1-4}$alkylene–$CONH_2$, or $–C_{1-4}$alkylene–OH;

R^{1f}, at each occurrence, is independently hydrogen, C_{1-6}alkyl, or C_{1-4}alkylene–CO_2H;

n, at each occurrence, is independently 0, 1, 2, or 3;

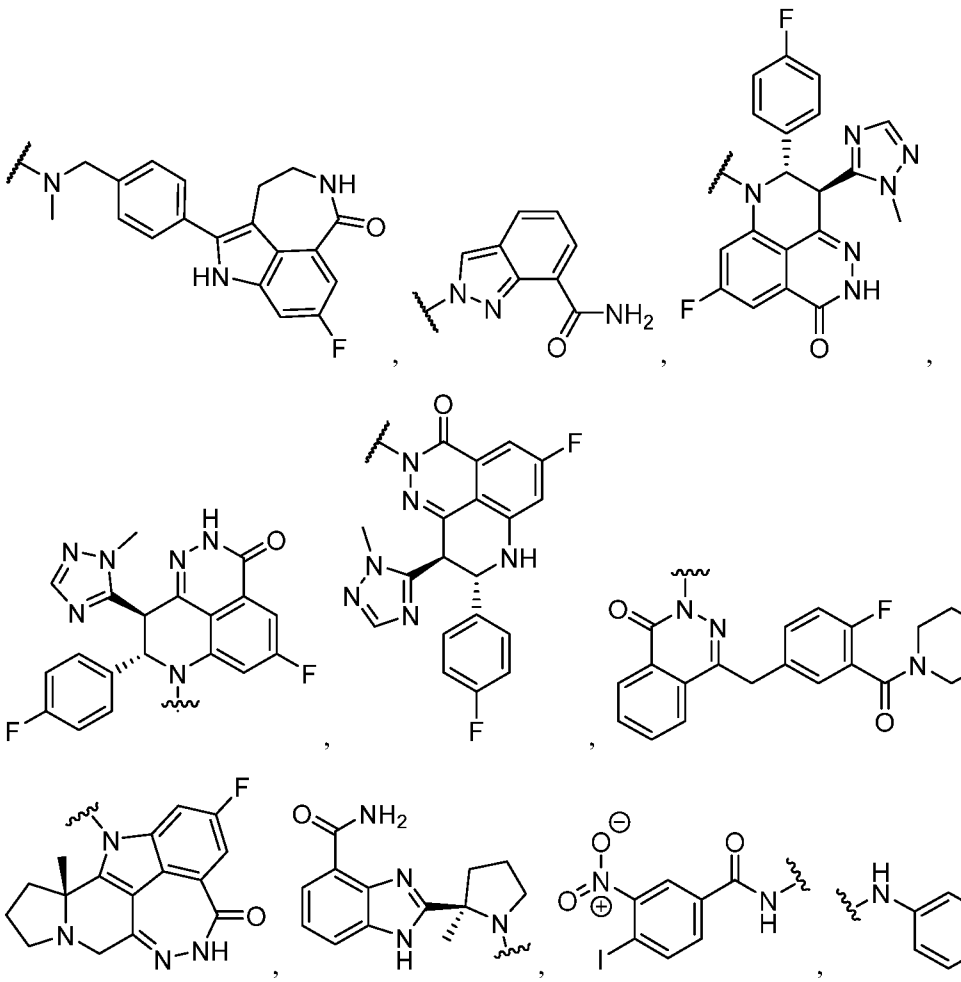
L^2, at each occurrence, is independently selected from the group consisting of –C(O)– and C_{1-3}alkylene; and

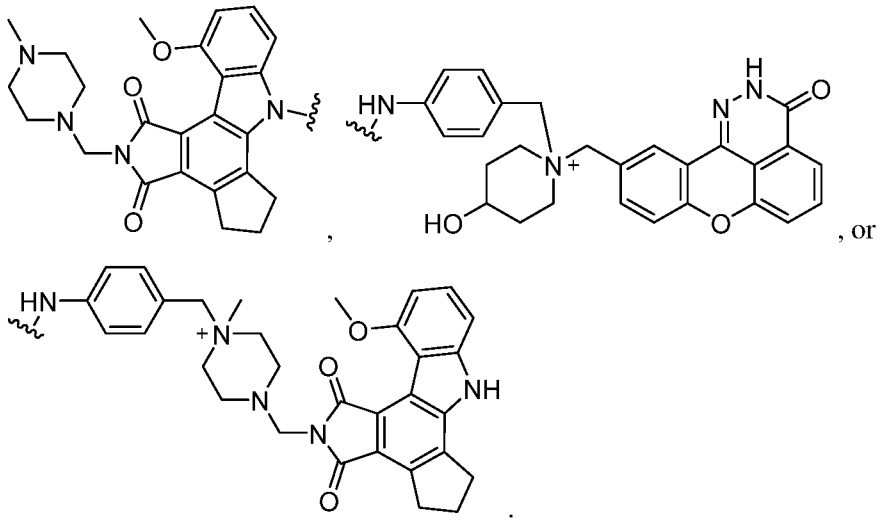
G^1, at each occurrence, is independently an optionally substituted heterocyclyl.

3. The conjugate of any one of claims 1-2, or a pharmaceutically acceptable salt thereof, wherein the payload is an inhibitor of poly (ADP-ribose) polymerase (PARP), or a derivative, or analog thereof.

4. The conjugate of claim 3, or a pharmaceutically acceptable salt thereof, wherein the inhibitor of poly (ADP-ribose) polymerase (PARP) is niraparib, talazoparib, olaparib, pamiparib, rucaparib, veliparib, iniparib, 3-aminobenzamide, CEP-9722, E7016, or a derivative, or analog thereof.

5. The conjugate of claim 3, or a pharmaceutically acceptable salt thereof, wherein D^1 is:

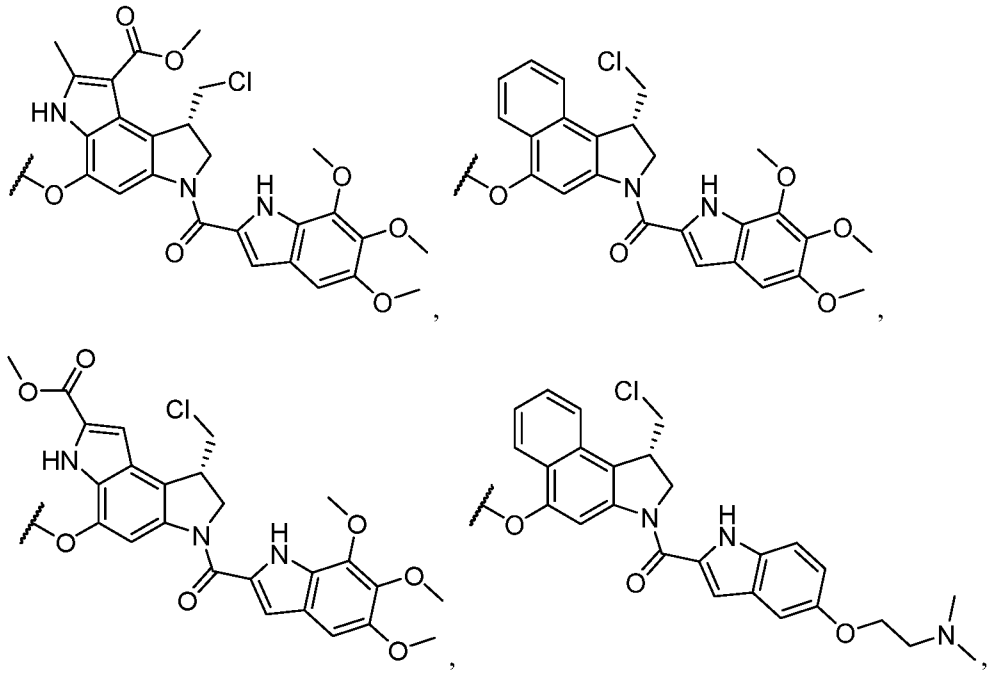


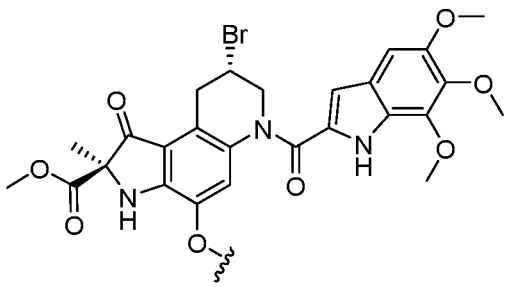
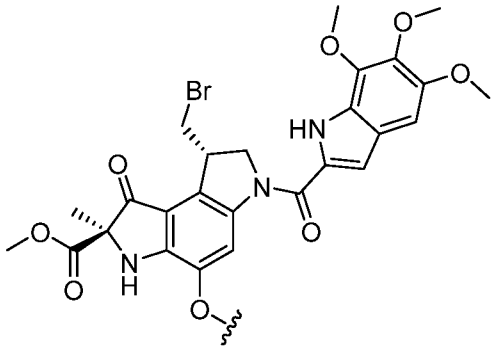
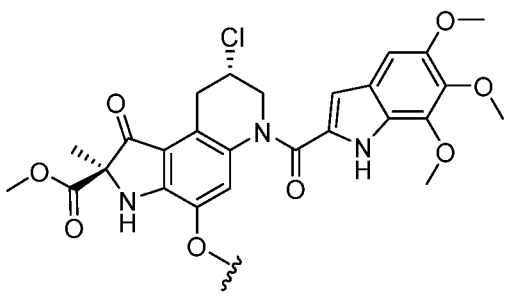
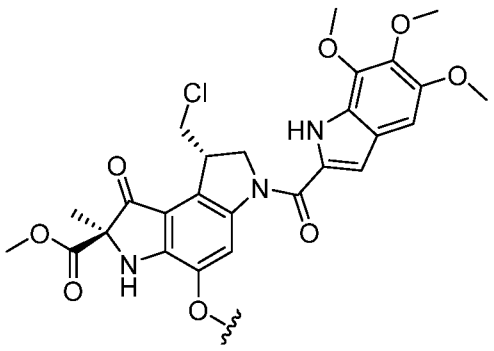
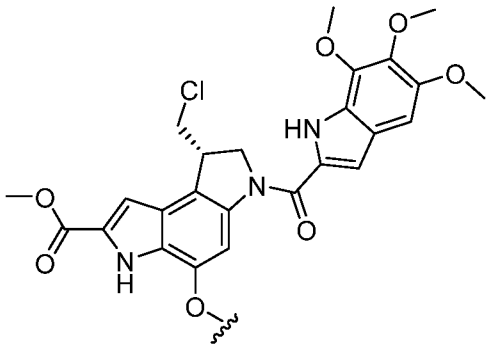
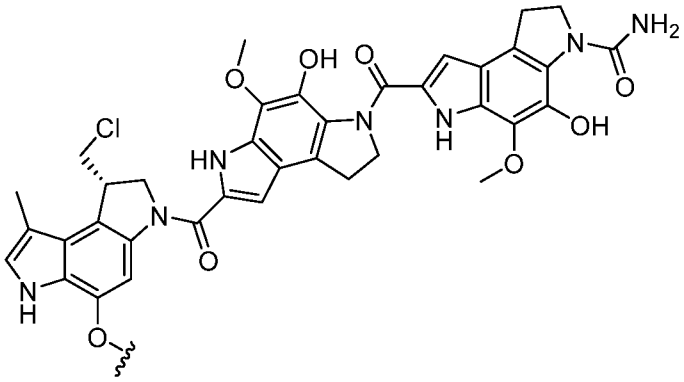
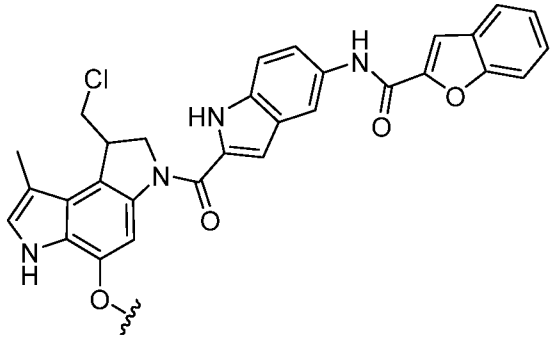
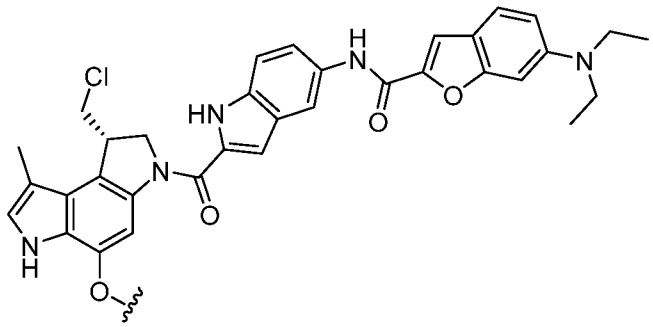


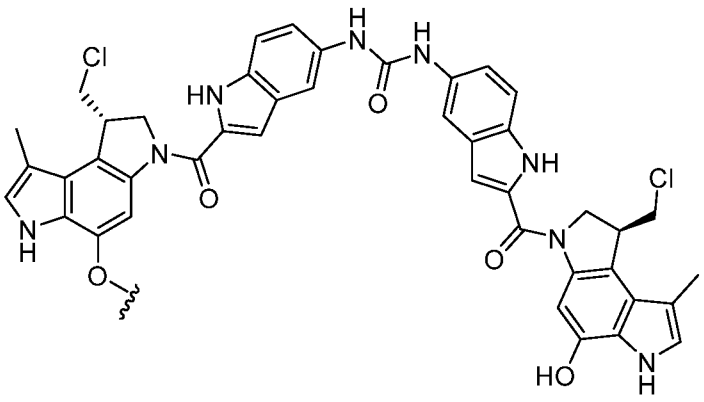
6. The conjugate of any one of claims 1-2, or a pharmaceutically acceptable salt thereof, wherein the payload is a duocarmycin, or a derivative, or analog thereof.

7. The conjugate of claim 6, or a pharmaceutically acceptable salt thereof, wherein the duocarmycin is duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, carzelesin, bizelesin, or a derivative, or analog thereof.

8. The conjugate of claim 6, or a pharmaceutically acceptable salt thereof, wherein D^1 is:




,

,

,

,

,

,

,

,

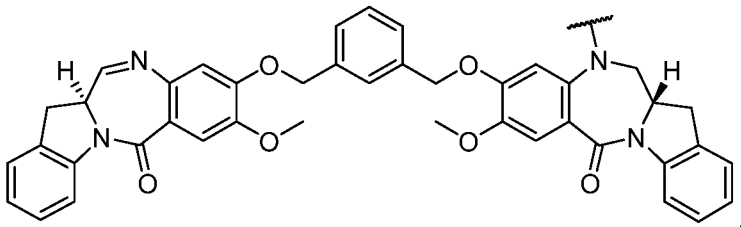


or .

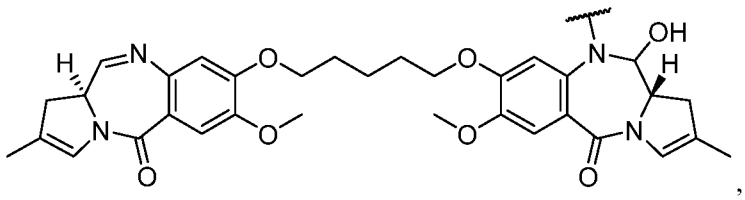
9. The conjugate of any one of claims 1-2, or a pharmaceutically acceptable salt thereof, wherein the payload is a pyrrolobenzodiazepine (PBD), or a derivative, or analog thereof.

10. The conjugate of claim 9, or a pharmaceutically acceptable salt thereof, wherein the pyrrolobenzodiazepine (PBD) is [1,2]diazepino[3,4-e]indole, or a derivative, or analog thereof:

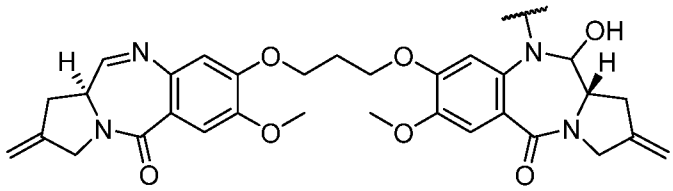
11. The conjugate of claim 9, or a pharmaceutically acceptable salt thereof, wherein D^1 is:



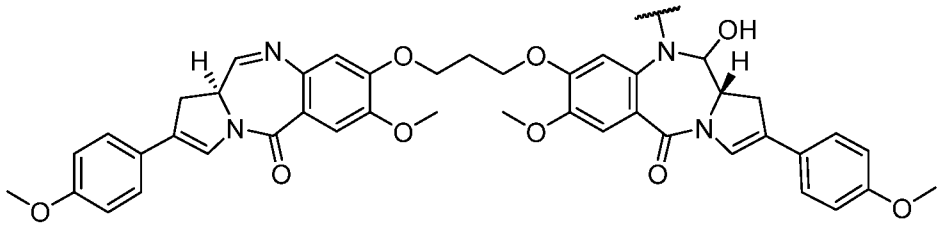
,



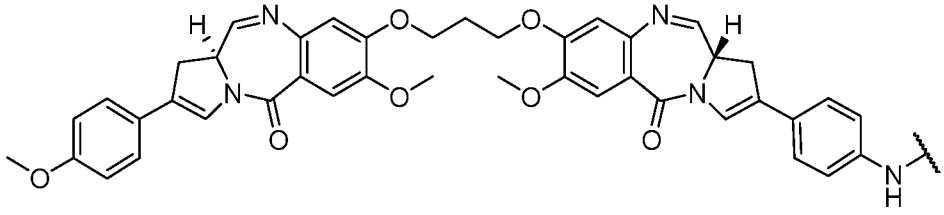
,



,



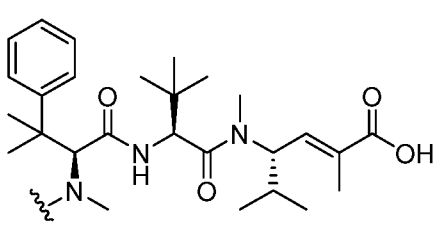
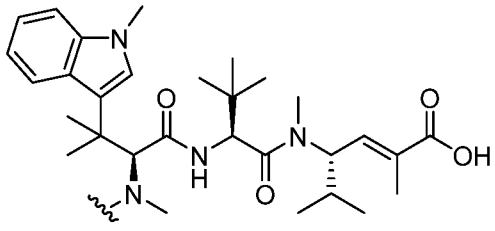
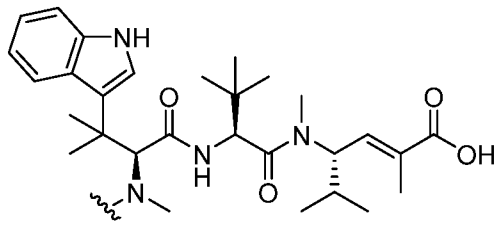
, or



.

12. The conjugate of any one of claims 1-2, or a pharmaceutically acceptable salt thereof, wherein the payload is hemiasterlin, HTI-286, or a derivative, or analog thereof.

13. The conjugate of claim 12, or a pharmaceutically acceptable salt thereof, wherein D^1 is:


,

, or

.

14. The conjugate of any one of claims 1-2, or a pharmaceutically acceptable salt thereof, wherein the payload is an anti-CD3 (αCD3) monoclonal antibody, or a derivative, or analog thereof.

15. The conjugate of claim 14, or a pharmaceutically acceptable salt thereof, wherein the anti-CD3 (αCD3) monoclonal antibody is OKT3, SP34, UCHT1, teplizumab, otelixizumab, visilizumab, or foralumab, or a derivative, or analog thereof.

16. The conjugate of any one of claims 1-15, wherein L^1 comprises 1 to 100 linking atoms, from 1 to 50 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms, or from 1 to 40 linking atoms, or from 1 to 30 linking atoms, or from 1 to 20 linking atoms, or from 1 to 10 linking atoms, or from 1 to 5 linking atoms, or from 5 to 30 linking atoms, or from 10 to 30 linking atoms, or from 5 to 40 linking atoms, or from 5 to 50 linking atoms, or from 10 to 50 linking atoms.

17. The conjugate of any one of claims 1-16, wherein L^1 comprises one or more chain heteroatoms and one or more alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene moieties; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene moiety, may be independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl.

18. The conjugate of any one of claims 1-16, wherein L^1 is an alkylene linker optionally comprising one or more -O-, -S-, amine, ester, amide, carbamate, carbonate, or ketone functional groups.

19. The conjugate of any one of claims 1-16, wherein L^1 is the formula:

$$-Y^{10}-(CH_2)_{n'}-Y^{20}-(CH_2)_{m''}-Y^{30}-$$

wherein:

each of Y^{10}, Y^{20}, and Y^{30} are independently a bond, -NR^{110}-, -O-, -$S(O)_{0-2}$-, -$NR^{110}C(O)$-, -$C(O)NR^{110}$-, -$NR^{110}S(O)_2$-, -$S(O)_2NR^{110}$-, -CR^{120}=N-NR^{110}-, -NR^{110}-N=CR^{120}-, -C(O)-, -OC(O)-, -OC(O)O-, alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene or heterocycloalkylene is independently optionally substituted with one to five substituents independently selected from oxo, halo, C_{1-4} alkyl, C_{1-4} alkoxy, and C_{1-4} haloalkyl;

each R^{110} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl;

each R^{120} is independently hydrogen, C_{1-4} alkyl, C_{1-4} haloalkyl, aryl, heteroaryl, cycloalkyl or heterocyclyl; and

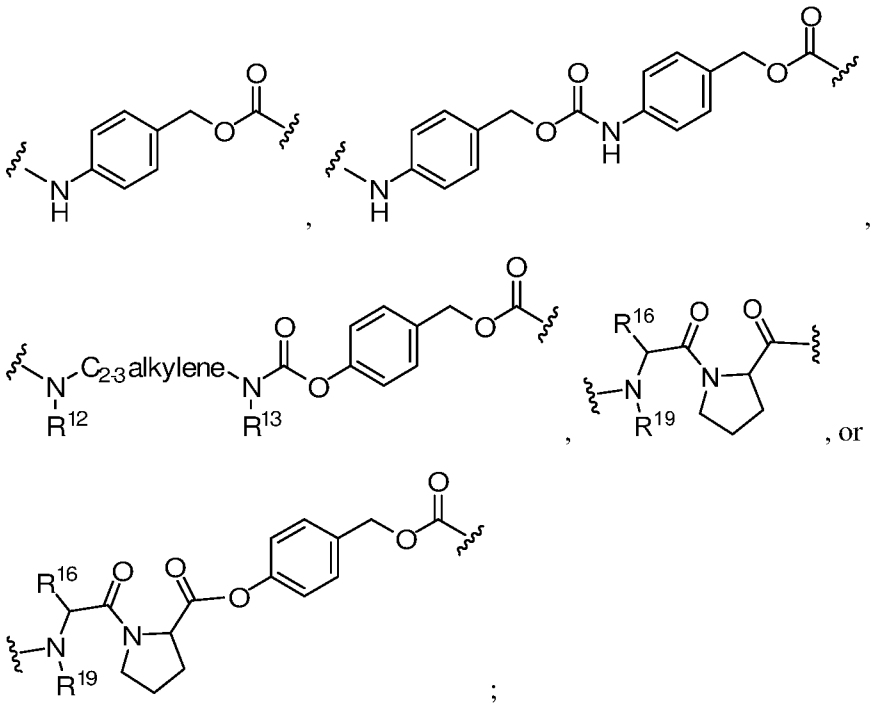
n' and m" are each independently 0, 1, 2, 3, 4, 5, 6, 7, or 8.

20. The conjugate of any one of claims 1-19, or a pharmaceutically acceptable salt thereof, wherein the linker comprises one or more amino acids.

21. The conjugate of any one of claims 1-16, or a pharmaceutically acceptable salt thereof, wherein:

L^1 is –OC(O)L^4– or –OC_{1-6}alkyleneC(O)L^4–;

L^4 is a bond, –N(R^{12})–C_{2-3}alkylene–N(R^{13})C(O)–,
–CH(NHC(O)R^{14})C_{1-4}alkylene–S–S–C_{1-4}alkylene–OC(O)–,
–NHNHC(O)CH(NHC(O)R^{15})CH_2C(O)–, –C_{1-6}alkylene–CH(G^x)OC(O)–,



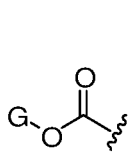
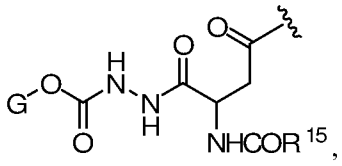
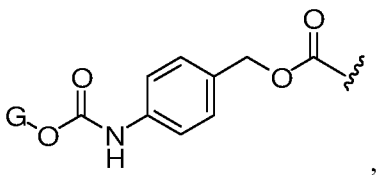
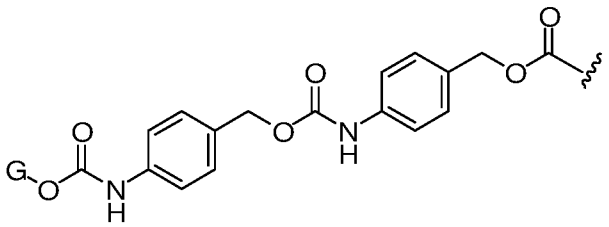
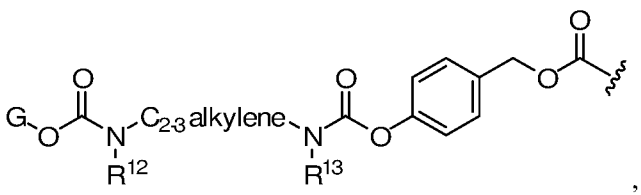
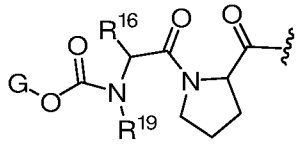
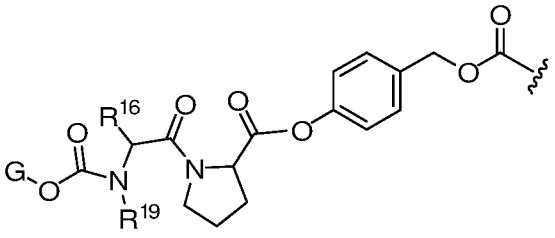
;

R^{12}, R^{13}, R^{14}, R^{15}, and R^{19} are each independently hydrogen or C_{1-4}alkyl;

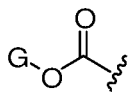
R^{16} is hydrogen, C_{1-4}alkyl, –C_{1-4}alkylene–OH, –C_{1-4}alkylene–OC_{1-4}alkyl, –C_{1-4}alkylene–CO_2H, or –C_{1-4}alkylene–$CONH_2$; and

G^x is phenyl optionally substituted with 1-5 substituents independently selected from the group consisting of halogen, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, cyano, and nitro.

22. The conjugate of any one of claims 1-16, or a pharmaceutically acceptable salt thereof, wherein:

G–L^1, at each occurrence, is independently  ,  ,  ,  ,  ,  ,  , or  .

23. The conjugate of any one of claims 1-22, or a pharmaceutically acceptable salt thereof, wherein

G–L^1, at each occurrence, is independently  .

24. The conjugate of any one of claims 4-23, or a pharmaceutically acceptable salt thereof, wherein:

R^{1B} is G^1, OH, –NR^{1c}–C_{1-4}alkylene–G^1, –NR^{1c}–C_{1-4}alkylene–$N(R^{1d})_2$, –$N(R^{1c})CHR^{1e}CO_2H$, –$N(R^{1c})CH_2CO_2H$, or –$N(R^{1f})$–CH_2CH_2–$(N(CH_2CO_2H)CH_2CH_2)_n$–$N(CH_2CO_2H)_2$;

R^{1e} is –CH_2CO_2H, –$CH_2CH_2CO_2H$, –CH_2CONH_2, –$CH_2CH_2CONH_2$, –CH_2OH, or –$CH(CH_3)OH$; and

R^{1f} is hydrogen or -CH_2CO_2H.

25. The conjugate of any one of claims 4-24, or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} is C_{1-4}alkyl;

R^{1B} is G^1, -OH, $–NR^{1c}–C_{1-4}$alkylene$–G^1$, $–NR^{1c}–C_{1-4}$alkylene$–N(R^{1d})_2$, $–N(R^{1c})CHR^{1e}CO_2H$, $–N(R^{1c})CH_2CO_2H$, or $–N(R^{1f})–CH_2CH_2–(N(CH_2CO_2H)CH_2CH_2)_n–N(CH_2CO_2H)_2$;

R^{1e} is $–C_{1-4}$alkylene$–CO_2H$;

R^{1f} is hydrogen or $-C_{1-4}$alkylene$–CO_2H$;

G^1 is a 4- to 8-membered monocyclic heterocyclyl containing a first nitrogen and optionally one additional heteroatom selected from nitrogen, oxygen, and sulfur, G^1 being attached at the first nitrogen and is optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, halo, cyano, -OH, $–OC_{1-4}$alkyl, and oxo; and

n is 0, 1, or 2.

26. The conjugate of any one of claims 4-25, or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} is CH_3;

R^{1e} is $–CH_2CO_2H$;

R^{1f} is hydrogen or $-CH_2CO_2H$; and

G^1 is a piperazinyl, morpholinyl, piperidinyl, azepanyl, or pyrrolidinyl, attached through a ring nitrogen atom and optionally substituted with 1-4 substituents independently selected from the group consisting of C_{1-4}alkyl, C_{1-4}haloalkyl, halo, cyano, -OH, $–OC_{1-4}$alkyl, and oxo.

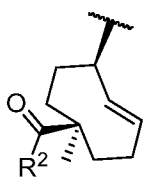
27. The conjugate of any one of claims 4-26, or a pharmaceutically acceptable salt thereof, wherein L^2 is –C(O)–.

28. The conjugate of claim 27, or a pharmaceutically acceptable salt thereof, wherein

R^{1B} is -OH, $N(H)CH_2CO_2H$, $–N(H)CHR^{1e}CO_2H$, $–N(H)–CH_2CH_2–(N(CH_2CO_2H)CH_2CH_2)_n–N(CH_2CO_2H)_2$, or $–N(CH_2CO_2H)–CH_2CH_2–N(CH_2CO_2H)_2$; and

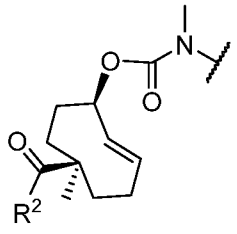
R^{1e} is $–CH_2CO_2H$.

29. The conjugate of any one of claims 1-22, or a pharmaceutically acceptable salt thereof wherein

G, at each occurrence, is independently 

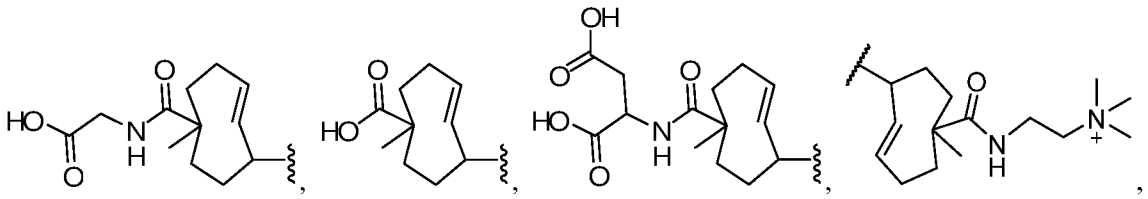
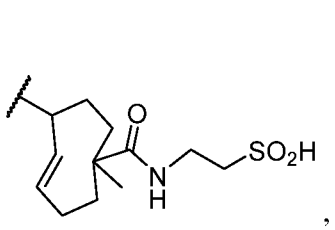
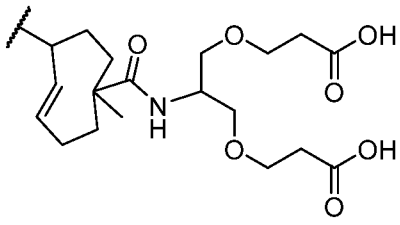
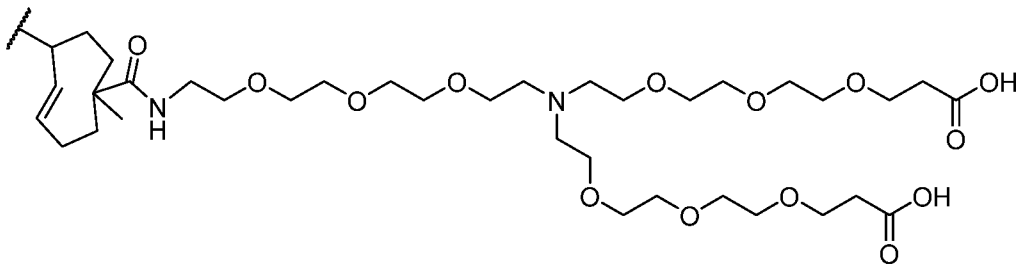
, and R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

30. The conjugate of any one of claims 1-22, or a pharmaceutically acceptable salt thereof, wherein

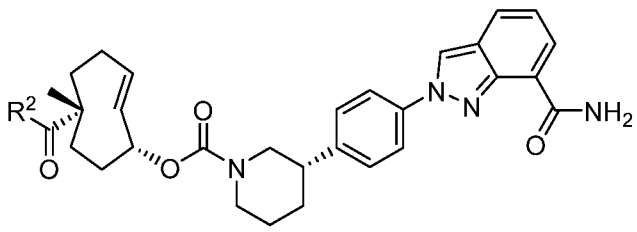
G–L[1], at each occurrence, is independently  , and R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

31. The conjugate of any one of claims 1-30, or a pharmaceutically acceptable salt thereof, wherein m is 1-20, or 1-10, or 1-5.

32. The conjugate of any one of claims 1-30, or a pharmaceutically acceptable salt thereof, wherein m is 1.

33. The conjugate of any one of claims 1-31, or a pharmaceutically acceptable salt thereof, wherein G is  ,  ,  , or  .

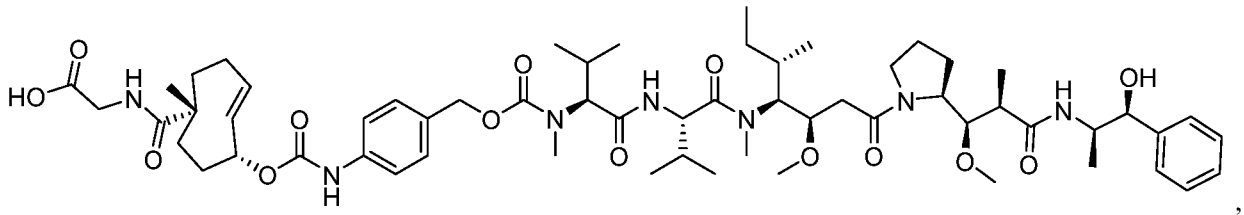
34. A conjugate of Formula IIA, or a pharmaceutically acceptable salt thereof, wherein



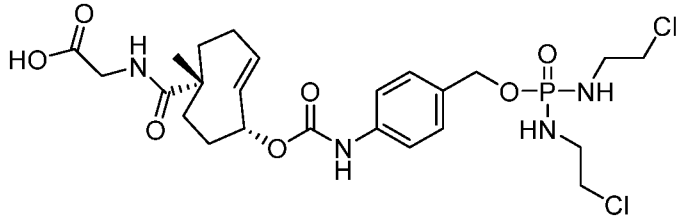
IIA

wherein R^2 is -OH, 2-aminoethanesulfonic acid, an N-linked natural or unnatural amino acid, or an optionally substituted ethylenediamine; wherein R^2 may be optionally further substituted with a polyether.

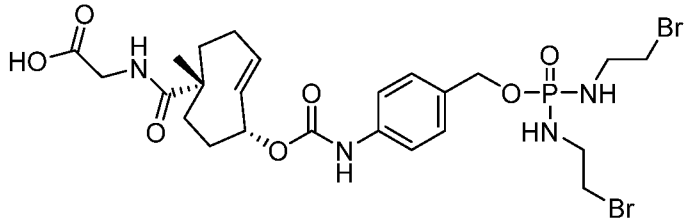
35. A conjugate, or a pharmaceutically acceptable salt thereof, where the conjugate has the structure:



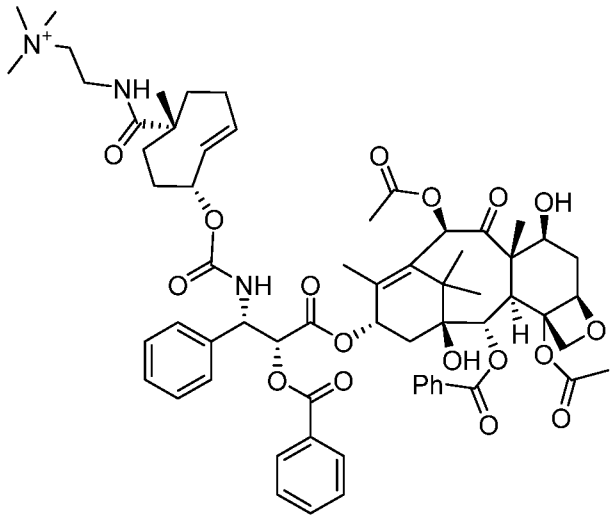
,



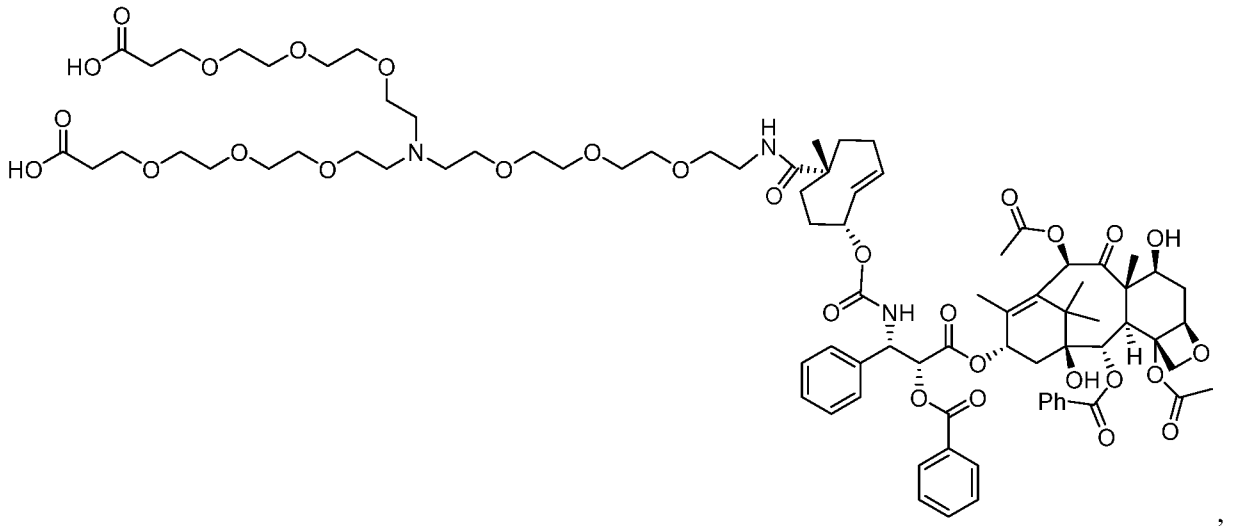
,



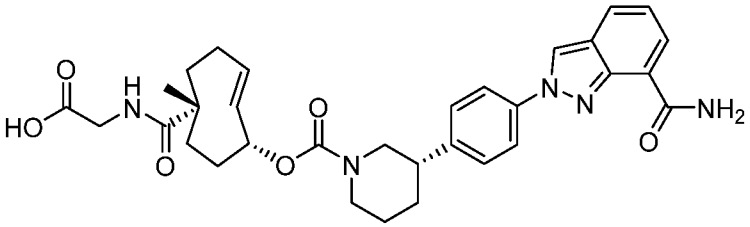
,



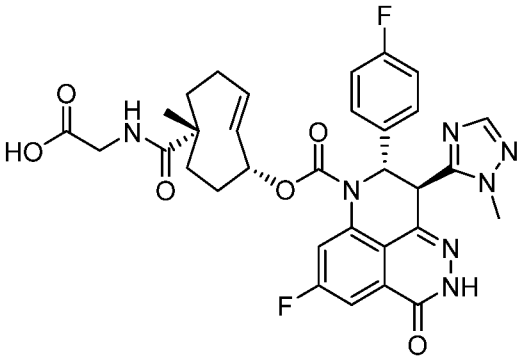
,



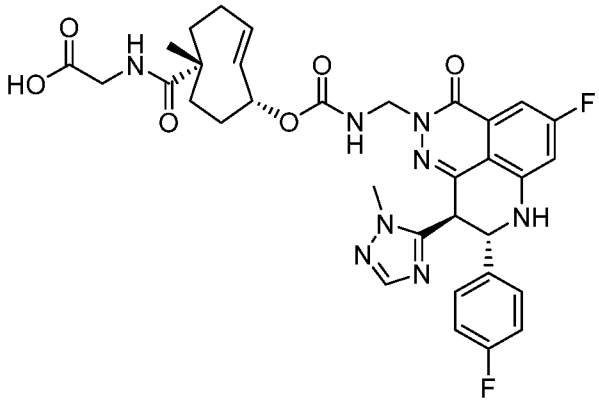
,



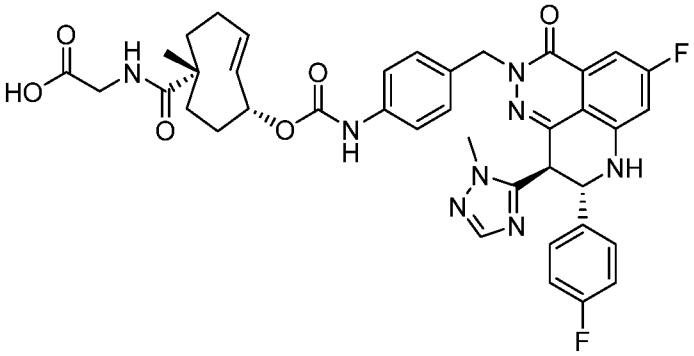
,



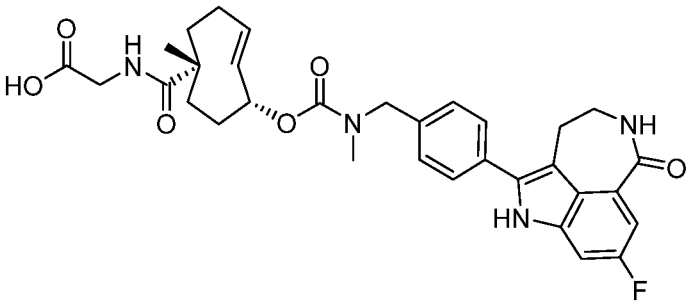
,



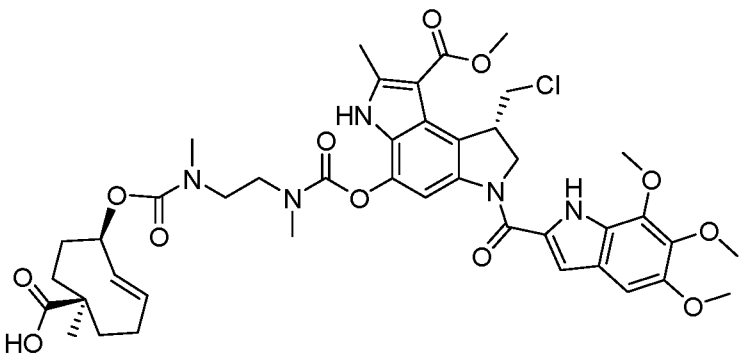
,



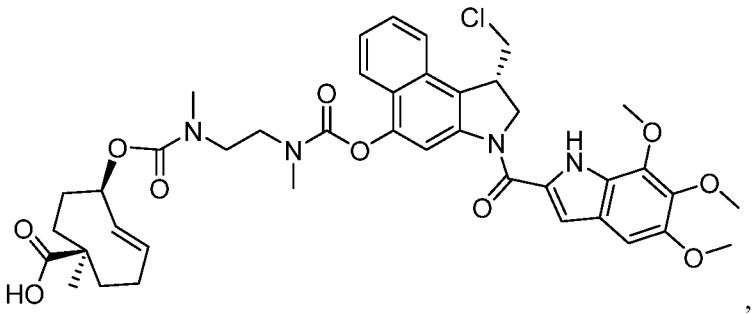
,



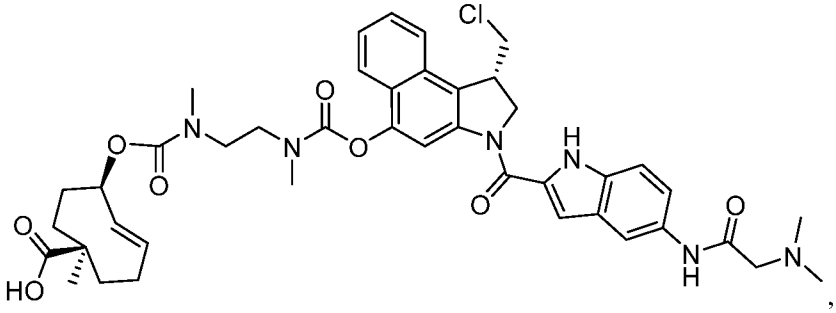
,



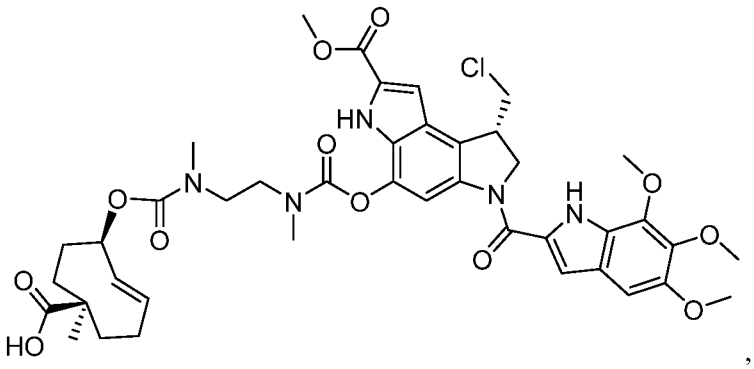
,



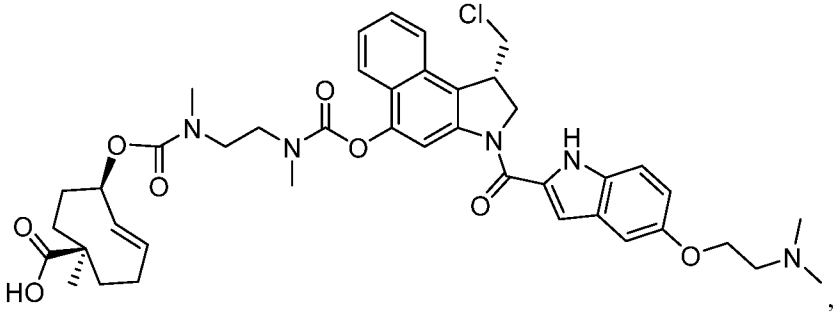
,



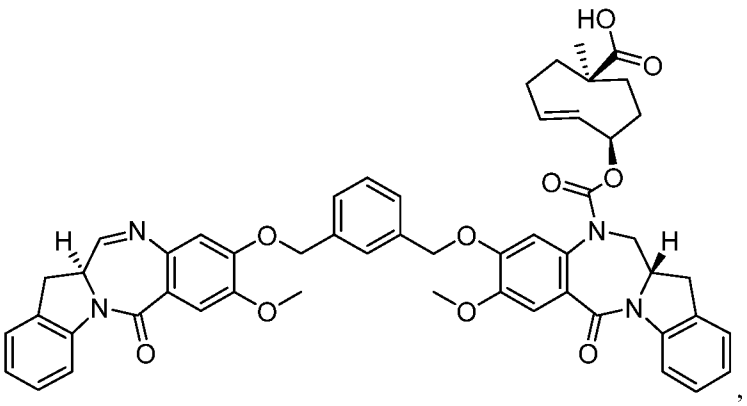
,



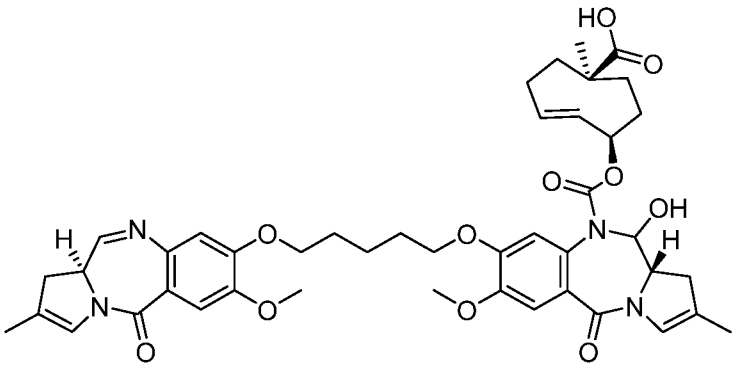
,



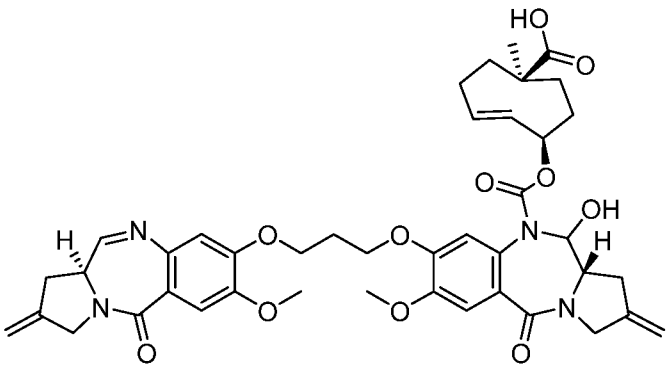
,



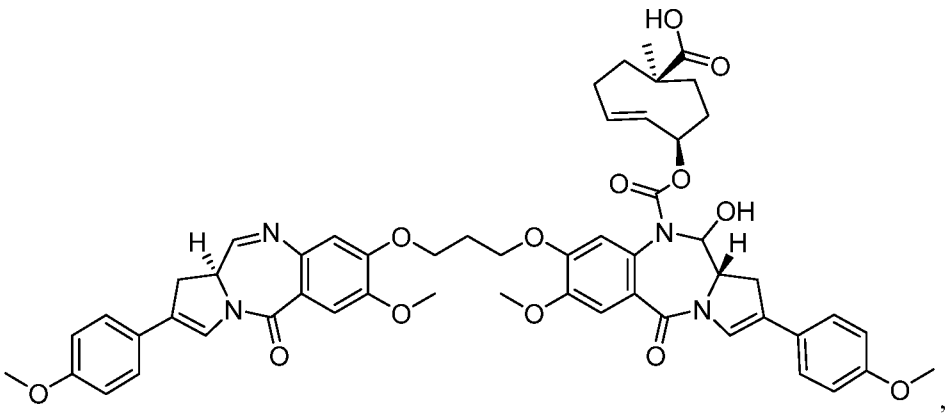
,



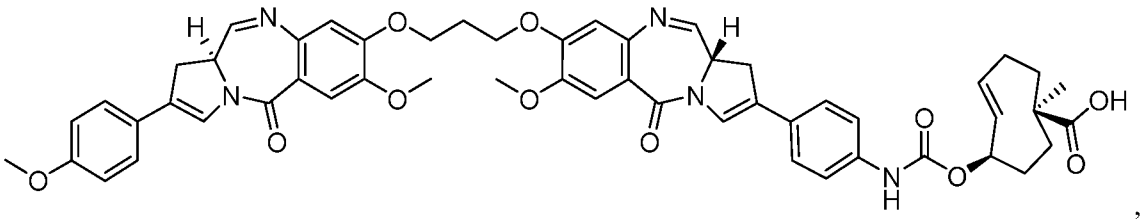
,



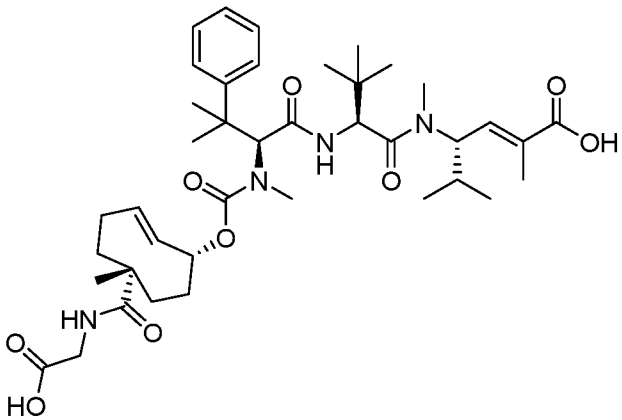
,



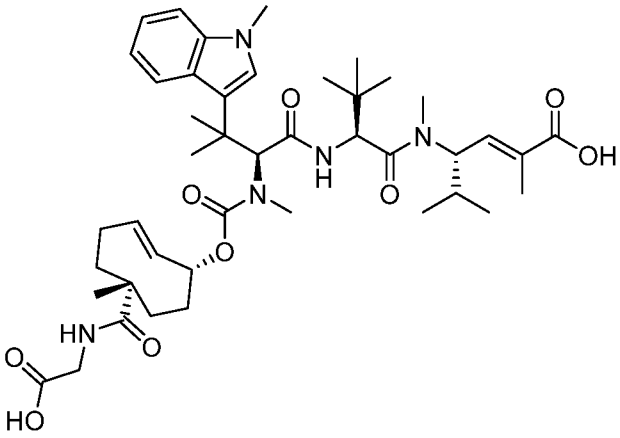
,



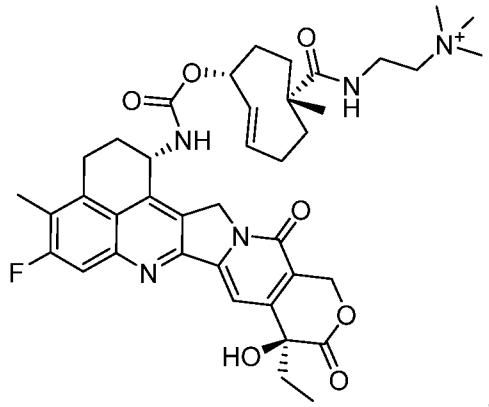
,



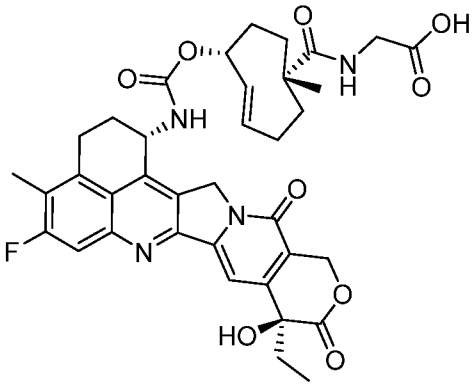
,



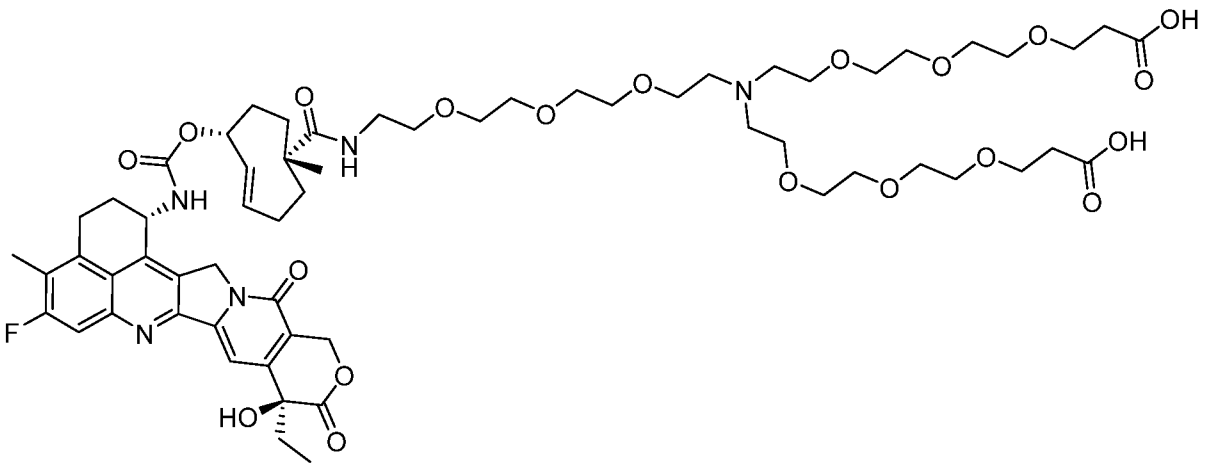
,



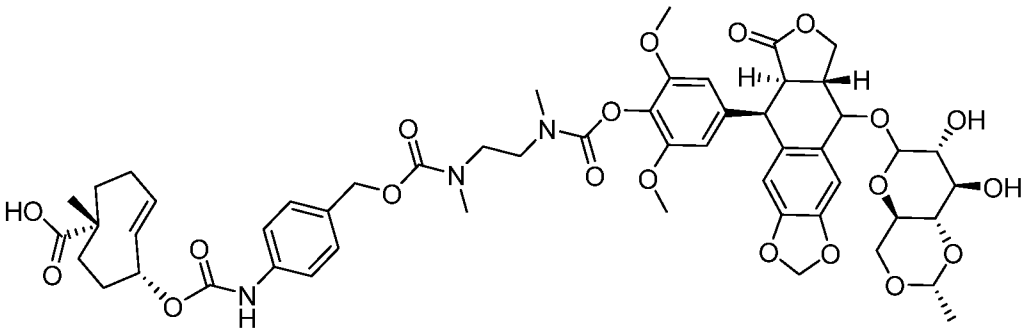
,



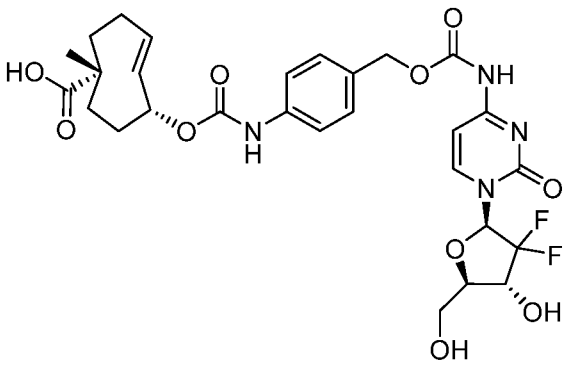
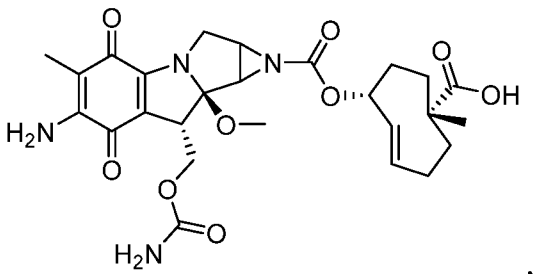
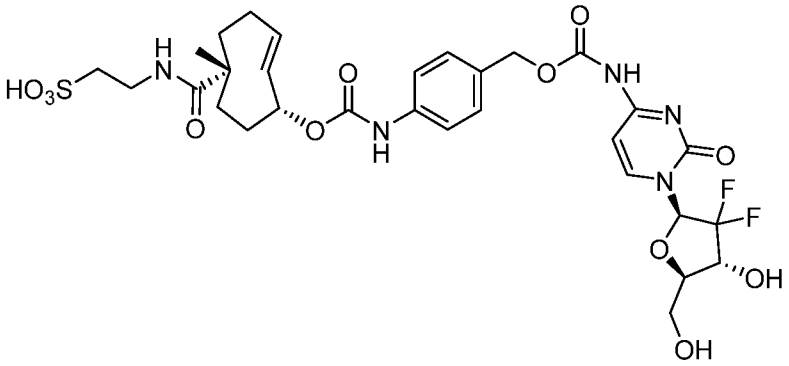
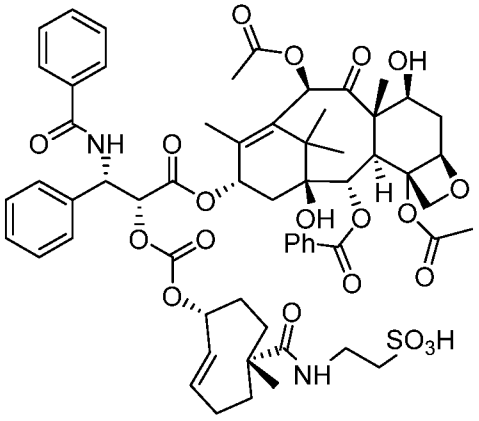
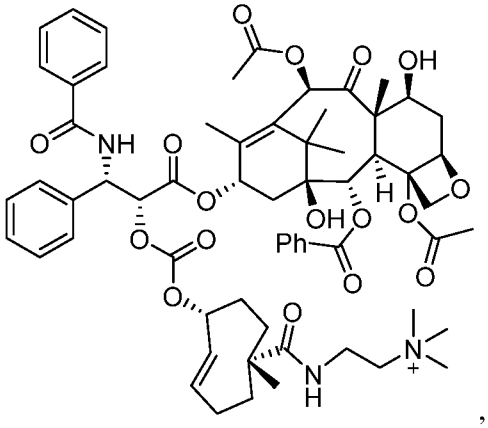
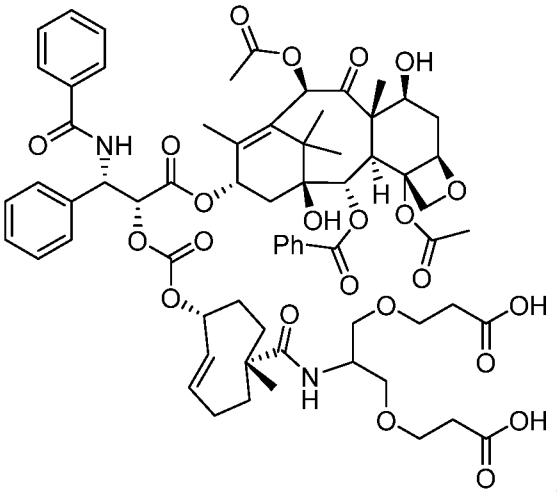
,

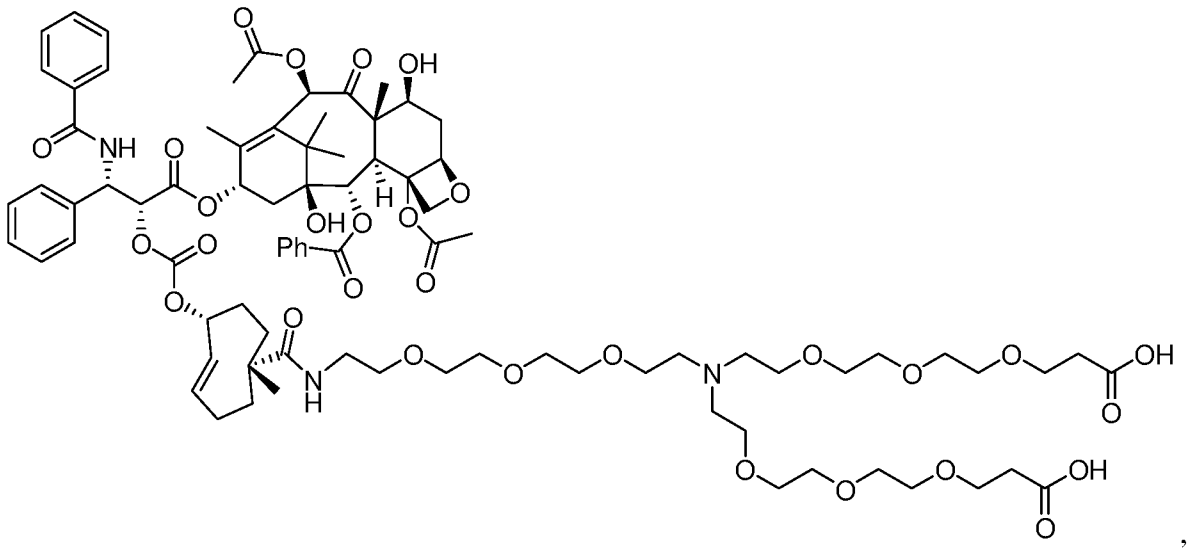


,

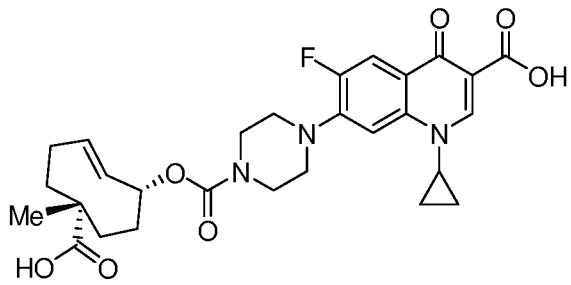


,

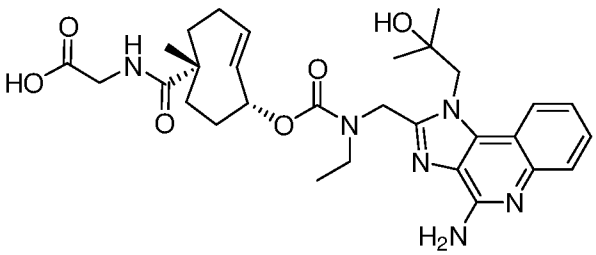

,

,

,

,

,

,



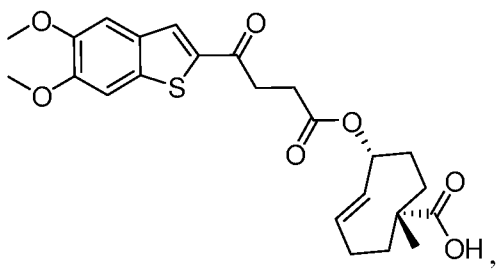
,



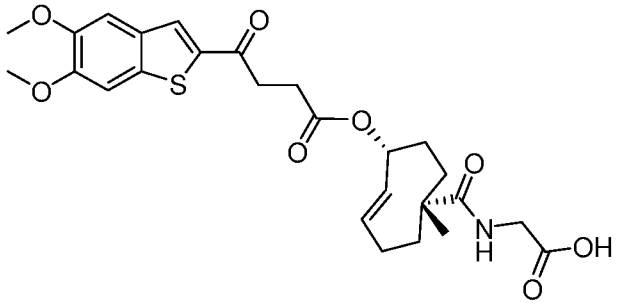
,



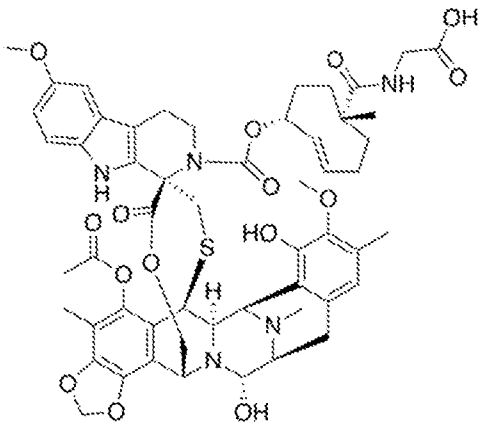
,



,



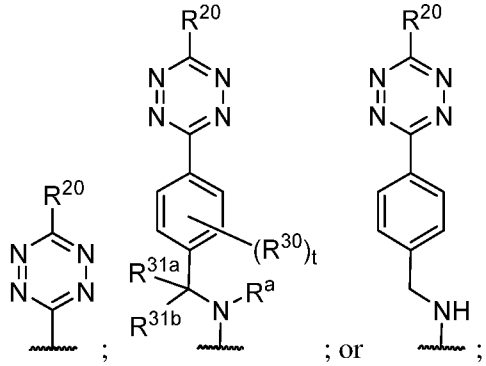
, or



.

36. A pharmaceutical composition comprising the conjugate of any of claims 1-35, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

37. A method of treating cancer, the method comprising administering to a subject in need thereof, a therapeutically effective amount of the conjugate of any of claims 1-35, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 36, and a therapeutic support composition, the therapeutic support composition comprising a biocompatible support and a tetrazine-containing group of formula:



wherein

R^{20} is selected from the group consisting of hydrogen, halogen, cyano, nitro, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, cycloalkenyl, CF_3, CF_2-R', NO_2, OR', SR', C(=O)R', C(=S)R', OC(=O)R"', SC(=O)R"', OC(=S)R"', SC(=S)R"', S(=O)R', $S(=O)_2$R"', $S(=O)_2$NR' R", C(=O)O-R', C(=O)S-R', C(=S)O-R', C(=S)S-R', C(=O)NR'R", C(=S)NR' R", NR'R", NR'C(=O)R", NR'C(=S)R", NR'C(=O)OR", NR'C(=S)OR", NR'C(=O)SR", NR'C(=S)SR", OC(=O)NR'R", SC(=O)NR'R", OC(=S) R'R"', SC(=S)R'R", NR'C(=O)NR"R", and NR'C(=S)NR"R";

R' and R" at each occurrence are independently selected from hydrogen, aryl and alkyl;

R"' at each occurrence is independently selected from aryl and alkyl;

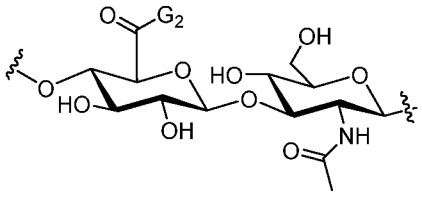
R^{30} is halogen, cyano, nitro, hydroxy, alkyl, haloalkyl; alkenyl, alkynyl, alkoxy; haloalkoxy; heteroalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or cycloalkenyl;

R^a, R^{31a} and R^{31b} are each independently hydrogen, C_1-C_6-alkyl, or C_1-C_6-haloalkyl; and

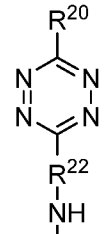
t is 0, 1, 2, 3, or 4.

38. The method of claim 37, wherein the tetrazine-containing group is linked or directly bonded to a hyaluronic acid biocompatible support.

39. The method of claim 37 or 38, wherein the therapeutic support composition comprises substituted hyaluronic acid units of formula (II),



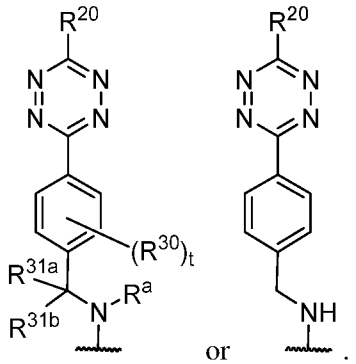
II



wherein G^2 is ; and

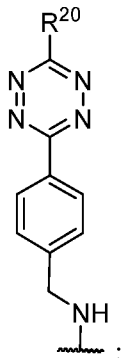
R^{22} is a linker of 1 to 100 linking atoms.

40. The method of claim 39, wherein:



G^2 is or .

41. The method of claim 40, wherein



G^2 is ; and R^{20} is hydrogen or $C_{1\text{-}4}$alkyl.

42. The method of any of claims 37-41, wherein the method is a method of treating cancer.

43. The method of claim 42, wherein the cancer is a melanoma, renal cancer , prostate cancer, ovarian cancer, endometrial carcinoma, breast cancer, glioblastoma, lung cancer, soft tissue sarcoma, fibrosarcoma, osteosarcoma, pancreatic cancer, gastric carcinoma, squamous cell carcinoma of head/neck, anal/vulvar carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, cervical carcinoma, hepatocellular carcinoma, kaposi's sarcoma, Non-Hodgkins lymphoma, Hodgkins lymphoma

Wilm's tumor/neuroblastoma, bladder cancer, thyroid adenocarcinoma, pancreatic neuroendocrine tumors, Prostatic adencocarcinoma, Nasopharyngeal carcinoma, or Cutaneous T-cell lymphoma.

44. The method of claim 42 or 43, wherein the cancer is a solid tumor.

45. The method of claim 42 or 43, wherein the cancer is a soft tissue sarcoma.

46. The method of claim 42, wherein the cancer is a hematological malignancy such as myelodysplastic syndrome, acute myeloid leukaemia, myeldysplastic syndroms, chronic myelogenous luekaemia, chronic myelomonocytic leukaemia, primary myelofibrosis, diffuse large B-cell lymphoma, chronic lymphocytic leukaemia, monoclonal gammopathy, plasma cell myeloma, follicular lymphoma, marginal zone lymphoma, classical Hodgkin lymphoma, monoclonal B-cell lymphocytosis, lymphoproliferative disorder NOS, T-cell lymphoma, precursor B-lymphoblastic leukaemia, mantle cell lymphoma, plasmacytoma, Burkitt lymphoma, T-cell leukaemia, hairy-cell leukaemia, precursor T-lymphobastic leukaemia, or nodular lymphocyte predominiant Hodgkin lymphoma.

47. The method of any of claims 37-41, wherein the method is a method of enhancing or eliciting an immune response.

48. The method of claim 47, wherein the immune response is an increase in one or more of leukocytes, lymphocytes, monocytes, and eosinophils.

49. The method of any of claims 37-48, further comprising administering a therapeutically effective amount of an additional therapeutic agent selected from the group consisting of an anticancer agent, an, or a trans-cyclooctene prodrug thereof.

50. A kit comprising the conjugate of any of claims 1-35, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 36, and instructions for use thereof.

51. The kit of claim 50, further comprising the therapeutic support composition as defined in any of claims 37-41.

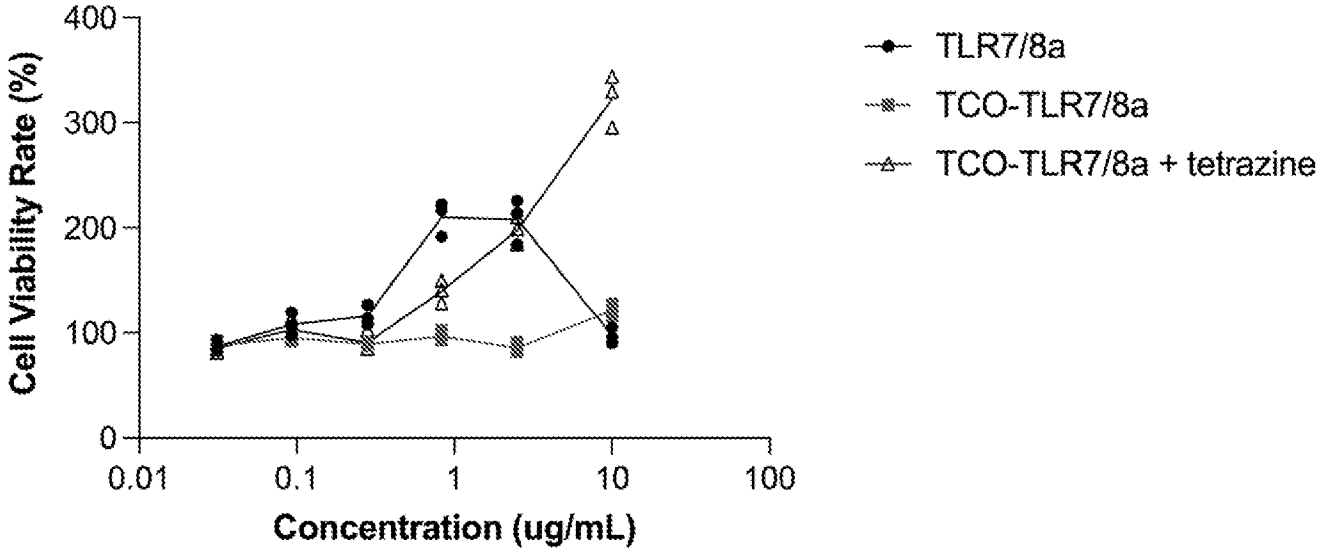

400
300
200
100
0
Cell Viability Rate (%)
0.01
0.1
1
10
100
Concentration (ug/mL)
TLR7/8a
TCO-TLR7/8a
TCO-TLR7/8a + tetrazine

FIG. 1

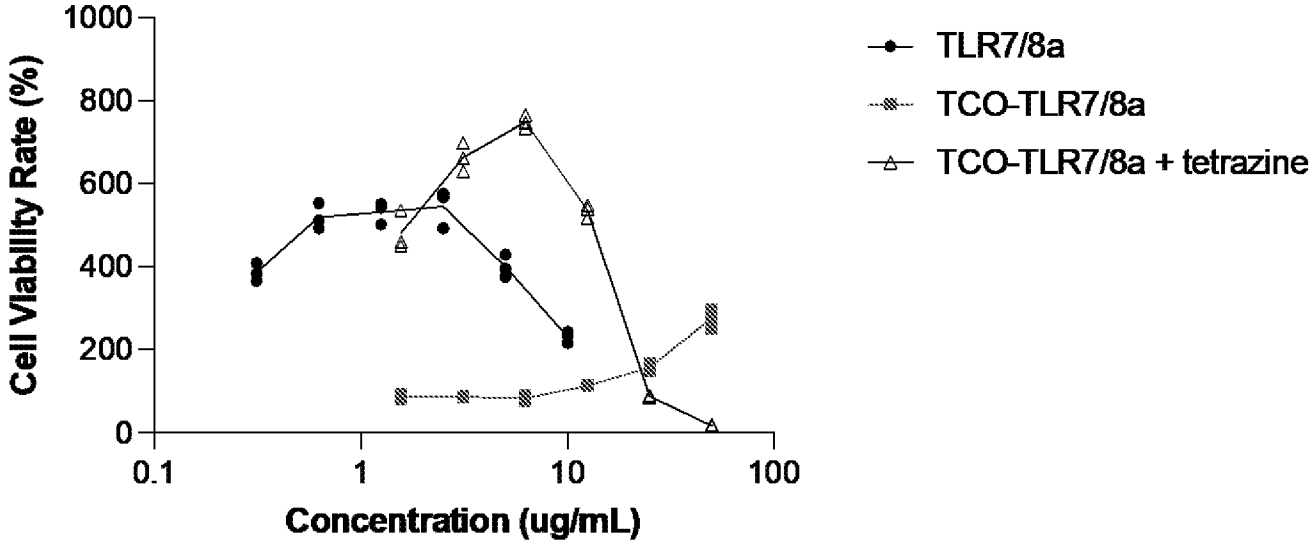

1000
800
600
400
200
0
Cell Viability Rate (%)
0.1
1
10
100
Concentration (ug/mL)
TLR7/8a
TCO-TLR7/8a
TCO-TLR7/8a + tetrazine

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/078991

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/078991

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/54 A61P35/00
ADD.

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)
A61K

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)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/187740 A1 (SHASQI INC [US]) 11 October 2018 (2018-10-11)	1-19, 21-51
Y	paragraphs [00148] - [00150], [00153], [00155], [00156], [00160], [00161] claims 1-16, 19, 21-40 scheme 1 on page 66 paragraphs [00167] - [00170] ----- -/--	20

[x] Further documents are listed in the continuation of Box C.

[x] 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
30 January 2023	09/02/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Birikaki, Lemonia

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YANG KATHERINE S. ET AL: "Bioorthogonal Approach to Identify Unsuspected Drug Targets in Live Cells", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 52, no. 40, 19 August 2013 (2013-08-19), pages 10593-10597, XP055860020, ISSN: 1433-7851, DOI: 10.1002/anie.201304096 whole document and especially Scheme 1 and page 10594, left-handed column	1-5,16, 22,23,36
X	WO 2012/074840 A2 (GEN HOSPITAL CORP [US]; REINER THOMAS [US] ET AL.) 7 June 2012 (2012-06-07) page 3, lines 8-13 compound 7 on page 19	1-5, 16-19, 21,23,36
X	REINER T ET AL: "Harnessing the Bioorthogonal Inverse Electron Demand Diels-Alder Cycloaddition for Pretargeted PET Imaging", JOURNAL OF VISUALIZED EXPERIMENTS, vol. 96, 3 February 2015 (2015-02-03), pages e52335-1, XP008181312, ISSN: 1940-087X, DOI: 10.3791/52335 [retrieved on 2015-02-01] figure 3	1,2,16, 22,36
X	CN 110 496 233 A (ZHONGSHAN HOSPITAL FUDAN UNIV) 26 November 2019 (2019-11-26) paragraph [0019]	1,2,16, 22,36
X	RAFFAELLA ROSSIN ET AL: "Trans -Cyclooctene Tag with Improved Properties for Tumor Pretargeting with the Diels?Alder Reaction", MOLECULAR PHARMACEUTICS, vol. 11, no. 9, 2 September 2014 (2014-09-02), pages 3090-3096, XP055648061, US ISSN: 1543-8384, DOI: 10.1021/mp500275a figure 2	1,2, 16-19,36
X	US 2021/308207 A1 (ROBILLARD MARC STEFAN [NL] ET AL) 7 October 2021 (2021-10-07) paragraphs [0122], [0123], [0129] page 39	1-13, 16-19, 21,22,36

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YAMAZAKI CHISATO M. ET AL: "Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance", NATURE COMMUNICATIONS, vol. 12, no. 1, 10 June 2021 (2021-06-10), XP055940303, DOI: 10.1038/s41467-021-23793-7 Retrieved from the Internet: URL:https://www.nature.com/articles/s41467-021-23793-7.pdf> figure 1	20

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/078991

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018187740 A1	11-10-2018	AU 2018250312 A1	31-10-2019
		CN 111093708 A	01-05-2020
		EP 3606559 A1	12-02-2020
		JP 2020516621 A	11-06-2020
		US 2021128733 A1	06-05-2021
		US 2022168426 A1	02-06-2022
		WO 2018187740 A1	11-10-2018
WO 2012074840 A2	07-06-2012	US 2013309170 A1	21-11-2013
		US 2018015186 A1	18-01-2018
		US 2019201562 A1	04-07-2019
		US 2021038748 A1	11-02-2021
		WO 2012074840 A2	07-06-2012
CN 110496233 A	26-11-2019	NONE	
US 2021308207 A1	07-10-2021	AU 2019262521 A1	07-01-2021
		CA 3099421 A1	07-11-2019
		EP 3788032 A1	10-03-2021
		US 2021308207 A1	07-10-2021
		WO 2019212357 A1	07-11-2019