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(54) **Title**: AURISTATIN ANALOGUES AND THEIR CONJUGATES WITH CELL-BINDING MOLECULES

(57) **Abstract**: This invention relates to analogues of auristatins, in particular monomethyl auristatin F (MMAF), as cytotoxic agents, conjugates of such cytotoxic agents with a cell-binding agent, the preparation and the therapeutic uses of these cytotoxic agents and conjugates thereof to arrest or retard abnormal cell growth and /or proliferation.

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AURISTATIN ANALOGUES AND THEIR CONJUGATES WITH CELL-BINDING MOLECULES

FIELD OF THE INVENTION

This invention relates to analogues of auristatins, in particular monomethyl auristatin F (MMAF), as cytotoxic agents, conjugates of such cytotoxic agents, and methods of using the conjugates thereof to arrest or retard cell growth and /or proliferation.

BACKGROUND OF THE INVENTION

Traditionally, chemotherapy is the predominant strategy for cancer treatment. The use of chemotherapeutic agents for the treatment of tumors is based on the premise that these agents would preferentially kill rapidly dividing cancer cells, while sparing normal cells. However, the lack of selectivity and the resulting systemic toxicity to the host remains a major drawback of chemotherapeutic agents.

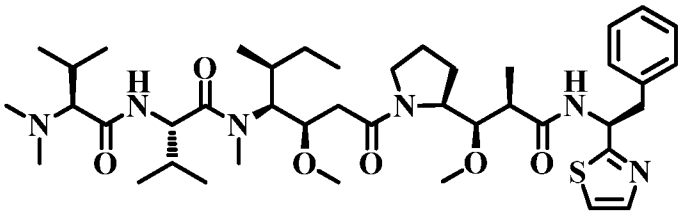
In order to improve the therapeutic activity of anticancer drugs, either the potency of the cytotoxic agents had to be improved, in order to achieve meaningful therapeutic effect with lower doses, while not causing serious adverse reactions to the patients; or the tumor selectivity had to be improved, by which means, the drugs could be administered at higher dose, thus providing greater therapeutic benefits.

Many industrious endeavors were undertaken aimed to discover new cytotoxic compounds with higher potency. A good part of such efforts was focused on those isolated from natural sources, such as plants, marine or microbial organisms. In 1984, Pettit and co-workers isolated a family of cytotoxic peptides, named the dolastatins, from sea hare, *dolabella auricularia*. In the following years, the structure of the most potent compound, dolastatin 10 was elucidated (Pettit, et al. J. Am. Chem. Soc. 1987, 109, 6883-6885; Pettit, et al. J. Am. Chem. Soc. 1989, 111: 5463-5465). Dolastatin 10 inhibits tubulin polymerization and exhibits powerful antiproliferative properties, with IC50 in the range of picomolar. Dolastatin 10 was rapidly advanced into human clinical evaluation. However, in phase I clinical studies, it was found that the maximum tolerated dose (0.4 mg m-2) was lower than that of anticancer drugs, such as doxorubicin (60-70 mg m-2). Phase II clinical trials also failed to demonstrate significant clinical benefit in a number of cancer indications, such as melanoma, renal cancer, sarcomas, breast cancer and prostate cancer (Pettit et al., Anticancer Drug Des., 1998, 13: 243-277; Poncet et al., Curr. Pharm. Des. 1999, 5: 139-162; Ali et al., Anticancer Res. 1998, 18: 1021-1026; Kalemkarian et al., Pharmacol.

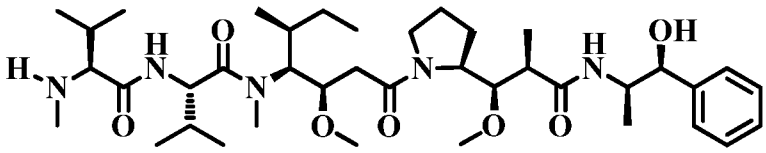
1999, 43: 507-515; Turner et al., Prostate 1998, 34: 175-181; Pitot, et al., Clin. Cancer Res. 1999, 5: 525-531; Vaishampayan et al. Clin. Cancer Res. 2000, 6: 4205-4208).

In the 1980s, the concept of antibody-drug conjugates (ADCs) emerged as a means to improve the tumor selectivity of cytotoxic drugs. Many antibodies bind preferentially to specific molecular markers, antigens on the cell surface, therefore could serves as a vehicle for selective delivery of the anticancer drug to the tumor. One important requirement of payload drugs used in ADCs is high potency. One factor that attributed to the failure of early ADCs, all of which used existing anticancer drugs, was their unsufficient in vitro potency. The unusually high cytotoxic potency of dolastatin 10 makes it an attractive candidate as payload for ADCs although it failed to display meaningful therapeutic activity by itself.

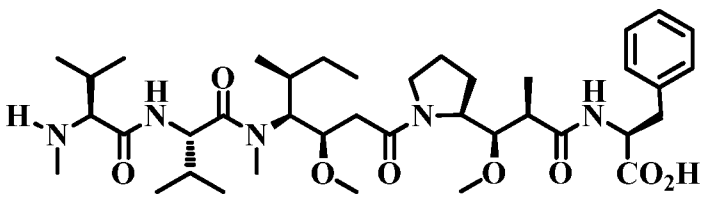
Extensive SAR studies based on dolastatin 10 was conducted. It was found that the C-terminal dolaphenine residue of dolastatin 10 could be replaced by phenethylamine and the N-terminal tertiary amine of dolastatin could be replaced by a primary or secondary amine without loss of potency (Pettit et al., Anticancer Drug Des., 1998, 13: 243-277; Doronina et al. Bioconjugate Chem. 2006, 17: 114-124). Monomethylauristatin E (MMAE) and monomethylauristatin F (MMAF) were then prepared for use as payloads for ADCs.



Dolastatin 10



Auristatin E (MMAE)



Auristatin F (MMAF)

In 2011, FDA approved the use of brentuximabvedotin (trade name Adecetris) in relapsed Hodgkin lymphoma (HL) and relapsed systemic anaplastic large cell lymphoma (sALCL). Brentuximabvedotin consists of the chimeric monoclonal antibody brentuximab (cAC10, which targets the cell-membrane protein CD30) linked to cathepsin cleavable linker (valine-citrulline), para-aminobenzylcarbamate spacer and three to five units of

MMAE. This approval has provided proof-of-concept for ADC approach of cancer therapies, and also testified the feasibility of the use of auristatin analogues, such as MMAE as a payload, in ADC drugs.

MMAF is less cytotoxic in vitro than MMAE; however the methyl ester derivative, MMAF-OMe is approximately 100 times more cytotoxic than MMAE on most of cell lines tested. It is believed that the C-terminal charge of MMAF impairs its intracellular access, thus attenuated its cytotoxicity. Its neutral derivative MMAF-OMe, on the other hand, can diffuse into cells where esterases in the plasma convert it to MMAF and display great cytotoxicity (Doronina et al. Bioconjugate Chem. 2006, 17: 114-124). Another advantage of using MMAF-OMe is that once it is hydrolyzed, the resultant MMAF is trapped in the cell, due to its more hydrophilicity, thus lower ability of passive intracellular traversing, resulting in reduced systemic toxicity.

We have worked on the modification of the γ- amino acids at the C-terminus of tubulysins (tubutyrosine (Tut) or tubuphenylalanine (Tup)), in order to improve their properties used as payloads for ADCs (PCT/IB2012/053554, WO2014009774). The incorporations of an epoxyl or ethylene group on Tut and Tup components dramatically increased the therapeutical windows of the antibody-tubulysin analog conjugates (Med Chem. #44, 249th ACS National Meeting, Dever, CO, Mar. 22~26, 2015; AACR national meeting, #4532, Philadelphia, Apr 18~22, 2015). Using the similar strategy, we have designed ADC payloads based on the structure of MMAF or MMAF-OMe and modified the side chains of the C- and N-terminal residues. These modified MMAF compounds demonstrated good potency against variety of cancer cells and were readily used for conjugation with antibodies, without any significant aggregation problem. Here this patent application disclosed these modified compounds and their use as payloads for cell-binding molecules conjugation.

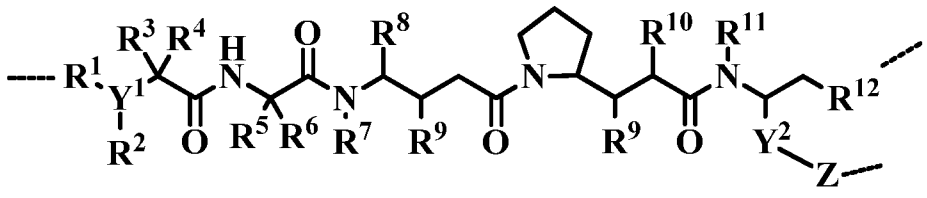
SUMMARY OF THE INVENTION

In one aspect, the present invention provides Drug-Linker-Ligand conjugates having the Formula (I):

G-Lm-Dn (I)

and pharmaceutical acceptable salts and solvates thereof;

wherein G is a cell surface binding molecule (ligand); L is a cleavable or uncleavable linker; n is 1-20; m is 1-10; D is a drug molecule having the Formula (II):



(II)

wherein --- is a linkage that connect a drug molecule to a linker independently; R^1, R^2, R^3, and R^4 are independently C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, alkylcarbonyl; or two R's, such as R^1R^2, R^1R^3, R^2R^3, R^3R^4, and R^5R^6 can be 3-7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; In addition, R^2, R^3, and R^4 can be H, and R^1 can be absent;

Y^1 is N or CH;

R^5 and R^6 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; or R^5 and R^6 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^7 is H, or C_1-C_8 alkyl;

R^8 is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

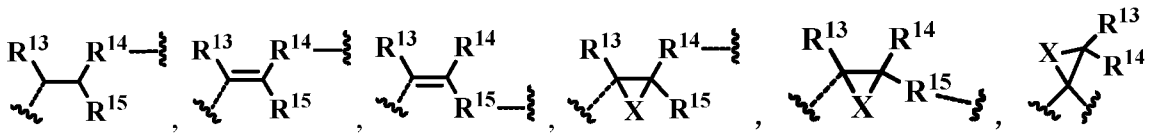
R^9 is H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle or O-(C_1-C_8 alkyl);

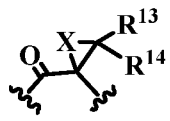
R^{10} is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

R^{11} is H, or C_1-C_8 alkyl;

R^{12} is aryl, alkylaryl, alkyl or C_3-C_8 heterocyles;

Y^2 is selected from:



or  ; wherein R^{13}, R^{14} and R^{15} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; and X is CH_2, O, S, NH, NR^{16}, S(O) or $S(O)_2$, wherein R^{16} is H, or C_1-C_8 alkyl;

Z is H, C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -NO_2, -CN, -COOH, -C(O)OR^{17}, -C(O)NHR^{18}, C(O)$NR^{18}R^{19}$, -CH_2R^{20}, -$CH_2OP(O)(OR^{21})_2$, wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle.

In another aspect, the linker L has the formula of $\mathbf{—W_w—(Aa)_r—V_v—}$, wherein: -W- is a stretcher unit, which links a targeted binding molecular unit (T) to an amino acid unit (Aa) when presents, or links V when an Aa is not present, and W may independently contain a self-immolative spacer, a peptidyl unit, a hydrazone, a disulfide, a thioether, an ester, or an amide bond; w is 0 or 1; wherein Aa is independently a natural or unnatural amino acid unit; r is independently an integer ranging from 0 to 12; $(Aa)_r$ represents a natural or unnatural amino acid, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit; wherein V is a spacer unit and is independently H, O, NH, S, C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, hetero-cyclic, carbocyclic; C_3-C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl, or one to four amino acid units, or $(CH_2CH_2O)_r$, r is an integer ranging from 0 to 12; and v is 0, 1 or 2.

In still another aspect, the cell surface binding molecule G may be of any kind presently known, or which becomes known, cell-binding molecule, such as a peptide and non-peptide. Generally the binding molecule G is an antibody; a single chain antibody; an antibody fragment that binds to the target cell; a monoclonal antibody; a single chain monoclonal antibody; or a monoclonal antibody fragment that binds the target cell; a chimeric antibody; a chimeric antibody fragment that binds to the target cell; a domain antibody; a domain antibody fragment that binds to the target cell; adnectins that mimic antibodies; DARPins; a lymphokine; a hormone; a vitamin; a growth factor; a colony stimulating factor; or a nutrient-transport molecule (a transferrin); a binding peptide, or protein, or antibody, or small molecule attached on albumin, polymers, dendrimers, liposomes, nanoparticles, vesicles, or (viral) capsids. Preferably the binding molecule G is a monoclonal antibody.

In yet another aspect, a compound of Formula (I) or a pharmaceutically acceptable salt or solvate thereof is used for treating cancers, autoimmune diseases or infectious diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Scheme 1 shows the synthesis of Dap unit ((2*R*,3*R*,4*S*)-dolaproine) of MMAF.

Scheme 2 shows the synthesis of Dil unit ((3*R*,4*S*,5*S*)-dolaisoleucine) of MMAF.

Scheme 3a and **3b** show the synthesis of a Drug-Linker compound that contains a maleimide unit, linking to MMAF-OMe.

Scheme 4 shows the synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

Scheme 5 shows the synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

Scheme 6 shows the synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

Scheme 7a and **7b** show the synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

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

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

"Carbocycle" refers to a saturated or unsaturated ring having 3 to 8 carbon atoms as a monocycle or 7 to 13 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring

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

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

"Alkenyl" refers to an aliphatic hydrocarbon group containing a carbon-carbon double bond which may be straight or branched having 2 to 8 carbon atoms in the chain. Exemplary alkenyl groups include ethenyl, propenyl, n-butenyl, i-butenyl, 3-methylbut-2-enyl, n-pentenyl, hexylenyl, heptenyl, octenyl.

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

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

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

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a

parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene, propargyl and 4-pentynyl.

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

"Heterocycle" refers to a ring system in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group of O, N, S, Se, and P. Preferable heteroatoms are O, N and S. Heterocycles are also described in *The Handbook of Chemistry and Physics*, 78th Edition, CRC Press, Inc., 1997-1998, p. 225 to 226, the disclosure of which is hereby incorporated by reference. Preferred nonaromatic heterocyclic include, but are not limited to epoxy, aziridinyl, thiiranyl, pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxiranyl, tetrahydrofuranyl, dioxolanyl, tetrahydropyranyl, dioxanyl, dioxolanyl, piperidyl, piperazinyl, morpholinyl, pyranyl, imidazolinyl, pyrrolinyl, pyrazolinyl, thiazolidinyl, tetrahydrothiopyranyl, dithianyl, thiomorpholinyl, dihydropyranyl, tetrahydropyranyl, dihydropyranyl, tetrahydropyridyl, dihydropyridyl, tetrahydropyrinidinyl, dihydrothiopyranyl, azepanyl, as well as the fused systems resulting from the condensation with a phenyl group.

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

"Alkyl", "cycloalkyl", "alkenyl", "alkynyl", "aryl", "heteroaryl", "heterocyclic" and the like refer also to the corresponding "alkylene", "cycloalkylene", "alkenylene", "alkynylene", "arylene", "heteroarylene", "heterocyclene" and the likes which are formed

by the removal of two hydrogen atoms.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl and the like.

Examples of a "hydroxyl protecting group" include, but are not limited to, methoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ether, benzyl ether, *p*-methoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, triisopropylsilyl ether, *t*-butyldimethylsilyl ether, triphenylmethylsilyl ether, acetate ester, substituted acetate esters, pivaloate, benzoate, methanesulfonate and *p*-toluenesulfonate.

"Leaving group" refers to a functional group that can be substituted by another functional group. Such leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, and iodide), methanesulfonyl (mesyl), *p*-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

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

"Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and

compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate.

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

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

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

2. Drug-Linker-Ligand conjugates

A drug-linker-ligand conjugate of the present invention is designed so that upon delivery of the conjugate to a cell, the conjugate will partially degrade to release the drug or perhaps with some residue from the linker attached to the ligand. The drug (or drug-linker residue) will then act in a cytotoxic manner to kill the tumor cell. The drug compound is of the dolastatin/auristatin type, which has been shown to interfere with microtubule

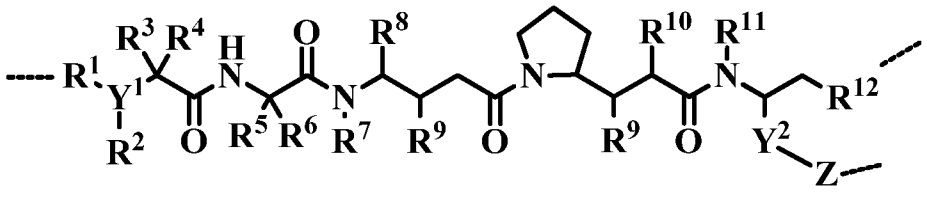
dynamics, GTP hydrolysis, and/or nuclear and cellular division and have anticancer and/or antifungal activity.

In one aspect, the present invention provides Drug-Linker-Ligand conjugates having the Formula (I):

$$\mathbf{G\text{-}L_m\text{-}D_n} \qquad (I)$$

and pharmaceutical acceptable salts and solvates thereof;

wherein G is a cell surface binding molecule (ligand); L is a cleavable or uncleavable linker; n is 1-20; m is 1-10; D is a drug molecule having the Formula (II):



(II)

Wherein --- is a linkage that connect a drug molecule to a linker independently; R^1, R^2, R^3, and R^4 are independently C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, alkylcarbonyl; or two R's, such as R^1R^2, R^1R^3, R^2R^3, R^3R^4, and R^5R^6 can be 3-7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; In addition, R^2, R^3, and R^4 can be H, and R^1 can be absent;

Y^1 is N or CH;

R^5 and R^6 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; or R^5 and R^6 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^7 is H, or C_1-C_8 alkyl;

R^8 is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

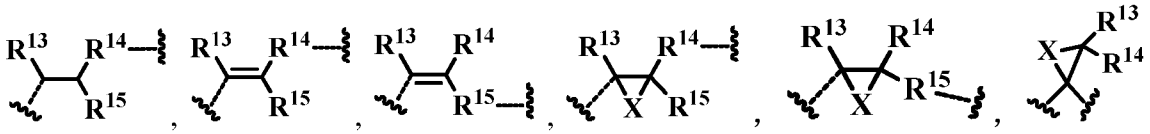
R^9 is H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle or O-(C_1-C_8 alkyl);

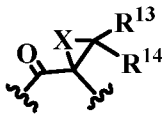
R^{10} is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

R^{11} is H, or C_1-C_8 alkyl;

R^{12} is aryl, alkylaryl, alkyl or C_3-C_8 heterocyles;

Y^2 is selected from:



or  wherein R^{13}, R^{14} and R^{15} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; and X is CH_2, O, S, NH, NR^{16}, S(O) or $S(O)_2$, wherein R^{16} is H, or C_1-C_8 alkyl; In addition, R_{14} can be absent.

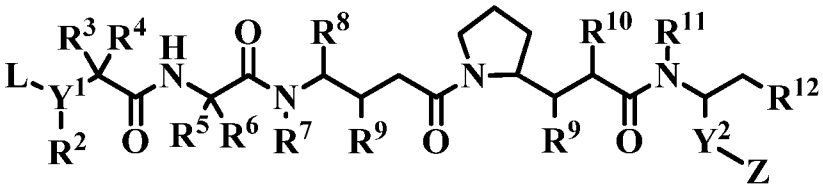
Z is H, C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, $-NO_2$, -CN, -COOH, $-C(O)OR^{17}$, $-C(O)NHR^{18}$, $C(O)NR^{18}R^{19}$, $-CH_2R^{20}$, $-CH_2OP(O)(OR^{21})_2$, wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle.

The linker L may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), *p*-aminobenzyloxy-carbonyl (PAB), 4-thiopentanoate (SPP), 4-(N-maleimidomethyl)-cyclohexane-1 carboxylate (MCC), (4-acetyl)aminobenzoate (SIAB), 4-thio-butyrate (SPDB), 4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB), ethyleneoxy (-CH_2CH_2O-)as one or more repeating units (EO or PEO), or a peptide unit, such as valine-citrulline (val-cit or vc), alanine-phenylalanine (ala-phe or af). Additional linker L components are known in the art, and it may be combined in all chemically relevant ways, such as forming alkylene, alkenylene, and alkynylene, ethers, polyoxyalkylene, esters, amines, imines, polyamines, hydrazines, hydrazones, amides, ureas, semicarbazides, carbazides, alkoxyamines, alkoxylamines, urethanes, amino acids, acyloxylamines, hydroxamic acids, and many others. In addition, it is to be understood that the atoms forming the linker (L) may be either saturated or unsaturated, or may be radicals, or may be cyclized upon each other to form divalent cyclic structures, including cyclo alkanes, cyclic ethers, cyclic amines, arylenes, heteroarylenes, and the like in the linker.

In another aspect, the linker L may have the formula of $\mathbf{—W_w—(Aa)_r—V_v—}$, wherein -W- is a stretcher unit, which links a targeted binding molecular unit (T) to an amino acid unit (Aa) when presents, or links V when an Aa is not present, and W may independently contain a self-immolative spacer, a peptidyl unit, a hydrazone, a disulfide, a thioether, an ester, or an amide bond; w is 0 or 1; wherein Aa is independently a natural or unnatural amino acid unit; r is independently an integer ranging from 0 to 12; $(Aa)_r$

represents a natural or unnatural amino acid, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit; wherein V is a spacer unit and is independently H, O, NH, S, C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, hetero-cyclic, carbocyclic; C_3-C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl, or one to four amino acid units, or $(CH_2CH_2O)_r$, r is an integer ranging from 0 to 12; and v is 0, 1 or 2.

In one aspect, a linker-cytotoxic agent (L_m-D_n) component for the conjugate of the Formula (I) is of the following Formula (III):



(III)

and pharmaceutical acceptable salts and solvates thereof,

Wherein L is a cleavable or uncleavable linker;

Y^1 is N or CH; R^2 is H, or C_1-C_8 alkyl;

R^3 and R^4 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle ; or R^3 and R^4 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^5 and R^6 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle ; or R^5 and R^6 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^7 is H, or C_1-C_8 alkyl;

R^8 is H, H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

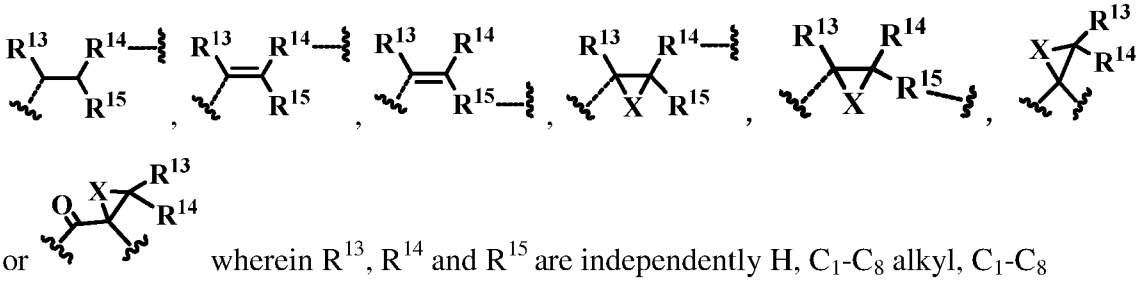
R^9 is H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle or O-(C_1-C_8 alkyl);

R^{10} is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

R^{11} is H, or C_1-C_8 alkyl;

R^{12} is aryl, alkylaryl, alkyl, or C_3-C_8 heterocyles;

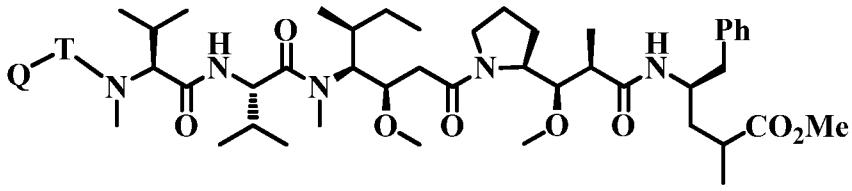
Y^2 is selected from:



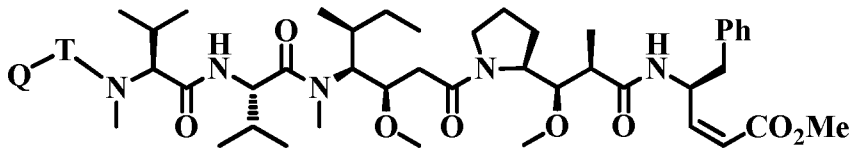
or wherein R^{13}, R^{14} and R^{15} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; and X is CH_2, O, S, NH, NR^{16}, S(O) or $S(O)_2$, wherein R^{16} is H, or C_1-C_8 alkyl; In addition, R^{14} can be absent.

Z is H, C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, $-NO_2$, -CN, -COOH, $-C(O)OR^{17}$, $-C(O)NHR^{18}$, $C(O)NR^{18}R^{19}$, $-CH_2R^{20}$, $-CH_2OP(O)(OR^{21})_2$, wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle.

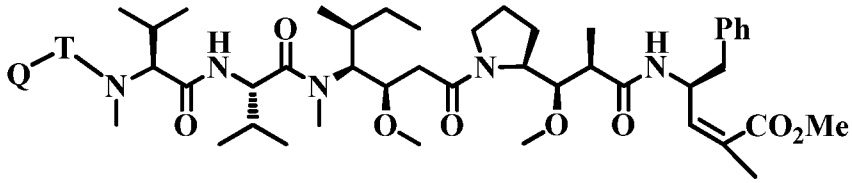
Examples of structures of the linker-cytotoxic agent (L_m-D_n) and their use in conjugation with a cell-binding molecule having the structure of Formula (III) include, but are not limited to, the following:



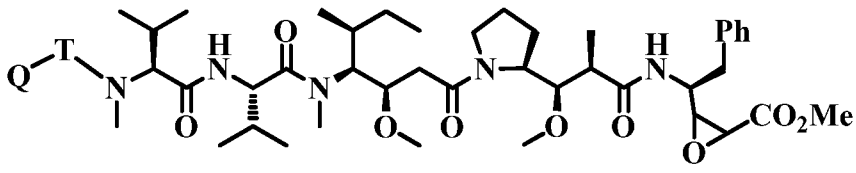
IIIa



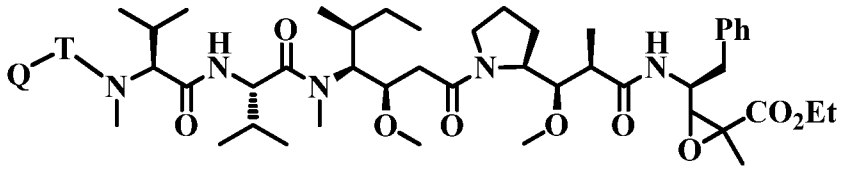
IIIb



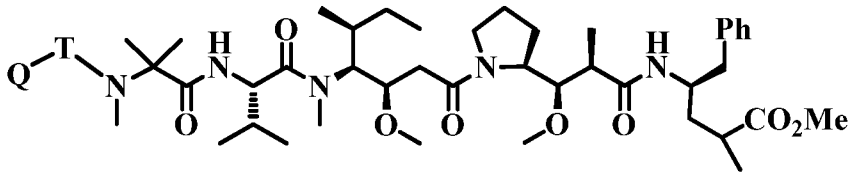
IIIc

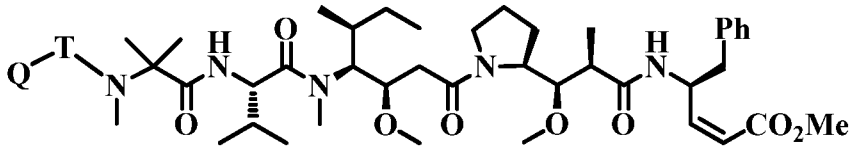


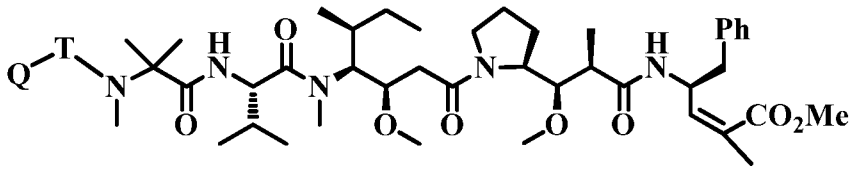
IIId

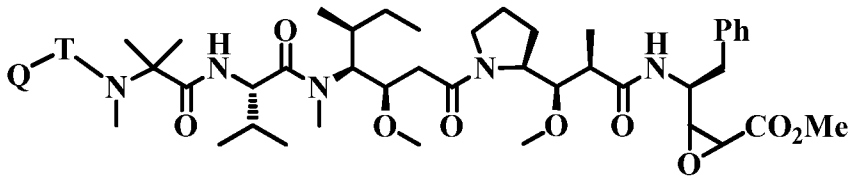


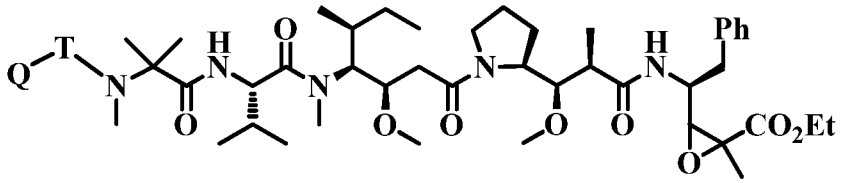
IIIe

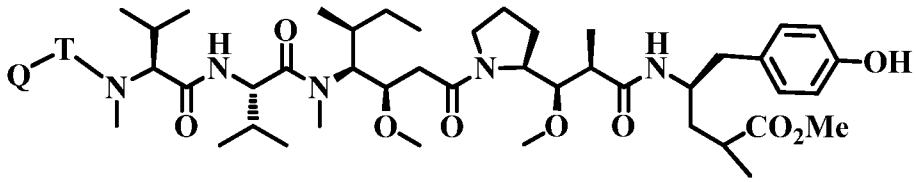

IIIf

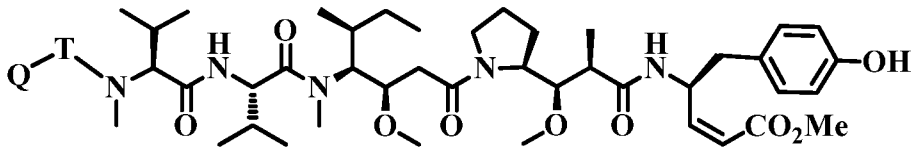

IIIg

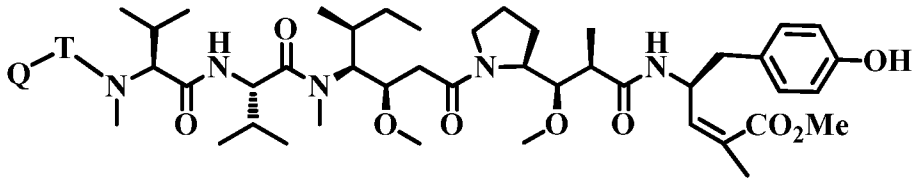

IIIh

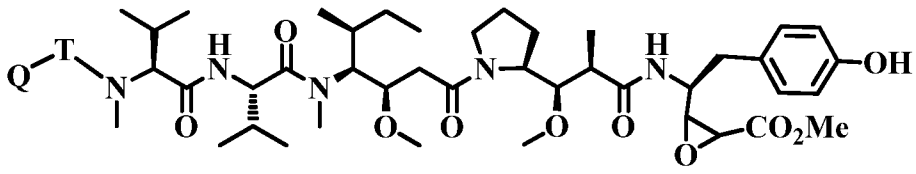

IIIi

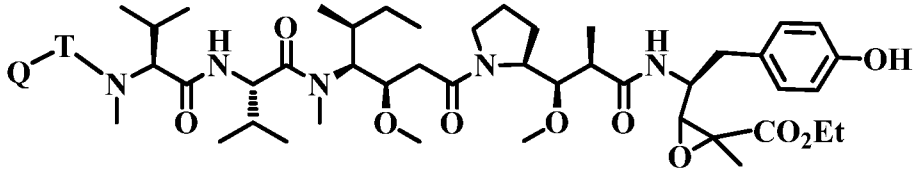

IIIj

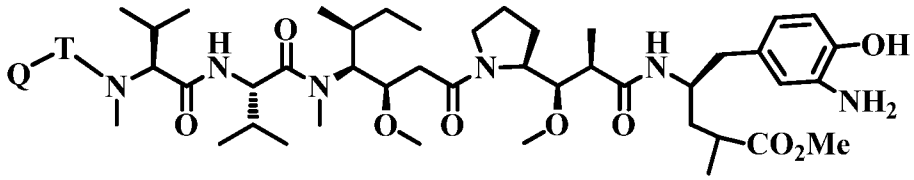

IIIk

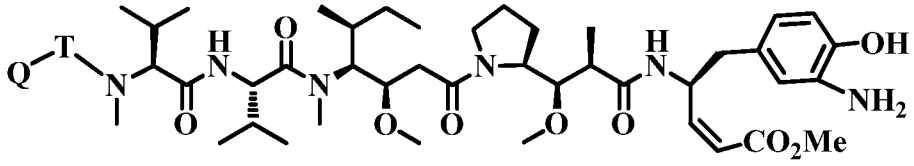

IIIl

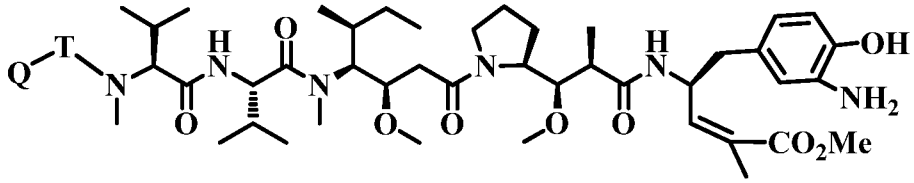

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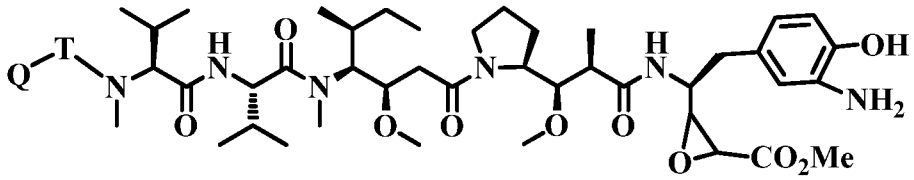

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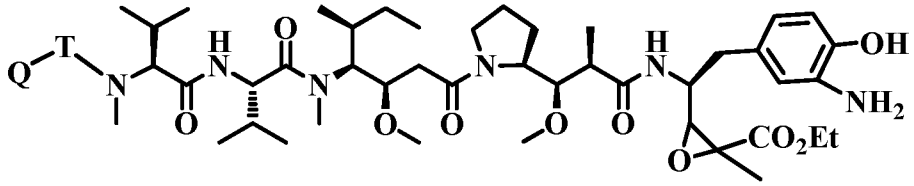

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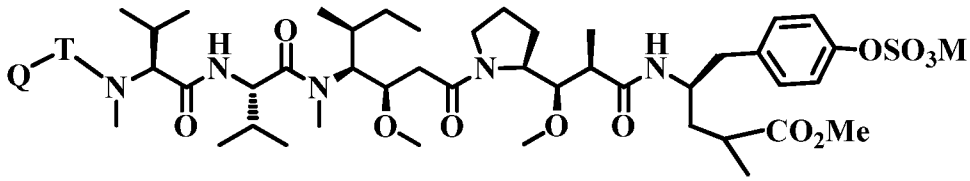

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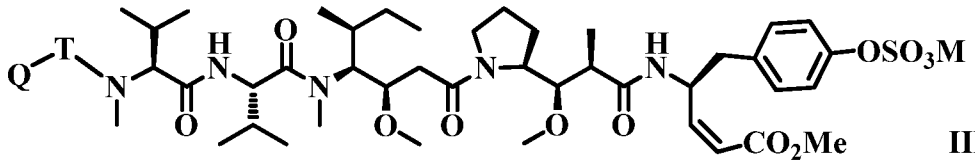

IIIq

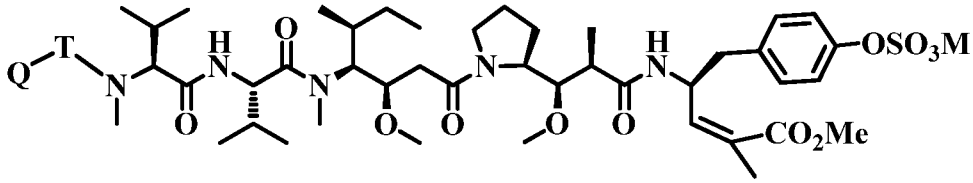

IIIr

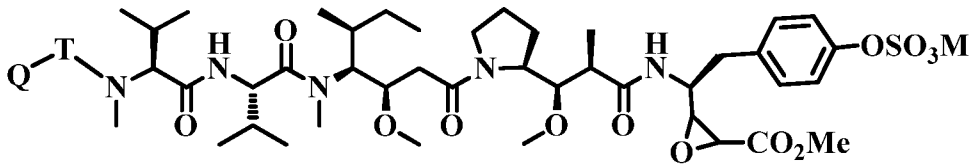

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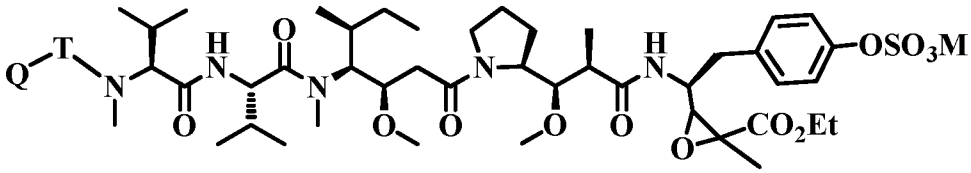

IIIt

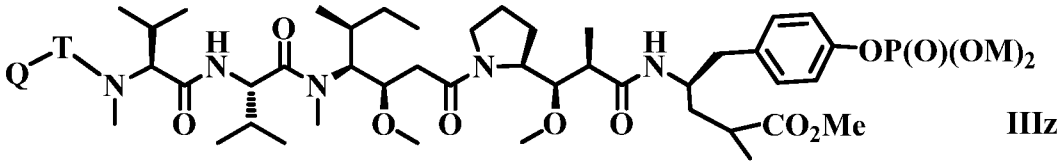

IIIu

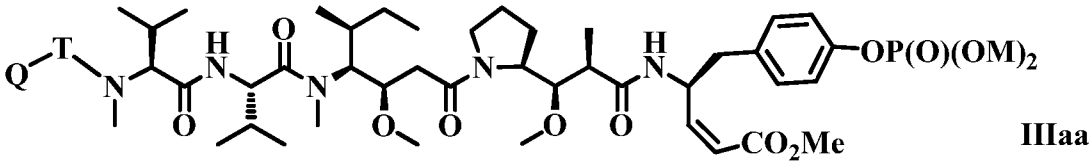

IIIv

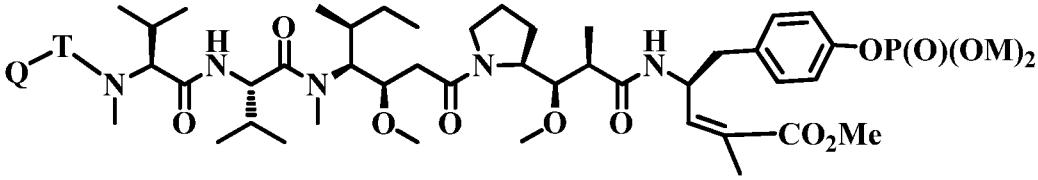

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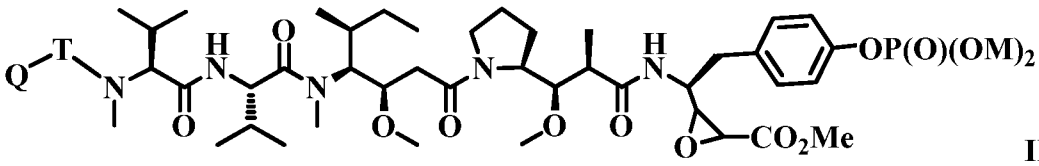

IIIx

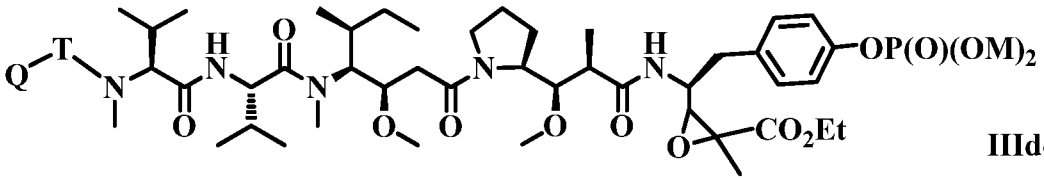

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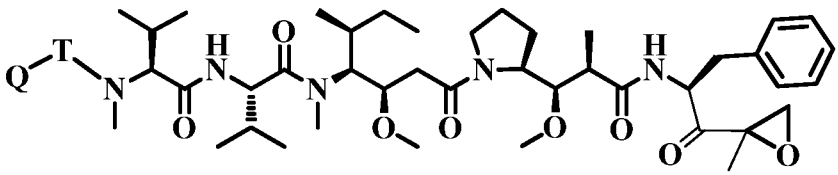

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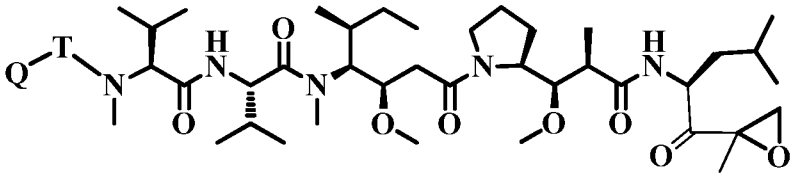

IIIaa


IIIbb


IIIcc


IIIdd


IIIee

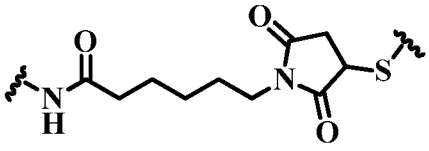

IIIff

Wherein M is H, or Na, or K, or $N^+R^1R^2R^3$ or a pharmaceutical salt; R^1, R^2 and R^3 are described above; T is independently L, R^1, -O-, -S-, -SS-, -NH-, =N-, =NNH-, -NH(R^1), -OR^1, -C(=O)-, -C(=O)R^1, -$COOR^1$-, -C(O)NH-, -C(=O)NHR^1-, -SR^1-, -S(=O)R^1-, -$P(=O)(OR^1)_2$-, -$P(=O)(NHR^1)_2$-, -$CH_2OP(=O)(OR^1)_2$-, -$CH_2NHP(=O)(NHR^1)_2$-, -$CH_2NHP(=O)(NHR^1)_2$-, -$CH_2NHP(=O)(NHR^1)(NHR^2)$-, -$SO_2R^1$-, $(CH_2CH_2O)_r$-, -$(CH(CH3)CH_2O)_r$-, C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3-C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl,

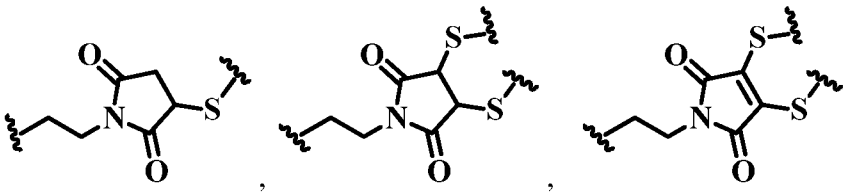
heteroalkylcycloalkyl, alkylcarbonyl, one to ten amino acid units; r is an integer ranging from 0 to 12; L, R^1 and R^2 are defined in Formula (I) and (II) above.

T may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit or vc), alanine-phenylalanine (ala-phe or af), *p*-aminobenzyloxycarbonyl (PAB), 4-thiopentanoate (SPP), 4-(*N*-maleimidomethyl)cyclohexane-1 carboxylate (MCC), (4-acetyl)aminobenzoate (SIAB), 4-thio-butyrate (SPDB), 4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB), ethyleneoxy (-CH_2CH_2O-) as one or more repeating units (EO or PEO), oxime (aldoxime, ketoxime), 1,2,3-triazoles (formed by a click chemistry), acetal (formed by a sugar or its analog). Additional linker components are known in the art and some are described herein.

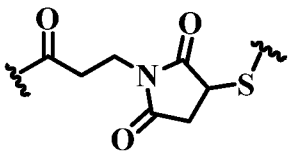
Examplary structures of these linker components are:



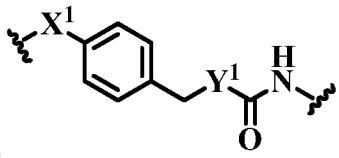
(MC, 6-maleimidocaproyl);



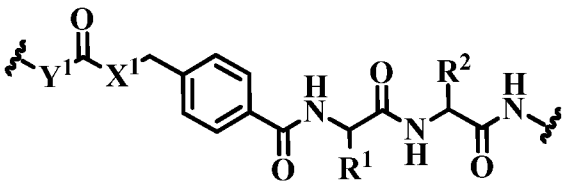
(ME, maleimidoethyl);



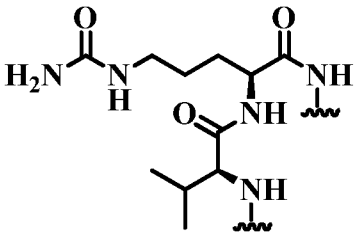
(MP, maleimidopropanoyl);



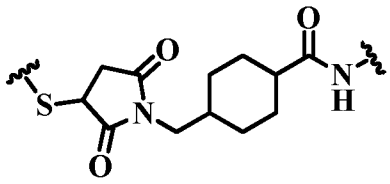
(PAB, *p*-aminobenzyloxycarbonyl analog, X^1 and Y^1 are independently O, S, NH or CH_2);



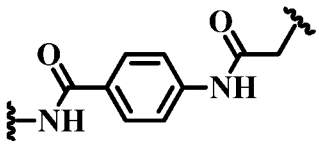
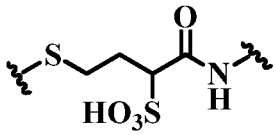
(carbonylbenzylpeptidyl analog);

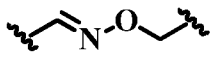
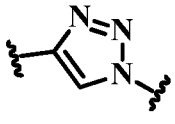


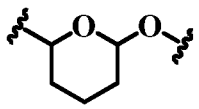
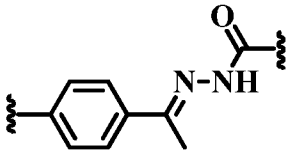
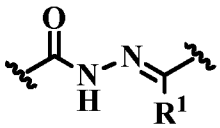
(valine-citrulline);

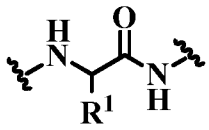
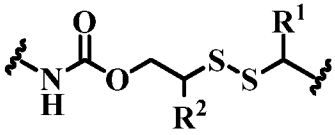


(MCC, 4-(*N*-maleimidomethyl) cyclohexane-1 carboxylate);


((4-acetyl)aminobenzoate);

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


(oxime);

(1,2,3-triazoles);

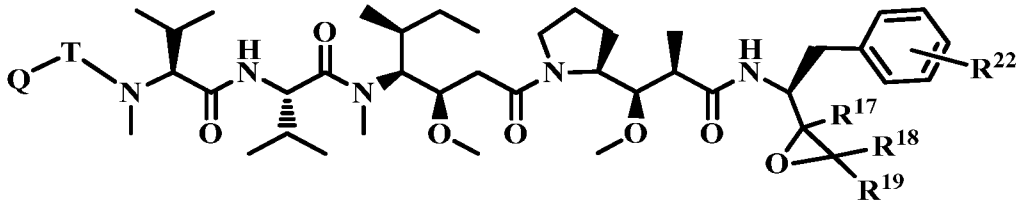

(acetal);

,

(hydrazone);

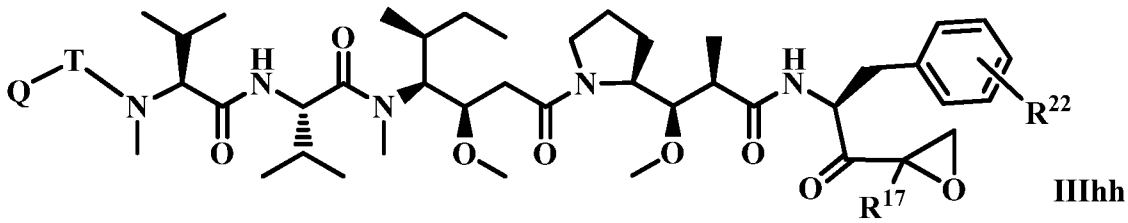

(stable peptide);

((2-disulfanyl) ethylcarbamate);

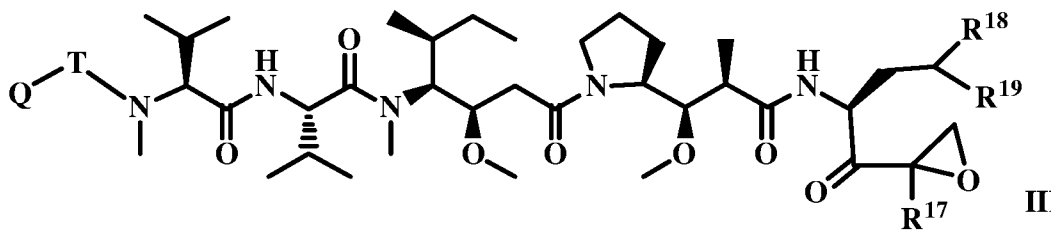
Wherein X^1 and Y^1 are independently O, S, NH or CH_2; R^1 and R^2 are defined above

Q is a cell-binding agent; or a function group readily link to a cell-binding molecule. The function group is selected, but not limited to, disulfide substituent, a maleimido, haloacetyl group, hydrazide, aldehyde, ketone, azide, amine, alkoxylamino, or an *N*-hydroxysuccinimide ester.

In another aspect, a conjugate of a cell-binding molecule bearing a linker-cytotoxic agent of formula (III) is of the following preferred structures:


IIIgg

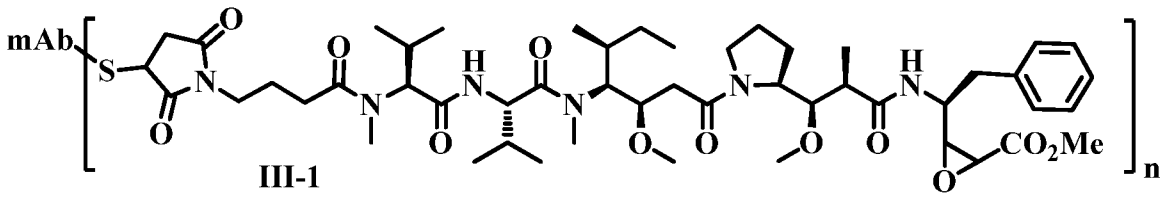

IIIhh


IIIii

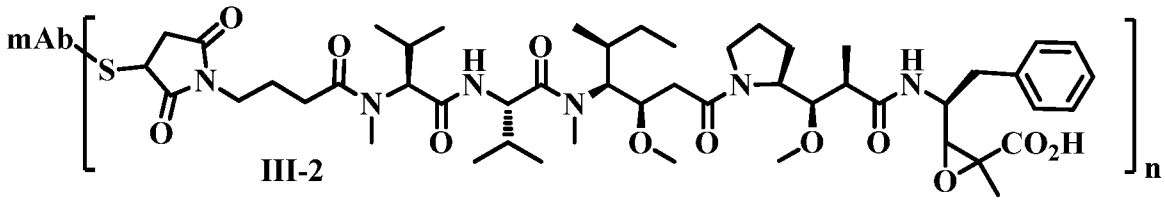
Wherein Q, T, R^1, R^2 and R^3 are defined the same above (IIIa~IIIff); R^{22} is H, OPO_3M_2, OSO_3M, OCH_2PO_3M, OH, NO_2, F, Cl, Br, I, SR^{13}, SSR^{13}, NH_2, NHR^{13},

$NR^{13}R^{14}$, or OR^{13}; M is H, Na, K, or $N^+R^1R^2R^3$; R^{13}, R^{14}, R^{15} , R^{17}, R^{18}, and R^{19} are defined the same above. In addition, R^{18} and R^{19} can jointly form a cycloalkyl, heterocycloalkyl, carbocyclic, heterocyclic ring system, aromatic system, or heteroaromatic group.

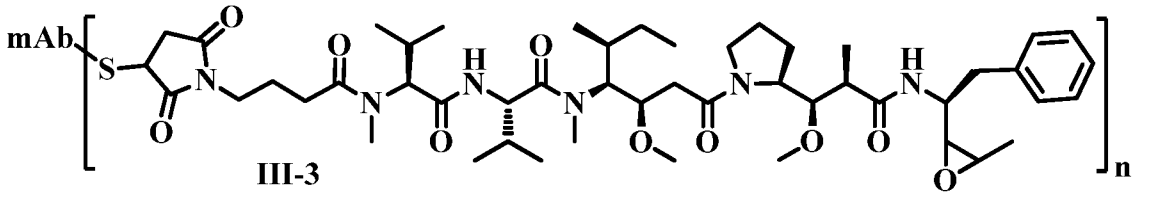
The preferred conjugate structures with an antibody bearing Formula (III) are, but not limited to, the following:



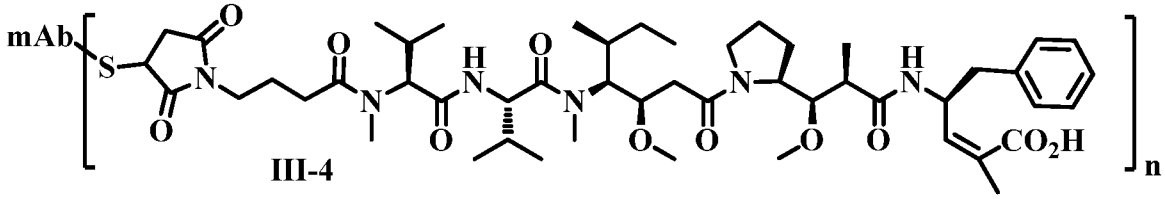
III-1



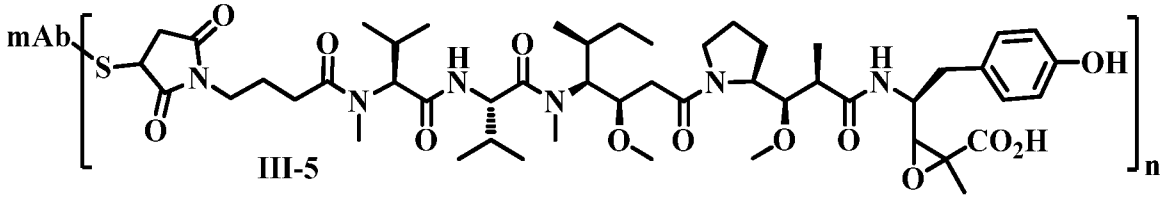
III-2



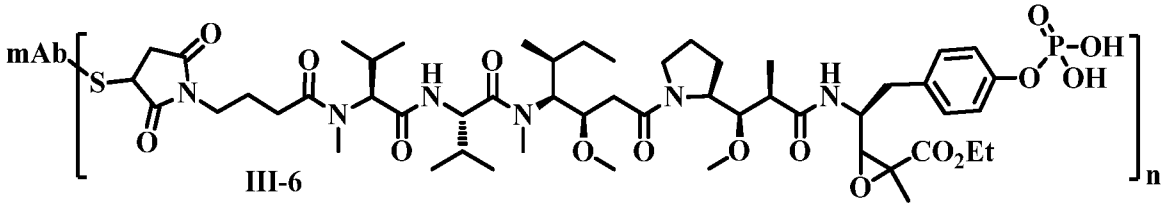
III-3



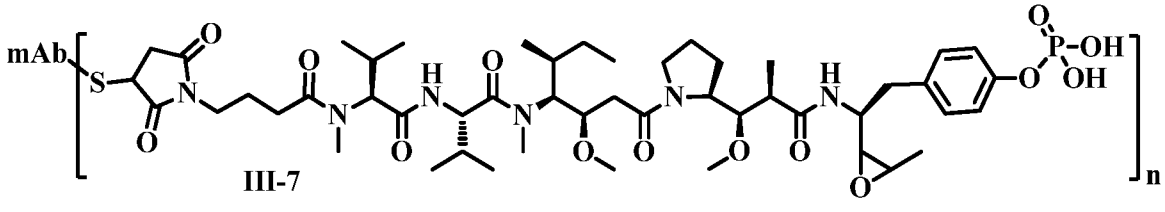
III-4



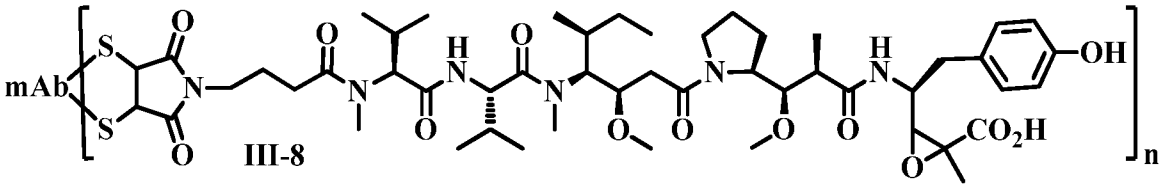
III-5



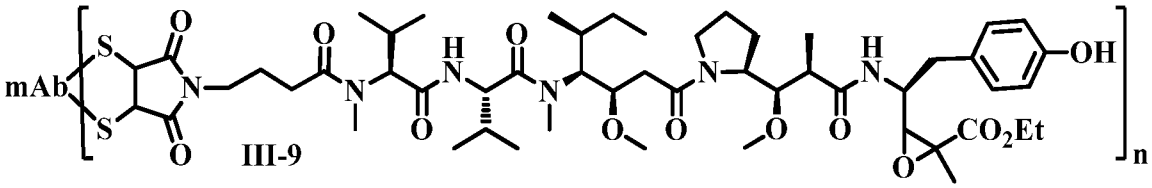
III-6



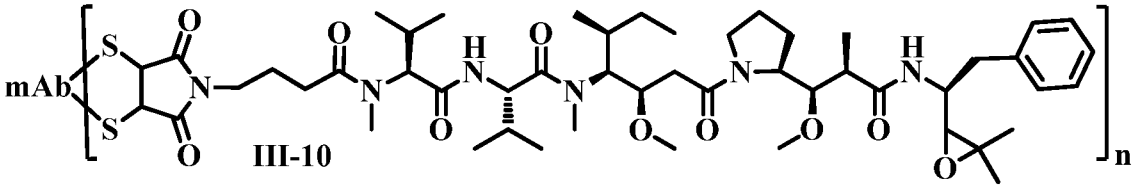
III-7



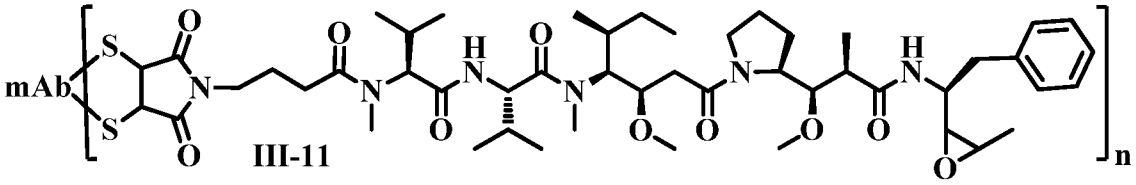
III-8



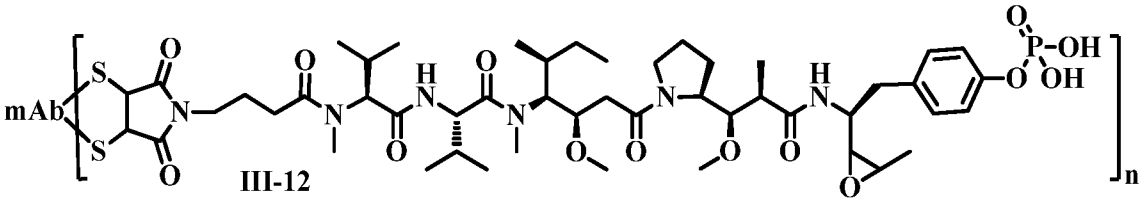
III-9



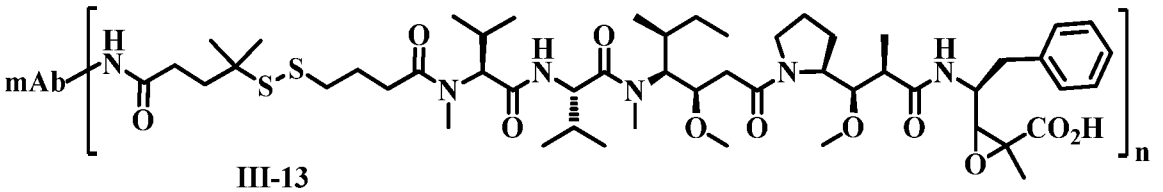
III-10



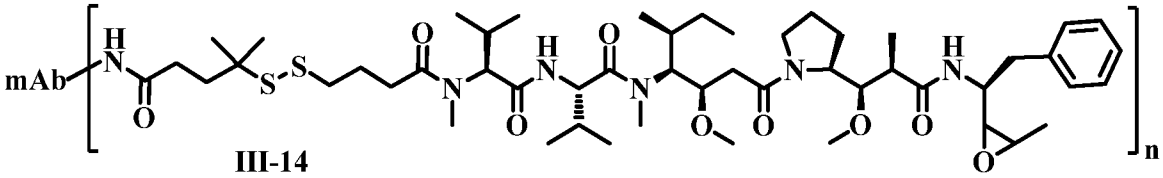
III-11



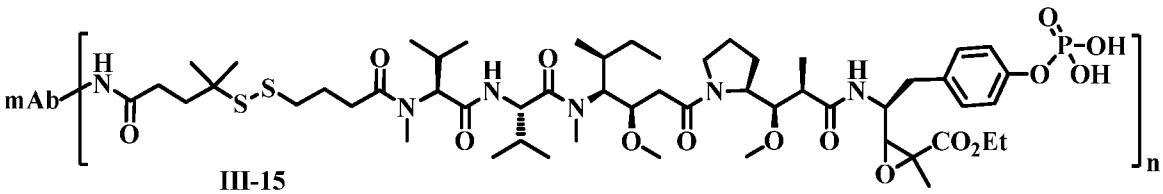
III-12



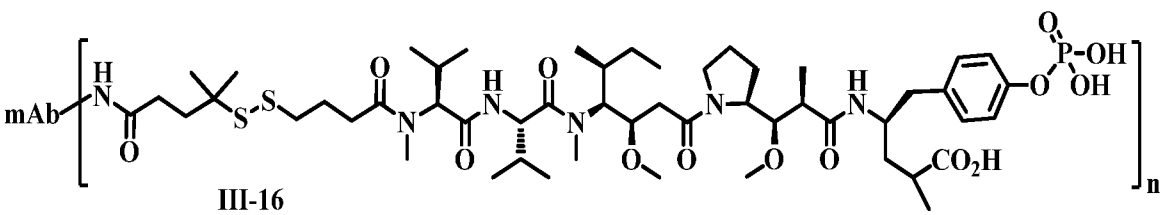
III-13



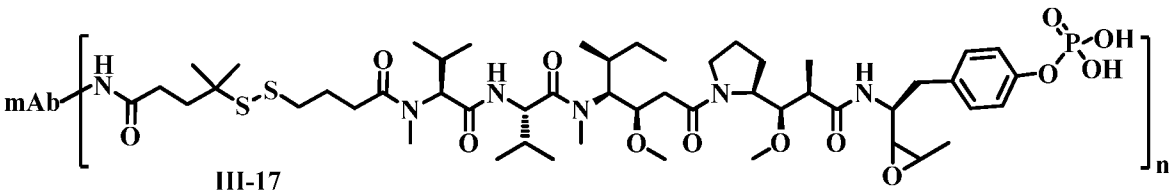
III-14



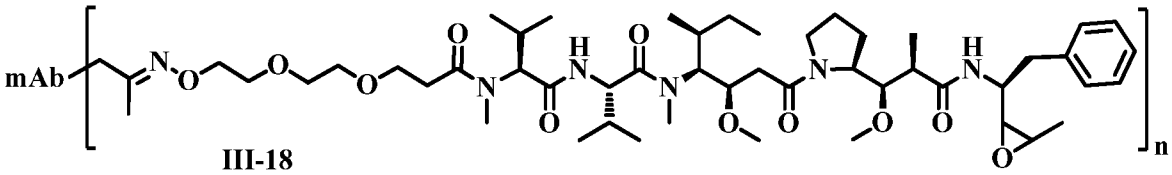
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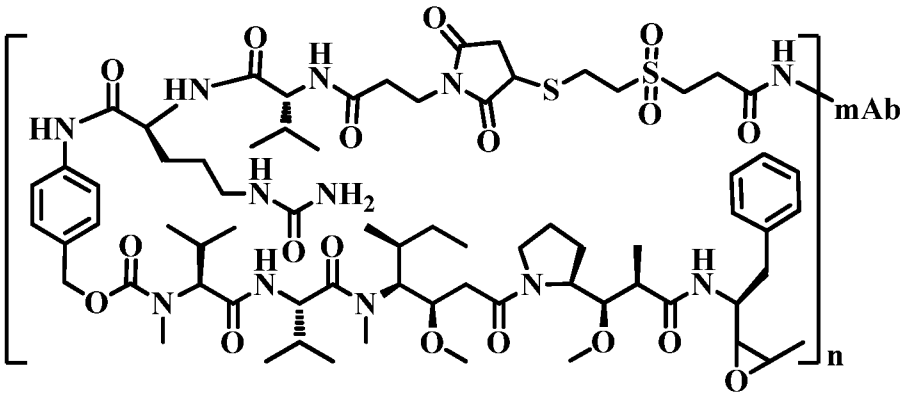
III-16



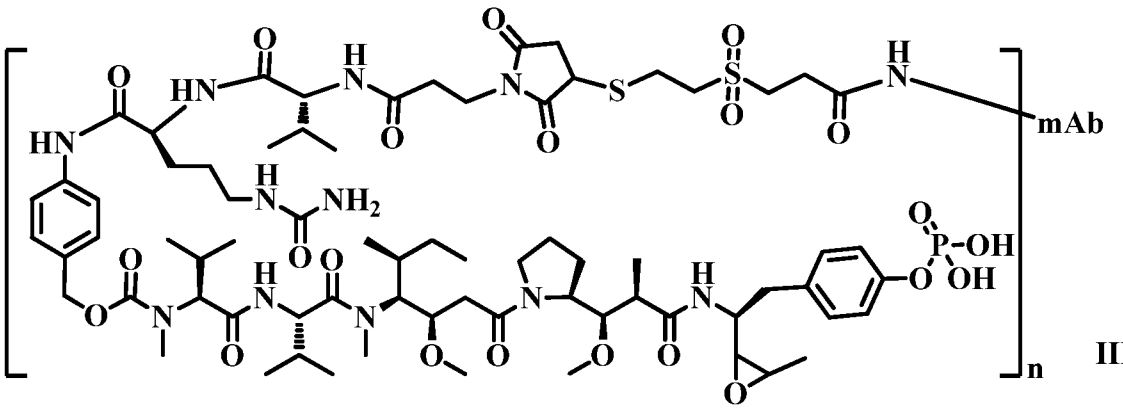
III-17



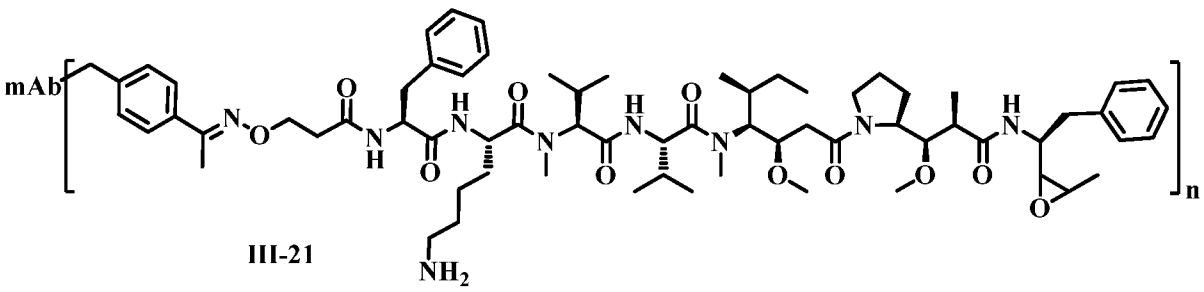
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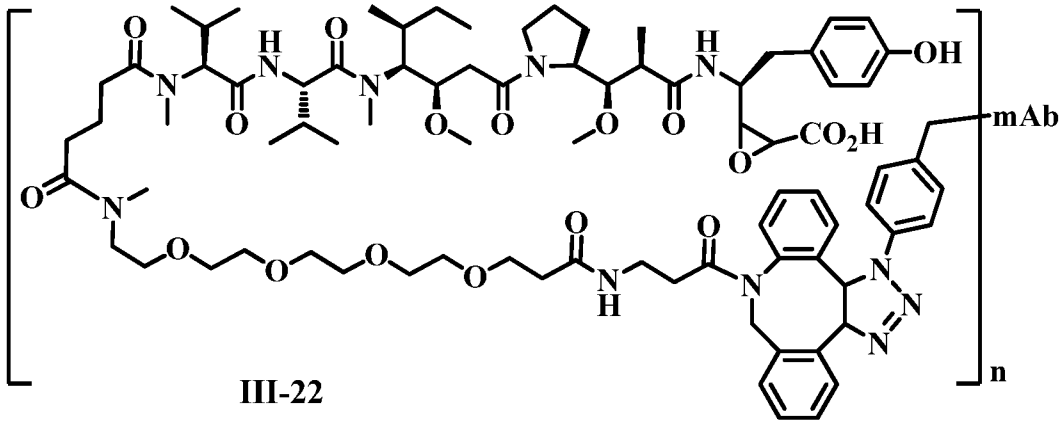
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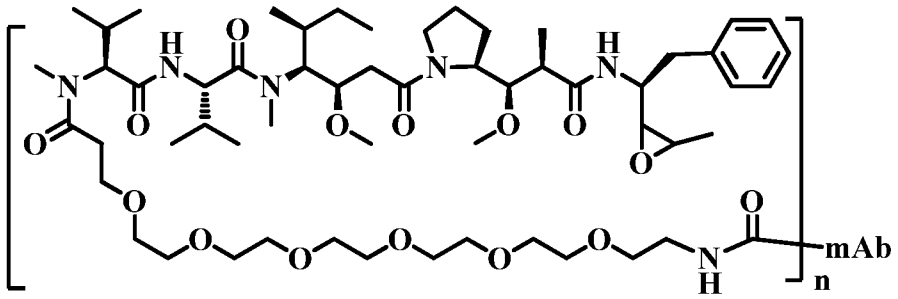
III-20



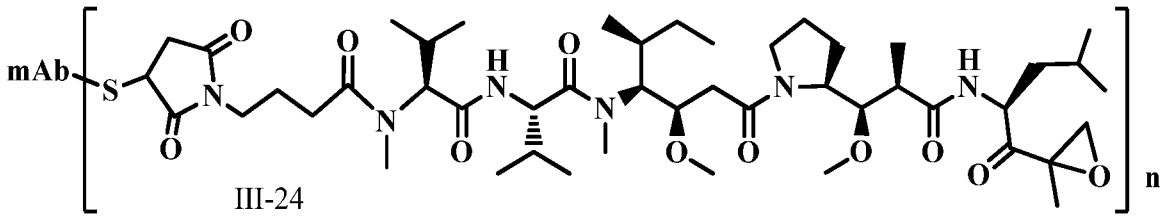
III-21



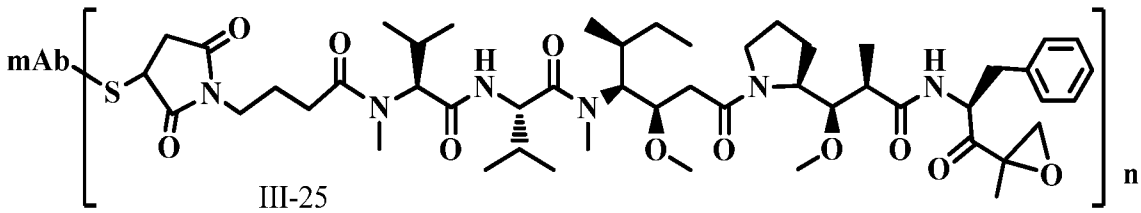
III-22



III-23



III-24

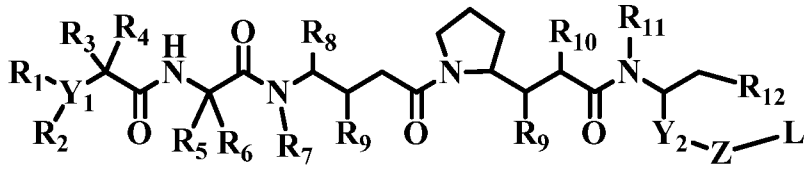


III-25

and pharmaceutical acceptable salts and solvates thereof,

wherein mAb is an antibody and n=1~30.

In another aspect, a linker-cytotoxic agent (L_m-D_n) for the conjugate of the Formula (I) is of the following Formula (IV):

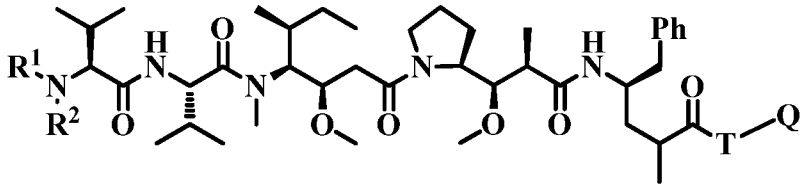


(IV)

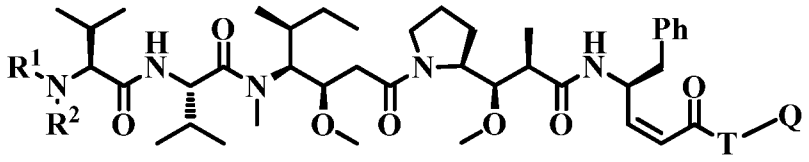
and pharmaceutical acceptable salts and solvates thereof.

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, Y^1, Y^2, Z and L are the same as described previously in Formula (III).

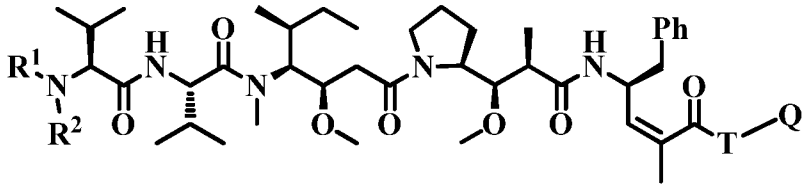
Examples of structures of the linker-cytotoxic agent (L_m-D_n) components or their conjugates with a cell-binding molecule bearing the structure of Formula (IV) include, but are not limited to, the following:



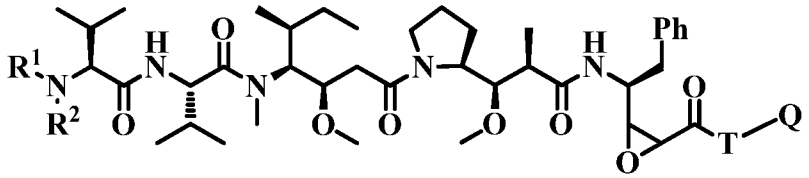
IVa



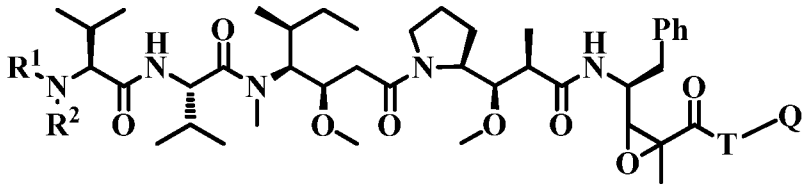
IVb



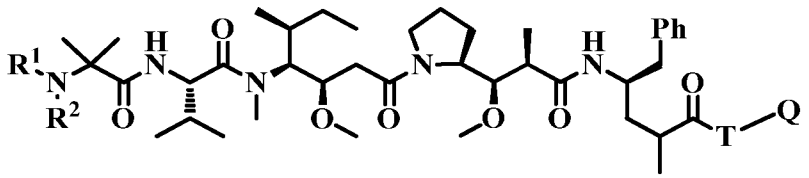
IVc



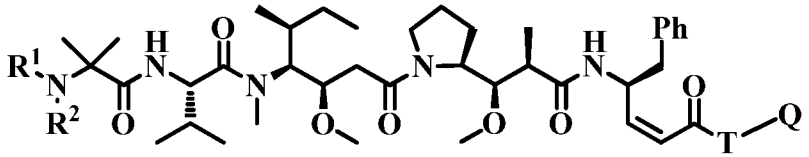
IVd



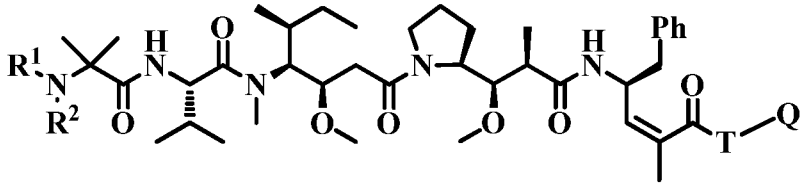
IVe



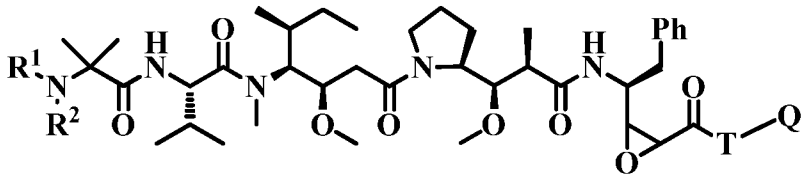
IVf



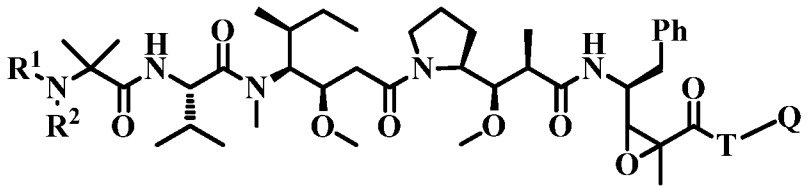
IVg



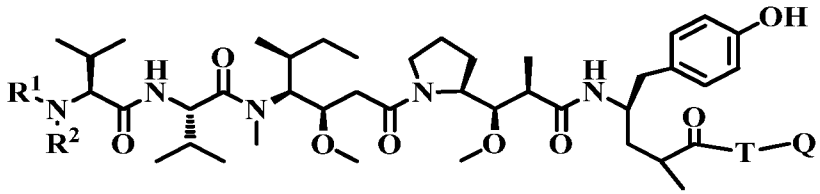
IVh



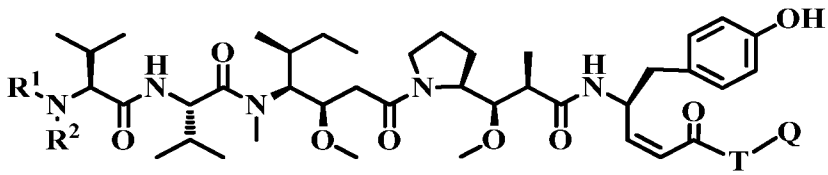
IVi



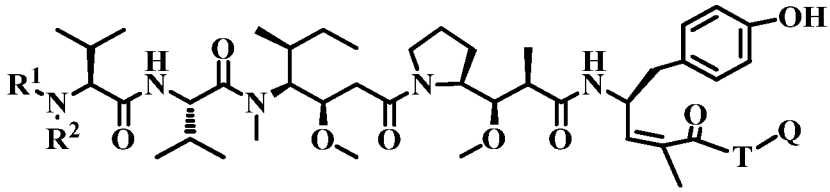
IVj



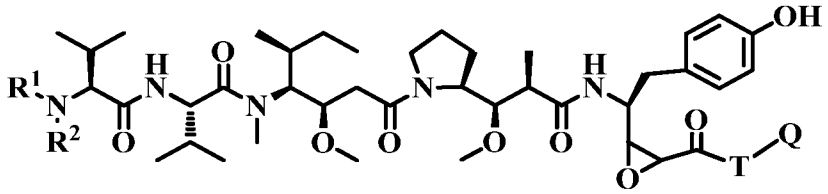
IVk



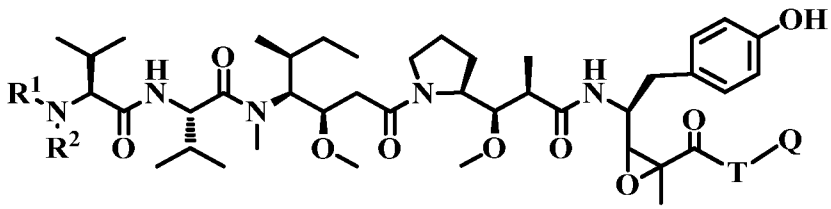
IVl



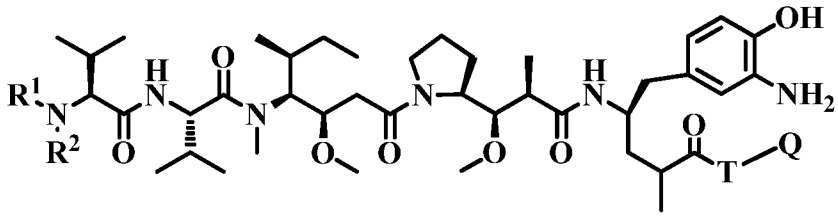
IVm



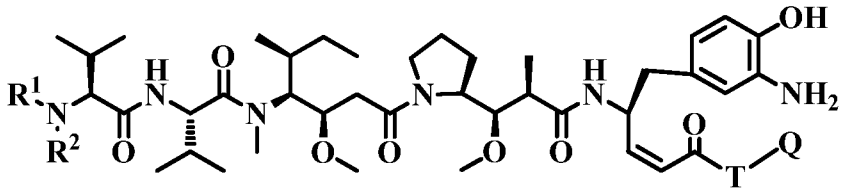
IVn



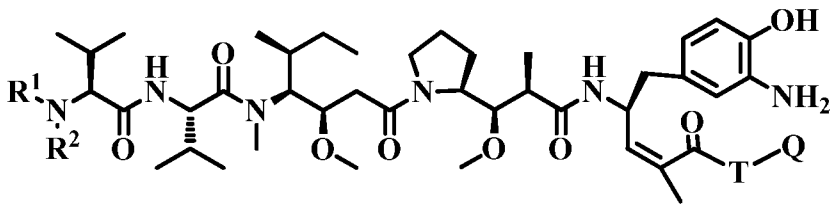
IVo

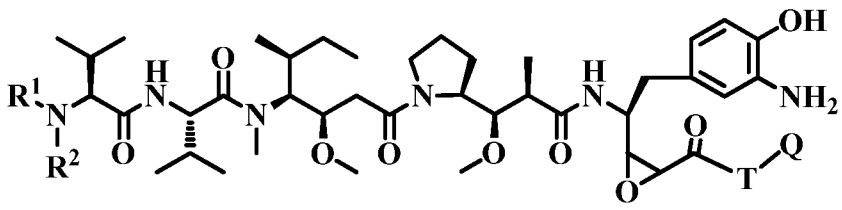


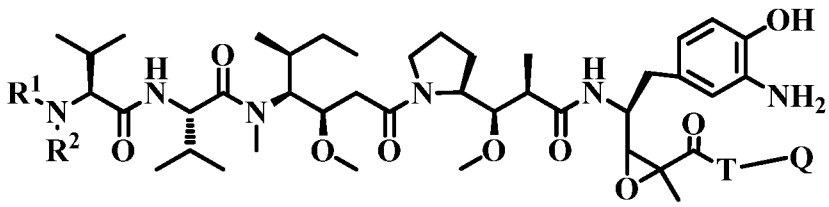
IVp

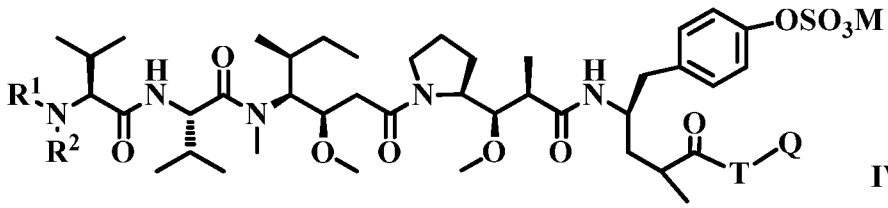


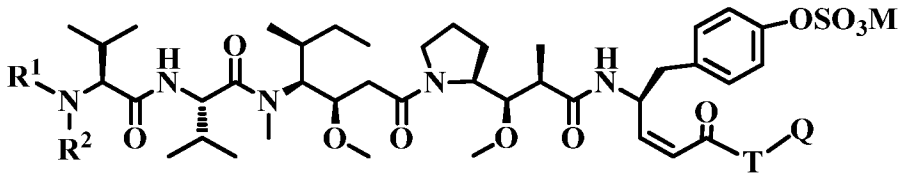
IVq

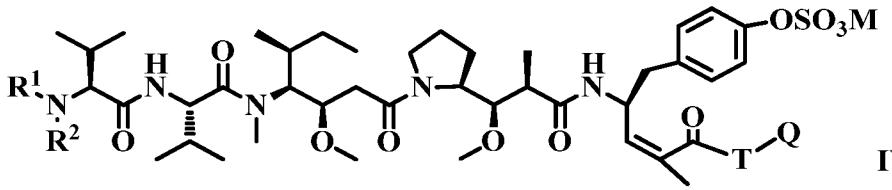

IVr

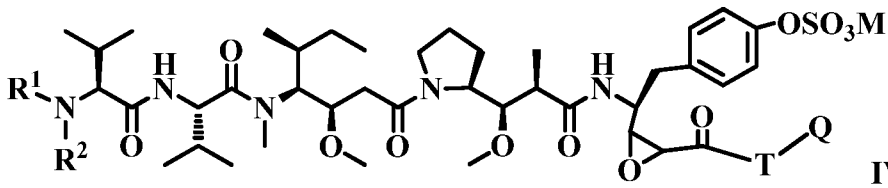

IVs

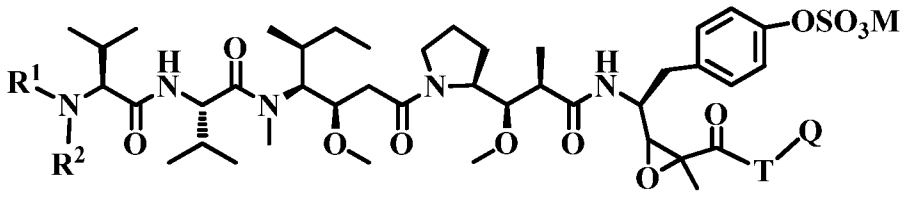

IVt

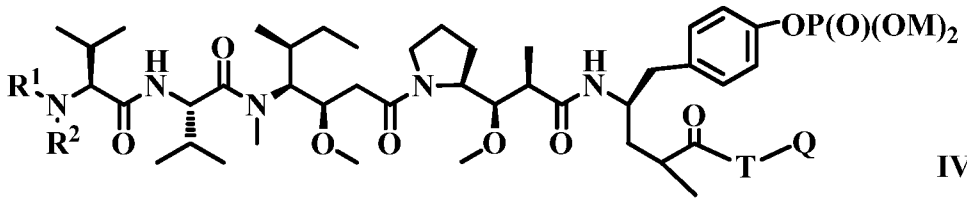

IVu

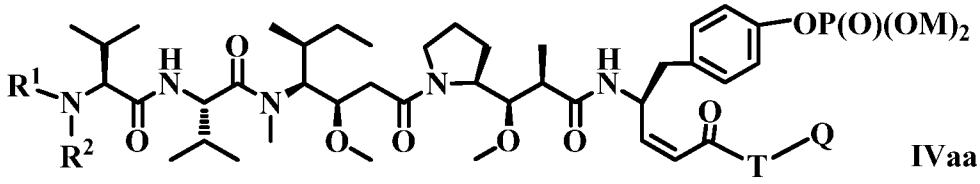

IVv

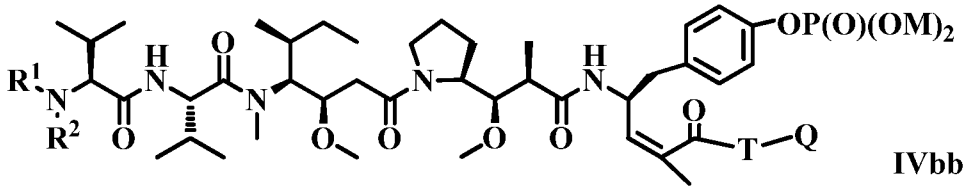

IVw

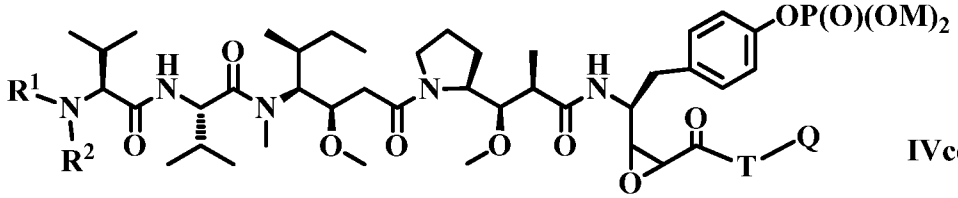

IVx

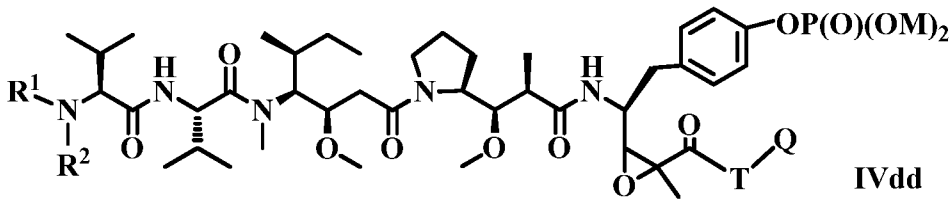

IVy


IVz


IVaa


IVbb

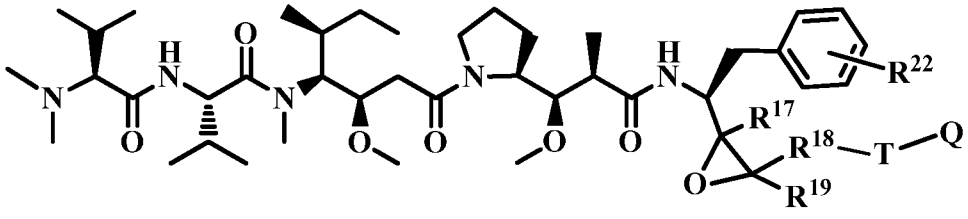

IVcc

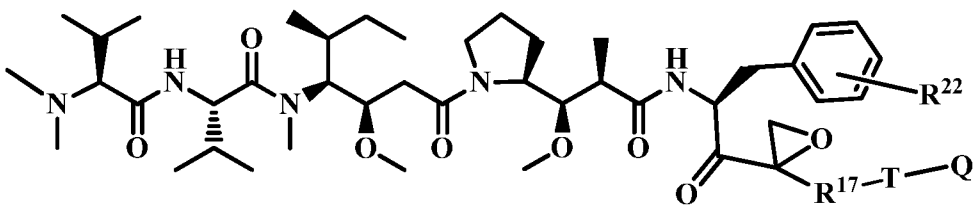

IVdd

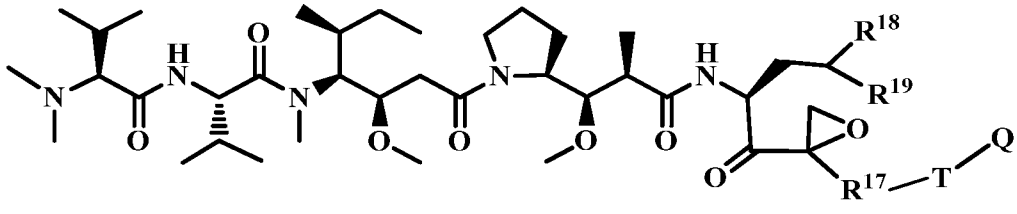
and pharmaceutical acceptable salts and solvates thereof.

Wherein R^1, R^2, T, Q and M are defined the same as in Formula (IIIa~IIIff).

In another aspect, a conjugate of a cell-binding molecule bearing a linker-cytotoxic agent of formula (IV) is of the following preferred structures:


IVee


IVff

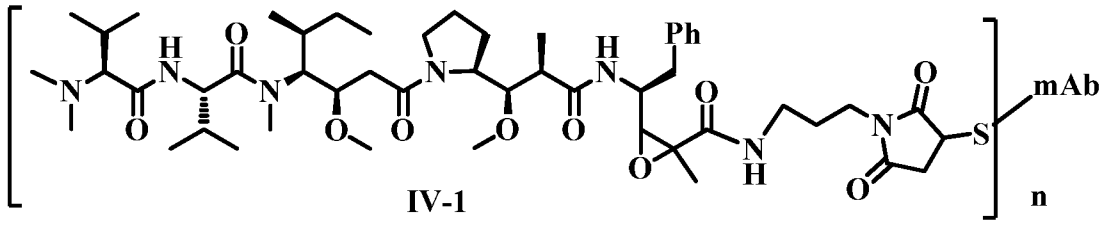


IVgg

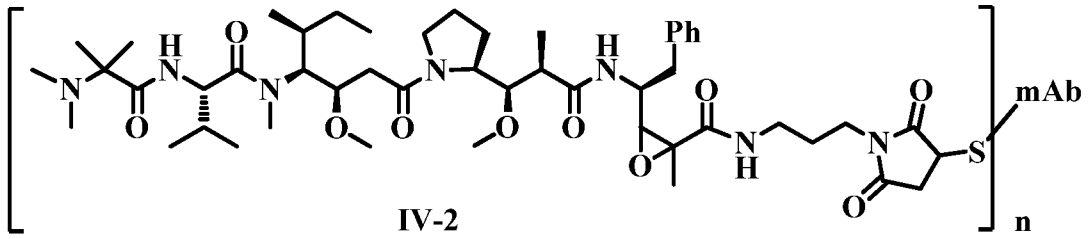
and pharmaceutical acceptable salts and solvates thereof.

Wherein Q, T, R^1, R^2, R^3, R^{17}, R^{18}, R^{19} and R^{22}, are defined the same above. In addition, R^{17} and R^{18} can be absent.

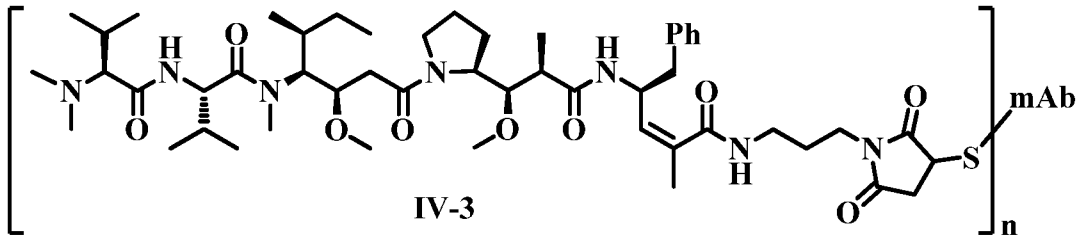
The preferred conjugate structures with an antibody bearing Formula (IV) are, but not limited to, the following:



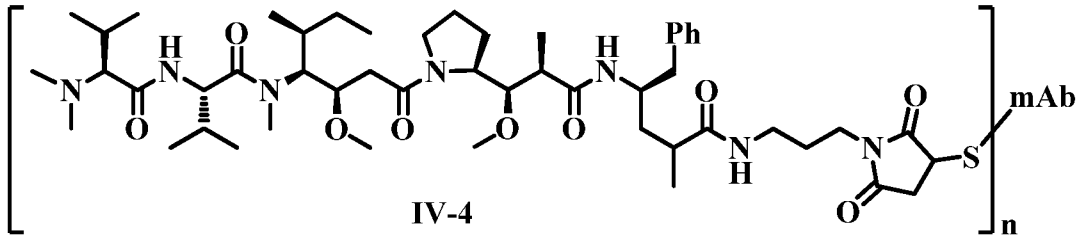
IV-1



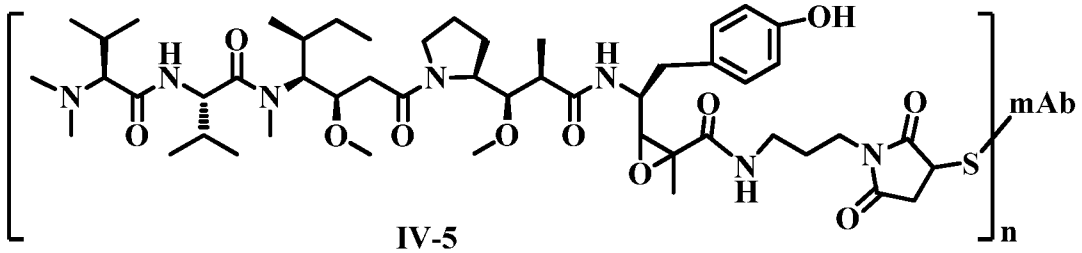
IV-2



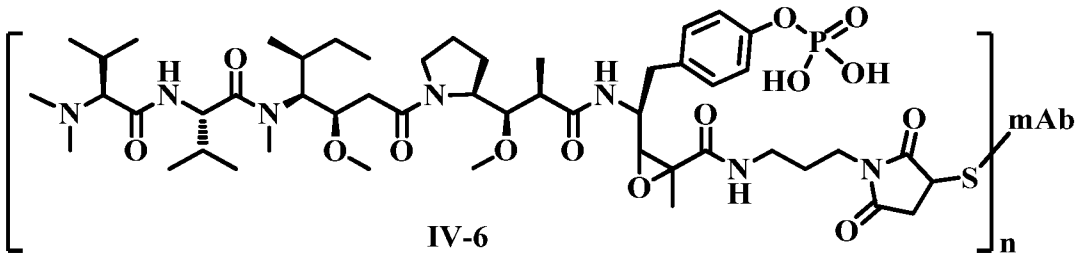
IV-3



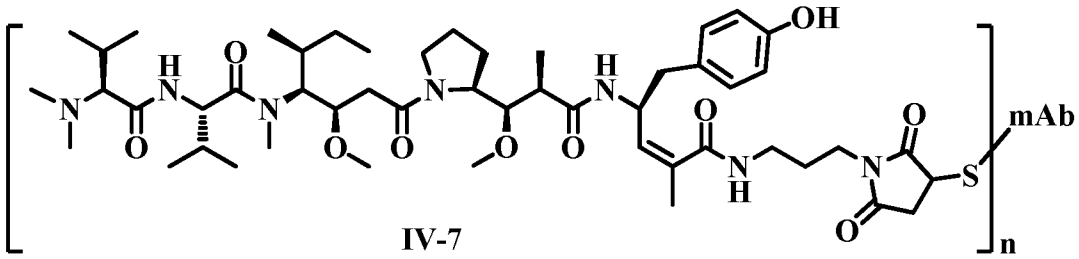
IV-4



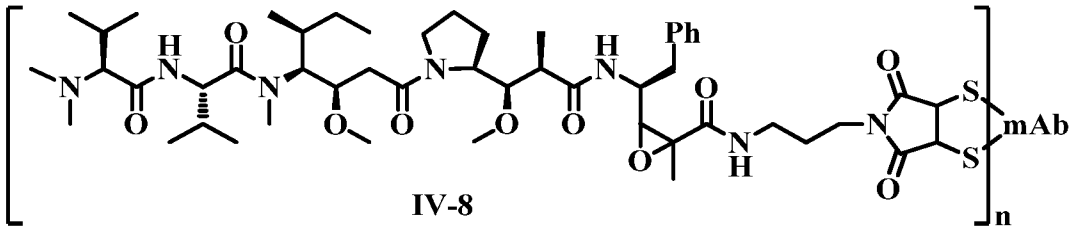
IV-5



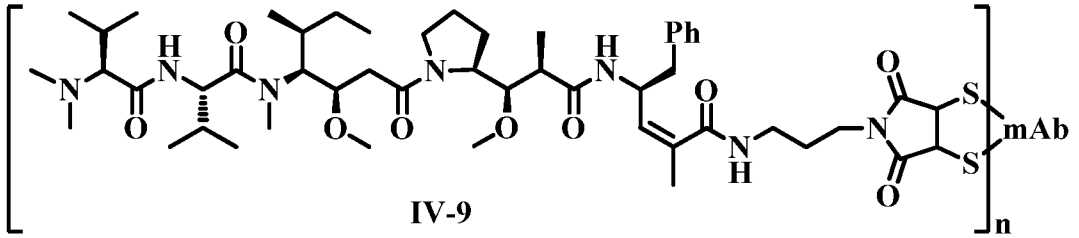
IV-6



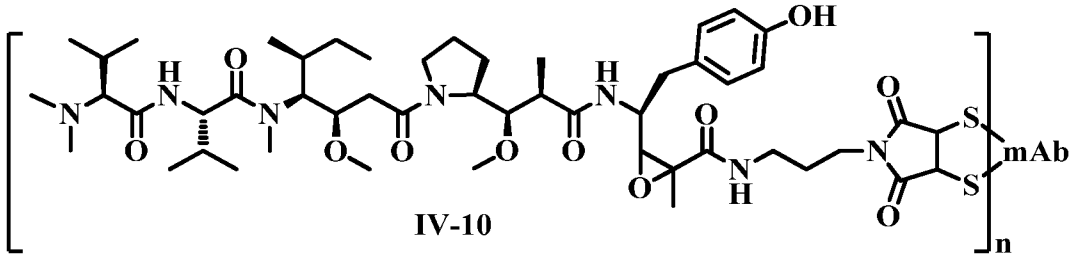
IV-7



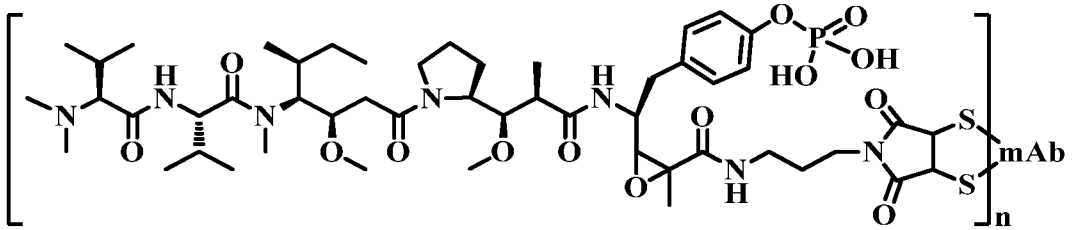
IV-8



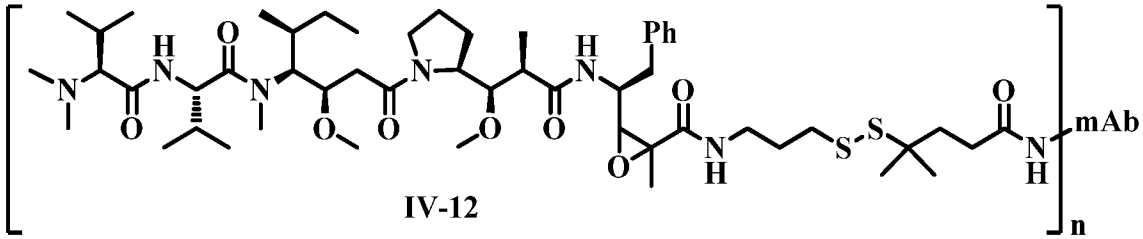
IV-9



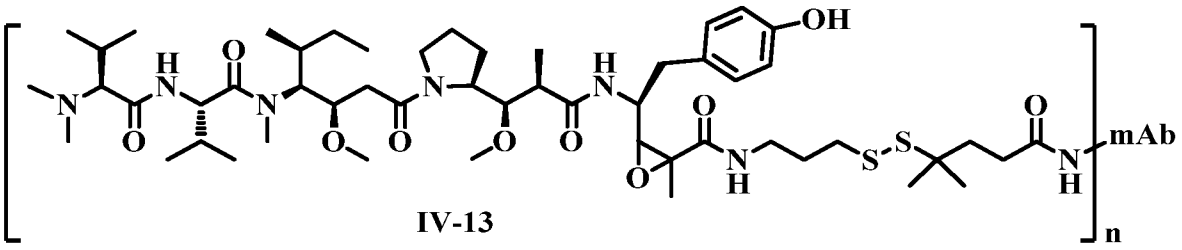
IV-10



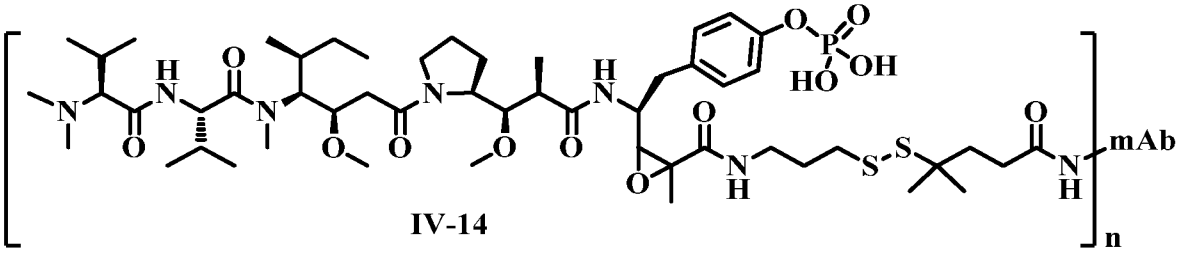
IV-11



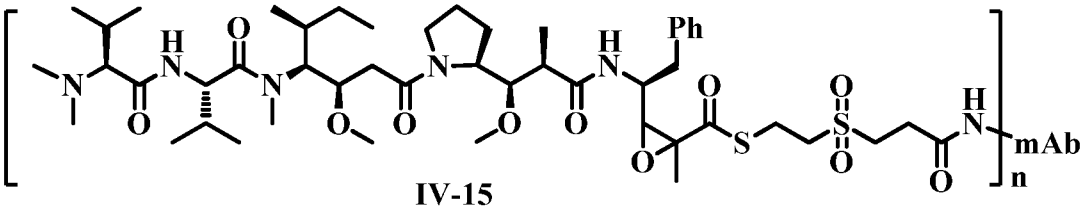
IV-12



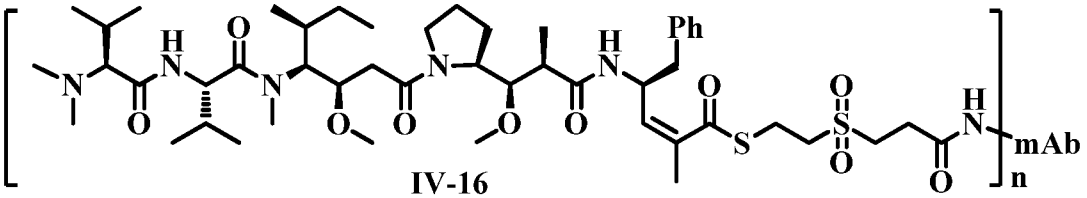
IV-13



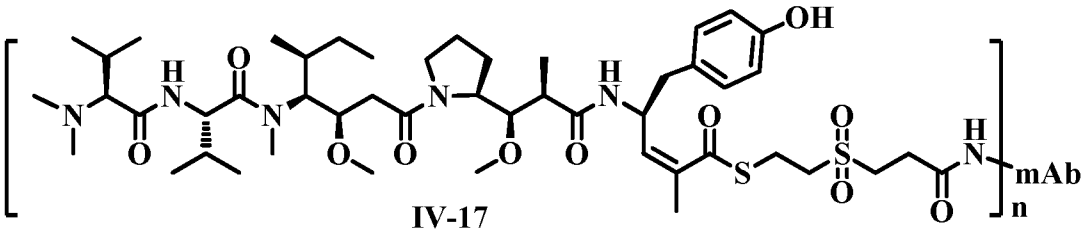
IV-14



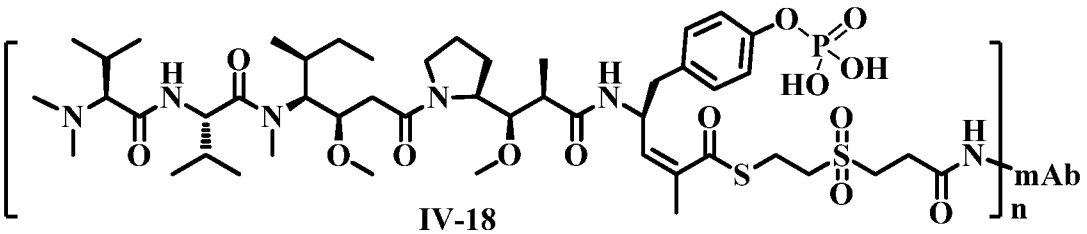
IV-15



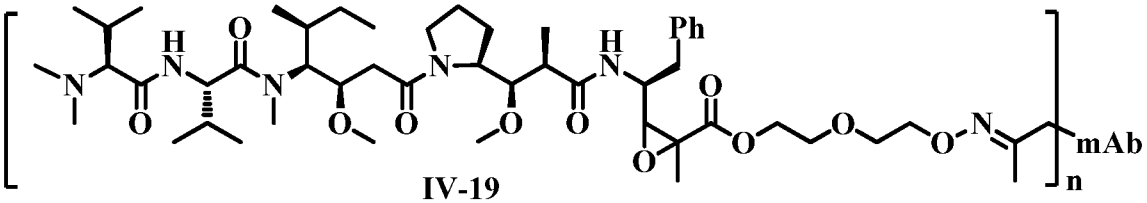
IV-16



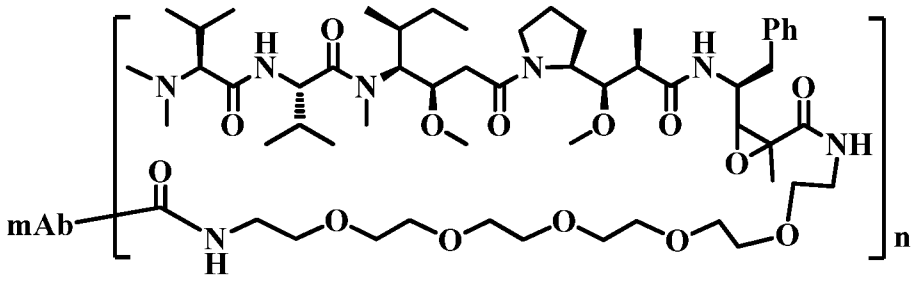
IV-17



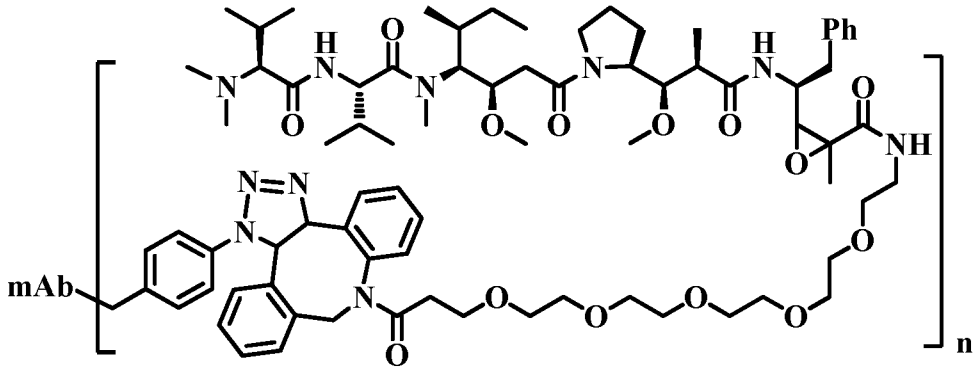
IV-18



IV-19



IV-20

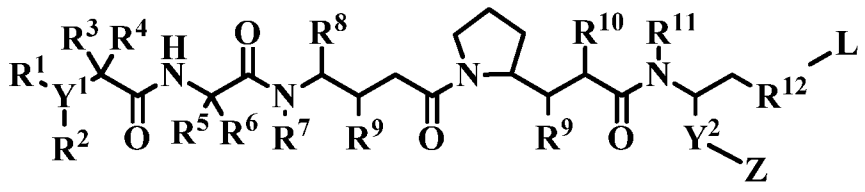


IV-21

and pharmaceutical acceptable salts and solvates thereof,

wherein mAb is an antibody and n=1~30.

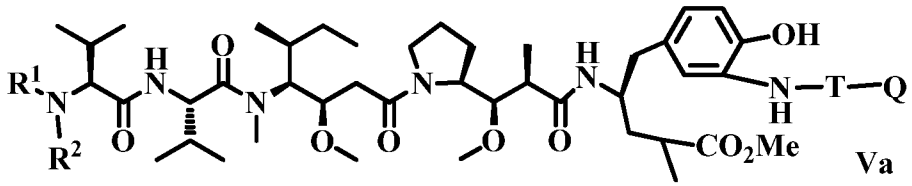
In still another aspect, a structure of Linker-Drug molecule (L_m-D_n) components of Formula (I) and its conjugate with a cell-binding molecule is, but not limited, of the Formula (V):



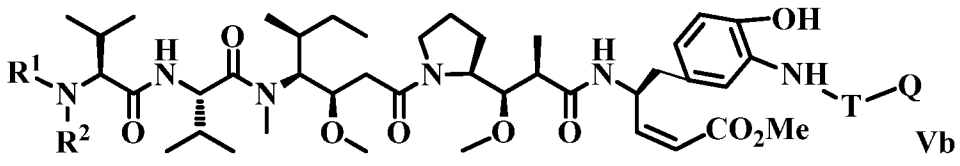
(V)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, Y^1, Y^2, Z and L are the same as described previously in Formula (III).

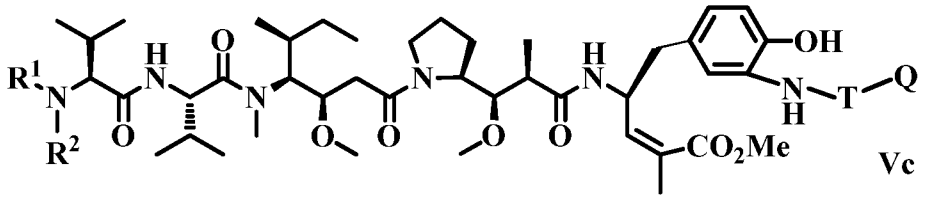
Examples of structures of the linker-cytotoxic agent (L_m-D_n) components or their conjugates with a cell-binding molecule bearing the structure of Formula (V) include, but are not limited to, the following:



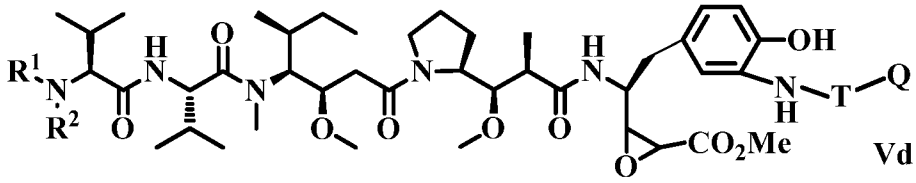
Va



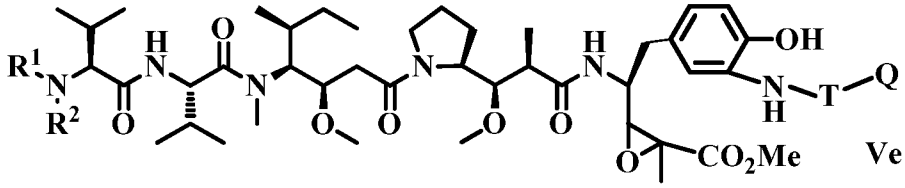
Vb



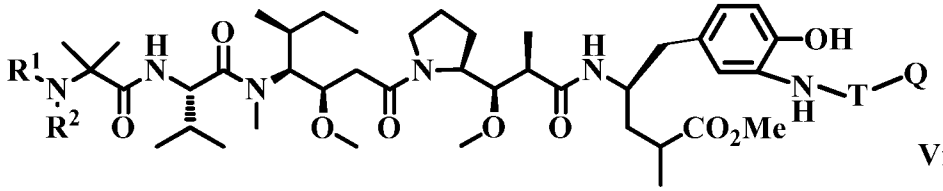
Vc



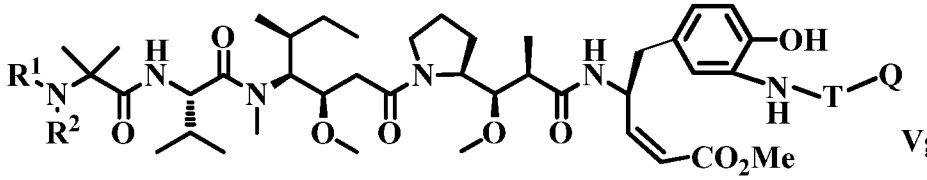
Vd



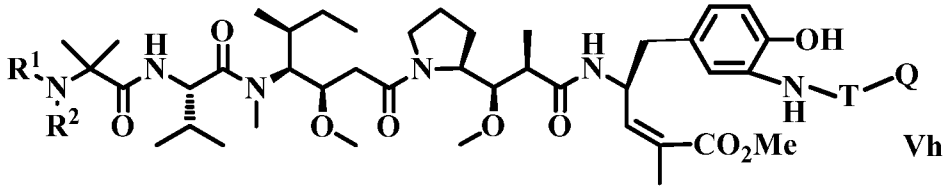
Ve



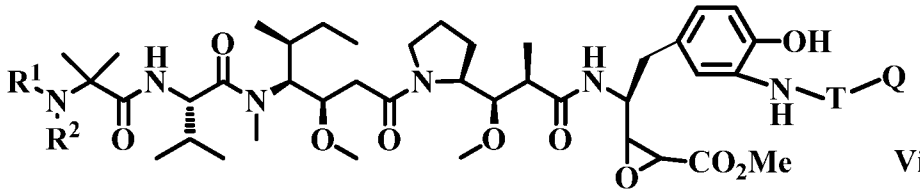
Vf



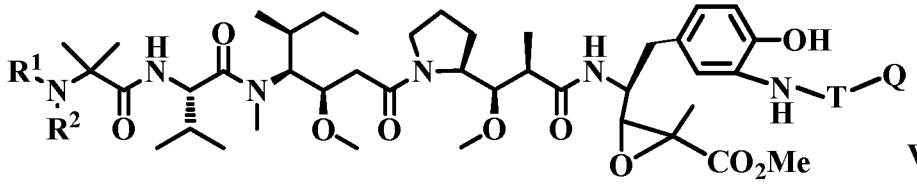
Vg



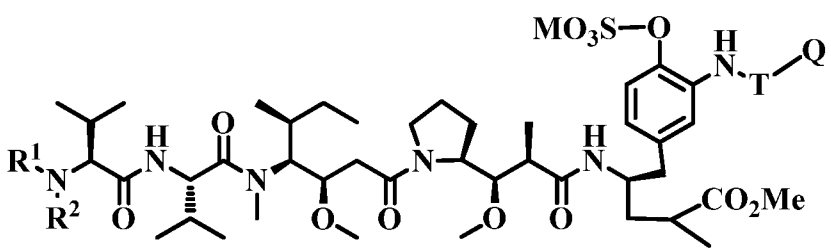
Vh



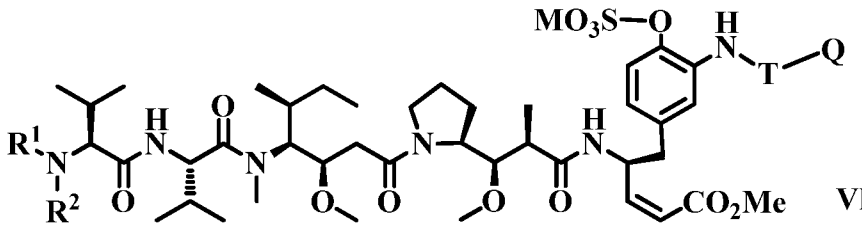
Vi



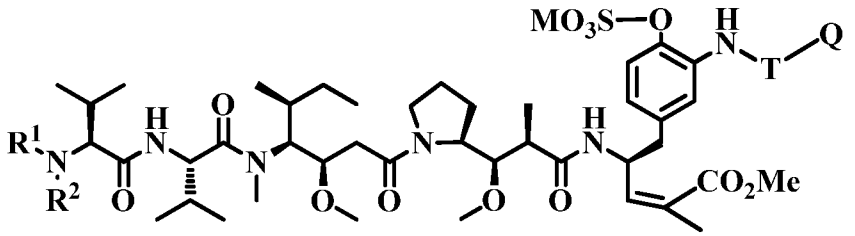
Vj



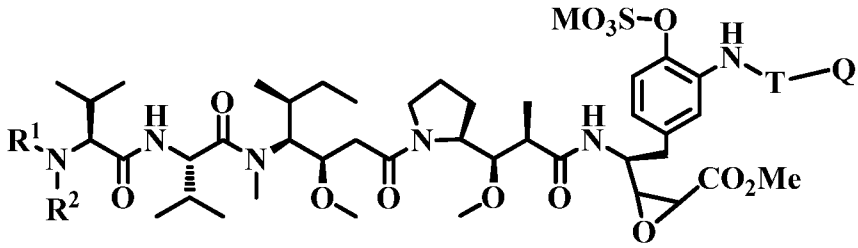
Vk



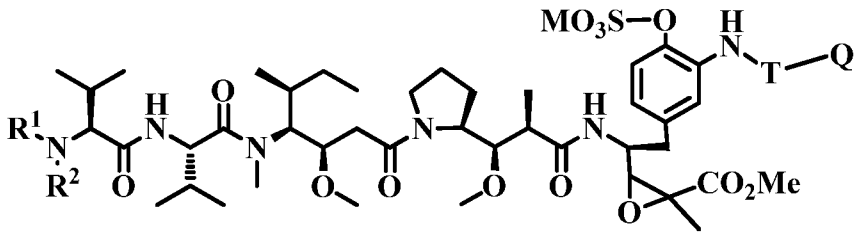
Vl



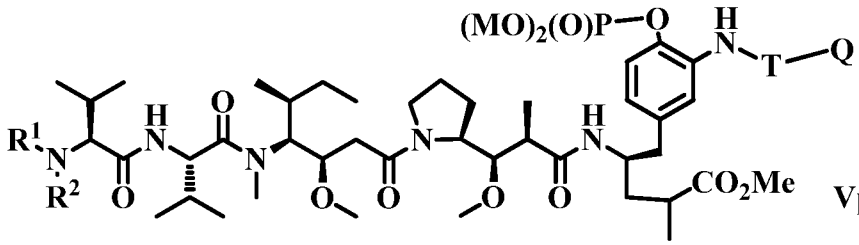
Vm



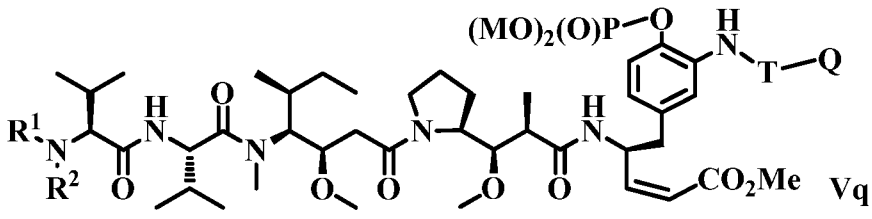
Vn



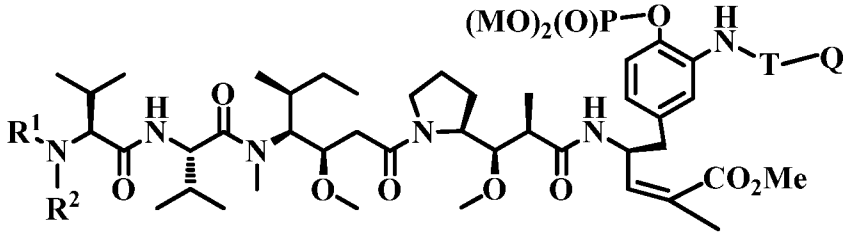
Vo



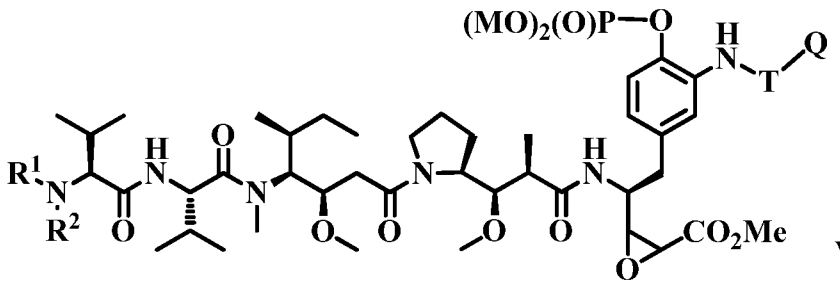
Vp



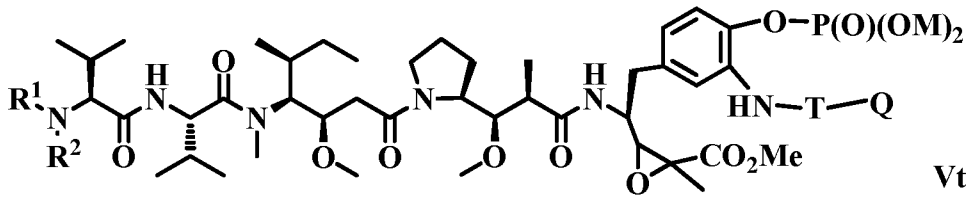
Vq



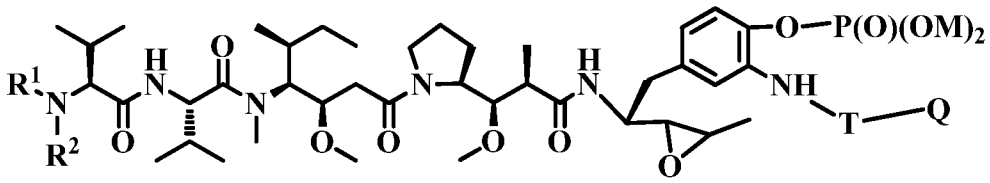
Vr



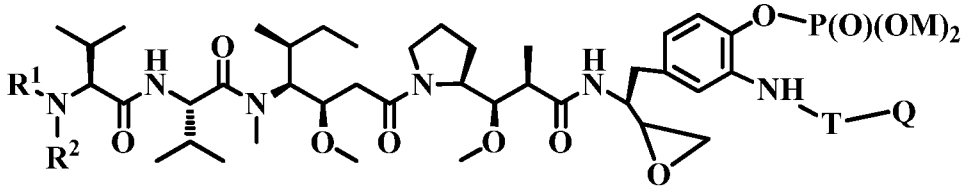
Vs



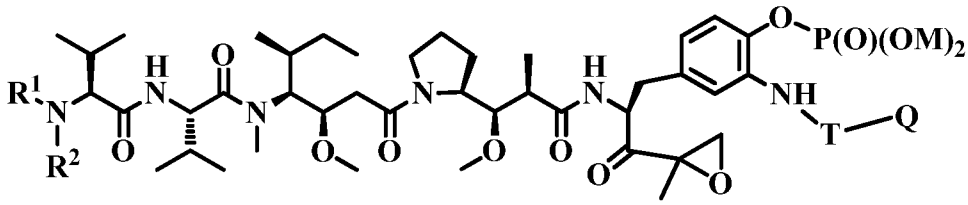
Vt



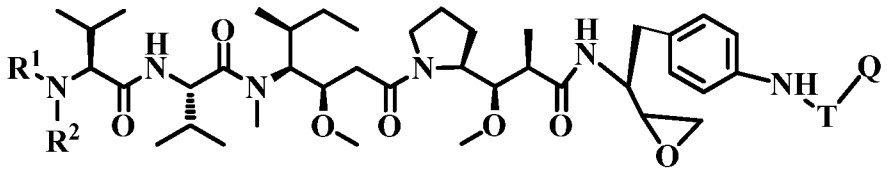
Vu



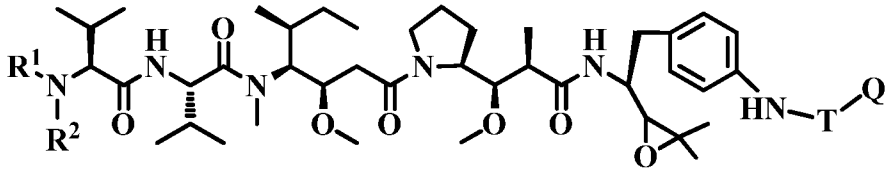
Vw



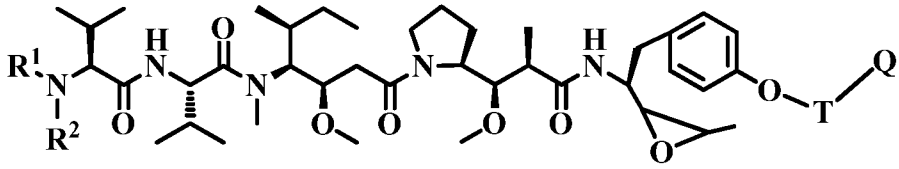
Vx



Vy



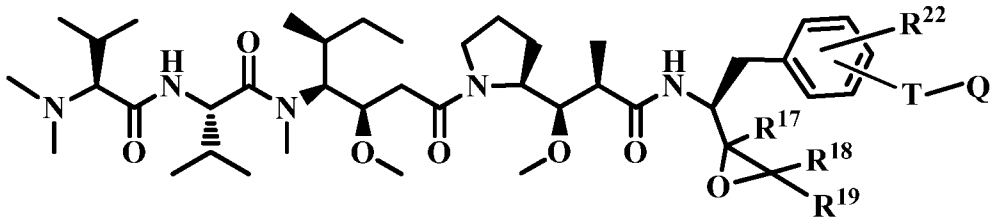
Vz



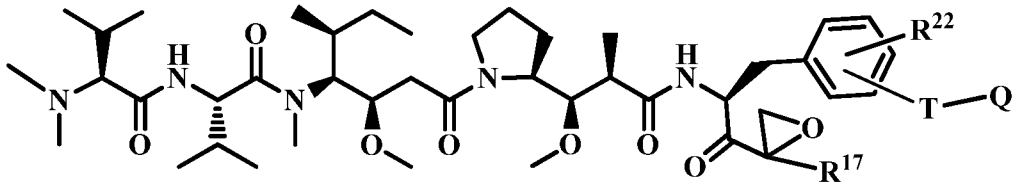
Vaa

wherein R^1, R^2, T, Q and M are defined the same as in Formula (IIIa~IIIff).

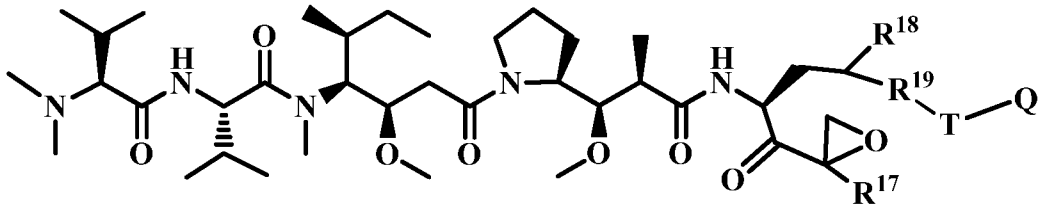
In another aspect, a conjugate of a cell-binding molecule bearing a linker-cytotoxic agent of formula (V) is of the following preferred structures:



Vbb



Vcc

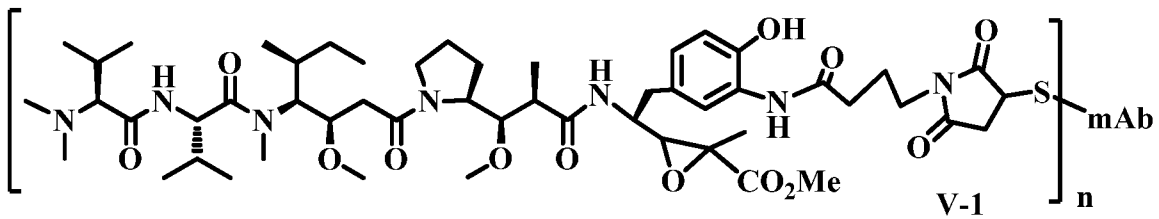


Vdd

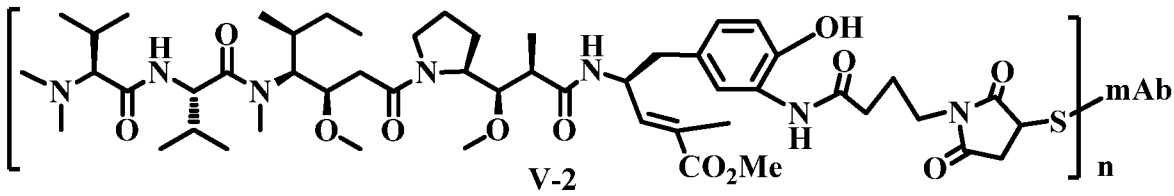
and pharmaceutical acceptable salts and solvates thereof,

Wherein Q, T, R^1, R^2, R^3, R^{17}, R^{18}, R^{19} and R^{22}, are defined the same above. In addition, R^{17} and R^{18} can be absent.

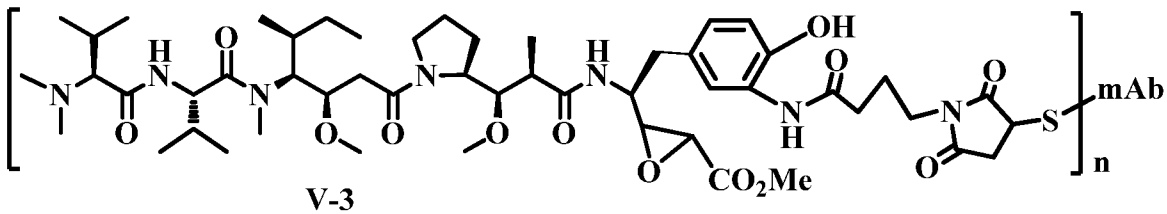
The preferred structures of conjugates bearing Formula (V) with an antibody are, but not limited to, the following:



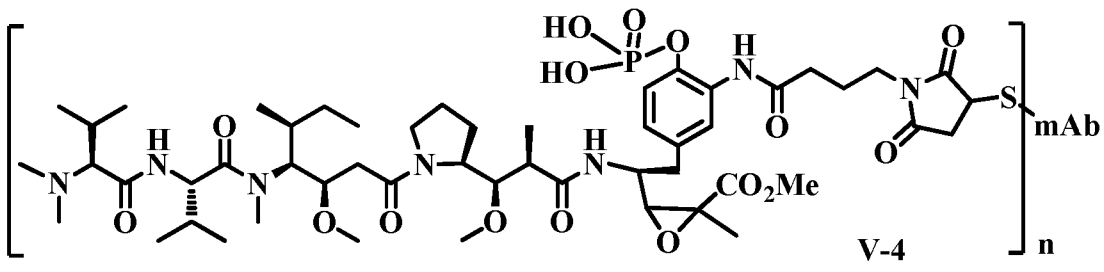
V-1



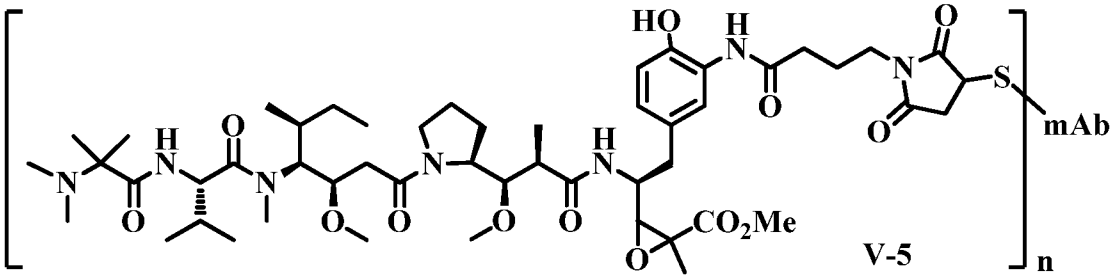
V-2



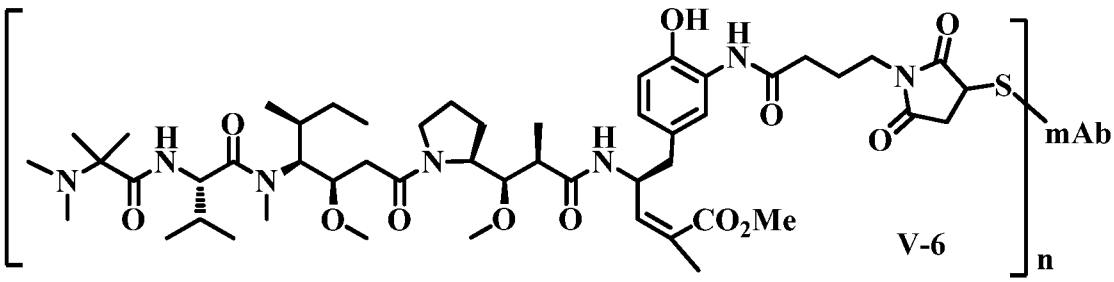
V-3



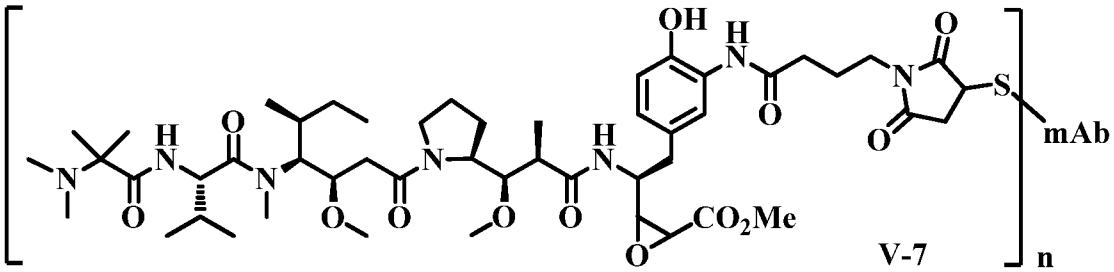
V-4



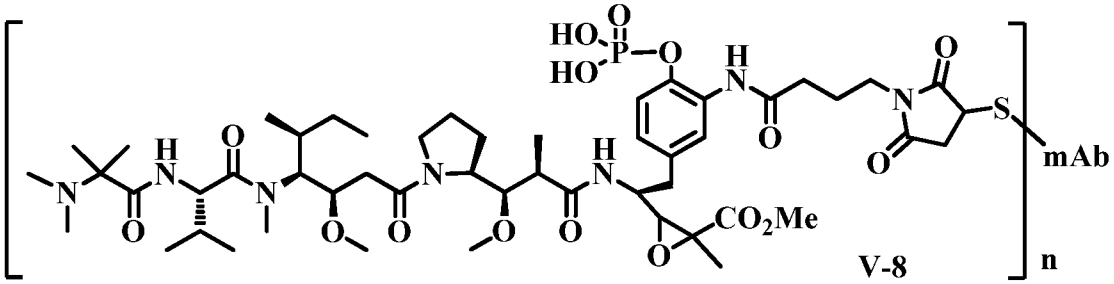
V-5



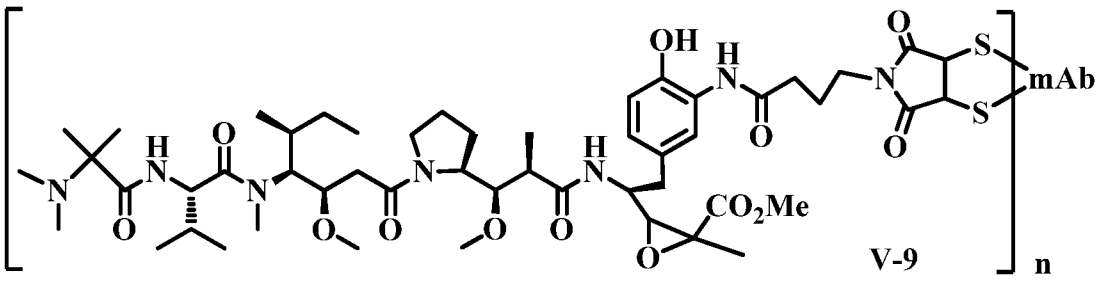
V-6



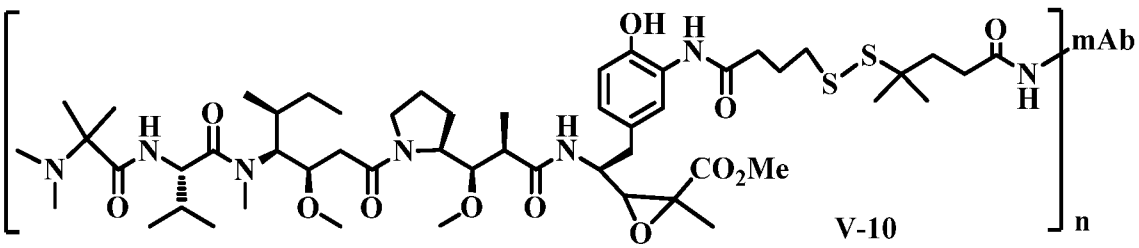
V-7



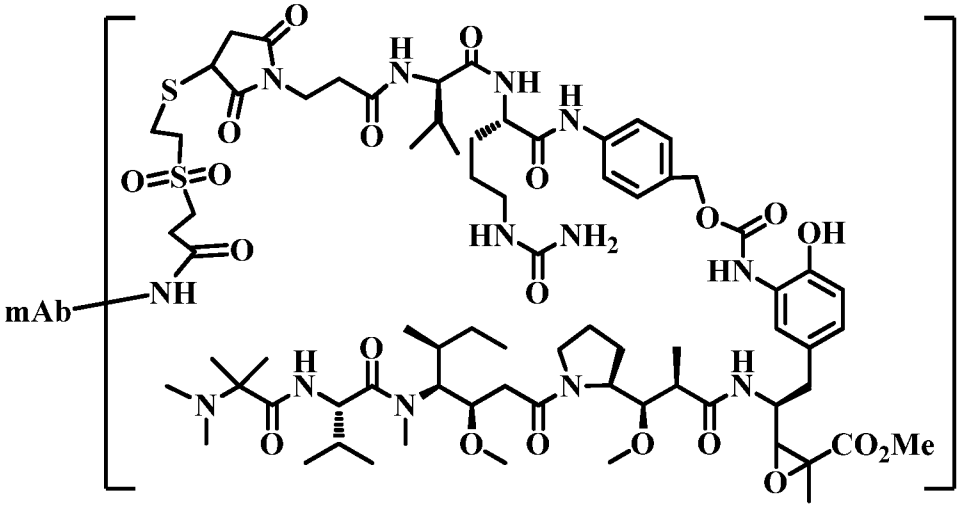
V-8



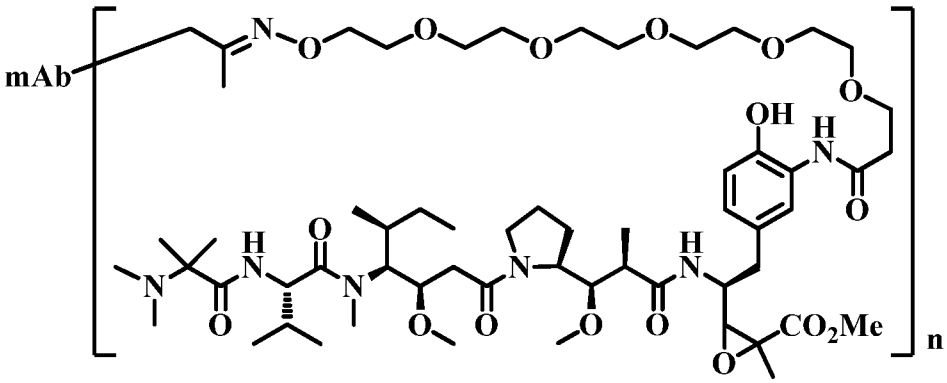
V-9



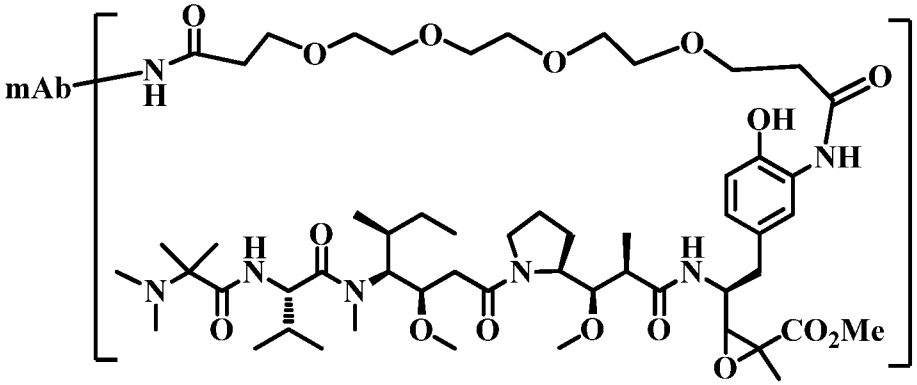
V-10



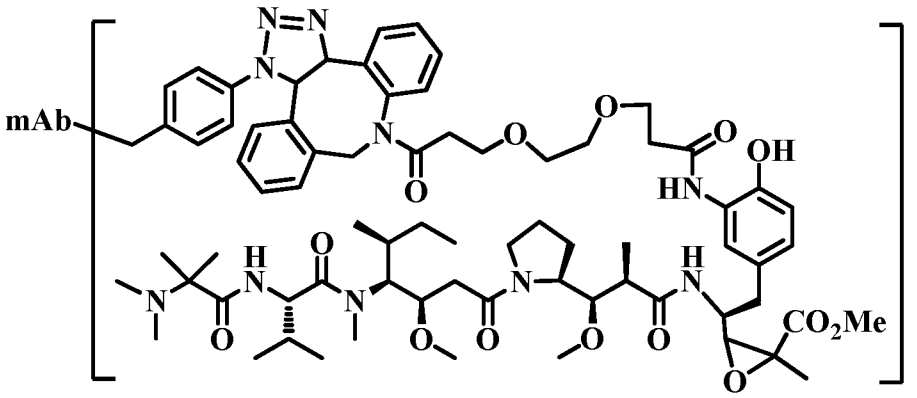
V-11



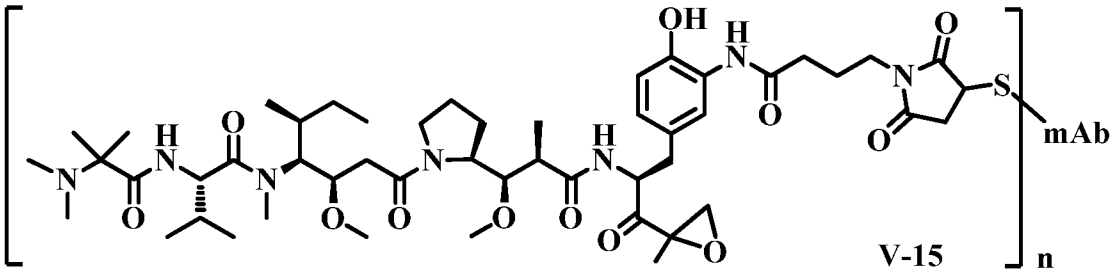
V-12



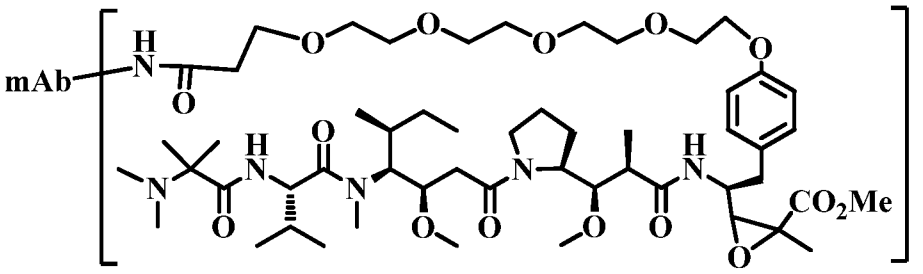
V-13



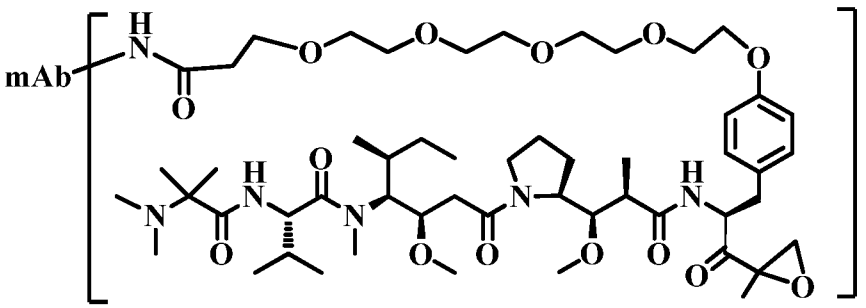
V-14



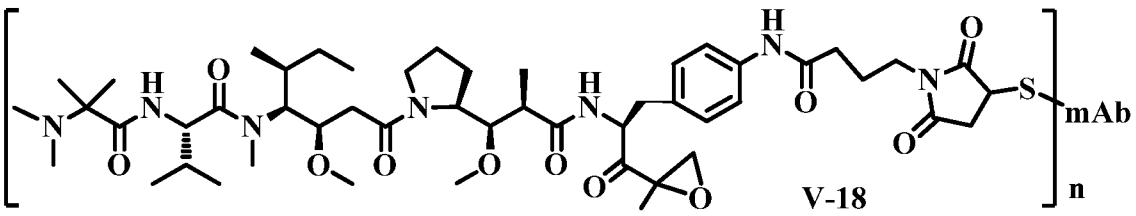
V-15



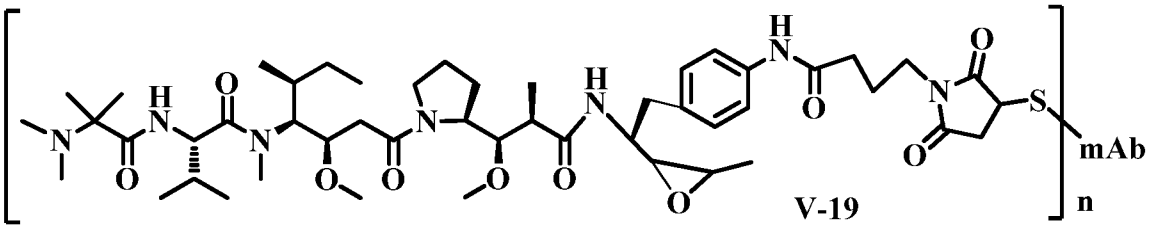
V-16



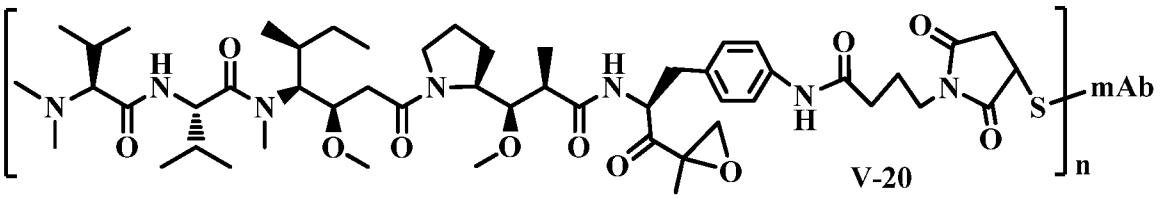
V-17



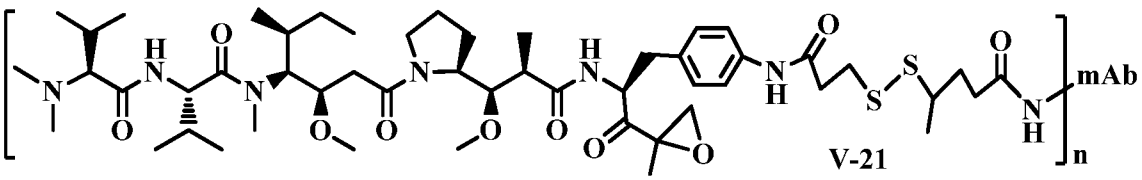
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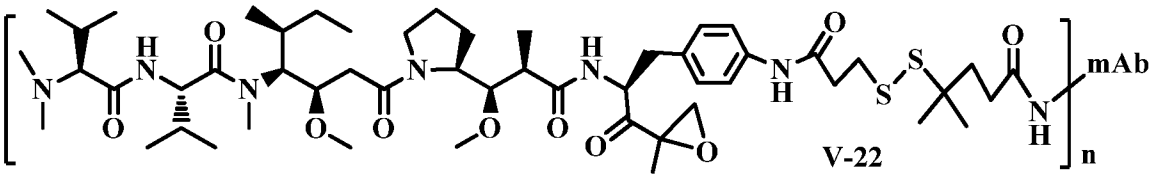
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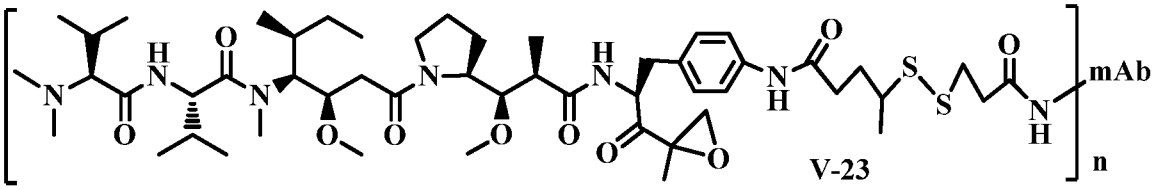
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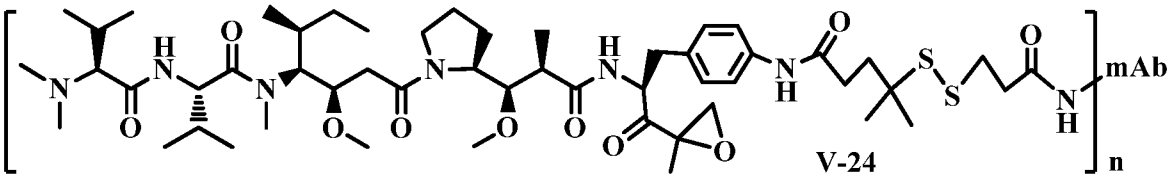
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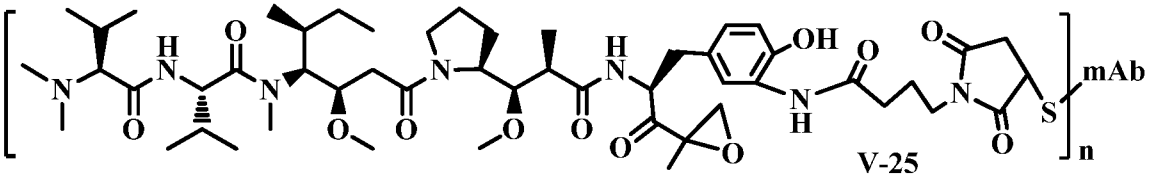
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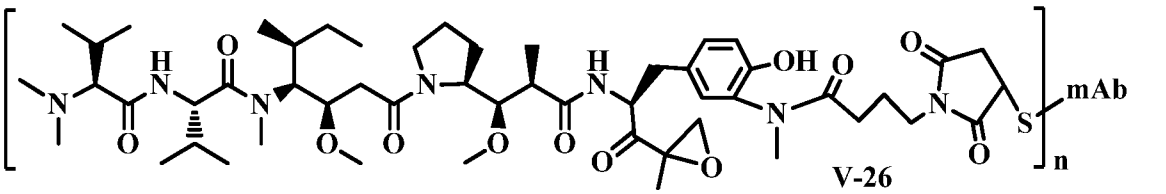
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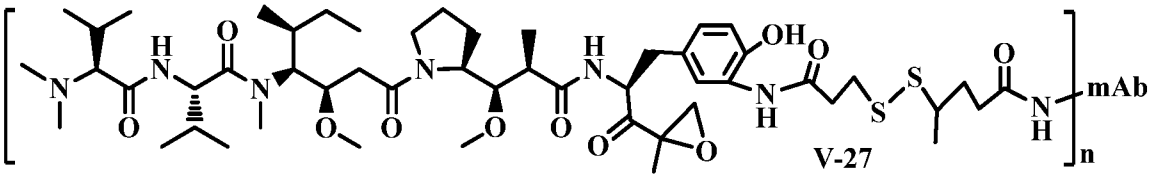
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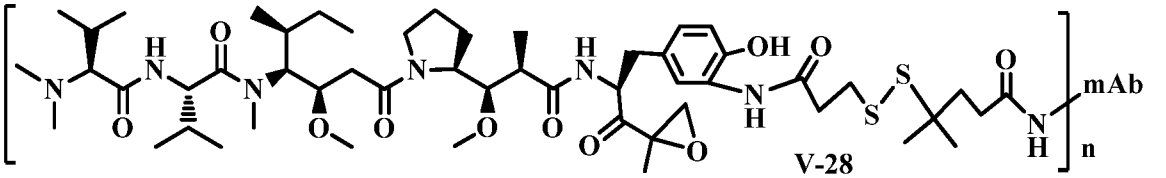
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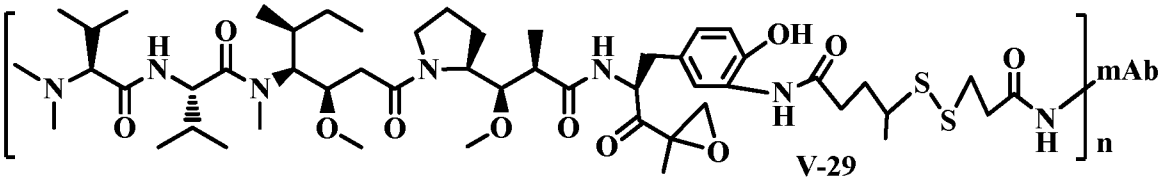
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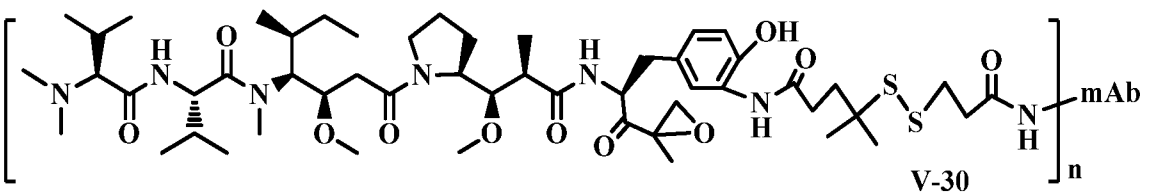
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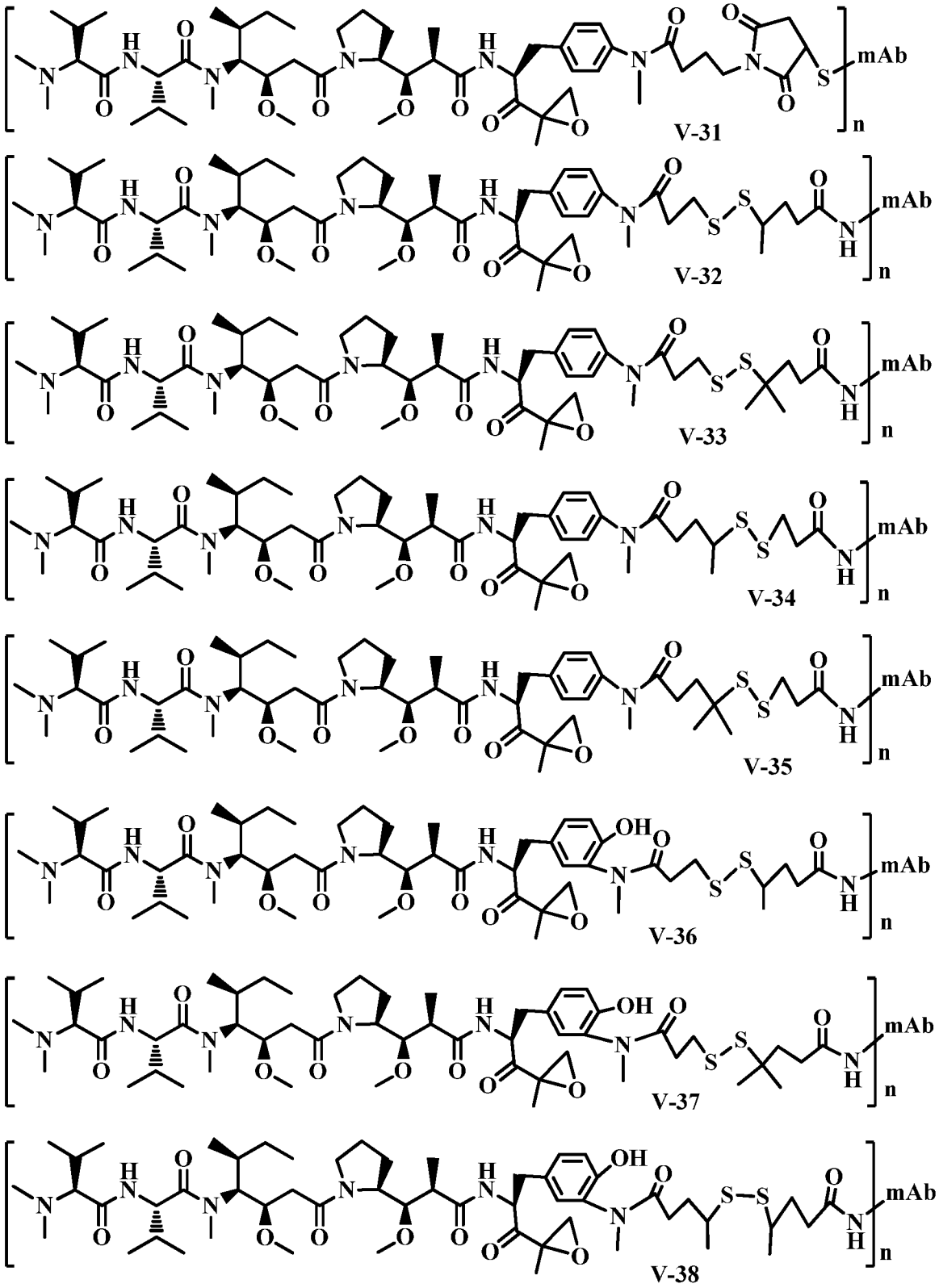
V-28



V-29



V-30



and pharmaceutical acceptable salts and solvates thereof.

Wherein mAb is an antibody and n = 1~30.

The cytotoxic agents (auristatin/dolastatin molecules) of this invention may be prepared according to the general methods of: U.S. Pat. No. 5,635, 483; U.S. Pat. No. 5,780, 588; Pettit et al., J. Am. Chem. Soc. 1989, 111: 5463-5465; Pettit et al., J. Chem. Soc.

Perkin Trans. 1996, 15: 859-863; Shioiri et al., Tetrahedron Lett. 1993, 49(9): 1913-1924. The preparation of Dap unit ((2*R*,3*R*,4*S*)-dolaproine) is illustrated in scheme **1**. The preparation of Dil unit ((3*R*,4*S*,5*S*)-dolaisoleucine) is illustrated in the scheme **2**.

Typically, peptide-based drugs can be prepared by forming a peptide bond between two or more amino acids and/or modified amino acids, and/or peptide fragments. Such peptide bonds can be prepared according to the standard liquid or solid phase synthetic methods that are well known in the field of peptide chemistry. The synthesis of MMAF analogue drugs and linker-drug compounds are illustrated in Scheme **3-7**.

3. Cell-binding molecules (ligand)

The cell-binding molecule that comprises the conjugates and the modified cell-binding molecule of the present invention may be of any kind presently known, or that become known, molecule that binds to, complexes with or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified.

The cell-binding agents include, but are not limited to, large molecular weight proteins such as, for example, full-length antibodies (polyconal antibodies, monoclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies); single chain antibodies; fragments of antibodies such as Fab, Fab', $F(ab')_2$, Fv, (Parham, J. Immunol. 1983, 131: 2895-2902), fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's, and epitope-binding fragments of any of the above which immuno-specifically bind to cancer cell antigens, viral antigens, microbial antigens or a protein generated by the immune system that is capable of recognizing, binding to a specific antigen or exhibiting the desired biological activity (Miller et al., J. Immunol. 2003, 170: 4854-4861); interferons (such as type I, II, III); peptides; lymphokines such as IL-2, IL-3, IL-4, IL-6, GM-CSF, interferon-gamma (IFN-γ); hormones such as insulin, TRH (thyrotropin releasing hormones), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens, melanocyte-stimulating hormone (MSH); growth factors and colony-stimulating factors such as epidermal growth factors (EGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factors (TGF), such as TGFα, TGFβ, insulin and insulin like growth factors (IGF-I, IGF-II) G-CSF, M-CSF and GM-CSF (Burgess, Immunol. Today, 1984, 5: 155-158); vaccinia growth factors (VGF); fibroblast growth factors (FGFs); smaller molecular weight proteins, poly-peptide, peptides and peptide hormones, such as bombesin, gastrin, gastrin-releasing peptide; platelet-derived growth factors; interleukin and cytokines, such as interleukin-2 (IL-2), interleukin-6 (IL-6), leukemia inhibitory factors, granulocyte-

macrophage colony-stimulating factor (GM-CSF); vitamins, such as folate; apoproteins and glycoproteins, such as transferrin (O'Keefe et al., J. Biol. Chem. 1985, 932-937); sugar-binding proteins or lipoproteins, such as lectins; cell nutrient-transport molecules; and small molecular inhibitors, such as prostate-specific membrane antigen (PSMA) inhibitors and small molecular tyrosine kinase inhibitors (TKI), non-peptides or any other cell-binding molecule or substance, such as bioactive polymers (Dhar, et al., Proc. Natl. Acad. Sci. 2008, 105: 17356-61); dendrimers (Lee, et al., Nat. Biotechnol. 2005, 23: 1517-26; Almutairi et al., Proc. Natl. Acad. Sci. 2009, 106: 685-90); nanoparticles (Liong et al., ACS Nano, 2008, 19: 1309-12; Medarova et al., Nat. Med. 2007, 13: 372-7; Javier et al., Bioconj. Chem. 2008, 19: 1309-12); liposomes (Medinai et al., Curr. Phar. Res. 2004, 10: 2981-9); viral capsides (Flenniken et al., Viruses Nanotechnol. 2009, 327: 71-93). In general monoclonal antibodies are preferred as a cell-surface binding agent if an appropriate one is available. And antibodies may be murine, human, humanized, chimeric, or derived from other species.

Production of antibodies used in the present invention involves in vivo or in vitro procedures or combinations thereof. Methods for producing polyclonal anti-receptor peptide antibodies are well-known in the art, such as in U.S. Pat. No. 4,493,795. A monoclonal antibody is typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen (Köhler and Milstein, Nature, 1975, 256: 495-497). The detailed procedures are described in *Antibodies--A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory Press, New York (1988), which is incorporated herein by reference. Particularly monoclonal antibodies are produced by immunizing mice, rats, hamsters or any other mammal with the antigen of interest such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus, and viral proteins. Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT (hypoxanthine-aminopterin-thymine). Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact specified receptors or inhibit receptor activity on target cells.

A monoclonal antibody used in the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected.

The antibody molecules can then be further isolated by well-known techniques, such as using protein-A affinity chromatography; anion, cation, hydrophobic, or size exclusive chromatographies (particularly by affinity for the specific antigen after Protein A, and sizing column chromatography); centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., Virol. 1959, 8: 396) supplemented with 4.5 gm/l glucose, 20 mm glutamine, 20% fetal calf serum and with an anti-foaming agent, such as polyoxyethylene-polyoxypropylene block copolymer.

In addition, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with an oncovirus, such as Epstein-Barr virus (EBV, also called human herpesvirus 4 (HHV-4)) or Kaposi's sarcoma-associated herpesvirus (KSHV). See U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890. A monoclonal antibody may also be produced via an anti-receptor peptide or peptides containing the carboxyl terminal as described well-known in the art. See Niman et al., Proc. Natl. Acad. Sci. 1983, 80: 4949-4953; Geysen et al., Proc. Natl. Acad. Sci. 1985, 82: 178-182; Lei et al. Biochemistry 1995, 34(20): 6675-6688. Typically, the anti-receptor peptide or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen for producing anti-receptor peptide monoclonal antibodies.

There are also a number of other well-known techniques for making monoclonal antibodies as binding molecules in this invention. Particularly useful are methods of making fully human antibodies. One method is phage display technology which can be used to select a range of human antibodies binding specifically to the antigen using methods of affinity enrichment. Phage display has been thoroughly described in the literature and the construction and screening of phage display libraries are well known in the art, see e.g., Dente et al., Gene 1994, 148(1): 7-13; Little et al., Biotechnol Adv. 1994, 12(3): 539-55; Clackson et al., Nature 1991, 352: 264-628; Huse et al., Science 1989, 246: 1275-1281.

Moncolonal antibodies derived by hybridoma technique from another species than human, such as mouse, can be humanized to avoid human anti-mouse antibodies when infused into humans. Among the more common methods of humanization of antibodies are complementarity-determining region grafting and resurfacing. These methods have

been extensively described, see e.g. U.S. Pat. Nos. 5,859,205 and 6,797,492; Liu et al., Immunol Rev. 2008, 222: 9-27; Almagro et al., Front Biosci. 2008, 13: 1619-33; Lazar et al., Mol. Immunol. 2007, 44(8): 1986-98; Li et al., Proc. Natl. Acad. Sci. 2006, 103(10): 3557-62. Fully human antibodies can also be prepared by immunizing transgenic mice, rabbits, monkeys, or other mammals, carrying large portions of the human immunoglobulin heavy and light chains, with an immunogen. Examples of such mice are: the Xenomouse (Abgenix/Amgen), the HuMAb-Mouse (UltiMab platform, Medarex/BMS), the VelociMouse (Regeneron), Kymab (Kymouse Technology), OmniRat™ and OmniMouse™ platform (Open Monoclonal Technology, Inc), see also U.S. Pat. Nos. 6,596,541 , 6,207,418, No. 6,150,584, No. 6,111,166, No. 6,075,181, No. 5,922,545, Nos. 5,661,016, 5,545,806, 5,436,149 and 5,569,825. In human therapy, murine variable regions and human constant regions can also be infused to construct called "chimeric antibodies" that are considerably less immunogenic in man than murine mAbs (Kipriyanov et al., Mol Biotechnol. 2004, 26: 39-60; Houdebine, Curr. Opin. Biotechnol. 2002, 13: 625-9). In addition, site-directed mutagenesis in the variable region of an antibody can result in an antibody with higher affinity and specificity for its antigen (Brannigan et al., Nat. Rev. Mol. Cell Biol. 2002, 3: 964-70); Adams et al., J. Immunol. Methods 1999, 231: 249-60) and exchanging constant regions of a mAb can improve its ability to mediate effector functions of binding and cytotoxicity.

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

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

limited, an albumin, a polymer, a liposome, a nano particle, as long as such attachment permits the peptide or protein to retain its antigen binding specificity.

Examples of antibodies used for conjugation of drugs of this prevention for treating cancer, autoimmune disease, and infectious disease include, but are not limited to, 3F8 (anti-GD2), Abagovomab (anti CA-125), Abciximab (anti CD41 (integrin alpha-IIb), Adalimumab (anti-TNF-α), Adecatumumab (anti-EpCAM, CD326), Afelimomab (anti-TNF-α); Afutuzumab (anti-CD20), Alacizumab pegol (anti-VEGFR2), ALD518 (anti-IL-6), Alemtuzumab (Campath, MabCampath, anti- CD52), Altumomab (anti-CEA), Anatumomab (anti-TAG-72), Anrukinzumab (IMA-638, anti-IL-13), Apolizumab (anti-HLA-DR), Arcitumomab (anti-CEA), Aselizumab (anti-L-selectin (CD62L), Atlizumab (tocilizumab, Actemra, RoActemra, anti-IL-6 receptor), Atorolimumab (anti-Rhesus factor), Bapineuzumab (anti-beta amyloid), Basiliximab (Simulect, antiCD25 (α chain of IL-2 receptor), Bavituximab (anti-phosphatidylserine), Bectumomab (LymphoScan, anti-CD22), Belimumab (Benlysta, LymphoStat-B, anti-BAFF), Benralizumab (anti-CD125), Bertilimumab (anti-CCL11 (eotaxin-1)), Besilesomab (Scintimun, anti-CEA-related antigen), Bevacizumab (Avastin, anti-VEGF-A), Biciromab (FibriScint, anti-fibrin II beta chain), Bivatuzumab (anti-CD44 v6), Blinatumomab (BiTE, anti-CD19), Brentuximab (cAC10, anti-CD30 TNFRSF8), Briakinumab (anti-IL-12, IL-23) Canakinumab (Ilaris, anti-IL-1), Cantuzumab (C242, anti-CanAg), Capromab, Catumaxomab (Removab, anti-EpCAM, anti-CD3), CC49 (anti-TAG-72), Cedelizumab (anti-CD4), Certolizumab pegol (Cimzia anti-TNF-α), Cetuximab (Erbitux, IMC-C225, anti-EGFR), Citatuzumab bogatox (anti-EpCAM), Cixutumumab (anti-IGF-1), Clenoliximab (anti-CD4), Clivatuzumab (anti-MUC1), Conatumumab (anti-TRAIL-R2), CR6261 (anti-Influenza A hemag-glutinin), Dacetuzumab (anti-CD40), Daclizumab (Zenapax, anti-CD25 (α chain of IL-2 receptor)), Daratumumab (anti-CD38 (cyclic ADP ribose hydrolase), Denosumab (Prolia, anti-RANKL), Detumomab (anti-B-lymphoma cell), Dorlimomab, Dorlixizumab, Ecromeximab (anti-GD3 ganglioside), Eculizumab (Soliris, anti-C5), Edobacomab (anti-endotoxin), Edrecolomab (Panorex, MAb17-1A, anti-EpCAM), Efalizumab (Raptiva, anti-LFA-1 (CD11a), Efungumab (Mycograb, anti-Hsp90), Elotuzumab (anti-SLAMF7), Elsilimomab (anti-IL-6), Enlimomab pegol (anti-ICAM-1 (CD54)), Epitumomab (anti-episialin), Epratuzumab (anti-CD22), Erlizumab (anti-ITGB2 (CD18)), Ertumaxomab (Rexomun, anti-HER2/neu, CD3), Etaracizumab (Abegrin, anti-integrin αvβ3), Exbi-virumab (anti-hepatitis B surface antigen), Fanolesomab (NeutroSpec, anti-CD15), Faralimomab (anti-interferon receptor), Farletuzumab (anti-folate receptor 1), Felvizumab

(anti-respiratory syncytial virus), Fezakinumab (anti-IL-22), Figitumumab (anti-IGF-1 receptor), Fontolizumab (anti-IFN-γ), Foravirumab (anti-rabies virus glycoprotein), Fresolimumab (anti-TGF-β), Galiximab (anti-CD80), Gantenerumab (anti- beta amyloid), Gavilimomab (anti-CD147 (basigin)), Gemtuzumab (anti-CD33), Girentuximab (anti-carbonic anhydrase 9), Glembatumumab (CR011, anti-GPNMB), Golimumab (Simponi, anti-TNF-α), Gomiliximab (anti-CD23 (IgE receptor)), Ibalizumab (anti-CD4), Ibritumomab (anti-CD20), Igovomab (Indimacis-125, anti-CA-125), Imciromab (Myoscint, anti-cardiac myosin), Infliximab (Remicade, anti-TNF-α), Intetumumab (anti-CD51), Inolimomab (anti-CD25 (α chain of IL-2 receptor)), Inotuzumab (anti-CD22), Ipilimumab (anti-CD152), Iratumumab (anti- CD30 (TNFRSF8)), Keliximab (anti-CD4), Labetuzumab (CEA-Cide, anti-CEA), Lebrikizumab (anti- IL-13), Lemalesomab (anti-NCA-90 (granulocyte antigen)), Lerdelimumab (anti-TGF beta 2), Lexatumumab (anti-TRAIL-R2), Libivirumab (anti-hepatitis B surface antigen), Lintuzumab (anti-CD33), Lucatumumab (anti-CD40), Lumiliximab (anti- CD23 (IgE receptor), Mapatumumab (anti-TRAIL-R1), Maslimomab (anti- T-cell receptor), Matuzumab (anti-EGFR), Mepolizumab (Bosatria, anti-IL-5), Metelimumab (anti-TGF beta 1), Milatuzumab (anti-CD74), Minretumomab (anti-TAG-72), Mitumomab (BEC-2, anti-GD3 ganglioside), Morolimumab (anti-Rhesus factor), Motavizumab (Numax, anti-respiratory syncytial virus), Muromonab-CD3 (Orthoclone OKT3, anti-CD3), Nacolomab (anti-C242), Naptumomab (anti-5T4), Natalizumab (Tysabri, anti-integrin α4), Nebacumab (anti-endotoxin), Necitumumab (anti-EGFR), Nerelimomab (anti-TNF-α), Nimotuzumab (Theracim, Theraloc, anti-EGFR), Nofetumomab, Ocrelizumab (anti-CD20), Odulimomab (Afolimomab, anti-LFA-1 (CD11a)), Ofatumumab (Arzerra, anti-CD20), Olaratumab (anti-PDGF-R α), Omalizumab (Xolair, anti-IgE Fc region), Oportuzumab (anti-EpCAM), Oregovomab (OvaRex, anti-CA-125), Otelixizumab (anti-CD3), Pagibaximab (anti-lipoteichoic acid), Palivizumab (Synagis, Abbosynagis, anti-respiratory syncytial virus), Panitumumab (Vectibix, ABX-EGF, anti-EGFR), Panobacumab (anti- Pseudomonas aeruginosa), Pascolizumab (anti-IL-4), Pemtumomab (Theragyn, anti-MUC1), Pertuzumab (Omnitarg, 2C4, anti-HER2/neu), Pexelizumab (anti-C5), Pintumomab (anti-adenocarcinoma antigen), Priliximab (anti-CD4), Pritumumab (anti-vimentin), PRO 140 (anti-CCR5), Racotumomab (1E10, anti-(N-glycolylneuraminic acid (NeuGc, NGNA)-gangliosides GM3)), Rafivirumab (anti-rabies virus glycoprotein), Ramucirumab (anti-VEGFR2), Ranibizumab (Lucentis, anti-VEGF-A), Raxibacumab (anti-anthrax toxin, protective antigen), Regavirumab (anti-cytomegalovirus glycoprotein B), Reslizumab

(anti-IL-5), Rilotumumab (anti-HGF), Rituximab (MabThera, Rituxanmab, anti-CD20), Robatumumab (anti-IGF-1 receptor), Rontalizumab (anti-IFN-α), Rovelizumab (LeukArrest, anti-CD11, CD18), Ruplizumab (Antova, anti-CD154 (CD40L)), Satumomab (anti-TAG-72), Sevirumab (anti-cytomegalovirus), Sibrotuzumab (anti-FAP), Sifalimumab (anti-IFN-α), Siltuximab (anti-IL-6), Siplizumab (anti-CD2), (Smart) MI95 (anti-CD33), Solanezumab (anti-beta amyloid), Sonepcizumab (anti-sphingosine-1-phosphate), Sontuzumab (anti-episialin), Stamulumab (anti-myostatin), Sulesomab (LeukoScan, (anti-NCA-90 (granulocyte antigen), Tacatuzumab (anti-alpha-fetoprotein), Tadocizumab (anti-integrin αIIbβ3), Talizumab (anti-IgE), Tanezumab (anti-NGF), Taplitumomab (anti-CD19), Tefibazumab (Aurexis, (anti-clumping factor A), Telimomab, Tenatumomab (anti-tenascin C), Teneliximab (anti-CD40), Teplizumab (anti-CD3), TGN1412 (anti-CD28), Ticilimumab (Tremelimumab, (anti-CTLA-4), Tigatuzumab (anti-TRAIL-R2), TNX-650 (anti-IL-13), Tocilizumab (Atlizumab, Actemra, RoActemra, (anti-IL-6 receptor), Toralizumab (anti-CD154 (CD40L)), Tositumomab (anti-CD20), Trastuzumab (Herceptin, (anti-HER2/neu), Tremelimumab (anti-CTLA-4), Tucotuzumab celmoleukin (anti-EpCAM), Tuvirumab (anti-hepatitis B virus), Urtoxazumab (anti-Escherichia coli), Ustekinumab (Stelara, anti-IL-12, IL-23), Vapaliximab (anti-AOC3 (VAP-1)), Vedolizumab, (anti-integrin α4β7), Veltuzumab (anti-CD20), Vepalimomab (anti-AOC3 (VAP-1), Visilizumab (Nuvion, anti-CD3), Vitaxin (anti-vascular integrin avb3), Volociximab (anti-integrin α5β1), Votumumab (HumaSPECT, anti-tumor antigen CTAA16.88), Zalutumumab (HuMax-EGFr, (anti-EGFR), Zanolimumab (HuMax-CD4, anti-CD4), Ziralimumab (anti-CD147 (basigin)), Zolimomab (anti-CD5), Etanercept (Enbrel®), Alefacept (Amevive®), Abatacept (Orencia®), Rilonacept (Arcalyst), 14F7 [anti-IRP-2 (Iron Regulatory Protein 2)], 14G2a (anti-GD2 ganglioside, from Nat. Cancer Inst. for melanoma and solid tumors), J591 (anti-PSMA, Weill Cornell Medical School for prostate cancers), 225.28S [anti-HMW-MAA (High molecular weight-melanoma-associated antigen), Sorin Radiofarmaci S.R.L. (Milan, Italy) for melanoma], COL-1 (anti-CEACAM3, CGM1, from Nat. Cancer Inst. USA for colorectal and gastric cancers), CYT-356 (Oncoltad®, for prostate cancers), HNK20 (OraVax Inc. for respiratory syncytial virus), ImmuRAIT (from Immunomedics for NHL), Lym-1 (anti-HLA-DR10, Peregrine Pharm. for Cancers), MAK-195F [anti-TNF (tumor necrosis factor; TNFA, TNF-alpha; TNFSF2), from Abbott / Knoll for Sepsis toxic shock], MEDI-500 [T10B9, anti-CD3, TRαβ (T cell receptor alpha/beta) complex, from MedImmune Inc for Graft-versus-host disease], RING SCAN [anti-TAG 72 (tumour associated glycoprotein 72), from Neoprobe

Corp. for breast, colon and rectal cancers], Avicidin (anti-EPCAM (epithelial cell adhesion molecule), anti-TACSTD1 (Tumor-associated calcium signal transducer 1), anti-GA733-2 (gastrointestinal tumor-associated protein 2), anti-EGP-2 (epithelial glycoprotein 2); anti-KSA; KS1/4 antigen; M4S; tumor antigen 17-1A; CD326, from NeoRx Corp. for Colon, Ovarian, Prostate cancers and NHL]; LymphoCide (Immunomedics, NJ), Smart ID10 (Protein Design Labs), Oncolym (Techniclone Inc, CA), Allomune (BioTransplant, CA), anti-VEGF (Genentech, CA); CEAcide (Immunomedics, NJ), IMC-1C11 (ImClone /Eli Lilly) and Cetuximab (ImClone/Eli Lilly) .

Other antibodies as binding molecules include, but are not limited to, are antibodies against the following antigens: Aminopeptidase N (CD13), Annexin A1, B7-H3 (CD276, various cancers), CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), CD2 (Hodgkin's disease, NHL lymphoma, multiple myeloma), CD3 epsilon (T cell lymphoma, lung, breast, gastric, ovarian cancers, autoimmune diseases, malignant ascites), CD19 (B cell malignancies), CD20 (non-Hodgkin's lymphoma), CD22 (leukemia, lymphoma, multiple myeloma, SLE), CD30 (Hodgkin's lymphoma), CD33 (leukemia, autoimmune diseases), CD38 (multiple myeloma), CD40 (lymphoma, multiple myeloma, leukemia (CLL)), CD51 (Metastatic melanoma, sarcoma), CD52 (leukemia), CD56 (small cell lung cancers, ovarian cancer, Merkel cell carcinoma, and the liquid tumor, multiple myeloma), CD66e (cancers), CD70 (metastatic renal cell carcinoma and non-Hodgkin lymphoma), CD74 (multiple myeloma), CD80 (lymphoma), CD98 (cancers), mucin (carcinomas), CD221 (solid tumors), CD227 (breast, ovarian cancers), CD262 (NSCLC and other cancers), CD309 (ovarian cancers), CD326 (solid tumors), CEACAM3 (colorectal, gastric cancers), CEACAM5 (carcinoembryonic antigen; CEA, CD66e) (breast, colorectal and lung cancers), DLL4 (Δ-like-4), EGFR (Epidermal Growth Factor Receptor, various cancers), CTLA4 (melanoma), CXCR4 (CD184, Heme-oncology, solid tumors), Endoglin (CD105, solid tumors), EPCAM (epithelial cell adhesion molecule, bladder, head, neck, colon, NHL prostate, and ovarian cancers), ERBB2 (Epidermal Growth Factor Receptor 2; lung, breast, prostate cancers), FCGR1 (autoimmune diseases), FOLR (folate receptor, ovarian cancers), GD2 ganglioside (cancers), G-28 (a cell surface antigen glyvolipid, melanoma), GD3 idiotype (cancers), Heat shock proteins (cancers), HER1 (lung, stomach cancers), HER2 (breast, lung and ovarian cancers), HLA-DR10 (NHL),

HLA-DRB (NHL, B cell leukemia), human chorionic gonadotropin (carcinoma), IGF1R (insulin-like growth factor 1 receptor, solid tumors, blood cancers), IL-2 receptor (interleukin 2 receptor,T-cell leukemia and lymphomas), IL-6R (interleukin 6 receptor, multiple myeloma, RA, Castleman's disease, IL6 dependent tumors), Integrins (αvβ3, α5β1, α6β4, αIIβ3, α5β5, αvβ5, for various cancers), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE 4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A member 1, Non-Hodgkin's B cell lymphoma, leukemia), MUC1 or MUC1-KLH (breast, ovarian, cervix, bronchus and gastrointestinal cancer), MUC16 (CA125) (Ovarian cancers), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), MPG (melanoma), MS4A1 (membrane-spanning 4-domains subfamily A, small cell lung cancers, NHL), Nucleolin, Neu oncogene product (carcinomas), P21 (carcinomas), Paratope of anti-(N-glycolylneuraminic acid, Breast, Melanoma cancers), PLAP-like testicular alkaline phosphatase (ovarian, testicular cancers), PSMA (prostate tumors), PSA (prostate), ROBO4, TAG 72 (tumour associated glycoprotein 72, AML, gastric, colorectal, ovarian cancers), T cell transmembrane protein (cancers), Tie (CD202b), TNFRSF10B (tumor necrosis factor receptor superfamily member 10B, cancers), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B, multiple myeloma, NHL, other cancers, RA and SLE), TPBG (trophoblast glycoprotein, Renal cell carcinoma), TRAIL-R1 (Tumor necrosis apoprosis Inducing ligand Receptor 1,lymphoma, NHL, colorectal, lung cancers), VCAM-1 (CD106, Melanoma), VEGF, VEGF-A, VEGF-2 (CD309) (various cancers). Some other tumor associated antigens recognized by antibodies have been reviewed (Gerber et al., mAbs 2009, 1(3): 247-253; Novellino et al., Cancer Immunol. Immunother. 2005, 54(3): 187-207; Franke et al., Cancer Biother. Radiopharm. 2000, 15: 459-76). Examples of these antigens that antibodies against are: Many other Cluster of Differentiations (CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD14, CD15, CD16, CDw17, CD18, CD21, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD31, CD32, CD34, CD35, CD36, CD37, CD41, CD42, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD53, CD54, CD55, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD68, CD69, CD71, CD72, CD79, CD81, CD82, CD83, CD86, CD87, CD88, CD89, CD90, CD91, CD95, CD96, CD100, CD103, CD105, CD106, CD109, CD117, CD120, CD127, CD133, CD134, CD135, CD138, CD141, CD142, CD143, CD144, CD147, CD151, CD152, CD154, CD156, CD158, CD163, CD166, .CD168, CD184, CDw186, CD195, CD202 (a, b), CD209, CD235a, CD271, CD303, CD304), Annexin A1,

Nucleolin, Endoglin (CD105), ROBO4, Amino-peptidase N, delta-like-4 (DLL4), VEGFR-2 (CD309), CXCR4 (9CD184), Tie2, B7-H3, WT1, MUC1, LMP2, HPV E6 E7, EGFRvIII, HER-2/neu, Idiotype, MAGE A3, p53 nonmutant, NY-ESO-1, GD2, CEA, MelanA/MART1, Ras mutant, gp100, p53 mutant, Proteinase3 (PR1), bcr-abl, Tyrosinase, Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, PAP, ML-IAP, AFP, EpCAM, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, PSCA, MAGE A1, sLe(a), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, ETV6-AML, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-β, MAD-CT-2, Fos-related antigen 1.

In another specific embodiment, the drug conjugates of this invention are used for the treatment of cancers. The cancers include, but are not limited, Adrenocortical Carcinoma, Anal Cancer, Bladder Cancer, Brain Tumor (Adult, Brain Stem Glioma, Childhood, Cerebellar Astrocytoma, Cerebral Astrocytoma, Ependymoma, Medulloblastoma, Supratentorial Primitive Neuroectodermal and Pineal Tumors, Visual Pathway and Hypothalamic Glioma), Breast Cancer, Carcinoid Tumor, Gastrointestinal, Carcinoma of Unknown Primary, Cervical Cancer, Colon Cancer, Endometrial Cancer, Esophageal Cancer, Extrahepatic Bile Duct Cancer, Ewings Family of Tumors (PNET), Extracranial Germ Cell Tumor, Eye Cancer, Intraocular Melanoma, Gallbladder Cancer, Gastric Cancer (Stomach), Germ Cell Tumor, Extragonadal, Gestational Trophoblastic Tumor, Head and Neck Cancer, Hypopharyngeal Cancer, Islet Cell Carcinoma, Kidney Cancer (renal cell cancer), Laryngeal Cancer, Leukemia (Acute Lymphoblastic, Acute Myeloid, Chronic Lymphocytic, Chronic Myelogenous, Hairy Cell), Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer (Non-Small Cell, Small Cell, Lymphoma (AIDS-Related, Central Nervous System, Cutaneous T-Cell, Hodgkin's Disease, Non-Hodgkin's Disease, Malignant Mesothelioma, Melanoma, Merkel Cell Carcinoma, Metasatic Squamous Neck Cancer with Occult Primary, Multiple Myeloma, and Other Plasma Cell Neoplasms, Mycosis Fungoides, Myelodysplastic Syndrome, Myeloproliferative Disorders, Nasopharyngeal Cancer, Neuroblastoma, Oral Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer (Epithelial, Germ Cell Tumor, Low Malignant Potential Tumor), Pancreatic Cancer (Exocrine, Islet Cell Carcinoma), Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pheochromocytoma Cancer, Pituitary Cancer, Plasma Cell Neoplasm, Prostate Cancer Rhabdomyosarcoma, Rectal Cancer, Renal Cell Cancer

(kidney cancer), Renal Pelvis and Ureter (Transitional Cell), Salivary Gland Cancer, Sezary Syndrome, Skin Cancer, Skin Cancer (Cutaneous T-Cell Lymphoma, Kaposi's Sarcoma, Melanoma), Small Intestine Cancer, Soft Tissue Sarcoma, Stomach Cancer, Testicular Cancer, Thymoma (Malignant), Thyroid Cancer, Urethral Cancer, Uterine Cancer (Sarcoma), Unusual Cancer of Childhood, Vaginal Cancer, Vulvar Cancer, Wilms' Tumor.

In another specific embodiment, the drug conjugates of this invention are used in accordance with the compositions and methods for the treatment or prevention of an autoimmune disease. The autoimmune diseases include, but are not limited, Achlorhydra Autoimmune Active Chronic Hepatitis, Acute Disseminated Encephalomyelitis, Acute hemorrhagic leukoence-phalitis, Addison's Disease, Agammaglobulinemia, Alopecia areata, Amyotrophic Lateral Sclerosis, Ankylosing Spondylitis, Anti-GBM/TBM Nephritis, Antiphospholipid syndrome, Antisynthetase syndrome, Arthritis, Atopic allergy, Atopic Dermatitis, Autoimmune Aplastic Anemia, Autoimmune cardiomyopathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune pancreatitis, Autoimmune polyendocrine syndrome Types I, II, & III, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Bechets Syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous Pemphigoid, Castleman's disease, Chagas disease, Chronic Fatigue Immune Dysfunction Syndrome, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal ostomyelitis, Chronic lyme disease, Chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial Pemphigoid, Coeliac Disease, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Cranial arteritis, CREST syndrome, Crohns Disease (a type of idiopathic inflammatory bowel diseases), Cushing's Syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Discoid lupus erythematosus, Eczema, Endometriosis, Enthesitis-related arthritis, Eosinophilic fasciitis, Epidermolysis bullosa acquisita, Erythema nodosum, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibromyositis, Fibrosing aveolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis, Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Haemolytic anaemia, Henoch-Schonlein purpura, Herpes

gestationis, Hidradenitis suppurativa, Hughes syndrome (See Antiphospholipid syndrome), Hypogammaglobulinemia, Idiopathic Inflammatory Demyelinating Diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura (See Autoimmune thrombocytopenic purpura), IgA nephropathy (Also Berger's disease), Inclusion body myositis, Inflammatory demyelinating polyneuopathy, Interstitial cystitis, Irritable Bowel Syndrome , Juvenile idiopathic arthritis, Juvenile rheumatoid arthritis, Kawasaki's Disease, Lambert-Eaton myasthenic syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease (LAD), Lou Gehrig's Disease (Also Amyotrophic lateral sclerosis), Lupoid hepatitis, Lupus erythematosus, Majeed syndrome, Ménière's disease, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed Connective Tissue Disease, Morphea, Mucha-Habermann disease, Muckle–Wells syndrome, Multiple Myeloma, Multiple Sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's Disease), Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord thyroiditis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis, Pemphigus, Pemphigus vulgaris, Pernicious anaemia, Perivenous encephalomyelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progressive inflammatory neuropathy, Psoriasis, Psoriatic Arthritis, Pyoderma gangrenosum, Pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syndrome, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatoid fever, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Spondyloarthropathy, Sticky blood syndrome, Still's Disease, Stiff person syndrome, Subacute bacterial endocarditis, Susac's syndrome, Sweet syndrome, Sydenham Chorea, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis (giant cell arteritis), Tolosa-Hunt syndrome, Transverse Myelitis, Ulcerative Colitis (a type of idiopathic inflammatory bowel diseases), Undifferentiated connective tissue disease, Undifferentiated spondyloarthropathy, Vasculitis, Vitiligo, Wegener's granulomatosis, Wilson's syndrome, Wiskott-Aldrich syndrome

In another specific embodiment, a binding molecule used for the conjugates of this invention for the treatment or prevention of an autoimmune disease includes, but are not limited to, anti-elastin antibody; Abys against epithelial cells antibody; Anti-Basement

Membrane Collagen Type IV Protein antibody; Anti-Nuclear antibody; Anti-ds DNA; Anti-ss DNA, Anti-Cardiolipin antibody IgM, IgG; Anti-celiac antibody; Anti-Phospholipid antibody IgK, IgG; Anti-SM Antibody; Anti-Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody, T-cells antibody; Thyroglobulin Antibody, Anti-SCL-70; Anti-Jo; Anti-U.sub.1RNP; Anti-La/SSB; Anti-SSA; Anti-SSB; Anti-Perital Cells Antibody; Anti-Histones; Anti-RNP; C-ANCA; P-ANCA; Anti-centromere; Anti-Fibrillarin, and Anti-GBM Antibody, Anti-ganglioside antibody; Anti-Desmogein 3 antibody; Anti-p62 antibody; Anti-sp100 antibody; Anti-Mitochondrial(M2) antibody; Rheumatoid factor antibody; Anti-MCV antibody; Anti-topoisomerase antibody; Anti-neutrophil cytoplasmic(cANCA) antibody;

In certain preferred embodiments, the binding molecule for the conjugate in the present invention, can bind to a receptor or a receptor complex expressed on an activated lymphocyte which is associated with an autoimmune disease. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member (e.g. CD2, CD3, CD4, CD8, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-1, or ICOS), a TNF receptor superfamily member (e.g. CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, INF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3), an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin (C-type, S-type, or I-type), or a complement control protein.

In another specific embodiment, useful binding molecules that are immunospecific for a viral or a microbial antigen are humanized or human monoclonal antibodies. As used herein, the term "viral antigen" includes, but is not limited to, any viral peptide, polypeptide protein (e.g. HIV gp120, HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g. gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacteria, fungi, pathogenic protozoa, or yeast polypeptide including, e.g., LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response. Examples of antibodies available l for the viral or microbial infection include, but are not limited to, Palivizumab which is a humanized anti-respiratory syncytial virus monoclonal antibody for the treatment of RSV infection; PRO542 which is a CD4 fusion antibody for the treatment of HIV infection; Ostavir which is a human antibody for the treatment of

hepatitis B virus; PROTVIR which is a humanized IgG.sub.1 antibody for the treatment of cytomegalovirus; and anti-LPS antibodies.

The drug conjugates of this invention can be used in the treatment of infectious diseases. These infectious diseases include, but are not limited to, Acinetobacter infections, Actinomycosis, African sleeping sickness (African trypanosomiasis), AIDS (Acquired immune deficiency syndrome), Amebiasis, Anaplasmosis, Anthrax, Arcanobacterium haemolyticum infection, Argentine hemorrhagic fever, Ascariasis, Aspergillosis, Astrovirus infection, Babesiosis, Bacillus cereus infection, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystis hominis infection, Blastomycosis, Bolivian hemorrhagic fever, Borrelia infection, Botulism (and Infant botulism), Brazilian hemorrhagic fever, Brucellosis, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Campylobacteriosis, Candidiasis (Moniliasis; Thrush), Cat-scratch disease, Cellulitis, Chagas Disease (American trypanosomiasis), Chancroid, Chickenpox, Chlamydia, Chlamydophila pneumoniae infection, Cholera, Chromoblastomycosis, Clonorchiasis, Clostridium difficile infection, Coccidioidomycosis, Colorado tick fever, Common cold (Acute viral rhinopharyngitis; Acute coryza), Creutzfeldt-Jakob disease, Crimean-Congo hemorrhagic fever, Cryptococcosis, Cryptosporidiosis, Cutaneous larva migrans, Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue fever, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Enterococcus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum, Fasciolopsiasis, Fasciolosis, Fatal familial insomnia, Filariasis, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann-Sträussler-Scheinker syndrome, Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group B streptococcal infection, Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome, Helicobacter pylori infection, Hemolytic-uremic syndrome, Hemorrhagic fever with renal syndrome, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Herpes simplex, Histoplasmosis, Hookworm infection, Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis, Human metapneumo-virus infection, Human monocytic ehrlichiosis, Human papillomavirus infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein-Barr Virus Infectious Mononucle-

osis (Mono), Influenza, Isosporiasis, Kawasaki disease, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Legionellosis (Legionnaires' disease), Legionellosis (Pontiac fever), Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever, Measles, Melioidosis (Whitmore's disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, Molluscum contagiosum, Mumps, Murine typhus (Endemic typhus), Mycoplasma pneumonia, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis, Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (Pubic lice, Crab lice), Pelvic inflammatory disease, Pertussis (Whooping cough), Plague, Pneumococcal infection, Pneumocystis pneumonia, Pneumonia, Poliomyelitis, Prevotella infection, Primary amoebic meningoencephalitis, Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Rat-bite fever, Respiratory syncytial virus infection, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsialpox, Rift Valley fever, Rocky mountain spotted fever, Rotavirus infection, Rubella, Salmonellosis, SARS (Severe Acute Respiratory Syndrome), Scabies, Schistosomiasis, Sepsis, Shigellosis (Bacillary dysentery), Shingles (Herpes zoster), Smallpox (Variola), Sporotrichosis, Staphylococcal food poisoning, Staphylococcal infection, Strongyloidiasis, Syphilis, Taeniasis, Tetanus (Lockjaw), Tinea barbae (Barber's itch), Tinea capitis (Ringworm of the Scalp), Tinea corporis (Ringworm of the Body), Tinea cruris (Jock itch), Tinea manuum (Ringworm of the Hand), Tinea nigra, Tinea pedis (Athlete's foot), Tinea unguium (Onychomycosis), Tinea versicolor (Pityriasis versicolor), Toxocariasis (Ocular Larva Migrans), Toxocariasis (Visceral Larva Migrans), Toxoplasmosis, Trichinellosis, Trichomoniasis, Trichuriasis (Whipworm infection), Tuberculosis, Tularemia, Ureaplasma urealyticum infection, Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, Viral pneumonia, West Nile Fever, White piedra (Tinea blanca), Yersinia pseudotuberculosis infection, Yersiniosis, Yellow fever, Zygomycosis.

The cell-binding molecules, which are more preferred to be antibodies described in this patent are against pathogenic strains include, but are not limited to, Acinetobacter baumannii, Actinomyces israelii, Actinomyces gerencseriae and Propionibacterium propionicus, Trypanosoma brucei, HIV (Human immunodeficiency virus), Entamoeba histolytica, Anaplasma genus, Bacillus anthracis, Arcanobacterium haemolyticum, Junin

virus, Ascaris lumbricoides, Aspergillus genus, Astroviridae family, Babesia genus, Bacillus cereus, multiple bacteria, Bacteroides genus, Balantidium coli, Baylisascaris genus, BK virus, Piedraia hortae, Blastocystis hominis, Blastomyces dermatitides, Machupo virus, Borrelia genus, Clostridium botulinum, Sabia, Brucella genus, usually Burkholderia cepacia and other Burkholderia species, Mycobacterium ulcerans, Caliciviridae family, Campylobacter genus, usually Candida albicans and other Candida species, Bartonella henselae, Group A Streptococcus and Staphylococcus, Trypanosoma cruzi, Haemophilus ducreyi, Varicella zoster virus (VZV), Chlamydia trachomatis, Chlamydophila pneumoniae, Vibrio cholerae, Fonsecaea pedrosoi, Clonorchis sinensis, Clostridium difficile, Coccidioides immitis and Coccidioides posadasii, Colorado tick fever virus, rhinoviruses, coronaviruses, CJD prion, Crimean-Congo hemorrhagic fever virus, Cryptococcus neoformans, Cryptosporidium genus, Ancylostoma braziliense; multiple parasites, Cyclospora cayetanensis, Taenia solium, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), Flaviviruses, Dientamoeba fragilis, Corynebacterium diphtheriae, Diphyllobothrium, Dracunculus medinensis, Ebolavirus, Echinococcus genus, Ehrlichia genus, Enterobius vermicularis, Enterococcus genus, Enterovirus genus, Rickettsia prowazekii, Parvovirus B19, Human herpesvirus 6 and Human herpesvirus 7, Fasciolopsis buski, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily, Clostridium perfringens, Fusobacterium genus, Clostridium perfringens; other Clostridium species, Geotrichum candidum, GSS prion, Giardia intestinalis, Burkholderia mallei, Gnathostoma spinigerum and Gnathostoma hispidum, Neisseria gonorrhoeae, Klebsiella granulomatis, Streptococcus pyogenes, Streptococcus agalactiae, Haemophilus influenzae, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71, Sin Nombre virus, Helicobacter pylori, Escherichia coli O157:H7, Bunyaviridae family, Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1, Herpes simplex virus 2, Histoplasma capsulatum, Ancylostoma duodenale and Necator americanus, Hemophilus influenzae, Human bocavirus, Ehrlichia ewingii, Anaplasma phagocytophilum, Human metapneumovirus, Ehrlichia chaffeensis, Human papillomavirus, Human parainfluenza viruses, Hymenolepis nana and Hymenolepis diminuta, Epstein-Barr Virus, Orthomyxoviridae family, Isospora belli, Kingella kingae, Klebsiella pneumoniae, Klebsiella ozaenas, Klebsiella rhinoscleromotis, Kuru prion, Lassa virus, Legionella pneumophila, Legionella pneumophila, Leishmania genus, Mycobacterium leprae and Mycobacterium lepromatosis, Leptospira genus, Listeria monocytogenes, Borrelia

burgdorferi and other Borrelia species, Wuchereria bancrofti and Brugia malayi, Lymphocytic choriomeningitis virus (LCMV), Plasmodium genus, Marburg virus, Measles virus, Burkholderia pseudomallei, Neisseria meningitides, Metagonimus yokagawai, Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Rickettsia typhi, Mycoplasma pneumoniae, numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma), parasitic dipterous fly larvae, Chlamydia trachomatis and Neisseria gonorrhoeae, vCJD prion, Nocardia asteroides and other Nocardia species, Onchocerca volvulus, Paracoccidioides brasiliensis, Paragonimus westermani and other Paragonimus species, Pasteurella genus, Pediculus humanus capitis, Pediculus humanus corporis, Phthirus pubis, Bordetella pertussis, Yersinia pestis, Streptococcus pneumoniae, Pneumocystis jirovecii, Poliovirus, Prevotella genus, Naegleria fowleri, JC virus, Chlamydophila psittaci, Coxiella burnetii, Rabies virus, Streptobacillus moniliformis and Spirillum minus, Respiratory syncytial virus, Rhinosporidium seeberi, Rhinovirus, Rickettsia genus, Rickettsia akari, Rift Valley fever virus, Rickettsia rickettsii, Rotavirus, Rubella virus, Salmonella genus, SARS coronavirus, Sarcoptes scabiei, Schistosoma genus, Shigella genus, Varicella zoster virus, Variola major or Variola minor, Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Staphylococcus aureus, Streptococcus pyogenes, Strongyloides stercoralis, Treponema pallidum, Taenia genus, Clostridium tetani, Trichophyton genus, Trichophyton tonsurans, Trichophyton genus, Epidermophyton floccosum, Trichophyton rubrum, and Trichophyton mentagrophytes, Trichophyton rubrum, Hortaea werneckii, Trichophyton genus, Malassezia genus, Toxocara canis or Toxocara cati, Toxoplasma gondii, Trichinella spiralis, Trichomonas vaginalis, Trichuris trichiura, Mycobacterium tuberculosis, Francisella tularensis, Ureaplasma urealyticum, Venezuelan equine encephalitis virus, Vibrio colerae, Guanarito virus, West Nile virus, Trichosporon beigelii, Yersinia pseudotuberculosis, Yersinia enterocolitica, Yellow fever virus, Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthoramycosis), Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Aeromonas hydrophila, Edwardsiella tarda, Yersinia pestis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospira icterohemorrhagiae, Pneumocystis carinii, Brucella abortus, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsia prowazeki, Rickettsia tsutsugumushi, Clamydia spp.; pathogenic fungi (Aspergillus fumigatus, Candida albicans, Histoplasma capsulatum); protozoa (Entomoeba histolytica, Trichomonas tenas, Trichomonas hominis, Tryoanosoma gambiense, Trypanosoma

rhodesiense, Leishmania donovani, Leishmania tropica, Leishmania braziliensis, Pneumocystis pneumonia, Plasmodium vivax, Plasmodium falciparum, Plasmodium malaria); or Helminiths (Schistosoma japonicum, Schistosoma mansoni, Schistosoma haematobium, and hookworms).

Other antibodies as cell-binding molecules used in this invention for the treatment of viral diseases include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxyiridae, Herpesviridae, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, Oncovirus [such as, HBV (Hepatocellular carcinoma), HPV (Cervical cancer, Anal cancer), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), Epstein-Barr virus (Nasopharyngeal carcinoma, Burkitt's lymphoma, Primary central nervous system lymphoma), MCPyV (Merkel cell cancer), SV40 (Simian virus 40), HCV (Hepatocellular carcinoma), HTLV-I (Adult T-cell leukemia/lymphoma)], Immune disorders caused virus [such as Human Immunodeficiency Virus (AIDS)]; Central nervous system virus [such as, JCV (Progressive multifocal leukoencephalopathy), MeV (Subacute sclerosing panencephalitis), LCV (Lymphocytic choriomeningitis), Arbovirus encephalitis, Orthomyxoviridae (probable) (Encephalitis lethargica), RV (Rabies), Chandipura virus, Herpesviral meningitis, Ramsay Hunt syndrome type II; Poliovirus (Poliomyelitis, Post-polio syndrome), HTLV-I (Tropical spastic paraparesis)]; Cytomegalovirus (Cytomegalovirus retinitis, HSV (Herpetic keratitis)); Cardiovascular virus [such as CBV (Pericarditis, Myocarditis)]; Respiratory system/acute viral nasopharyngitis/viral pneumonia [Epstein-Barr virus (EBV infection/Infectious mononucleosis), Cytomegalovirus; SARS coronavirus (Severe acute respiratory syndrome) Orthomyxoviridae: Influenzavirus A/B/C (Influenza/Avian influenza), Paramyxovirus: Human parainfluenza viruses (Parainfluenza), RSV (Human respiratory syncytial virus), hMPV]; Digestive system virus [MuV (Mumps), Cytomegalovirus (Cytomegalovirus esophagitis); Adenovirus (Adenovirus infection); Rotavirus, Norovirus, Astrovirus, Coronavirus; HBV (Hepatitis B virus), CBV, HAV (Hepatitis A virus), HCV (Hepatitis C virus), HDV (Hepatitis D virus), HEV (Hepatitis E virus), HGV (Hepatitis G virus)]; Urogenital virus [such as, BK virus, MuV (Mumps)].

The present invention also concerns pharmaceutical compositions comprising the conjugate of the invention together with a pharmaceutically acceptable carrier for the

treatment of cancer and autoimmune disorders. The method for the treatment of cancer and autoimmune disorders can be practiced in vitro, in vivo, or ex vivo. Examples of in vitro uses include treatments of cell cultures in order to kill all cells except for desired variants that do not express the target antigen; or to kill variants that express undesired antigen. Examples of ex vivo uses include treatments of hematopoietic stem cells (HSC) prior to the performance of the transplantation (HSCT) into the same patient in order to kill diseased or malignant cells. For instance, clinical ex vivo treatment to remove tumour cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from allogeneic bone marrow or tissue prior to transplant in order to prevent graft-versus-host disease, can be carried out as follows. Bone marrow is harvested from the patient or other individual and then incubated in medium containing serum to which is added the conjugate of the invention, concentrations range from about 1 pM to 0.1 mM, for about 30 minutes to about 48 hours at about 37 °C. The exact conditions of concentration and time of incubation are readily determined by the skilled clinicians. After incubation the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or totalbody irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

For clinical in vivo use, the conjugate will be supplied as solutions or as a lyophilized solid that can be redisolved in sterile water for injection. Examples of suitable protocols of conjugate administration are as follows. Conjugates are given weekly for 8 weeks as an i.v. bolus. Bolus doses are given in 50 to 500 ml of normal saline to which human serum albumin (e.g. 0.5 to 1 mL of a concentrated solution of human serum albumin, 100 mg/mL) can be added. Dosages will be about 50 μg to 20 mg/kg of body weight per day, or per week, or biweek, or triweek, or per month, i.v. (range of 10 μ g to 200 mg/kg per injection). 4~20 weeks after treatment, the patient may receive a second course of treatment. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by the skilled clinicians.

Examples of medical conditions that can be treated according to the in vivo or ex vivo methods of killing selected cell populations include malignancy of any types of cancer, autoimmune diseases, graft rejections, and infections (viral, bacterial or parasite).

The amount of a conjugate which is required to achieve the desired biological effect, will vary depending upon a number of factors, including the chemical characteristics, the potency, and the bioavailability of the conjugates, the type of disease, the species to which the patient belongs, the diseased state of the patient, the route of administration, all factors which dictate the required dose amounts, delivery and regimen to be administered.

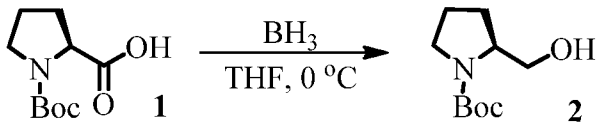
In general terms, the conjugates of this invention may be provided in an aqueous physiological buffer solution containing 0.1 to 10% w/v conjugates for parenteral administration. Typical dose ranges are from 1 μg/kg to 1 g/kg of body weight per day, or per week, or biweek, or triweek, or per month; a preferred dose range is from 0.01 mg/kg to 20 mg/kg of body weight per day or an equivalent dose in a human child. The preferred dosage of drug to be administered is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound, the route of administration (intravenous, intramuscular, or other), the pharmacokinetic properties of the compound by the chosen delivery route, and the speed (bolus or continuous infusion) and schedule of administrations (number of repetitions in a given period of time).

The conjugates of the present invention are also capable of being administered in unit dose forms, wherein the term "unit dose" means a single dose which is capable of being administered to a patient, and which can be readily handled and packaged, remaining as a physically and chemically stable unit dose comprising either the active conjugate itself, or as a pharmaceutically acceptable composition, as described hereinafter. As such, typical total daily dose ranges are from 0.01 to 100 mg/kg of body weight. By way of general guidance, unit doses for humans range from 1 mg to 3000 mg per day, or per week, or biweek, or triweek, or per month. Preferably the unit dose range is from 1 to 500 mg administered one to four times a day, and even more preferably from 10 mg to 500 mg, once a day. Conjugatess provided herein can be formulated into pharmaceutical compositions by admixture with one or more pharmaceutically acceptable excipients. Such unit dose compositions may be prepared for use by oral administration, particularly in the form of tablets, simple capsules or soft gel capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols; or dermally, for example, topically in ointments, creams, lotions, gels or sprays, or via trans-dermal patches.

EXAMPLES

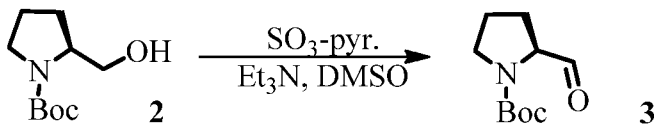
The invention is further described in the following examples, which are not intended to limit the scope of the invention. Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ), unless otherwise specified. Cell culture reagents were obtained from Invitrogen Corp., unless otherwise specified. All anhydrous solvents were commercially obtained and stored in Sure-seal bottles under nitrogen. All other reagents and solvents were purchased as the highest grade available and used without further purification. NMR spectra were recorded on Bruker 500 MHz Instrument. Chemical shifts (δ) are reported in parts per million (ppm) referenced to tetramethylsilane at 0.00 ppm and coupling constants (J) are reported in Hz. High resolution mass spectral data were acquired on a Waters Xevo QTof MS with Waters Acquity UPLC separations module.

Example 1 Preparation of compound **2**



Boc-L-proline (10.0 g, 46.4 mmol) dissolved in 50 mL THF was cooled to 0 °C, to which BH_3 in THF (1.0 M, 46.4 mL) was added carefully. The mixture was stirred at 0 °C for 1.5 h then poured onto ice water and extracted with ethyl acetate. The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give known compound **2** (8.50 g, 91% yield) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 3.94 (dd, J = 4.9, 2.7 Hz, 2H), 3.60 (ddd, J = 18.7, 11.9, 9.3 Hz, 2H), 3.49 – 3.37 (m, 1H), 3.34 – 3.23 (m, 1H), 2.06 – 1.91 (m, 1H), 1.89 – 1.69 (m, 2H), 1.65 – 1.51 (m, 1H), 1.49 – 1.40 (m, 9H).

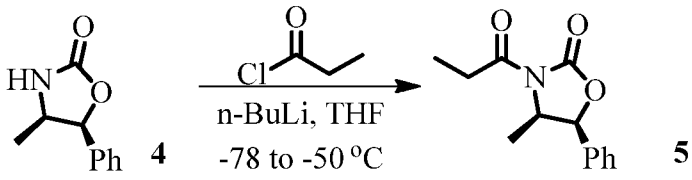
Example 2 Preparation of compound **3**



To a solution of alcohol **2** (13.0 g, 64.6 mmol) in dimethyl sulfoxide (90 mL) was added triethylamine (40 mL) and the stirring was continued for 15 min. The mixture was cooled over ice bath and sulfur trioxide-pyridine complex (35.98 g, 226 mmol) was added in portions over a 40 min period. The reaction was warmed to r.t. and stirred for 2.5 h. After addition of ice (250 g), the mixture was extracted with dichloromethane (150 mL × 3). The organic phase was washed with 50% citric acid solution (150 mL), water (150

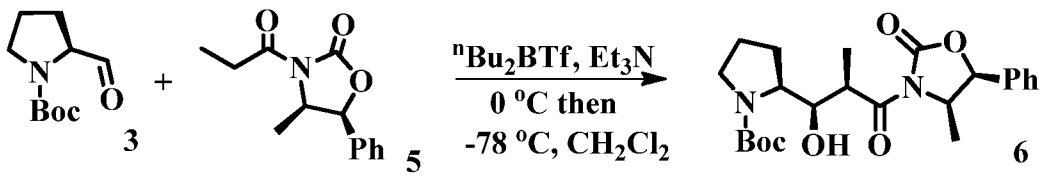
mL), saturated sodium bicarbonate solution (150 mL), and brine (150 mL), dried over anhydrous Na_2SO_4. Removal of solvent in vacuo yielded aldehyde **3** (10.4 g, 81% yield) as a dense oil which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 4.04 (s, 1H), 3.53 (dd, J = 14.4, 8.0 Hz, 2H), 2.00 – 1.82 (m, 4H), 1.44 (d, J = 22.6 Hz, 9H).

Example 3 Preparation of compound **5**



n-Butyllithium in hexane (21.6 mL, 2.2 M, 47.43 mmol) was added dropwise at -78 °C to a stirred solution of 4-methyl-5-phenyloxazolidin-2-one (8.0 g, 45.17 mmol) in THF (100 mL) under N_2. The solution was maintained at -78 ℃ for 1 h then propionyl chloride (4.4 mL, 50.59 mmol) was added slowly. The reaction mixture was warmed to -50 °C, stirred for 2 h then quenched by addition of a saturated solution of ammonium chloride (100 mL). The organic solvent was removed in vacuo and the resultant solution was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with saturated sodium bicarbonate solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (20% ethyl acetate/hexanes) to afford the compound **5** as dense oil (10.5 g, 98% yield). 1H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.34 (m, 3H), 7.30 (d, J = 7.0 Hz, 2H), 5.67 (d, J = 7.3 Hz, 1H), 4.82 – 4.70 (m, 1H), 2.97 (dd, J = 19.0, 7.4 Hz, 2H), 1.19 (t, J = 7.4 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H).

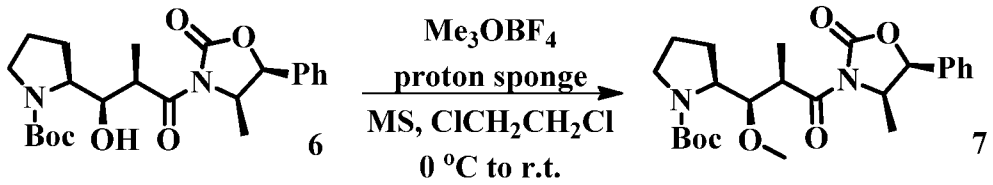
Example 4 Preparation of compound **6**



To a solution of **5** (9.40 g, 40.4 mmol) in dichloromethane (60 mL) was added Et_3N (6.45 mL, 46.64 mmol) at 0 °C, followed by 1M dibutylboron triflate in dichloromethane (42 mL, 42 mmol). The mixture was stirred at 0 °C for 45 min, cooled to -70 °C, aldehyde **3** (4.58 g, 22.97 mmol) in dichloromethane (40 mL) was then added slowly over a 30 min period. The reaction was stirred at -70 °C for 2 h, 0 °C 1 h, and r.t. 15 min, and then quenched with phosphate buffer solution (pH 7, 38 mL). After the addition of MeOH-30% H_2O_2 (2:1, 100 mL) at below 10 °C and stirring for 20 min, water (100 mL) was added and

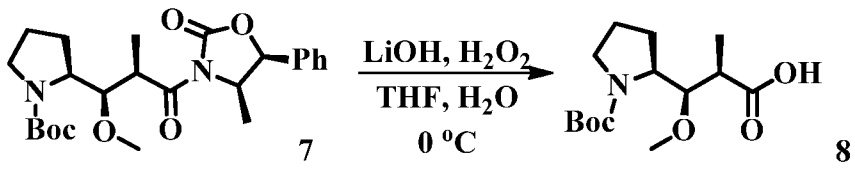
the mixture was concentrated in vacuo. More water (200 mL) was added to the residue and the mixture was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with 1N $KHSO_4$ (100 mL), sodium bicarbonate solution (100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (10% - 50% ethyl acetate/hexanes) to afford the compound **6** as a white solid (7.10g, 71% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (dt, *J* = 23.4, 7.1 Hz, 3H), 7.30 (d, *J* = 7.5 Hz, 2H), 5.67 (d, *J* = 7.1 Hz, 1H), 4.84 – 4.67 (m, 1H), 4.08 – 3.93 (m, 3H), 3.92 – 3.84 (m, 1H), 3.50 (d, *J* = 9.0 Hz, 1H), 3.24 (d, *J* = 6.7 Hz, 1H), 2.15 (s, 1H), 1.89 (dd, *J* = 22.4, 14.8 Hz, 3H), 1.48 (d, *J* = 21.5 Hz, 9H), 1.33 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H).

Example 5 Preparation of compound **7**



To a mixture of **6** (5.1 g 11.9 mmol) and molecular sieves (4 Å, 5 g) was added anhydrous dichloroethane (30 mL) under N_2. The mixture was stirred at room temperature for 20 min and cooled to 0 °C. Proton sponge (6.62 g, 30.9 mmol) was added, followed by trimethyloxonium tetrafluoroborate (4.40 g, 29.7 mmol). Stirring was continued for 2 h at 0 °C and 48 h at r.t. The reaction mixture was filtrated and the filtrate was concentrated and purified by column chromatography (20-70% ethyl acetate/hexanes) to afford compound **7** as a colorless solid (1.80 g, 35% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 – 7.27 (m, 5H), 5.65 (s, 1H), 4.69 (s, 1H), 3.92 (s, 1H), 3.83 (s, 1H), 3.48 (s, 3H), 3.17 (s, 2H), 2.02 – 1.68 (m, 5H), 1.48 (d, *J* = 22.3 Hz, 9H), 1.32 (t, *J* = 6.0 Hz, 3H), 0.91 – 0.84 (m, 3H).

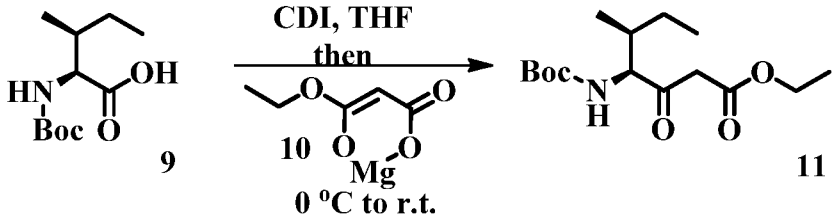
Example 6 Preparation of compound **8**



To a solution of **7** (1.80 g, 4.03 mmol) in THF (30 mL) and H_2O (7.5 mL), 30% H_2O_2 (1.44 mL, 14.4 mmol) was added over a 5 min period at 0 °C , followed by a solution of LiOH (0.27 g, 6.45 mmol) in water (5 mL). After stirring at 0 °C for 3 h, 1 N sodium sulfite (15.7 mL) was added and the mixture was allowed to warm to r.t. and stirred overnight. THF was removed in vacuo and the aqueous phase was wash with dichloromethane

(3 × 50 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified to pH 3 with 1N HCl and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to afford the compound **8** as a colorless oil (1.15 g, 98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 – 3.74 (m, 2H), 3.44 (d, J = 2.6 Hz, 3H), 3.23 (s, 1H), 2.60 – 2.45 (m, 1H), 1.92 (tt, J = 56.0, 31.5 Hz, 3H), 1.79 – 1.69 (m, 1H), 1.58 – 1.39 (m, 9H), 1.30 – 1.24 (m, 3H).

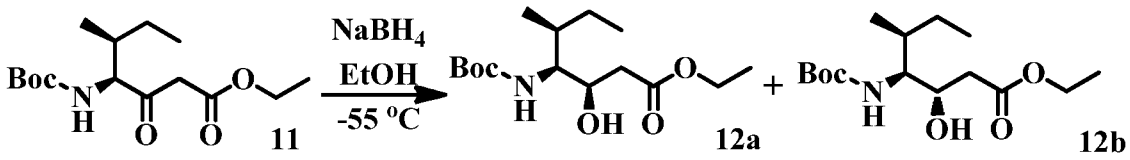
Example 7 Preparation of compound **11**



To an ice-cooled solution of **9** (4.55 g, 19.67 mmol) in THF (20 mL) was added 1,1'-carbonyldiimidazole (3.51 g, 21.63 mmol). After evolution of gas ceased, the resultant mixture was stirred at r.t. for 3.5 h.

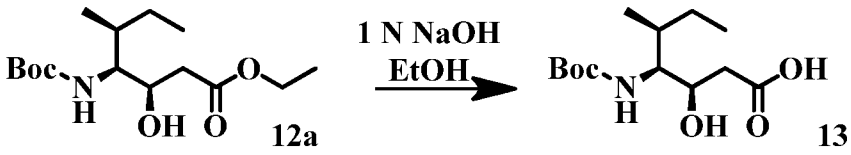
A solution of freshly prepared isopropylmagnesium bromide in THF (123 mmol, 30mL) was added dropwise to a pre-cooled (0 °C) solution of ethyl hydrogen malonate (6.50 g, 49.2 mmol) at such a rate to keep the internal temperature below 5 °C. The mixture was stirred at r.t. for 1.5 h. This solution of the magnesium enolate was then cooled over an ice-water bath, followed by the gradual addition of the imidazolide solution over a 1 h period via a double-ended needle at 0 °C. The resultant mixture was stirred at 0 °C for 30 min then r.t. 64 h. The reaction mixture was quenched by addition of 10% aqueous citric acid (5 mL), and acidified to pH 3 with an additional 10% aqueous citric acid (110 mL). The mixture was extracted with ethyl acetate (150 mL x 3). The organic extracts were washed with water (50 mL), saturated aqueous sodium hydrogen carbonate (50 mL), and saturated aqueous sodium chloride (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane (1:4) as an eluent to give compound **11** (5.50 g, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.04 (d, J = 7.8 Hz, 1H), 4.20 (p, J = 7.0 Hz, 3H), 3.52 (t, J = 10.7 Hz, 2H), 1.96 (d, J = 3.7 Hz, 1H), 1.69 (s, 2H), 1.44 (s, 9H), 1.28 (dd, J = 7.1, 2.9 Hz, 3H), 0.98 (t, J = 6.9 Hz, 3H), 0.92 – 0.86 (m, 3H).

Example 8 Preparation of compound **12a**



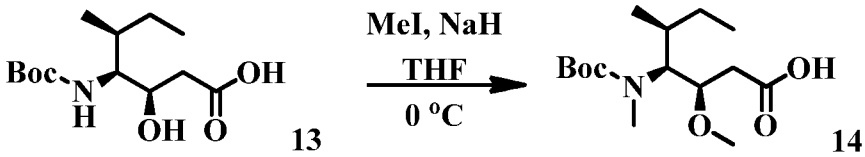
To a solution of the compound **11** (5.90 g, 19.83 mmol) in ethanol (6 mL) at -60 °C was added sodium borohydride (3.77 g, 99.2 mmol) in one portion. The reaction mixture was stirred for 5.5 h below -55 °C then quenched with 10% aqueous citric acid (100 mL). The resultant solution was acidified to pH 2 with an additional 10% aqueous citric acid, followed by extraction with ethyl acetate (100 mL x 3). The organic extracts were washed with saturated aqueous sodium chloride (100 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (10-50% ethyl acetate/hexane) to give pure diastereomer **12a** (2.20 g, 37% yield) and a mixture of **12a** and **12b** (2.0g, 34% yield, about 9:1 ratio). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.41 (d, J = 9.3 Hz, 1H), 4.17 (tt, J = 7.1, 3.6 Hz, 2H), 4.00 (t, J = 6.9 Hz, 1H), 3.55 (dd, J = 11.7, 9.3 Hz, 1H), 2.56 – 2.51 (m, 2H), 2.44 (dd, J = 16.4, 9.0 Hz, 1H), 1.79 (d, J = 3.8 Hz, 1H), 1.60 – 1.53 (m, 1H), 1.43 (s, 9H), 1.27 (dd, J = 9.3, 5.0 Hz, 3H), 1.03 – 0.91 (m, 7H).

Example 9 Preparation of compound **13**



To a solution of compound **12a** (2.20 g, 7.20 mmol) in ethanol (22 mL) was added 1 N aqueous sodium hydroxide (7.57 mL, 7.57 mmol). The mixture was stirred at 0 °C for 30 min then r.t. 2 h. The resultant solution was acidified to pH 4 by addition of 1 N aqueous hydrochloric acid, which was then extracted with ethyl acetate (50 mL x 3). The organic extracts were washed with 1 N aqueous potassium hydrogen sulfate (50 mL), and saturated aqueous sodium chloride (50 mL), dried over Na_2SO_4, and concentrated in vacuo to give compound **13** (1.90 g, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.50 (d, J = 8.7 Hz, 1H), 4.07 (d, J = 5.5 Hz, 1H), 3.59 (d, J = 8.3 Hz, 1H), 2.56 – 2.45 (m, 2H), 1.76 – 1.65 (m, 1H), 1.56 (d, J = 7.1 Hz, 1H), 1.45 (s, 9H), 1.26 (t, J = 7.1 Hz, 3H), 0.93 (dd, J = 14.4, 7.1 Hz, 6H).

Example 10 Preparation of compound **14**

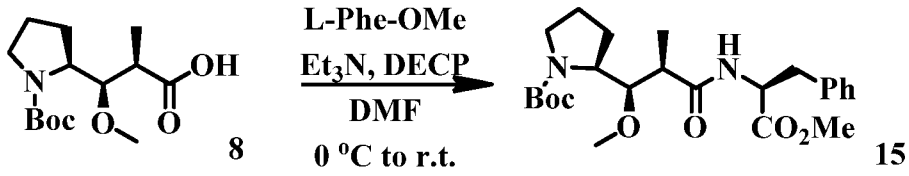


To a solution of compound **13** (1.90 g, 6.9 mmol) in THF (40 mL) was added sodium hydride (60% oil suspension, 1.93 g, 48.3 mmol) at 0 °C. After stirring for 1h, methyl iodide (6.6 mL, 103.5 mmol) was added. The stirring was continued at 0 °C for 40 h before saturated aqueous sodium hydrogen carbonate (50 mL) was added, followed by water (100 mL). The mixture was washed with diethyl ether (50 mL × 2) and the aqueous layer was acidified to pH 3 by 1 N aqueous potassium hydrogen sulfate, then extracted with ethyl acetate (50 mL x 3). The combined organic extracts were washed with 5% aqueous sodium thiosulfate (50 mL) and saturated aqueous sodium chloride (50 mL), dried over Na_2SO_4, and concentrated in vacuo to give compound **14** (1.00 g, 48% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.95 (d, *J* = 75.4 Hz, 2H), 3.42 (d, *J* = 4.4 Hz, 3H), 2.71 (s, 3H), 2.62 (s, 1H), 2.56 – 2.47 (m, 2H), 1.79 (s, 1H), 1.47 (s, 1H), 1.45 (d, *J* = 3.3 Hz, 9H), 1.13 – 1.05 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.89 (td, *J* = 7.2, 2.5 Hz, 3H).

General procedure for the removal of the Boc **function with trifluoroacetic acid.**

To a solution of the *N*-Boc amino acid (1.0 mmol) in methylene chloride (2.5 mL) was added trifluoroacetic acid (1.0 mL). After being stirred at room temperature for 1-3 h, the reaction mixture was concentrated in vacuo. Co-evaporation with toluene gave the deprotected product, which was used without any further purification.

Example 11 Preparation of compound **15**

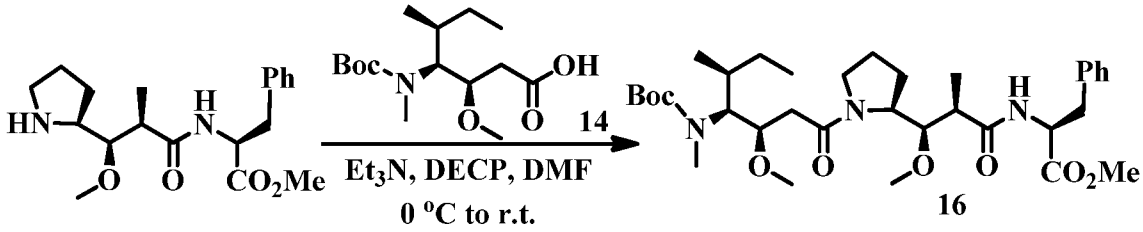


To a solution of compound **8** (100 mg, 0.347 mmol) and L-Phenylalanine methyl ester hydrochloride (107.8 mg, 0.500 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (75.6 μL, 0.451 mmol), followed by Et_3N (131 μL, 0.94 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight.

The reaction mixture was then diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **15** (130 mg, 83% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 7.9, 6.5 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.16 (s, 2H), 4.81 (s, 1H), 3.98 – 3.56 (m, 5H), 3.50 (s, 1H), 3.37 (d, *J* = 2.9 Hz, 3H),

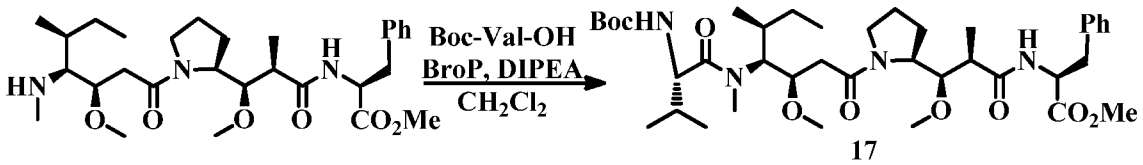
3.17 (dd, J = 13.9, 5.4 Hz, 2H), 3.04 (dd, J = 14.0, 7.7 Hz, 1H), 2.34 (s, 1H), 1.81 – 1.69 (m, 2H), 1.65 (s, 3H), 1.51 – 1.40 (m, 9H), 1.16 (d, J = 7.0 Hz, 3H).

Example 12 Preparation of compound **16**



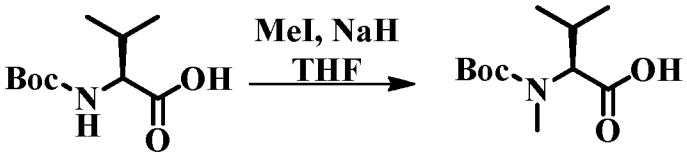
To a solution of the deprotected product from compound **15** (0.29 mmol) and compound **14** (96.6 mg, 0.318 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (58 μL, 0.347 mmol), followed by Et_3N (109 μL, 0.78 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **16** (150 mg, 81% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{34}H_{55}N_3O_8$ $[M+H]^+$: 634.40, found: 634.40.

Example 13 Preparation of compound **17**



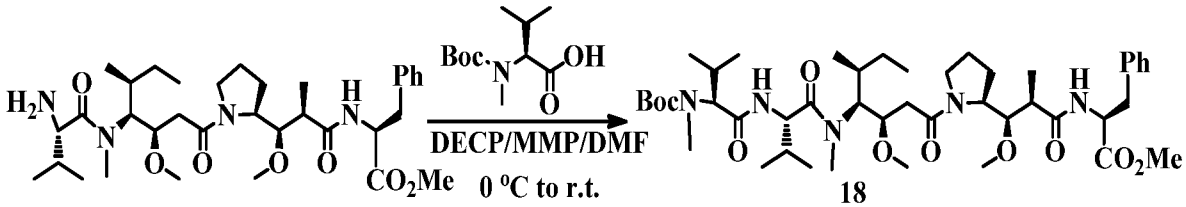
To a solution of the deprotected product from compound **16** (0.118 mmol) and Boc-Val-OH (51.8 mg, 0.236 mmol) in DCM (5 mL) at 0 °C was added bromotris(dimethylamino)- phosphonium hexafluorophosphate (BroP, 70.1 mg, 0.184 mmol), followed by diisopropylethylamine (70 μL, 0.425 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 2 days. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **17** (67 mg, 77% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{39}H_{64}N_4O_9$ $[M+H]^+$: 733.47, found: 733.46.

Example 14 Preparation of compound **Boc-N-Me-Val-OH**



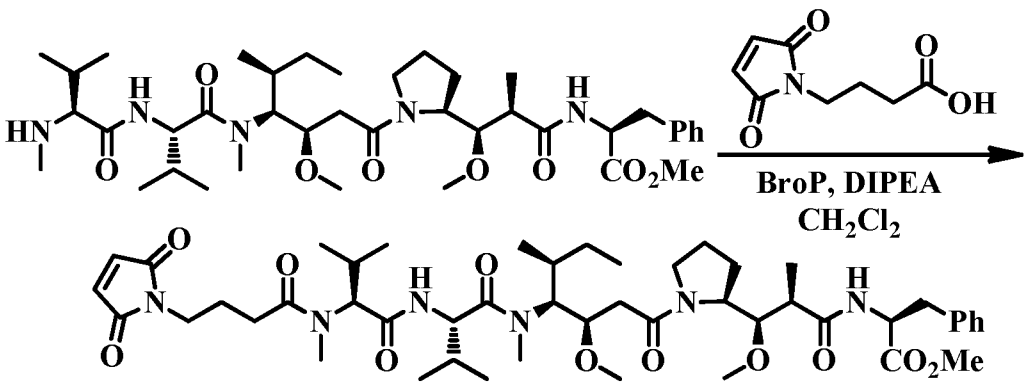
To a solution of Boc-L-Val-OH (2.00 g, 9.2 mmol) and methyl iodide (5.74 mL, 92 mmol) in anhydrous THF (40 mL) was added sodium hydride (3.68 g, 92 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h, then warmed to r.t. and stirred for 24 h. The reaction was quenched by ice water (50 mL). After addition of water (100 mL), the reaction mixture was washed with ethyl acetate (50 mL × 3) and the aqueous solution was acidified to pH 3 then extracted with ethyl acetate (50 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated to afford Boc-N-Me-Val-OH (2.00 g, 94% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (d, J = 10.0 Hz, 1H), 2.87 (s, 3H), 2.37 – 2.13 (m, 1H), 1.44 (d, J = 26.7 Hz, 9H), 1.02 (d, J = 6.5 Hz, 3H), 0.90 (t, J = 8.6 Hz, 3H).

Example 15 Preparation of compound **18**



To a solution of the deprotected product from compound **17** (0.091 mmol) and Boc-N-Me-Val-OH (127 mg, 0.548 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (18.2 μL, 0.114 mmol), followed by 4-methylmorpholine (59 μL, 0.548 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **18** (30 mg, 39% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{45}H_{75}N_5O_{10}$ $[M+H]^+$: 846.55, found: 846.56.

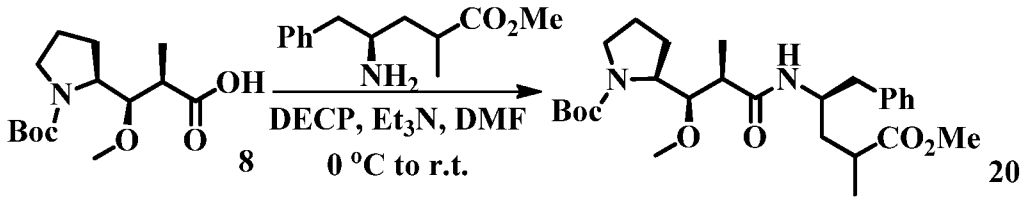
Example 16 Preparation of compound **19**



19

To a solution of the deprotected product from compound **18** (0.071 mmol) and 4-maleimidobutyric acid (26 mg, 0.142 mmol) in DCM (5 mL) at 0 °C was added BroP (81 mg, 0.213 mmol), followed by diisopropylethylamine (42 μL, 0.256 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at room temperature for 2 days. The reaction mixture was diluted with ethyl acetate (40 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (50% ethyl acetate/hexanes to 10% MeOH/ethyl acetate) to afford compound **19** (19 mg, 29% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{48}H_{74}N_6O_{11}$ $[M+H]^+$: 911.54, found: 911.53.

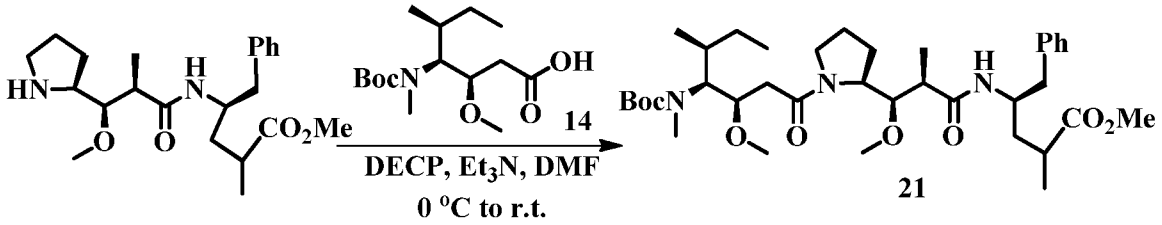
Example 17 Preparation of compound **20**



8 20

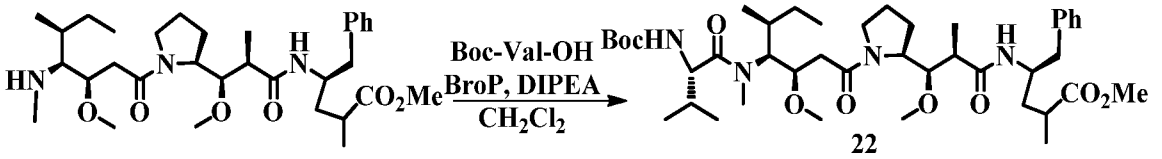
To a solution of compound **8** (58 mg, 0.201 mmol) and (4*R*)-methyl-4-amino-2-methyl-5-phenylpentanoate (64 mg, 0.29 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (43.9 μL, 0.262 mmol), followed by Et_3N (76 μL, 0.544 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **20** (83 mg, 84% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{27}H_{42}N_2O_6$ $[M+H]^+$: 491.30, found: 491.29.

Example 18 Preparation of compound **21**



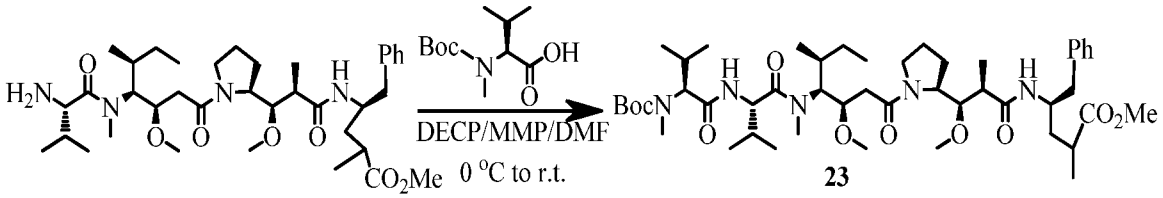
To a solution of the deprotected product from compound **20** (0.17 mmol) and compound **14** (57 mg, 0.187 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (35 µL, 0.204 mmol), followed by Et_3N (64 µL, 0.459 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **21** (100 mg, 87% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{37}H_{61}N_3O_8$ $[M+H]^+$: 676.45, found: 676.43.

Example 19 Preparation of compound **22**



To a solution of the deprotected product from compound **21** (0.124 mmol) and Boc-Val-OH (77 mg, 0.354 mmol) in DCM (5 mL) at 0 °C was added BroP (68.5 mg, 0.176 mmol), followed by diisopropylethylamine (96 µL, 0.584 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 24 h. TLC analysis indicates that the starting material was still present. More Boc-Val-OH (77 mg), BroP (68.5 mg) and diisopropylethylamine (96 µL) were added and the reaction was stirred for additional 24h, then diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **22** (65 mg, 67% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{42}H_{70}N_4O_9$ $[M+H]^+$: 775.51, found: 775.49.

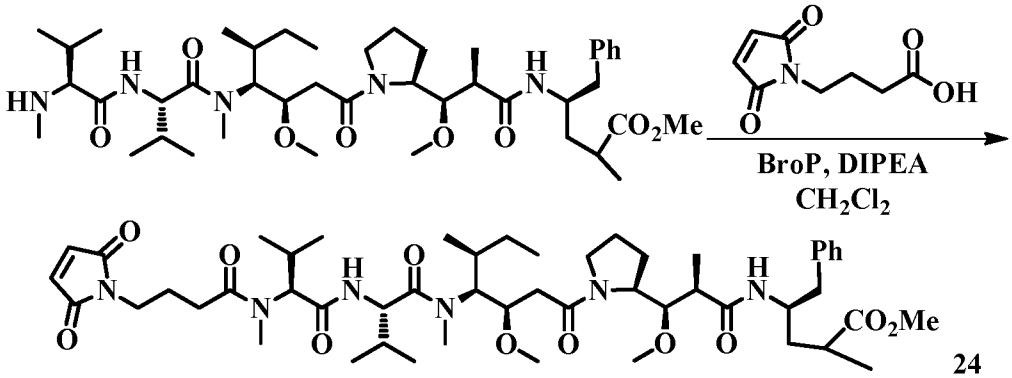
Example 20 Preparation of compound **23**



23

To a solution of the deprotected product from compound **22** (0.084 mmol) and Boc-N-Me-Val-OH (116 mg, 0.503 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (16.8 μL, 0.105 mmol), followed by 4-methylmorpholine (54 μL, 0.503 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (80 mL), washed with 1 N aqueous potassium hydrogen sulfate (40 mL), water (40 mL), saturated aqueous sodium hydrogen carbonate (40 mL), and saturated aqueous sodium chloride (40 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **23** (60 mg, 80% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{48}H_{81}N_5O_{10}$ $[M+H]^+$: 888.60, found: 888.60.

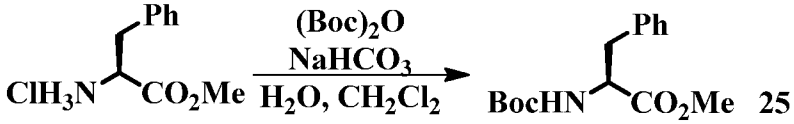
Example 21 Preparation of compound **24**



24

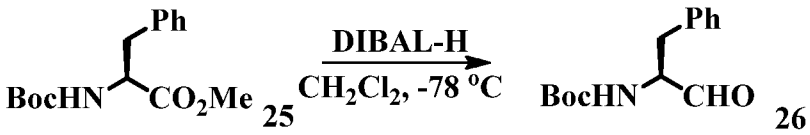
To a solution of the deprotected product from compound **23** (0.034 mmol) and 4-maleimidobutyric acid (17.7 mg, 0.097 mmol) in DCM (5 mL) at 0 °C was added BroP (18.7 mg, 0.0483 mmol), followed by diisopropylethylamine (26 μL, 0.159 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at room temperature for 24 h. The reaction mixture was diluted with ethyl acetate (40 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (50% ethyl acetate/hexanes to 10% MeOH/ethyl acetate) to afford compound **24** (23 mg, 70% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{51}H_{80}N_6O_{11}$ $[M+H]^+$: 953.59, found: 953.63.

Example 22 Preparation of compound **25**



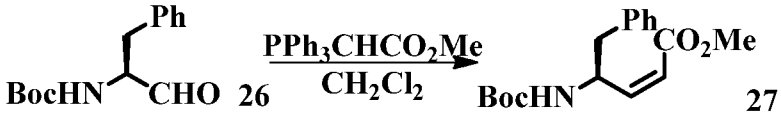
A mixture of L-phenylalanine methyl ester hydrochloride (7.90 g, 36.6 mmol), di-*tert*-butyl dicarbonate (12.9 g) in DCM (100 mL) and saturated aqueous $NaHCO_3$ solution (80 mL) was stirred at r.t. for 2 h. The organic phase was separated and the aqueous phase was extracted with DCM twice. The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a colorless oil (9.76 g, 95% yield). The crude product was used directly in the next step.

Example 23 Preparation of compound **26**



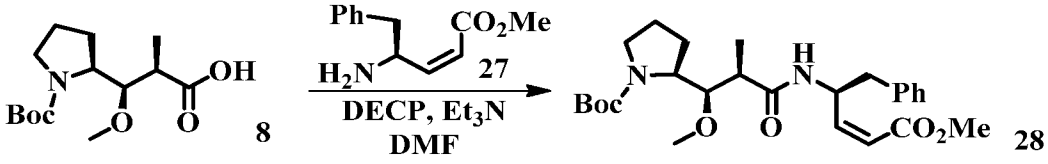
A solution of Boc-L-phenylalanine methyl ester (9.76 g, 35.0 mmol) in DCM (60 mL) was cooled to -65 °C, DIBAL-H (1.0 M in hexanes, 70 mL) was added slowly over 1 h. After being stirring for 2 h, the reaction was quenched by MeOH (20 mL) and warmed to r.t. 1N HCl (120 mL) was then added and the organic phase was separated. The aqueous phase was extracted with DCM twice. The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a colorless oil (7.0 g, 80% yield). The crude product was used directly in the next step.

Example 24 Preparation of compound **27**



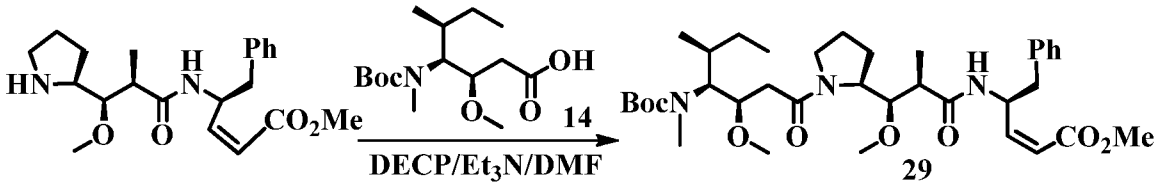
A mixture of aldehyde (7.0 g, 28 mmol) and methyl (triphenylphosphoranylidene)-acetate (11.7 g, 35 mmol) in DCM (100 mL) was stirred at r.t. for 2 h. After the removal of solvent, the residue was purified by column chromatography (85% ethyl acetate/hexanes) to afford a white solid (6.9 g, 81% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.28 (m, 2H), 7.24 (dt, J = 4.2, 1.8 Hz, 1H), 7.18 – 7.14 (m, 2H), 6.91 (dd, J = 15.7, 4.9 Hz, 1H), 5.86 (dd, J = 15.7, 1.3 Hz, 1H), 4.60 (s, 1H), 4.58 (s, 1H), 3.72 (s, 3H), 2.89 (d, J = 6.4 Hz, 2H), 1.39 (s, 9H).

Example 25 Preparation of compound **28**



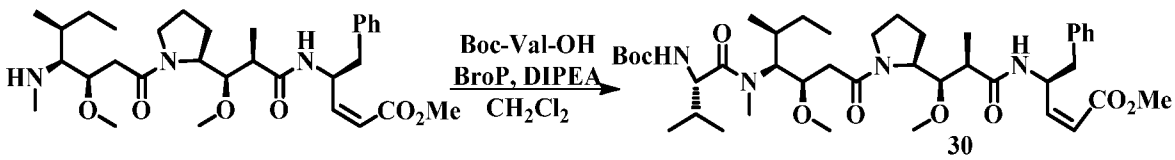
To a solution of amine (0.194mmol) in DMF (2 mL) at 0 °C was added Et_3N (54.1 μL, 0.388 mmol), followed by compound **8** (37.2 mg, 0.129 mmol) in DMF (1 mL) and diethyl cyanophosphonate (29.0 μL, 0.194 mmol). The reaction mixture was stirred at 0 °C for 30 min, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (30-50% ethyl acetate/hexanes) to afford compound **28** (58.2 mg, 95% yield) as a colorless oil. LC-MS (ESI) m/z calcd. for $C_{26}H_{38}N_2O_6$ $[M+H]^+$: 475.27, found: 475.26.

Example 26 Preparation of compound **29**



To a solution of the deprotected product from compound 28 (0.123 mmol) in DMF (2 mL) at 0 °C was added Et_3N (51.4 μL, 0.369 mmol), followed by compound 14 (37.3 mg, 0.123 mmol) in DMF (1 mL) and diethyl cyanophosphonate (22.1 μL, 0.148 mmol). The reaction mixture was stirred at 0 °C for 30 min, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (70-90% ethyl acetate/hexanes) to afford compound **29** (81.0 mg, 99% yield) as a light yellow oil. LC-MS (ESI) m/z calcd. for $C_{36}H_{57}N_3O_8$ $[M+H]^+$: 660.41, found: 660.41.

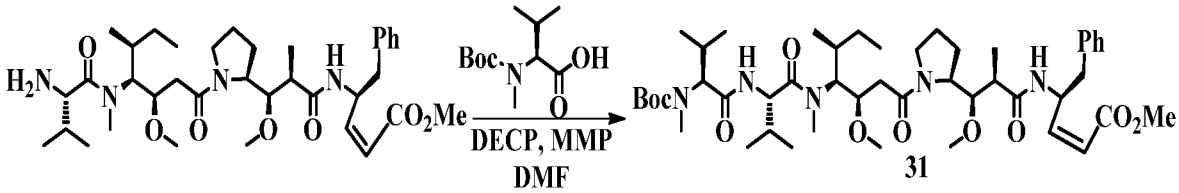
Example 27 Preparation of compound **30**



To a solution of the deprotected product from compound 29 (0.123 mmol) and Boc-Val-OH (80.2 mg, 0.369 mmol) in DCM (5 mL) at 0 °C was added BroP (71.6 mg, 0.185 mmol), followed by addition of diisopropylethylamine (107.4 μL, 0.615 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 24 h. TLC analysis indicates that the starting material was still present. More Boc-Val-OH (80.2 mg),

BroP (71.6 mg) and diisopropylethylamine (107.4 μL) were added and the reaction was stirred for additional 24h, then diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography to afford compound 30 (86.3 mg, 92% yield) as a white foam. LC-MS (ESI) m/z calcd. for $C_{41}H_{66}N_4O_9$ $[M+H]^+$: 759.48, found: 759.48.

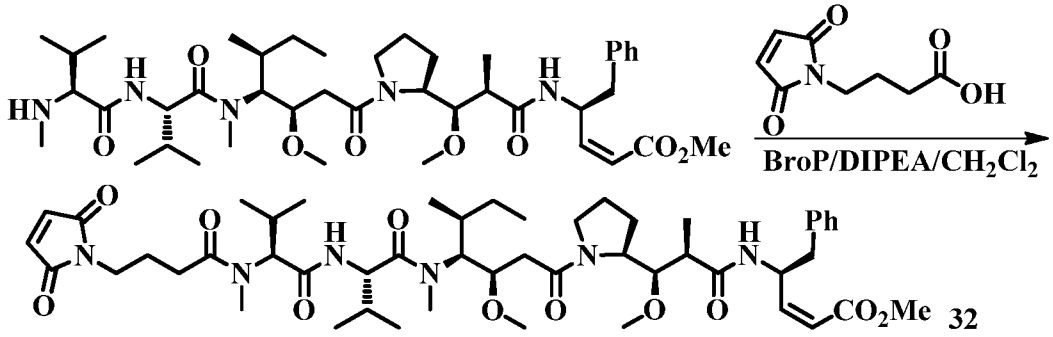
Example 28 Preparation of compound **31**



31

To a solution of the deprotected product from compound 30 (0.107mmol) in DMF (2 mL) at 0 °C was added 4-methylmorpholine (70.6 μL, 0.642 mmol), followed by Boc-N-Me-Val-OH (148.5 mg, 0.642 mmol) in DMF (1 mL) and diethyl cyanophosphonate (32.0 μL, 0.214 mmol). The reaction mixture was stirred at 0 °C for 30 min, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (75% ethyl acetate/hexanes) to afford compound 31 (57.9 mg, 62% yield) as a colorless oil. LC-MS (ESI) m/z calcd. for $C_{47}H_{77}N_5O_{10}$ $[M+H]^+$: 872.57, found: 872.57.

Example 29 Preparation of compound **32**

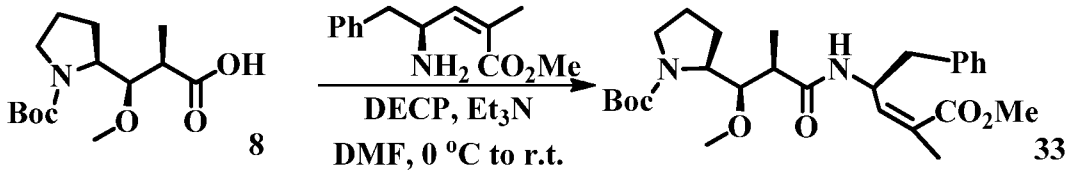


32

To a solution of the deprotected product from compound **31** (0.0316 mmol) and 4-maleimidobutyric acid (17.4 mg, 0.0949 mmol) in DCM (3 mL) at 0 °C was added BroP (18.4 mg, 0.0474 mmol), followed by diisopropylethylamine (27.6 μL, 0.158 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at room temperature

for 16 h. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (pure ethyl acetate to 10% MeOH/ethyl acetate) to afford compound **32** (30.0 mg, 99% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{50}H_{76}N_6O_{11}$ $[M+H]^+$: 938.17, found: 938.17.

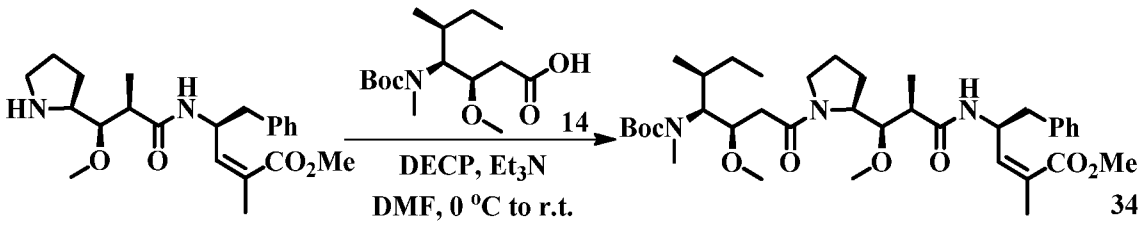
Example 30 Preparation of compound **33**



(S,E)-methyl 4-amino-2-methyl-5-phenylpent-2-enoate is prepared from L-Phe-OMe in the same manner as described in example 24.

To a solution of compound **8** (32 mg, 0.112 mmol) and (S,E)-methyl 4-amino-2-methyl-5-phenylpent-2-enoate (34 mg,0.157 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (23 μL, 0.146 mmol), followed by Et3N (42 μL, 0.302 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **33** (54 mg, 98% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{27}H_{40}N_2O_6$ $[M+H]^+$: 489.29, found: 489.28.

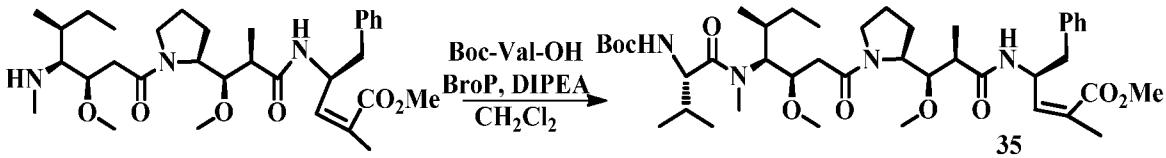
Example 31 Preparation of compound **34**



To a solution of the deprotected product from compound **33** (0.112 mmol) and compound **14** (37.6 mg, 0.124 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (21 μL, 0.135 mmol), followed by Et_3N (42 μL, 0.304 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N

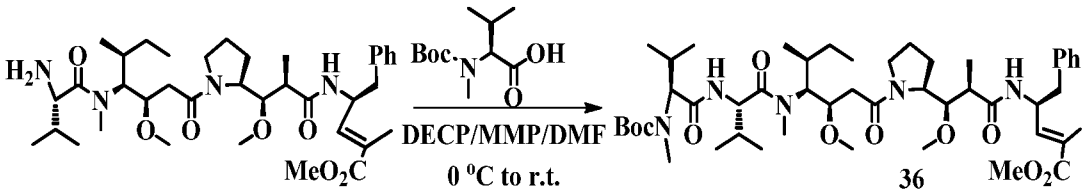
aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **34** (61 mg, 80% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{37}H_{59}N_3O_8$ $[M+H]^+$: 674.43, found: 674.43.

Example 32 Preparation of compound **35**



To a solution of the deprotected product from compound **34** (0.091 mmol) and Boc-Val-OH (56 mg, 0.258 mmol) in DCM (5 mL) at 0 °C was added BroP (50 mg, 0.129 mmol), followed by addition of diisopropylethylamine (70 μL, 0.426 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 24 h. TLC analysis indicates that the starting material was still present. More Boc-Val-OH (56 mg), BroP (50 mg) and diisopropylethylamine (70 μL) were added and the reaction was stirred for additional 24 h, then diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **35** (62 mg, 88% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{42}H_{68}N_4O_9$ $[M+H]^+$: 773.50, found: 773.50

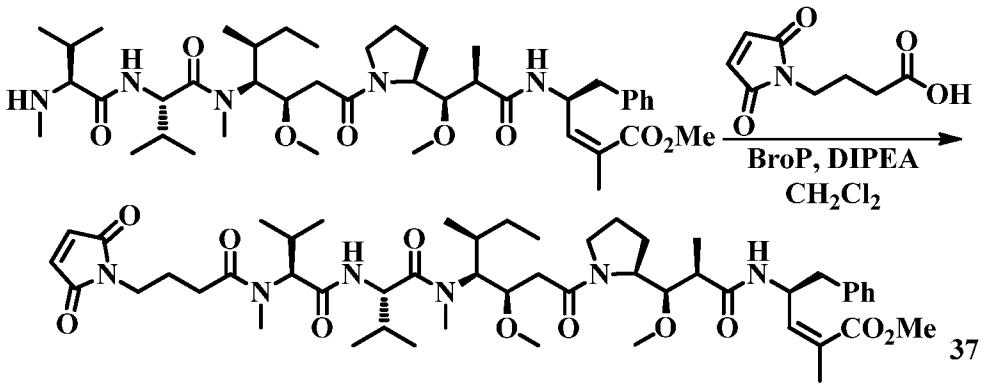
Example 33 Preparation of compound **36**



To a solution of the deprotected product from compound **35** (0.0804 mmol) and Boc-N-Me-Val-OH (111.4 mg, 0.482 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (16.0 μL, 0.100 mmol), followed by addition of 4-methylmorpholine (52 μL, 0.482 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by

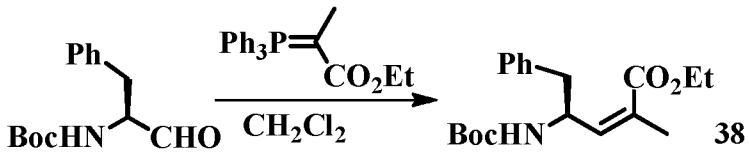
column chromatography (20-100% ethyl acetate/hexanes) to afford compound **36** (56 mg, 78% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{48}H_{79}N_5O_{10}$ $[M+H]^+$: 886.58, found: 886.59.

Example 34 Preparation of compound **37**



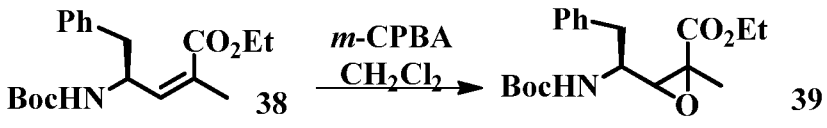
To a solution of the deprotected product from compound **36** (0.0316 mmol) and 4-maleimidobutyric acid (16.5 mg, 0.090 mmol) in DCM (5 mL) at 0 °C was added BroP (17.4 mg, 0.0450 mmol), followed by addition of diisopropylethylamine (24 μL, 0.149 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at room temperature for 24 h. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (50% ethyl acetate/hexanes to 10% MeOH/ethyl acetate) to afford compound **37** (15 mg, 50% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{51}H_{78}N_6O_{11}$ $[M+H]^+$: 951.57, found: 951.58.

Example 35 Preparation of compound **38**



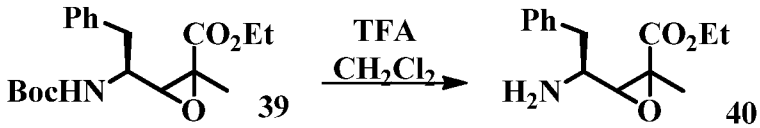
A mixture of aldehyde (0.18 g, 0.722 mmol) and ethyl 2-(triphenylphosphoranylidene)propionate (0.31 g, 0.866 mmol) in DCM (3.6 mL) was stirred at r.t. for 16 h then concentrated. The residue was purified by column chromatography (20% ethyl acetate/hexanes) to afford a white solid (0.19 g, 80% yield).

Example 36 Preparation of compound **39**



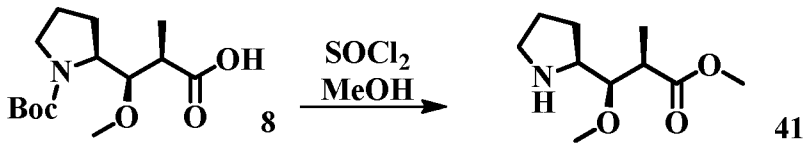
To a solution of α,β-unsaturated olefin (0.19 g, 0.57 mmol) in DCM (6 mL) over ice-water bath, *m*-CPBA (75 wt%, 0.22 g, 0.90 mmol) was added in portions. The mixture was stirred at 0 °C for 30 min then warmed to r.t. and stirred for 16 h. TLC analysis indicated that the starting material is still present. The reaction was further warmed to 30 °C and stirred for 40 h then cooled to r.t. A white solid precipitated from the reaction mixture was filtered off and the filtrate was washed with saturated $NaHSO_3$, saturated $NaHCO_3$ and brine. After the solvent was removed the residue was purified by column chromatography (10-50% ethyl acetate/hexanes) to afford a white solid (0.10 g, 50% yield).

Example 37 Preparation of compound **40**



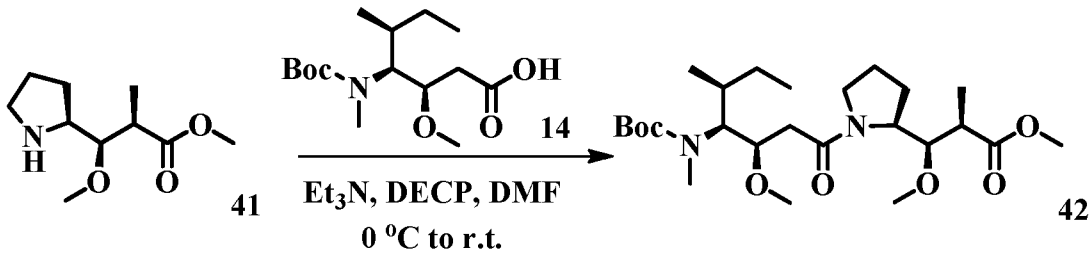
To a solution of epoxide (28 mg, 0.08 mmol) in DCM (5 mL) was added TFA (0.3 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h then diluted with DCM (10 mL) and washed with saturated aqueous sodium hydrogen carbonate (10 mL), saturated aqueous sodium chloride (10 mL), dried over Na_2SO_4 and concentrated in vacuo to give compound **36** (16.9 mg, 84% yield) as a colorless oil, which was used without any further purification. LC-MS (ESI) m/z calcd. for $C_{14}H_{19}NO_3$ $[M+H]^+$: 250.14, found: 250.14.

Example 38 Preparation of compound **41**



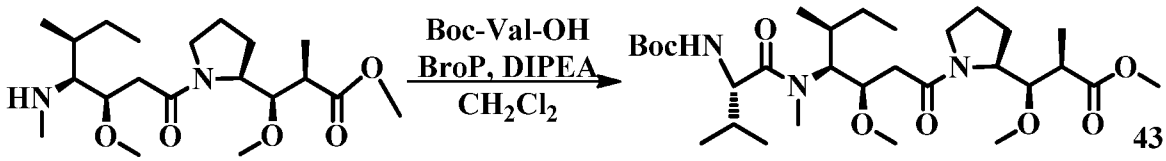
To a solution of compound **8** (63 mg, 0.219 mmol) in MeOH (2 mL) was added thionyl chloride (79.5 μL, 1.09 mmol) carefully at 0 °C. The reaction was then warmed to room temperature and stirred overnight. The mixture was concentrated in vacuo and co-evaporation with toluene gave compound **41** (44 mg) as a colorless oil, which was immediately used for the next step without further purification. LC-MS (ESI) m/z calcd. for $C_{10}H_{19}NO_3$ $[M+H]^+$: 202.14, found: 202.14.

Example 39 Preparation of compound **42**



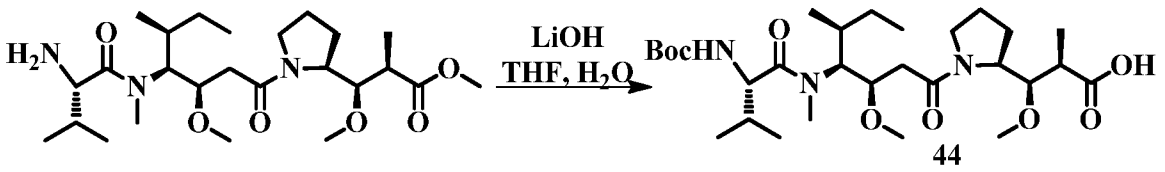
To a solution of the compound **41** (0.219 mmol) and compound **14** (73 mg, 0.24 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (45 µL, 0.263 mmol), followed by addition of Et_3N (152.3 µL, 1.09 mmol). The reaction mixture was stirred at 0 °C for 2 h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (15-75% ethyl acetate/hexanes) to afford compound **42** (84 mg, 78% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{25}H_{46}N_2O_7$ $[M+H]^+$: 487.33, found: 487.32.

Example 40 Preparation of compound **43**



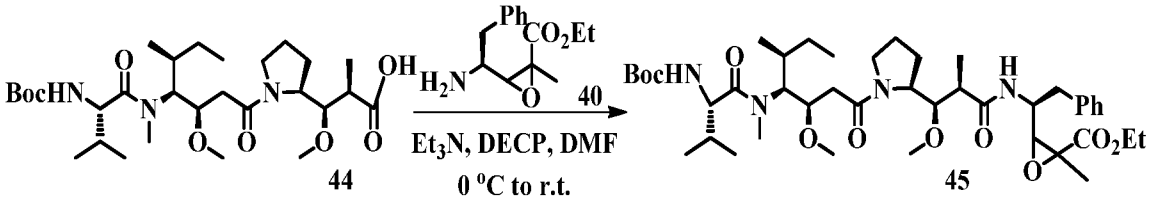
To a solution of the deprotected product from compound **42** (0.173 mmol) and Boc-Val-OH (107.1 mg, 0.493 mmol) in DCM (5 mL) at 0 °C was added BroP (95.4 mg, 0.246 mmol), followed by addition of diisopropylethylamine (133.7 µL, 0.813 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at r.t. for 48 h. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (15-80% ethyl acetate/hexanes) to afford compound **43** (80 mg, 78% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{30}H_{55}N_3O_8$ $[M+H]^+$: 586.40, found: 586.37.

Example 41 Preparation of compound **44**



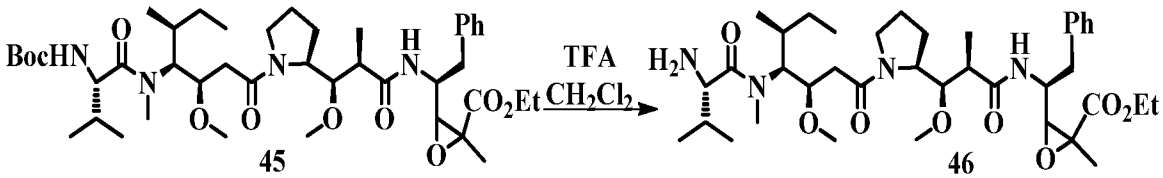
To a solution of the deprotected product from compound **43** (72 mg, 0.123 mmol) in THF (2 mL), was added a solution of lithium hydroxide monohydrate (26 mg, 0.614 mmol) in H_2O (1 mL) at 0 °C. The reaction mixture was warmed to 25 °C and stirred overnight. THF was removed in vacuo and the residue was diluted with water (50 mL) and adjusted to pH 3 with 1N aqueous potassium hydrogen sulfate then extracted with ethyl acetate (20 mL × 3). The organic phase was washed with saturated aqueous sodium chloride, dried over Na_2SO_4, and concentrated in vacuo to give compound **44** (70 mg, 98% yield) as a white solid, which was used without any further purification. LC-MS (ESI) m/z calcd. for $C_{29}H_{53}N_3O_8$ $[M+H]^+$: 572.38, found: 572.38.

Example 42 Preparation of compound **45**



To a solution of the compound **44** (27.7 mg, 0.048 mmol) and compound 40 (16.9 mg, 0.068 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (10 μL, 0.063 mmol), followed by addition of Et_3N (18.2 μL, 0.131 mmol). The reaction mixture was stirred at 0 °C for 2h, then warmed to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), saturated aqueous sodium hydrogen carbonate (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **45** (10 mg, 26% yield) as a colorless oil. LC-MS (ESI) m/z calcd. for $C_{43}H_{70}N_4O_{10}$ $[M+H]^+$: 803.51, found: 803.47.

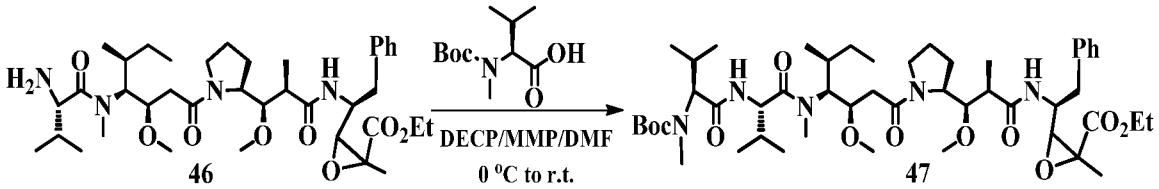
Example 43 Preparation of compound **46**



To a solution of the compound **45** (10 mg, 0.0124 mmol) in DCM (5 mL) was added TFA (0.3 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 2 h then diluted with DCM (10 mL) and washed with saturated aqueous sodium hydrogen carbonate (10 mL), saturated aqueous sodium chloride (10 mL), dried over Na_2SO_4, and concentrated in vacuo to give the compound **46** (8 mg, 99% yield) as a colorless oil, which

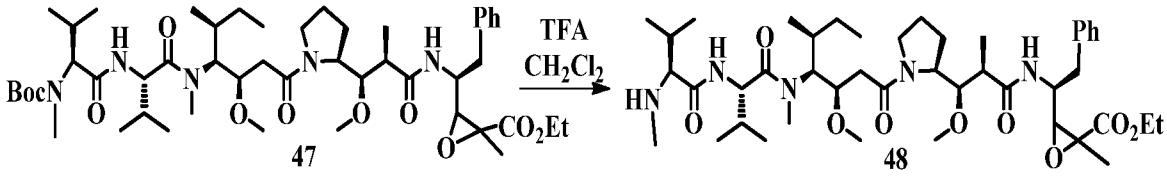
was used without any further purification. LC-MS (ESI) m/z calcd. for $C_{38}H_{62}N_4O_8$ $[M+H]^+$: 703.46, found: 703.44.

Example 44 Preparation of compound **47**



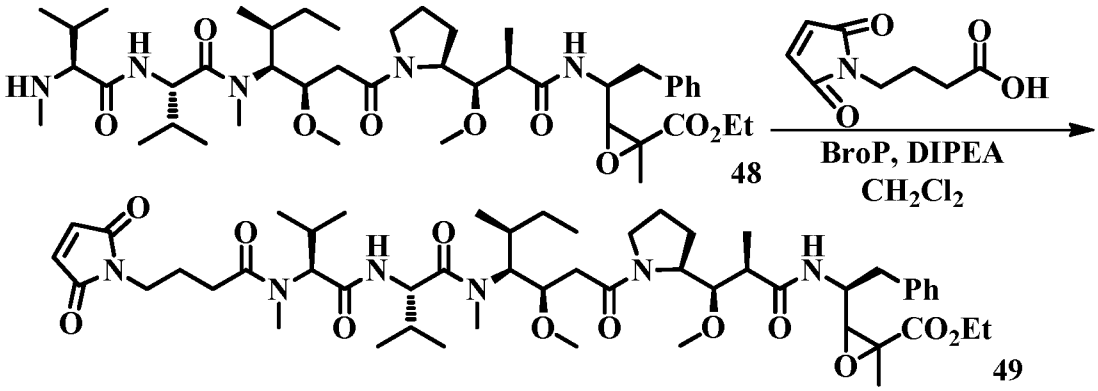
To a solution of compound 46 (8 mg, 0.0113 mmol) and Boc-N-Me-Val-OH (15.7 mg, 0.0682 mmol) in DMF (5 mL) at 0 °C was added diethyl cyanophosphonate (2 μL, 0.0142 mmol), followed by addition of 4-methylmorpholine (7.3 μL, 0.0682 mmol). The reaction mixture was stirred at 0 °C for 2h, then warmed to r.t. and stirred overnight. The reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (20-100% ethyl acetate/hexanes) to afford compound **47** (8 mg, 76% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{49}H_{81}N_5O_{11}$ $[M+H]^+$: 916.59, found: 916.60.

Example 45 Preparation of compound **48**



To a solution of the compound **47** (8 mg, 0.0087 mmol) in DCM (5 mL) was added TFA (0.3mL) at 0 °C. The mixture was warmed to room temperature and stirred for 2 h then diluted with DCM (10 mL) and washed with saturated aqueous sodium hydrogen carbonate (10 mL), saturated aqueous sodium chloride (10 mL), dried over Na_2SO_4, and concentrated in vacuo to give compound **48** (7 mg, 99% yield) as a white solid, which was used without any further purification. LC-MS (ESI) m/z calcd. for $C_{44}H_{73}N_5O_9$ $[M+H]^+$: 816.54, found: 816.53.

Example 46 Preparation of compound **49**

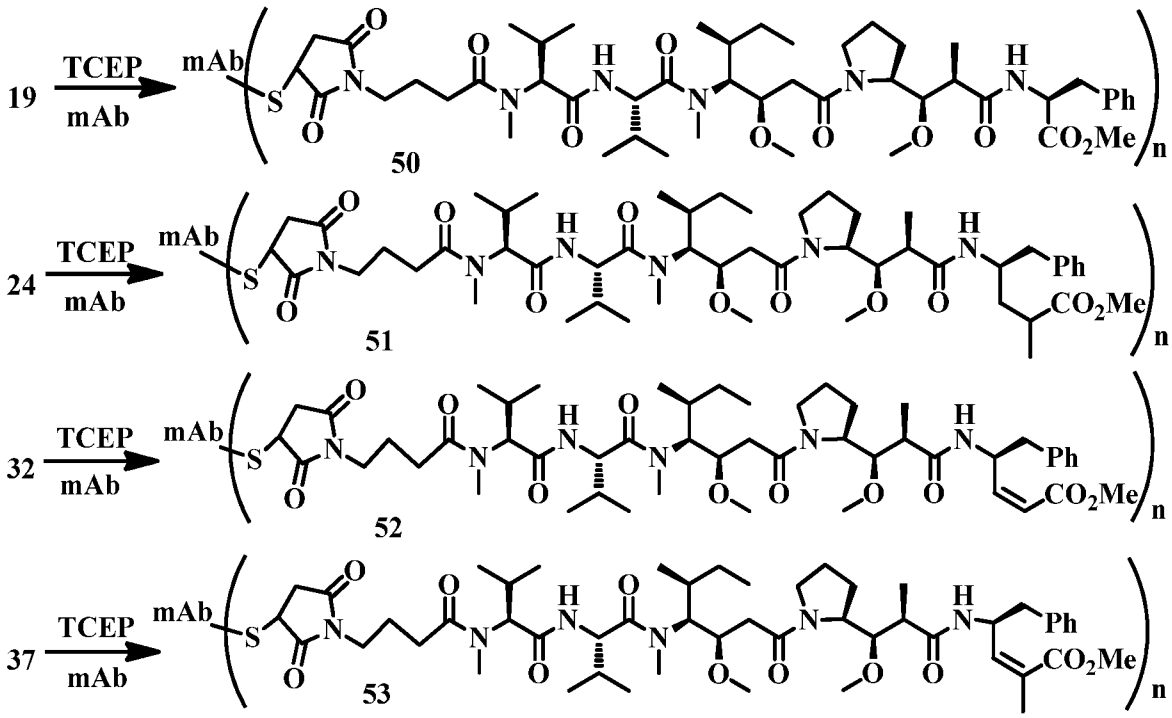


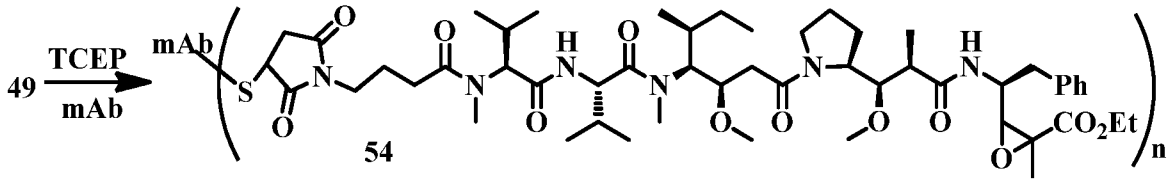
48

49

To a solution of the compound **48** (0.0087 mmol) and 4-maleimidobutyric acid (4.6 mg, 0.025 mmol) in DCM (5 mL) at 0 °C was added BroP (4.8 mg, 0.0124 mmol), followed by addition of DIPEA (6.7 μL, 0.041 mmol). The mixture was shielded from light and stirred at 0 °C for 30 min then at room temperature for 24 h. The reaction mixture was diluted with ethyl acetate (50 mL), washed with 1 N aqueous potassium hydrogen sulfate (20 mL), water (20 mL), and saturated aqueous sodium chloride (20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (50% ethyl acetate/hexanes to 10% MeOH/ethyl acetate) to afford compound **49** (3.2 mg, 37% yield) as a white solid. LC-MS (ESI) m/z calcd. for $C_{52}H_{80}N_6O_{12}$ $[M+H]^+$: 981.58, found: 981.57.

Example 47 Preparation of an antibody conjugate with compounds **19, 24, 32, 37** and **49.**





The mAbs (such as Her2 antibody, Herceptin 5 mg/mL) in PBS buffer containing 50 mM sodium borate, pH 7.8, were treated with TCEP (2 ~10 eq of the antibody) at 25 °C was added the compound 19, 24, 32, 37 or 49 at the ratio of 4~10 eq of the antibody. The reaction was stirred for 2~24 hours at 25 °C. The reactions were quenched with excess cysteine, or N-ethyl malimide, or dehydroascorbic acid. The conjugate was purified and buffer exchanged into 50 mM Glycine, 10 mM Histidine, 1% sucrose, 0.01% Tween-20, 100 mM sodium phosphate formulation buffer, pH 6.0, using NAP desalting columns (Illustra Sephadex G-25 DNA Grade, GE Healthcare). Dialysis was performed in the same buffer for 4 hours at room temperature utilizing Slide-a-Lyzer dialysis cassettes (ThermoScientific 20,000 MWCO). The purified conjugate was found to have an average of 3~4 cytotoxic molecules linked per antibody (by LC-MS), 99% monomer (by size exclusion chromatography), <0.2% unconjugated cysteine-quenched drug (by dual-column reverse-phase HPLC analysis) and a final protein concentration of 0.8 ~2.5 mg/ml (Protein concentration and drug loading were determined by spectral analysis at 280 and 254 nm, respectively)

Example 47 In vitro cytotoxic assay of the antiHer2 antibody conjugates of **50, 51, 52, 53** and **54.**

The cell lines used in the cytotoxicity assays were NCI-N87, a human gastric carcinoma cell line; BT-474, a human invasive ductal carcinoma cell line; and SKOV3, a human ovarian carcinoma cell line. For NCI-N87, and BT-474 cells, the cells were grown in RPMI-1640 with 10% FBS. For SKOV3 cells, the cells were grown in McCoy's 5A Medium with 10% FBS. To run the assay, the cells (180 µl, 6000 cells) were added to each well in a 96-well plate and incubated for 24 hours at 37°C with 5% CO_2. Next, the cells were treated with test compounds (**50, 51, 52, 53** and **54**) (20 µl) at various concentrations in appropriate cell culture medium (total volume, 0.2 mL). The control wells contain cells and the medium but lack the test compounds. The plates were incubated for 120 hours at 37°C with 5% CO_2. 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetra zoliumromide (MTT) (5 mg/ml) was then added to the wells (20 µl) and the plates were incubated for 1.5 hr at 37°C. The medium was carefully removed and DMSO (180 µl) was

added afterward. After it was shaken for 15min, the absorbance was measured at 490nm and 570nm with a reference filter of 620nm. The inhibition% was calculated according to the following equation: inhibition% = [1-(assay-blank)/(control-blank)] × 100.

Table 1. In vitro potency measurement of the conjugates along with the conjugates plus saturate naked antiHer2 antibody (for shielding the antigens) against N-87 cells (Ag+) at 37 °C, incubation.

conjugate	Conjugate Drug/mAb ratio	IC_{50} values	IC_{50} with 4 μM un-conjugated Herceptin blocking	Specific win-dows of cyto-toxicity
50	3.53	0.21~0.68 nM	0.23 ~0.45 mM	338~1956
51	3.51	0.62 ~1.73 nM	0.41 ~0.72 mM	236~1161
52	3.55	1.92~4.87 nM	0.55 ~0.71 mM	112~369
53	3.52	0.93~2.16 nM	0.53 ~0.76 mM	245~817
54	3.53	0.17~0.61 nM	0.23 ~0.61 mM	377~3588

Compounds **50, 51, 53** and **54** had the similar ranges of potency as well as the similar specific window. The conjugate **54** bearing an epoxide was better than other four tested conjugates in both potency and specific window against antigen positive tumor cells.

Claims

What is claimed is:

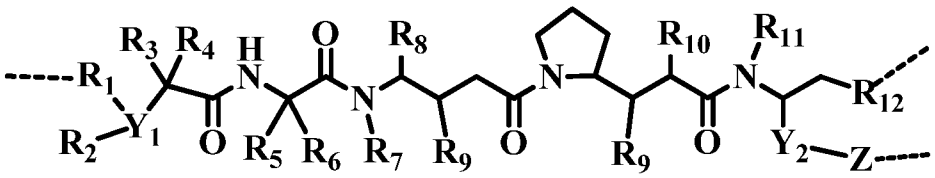
1. A conjugate of a cell binding molecule with the cytotoxic agents (auristatin analogues) of the invention having a structure of Formula (I):

$$G\text{-}L_m\text{-}D_n \qquad (I)$$

and pharmaceutical acceptable salts and solvates thereof.

Wherein G is a cell surface binding molecule (ligand); n is 1-20; m is 1-10.

L is a cleavable or uncleavable linker; D is a cytotoxic agent (drug) molecule having the Formula (II):



(II)

Wherein --- is a linkage that connect a drug molecule to a linker independently; R^1, R^2, R^3, and R^4 are independently C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of heterocyclic, carbocyclic, cycloalkyl, alkylcycloalkyl, heterocycloalkyl; C_3-C_8 of aryl, Ar-alkyl, heteroalkylcycloalkyl, alkylcarbonyl; or two R's, such as R^1R^2, R^1R^3, R^2R^3, R^3R^4, and R^5R^6 can be 3-7 members of a carbocyclic, cycloalkyl, or heterocyclic, heterocycloalkyl ring system; In addition, R^2, R^3, and R^4 can be H, and R^1 can be absent;

Y^1 is N or CH;

R^5 and R^6 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; or R^5 and R^6 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^7 is H, or C_1-C_8 alkyl;

R^8 is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

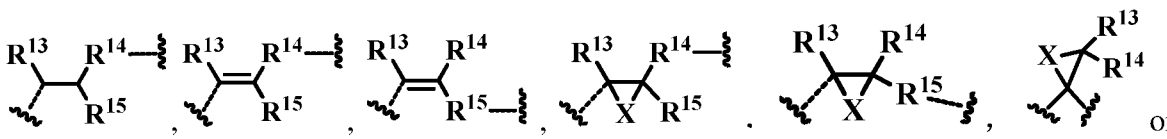
R^9 is H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle or O-(C_1-C_8 alkyl);

R^{10} is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

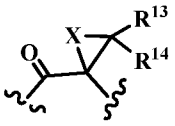
R^{11} is H, or C_1-C_8 alkyl;

R^{12} is aryl, alkylaryl, alkyl or C_3-C_8 heterocyles;

Y^2 is selected from:



or



; wherein R^{13}, R^{14} and R^{15} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle; and X is CH_2, O, S, NH, NR^{16}, S(O) or $S(O)_2$, wherein R^{16} is H, or C_1-C_8 alkyl; In addition, R_{14} can be absent.

Z is H, C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -NO_2, -CN, -COOH, -$C(O)OR^{17}$, -$C(O)NHR^{18}$, $C(O)NR^{18}R^{19}$, -CH_2R^{20}, -$CH_2OP(O)(OR^{21})_2$, wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle.

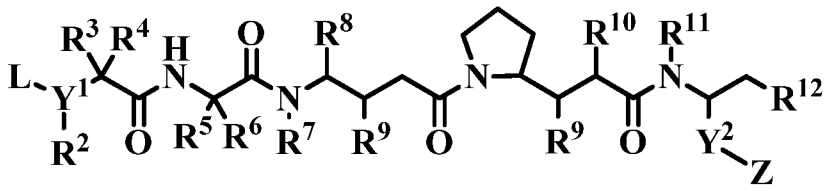
The linker L has the Formula of: $\mathbf{—W_w—(Aa)_r—V_v—}$,

Wherein: -W- is a stretcher unit, which links a targeted binding molecular unit (G) to an amino acid unit (Aa) when presents, or links V when an Aa is not present, and W may independently contain a self-immolative spacer, a peptidyl unit, a hydrazone, a disulfide, a thioether, an ester, or an amide bond; w is 0 or 1; Wherein Aa is independently a natural or unnatural amino acid unit; r is independently an integer ranging from 0 to 12; $(Aa)_r$ represents a natural or unnatural amino acid, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit.

Wherein V is a spacer unit and is independently selected from H, O, NH, S, C_1~C_8 of alkyl, heteroalkyl; C_2~C_8 of alkenyl, alkynyl, hetero-cyclic, carbocyclic; C_3~C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl, or one to four amino acid units, or $(CH_2CH_2O)_r$, r is an integer ranging from 0 to 12; and v is 0, 1 or 2.

The cell surface binding molecule G is an antibody; a single chain antibody; an antibody fragment that binds to the target cell; a monoclonal antibody; a single chain monoclonal antibody; or a monoclonal antibody fragment that binds the target cell; a chimeric antibody; a chimeric antibody fragment that binds to the target cell; a domain antibody; a domain antibody fragment that binds to the target cell; adnectins that mimic antibodies; DARPins; a lymphokine; a hormone; a vitamin; a growth factor; a colony stimulating factor; or a nutrient-transport molecule (a transferrin); a binding peptide, or protein, or antibody, or small molecule attached on albumin, polymers, dendrimers, liposomes, nanoparticles, vesicles, or (viral) capsids.

2. A linker-cytotoxic agent (L_m-D_n) for the conjugate according to claim **1** having Formula (III):



(III)

and pharmaceutical acceptable salts and solvates thereof.

Wherein

L is a cleavable or uncleavable linker; L can have a function group that enables linkage of a cytotoxic drug via a disulfide, thioether, thioester, peptide, hydrazone, ether, ester, carbamate, carbonate, amine (secondary, tertiary, or quartary), imine, cycloheteroalkyane, heteroaromatic, alkoxime, or amide bond;

Y^1 is N or CH;

R^2 is H, or C_1-C_8 alkyl;

R^3 and R^4 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle ; or R^3 and R^4 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^5 and R^6 are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle ; or R^5 and R^6 jointly form a cycloalkyl, heterocycloalky, carbocyclic or heterocyclic ring system;

R^7 is H, or C_1-C_8 alkyl;

R^8 is H, H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

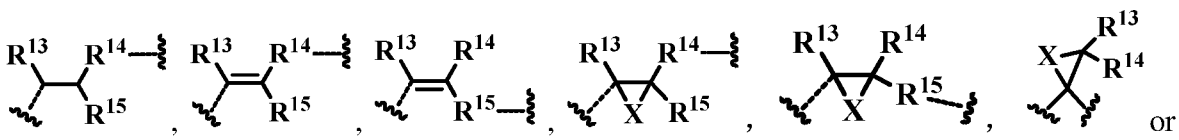
R^9 is H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle or O-(C_1-C_8 alkyl);

R^{10} is H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle;

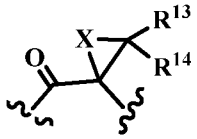
R^{11} is H, or C_1-C_8 alkyl;

R^{12} is aryl, alkylaryl, alkyl, or C_3-C_8 heterocyles;

Y^2 is selected from:



or

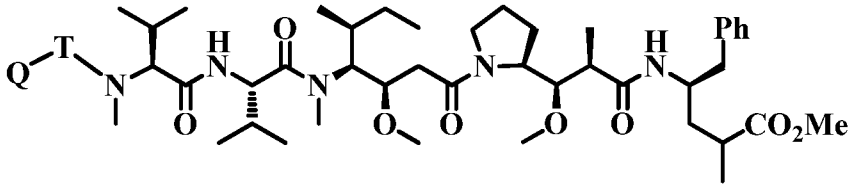


wherein R^{13}, R^{14} and R^{15} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8

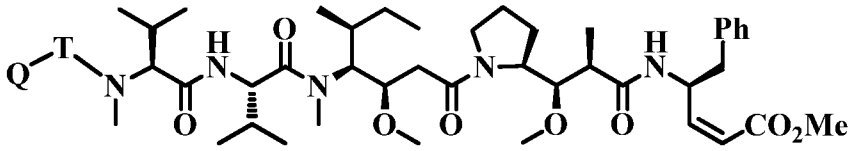
alkylcarbocycle, or C_1-C_8 alkylheterocycle; and X is CH_2, O, S, NH, NR^{16}, S(O) or $S(O)_2$, wherein R^{16} is H, or C_1-C_8 alkyl; In addition, R^{14} can be absent.

Z is H, C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -NO_2, -CN, -COOH, -$C(O)OR^{17}$, -$C(O)NHR^{18}$, $C(O)NR^{18}R^{19}$, -CH_2R^{20}, -$CH_2OP(O)(OR^{21})_2$, wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently H, C_1-C_8 alkyl, C_1-C_8 heteroalkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 heterocycle, C_3-C_8 carbocycle, aryl, C_1-C_8 alkylaryl, C_1-C_8 alkylcarbocycle, or C_1-C_8 alkylheterocycle.

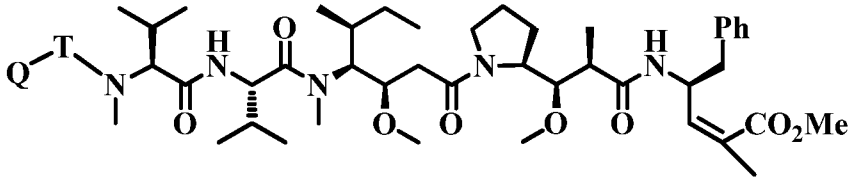
3. A conjugate of a cell binding molecule with the cytotoxic agents of the invention according to claim **2** having the following structures:



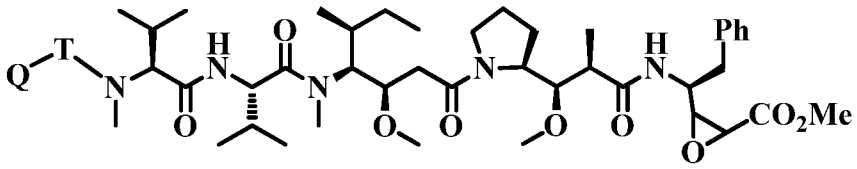
IIIa



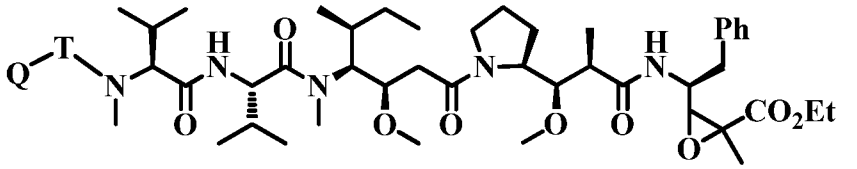
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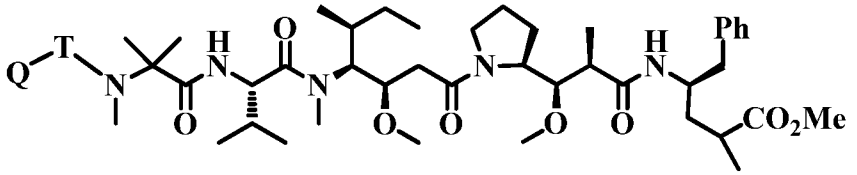
IIIc



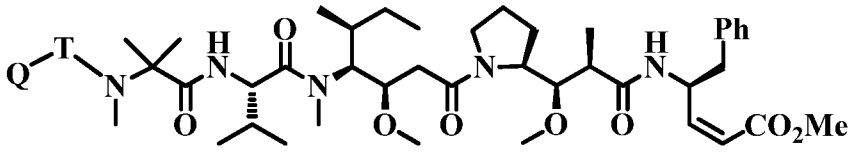
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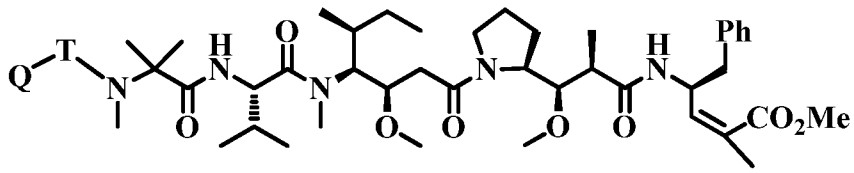
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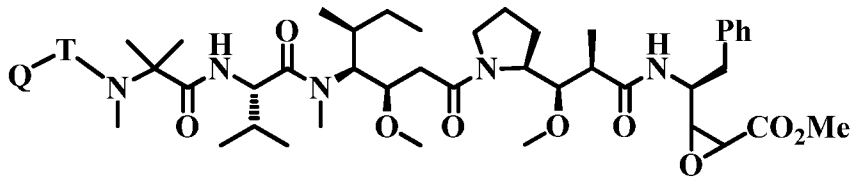


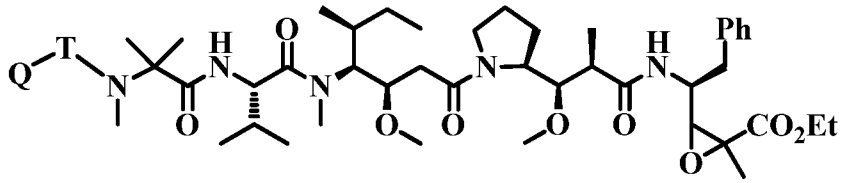
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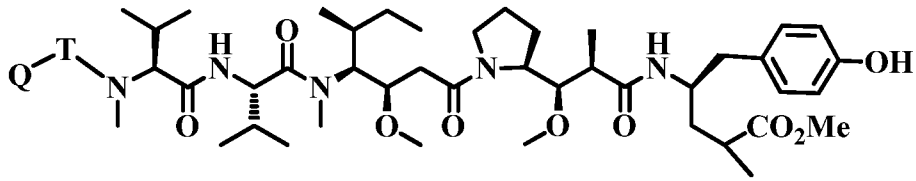


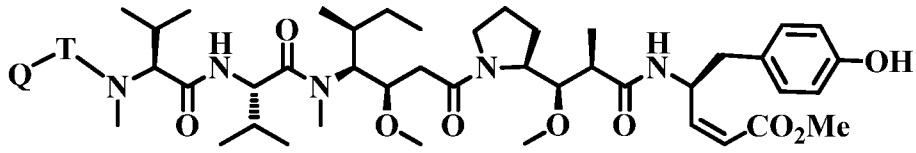
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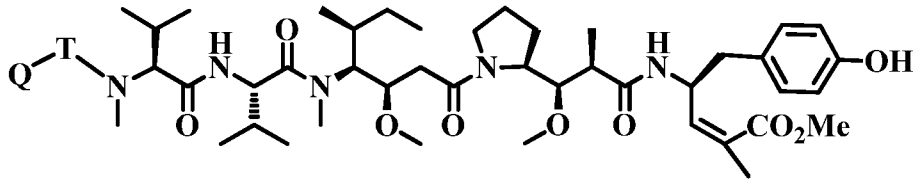

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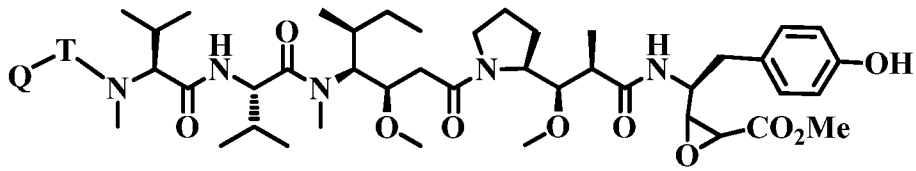

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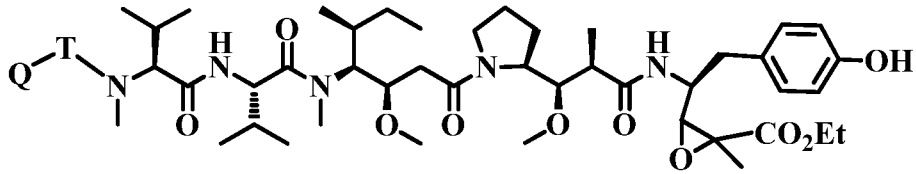

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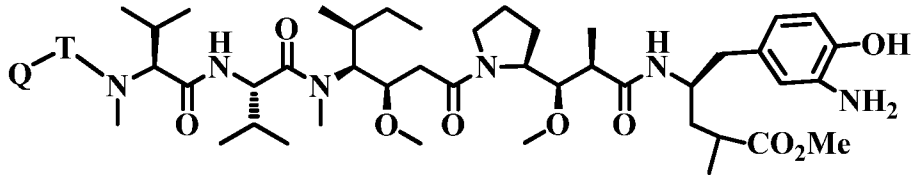

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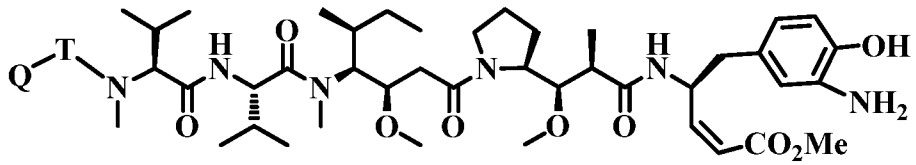

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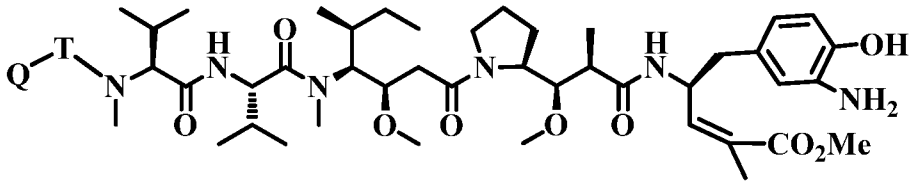

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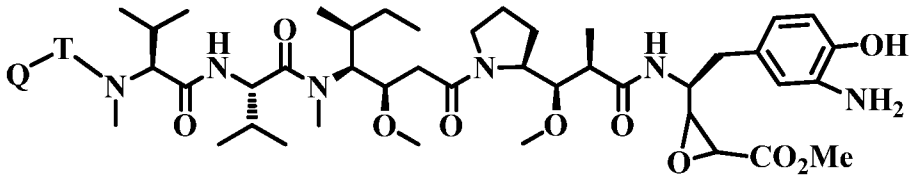

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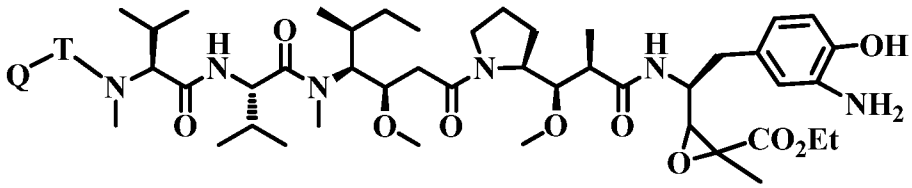

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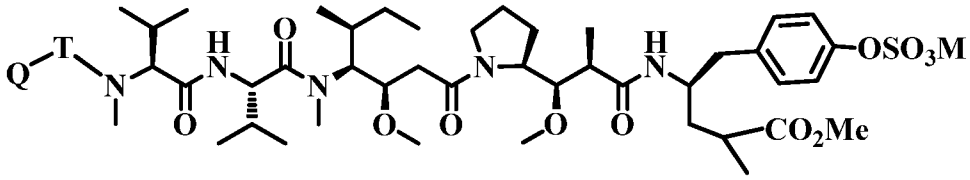

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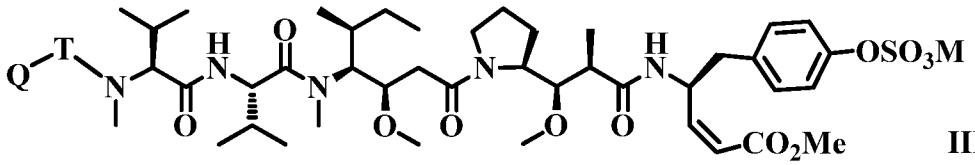

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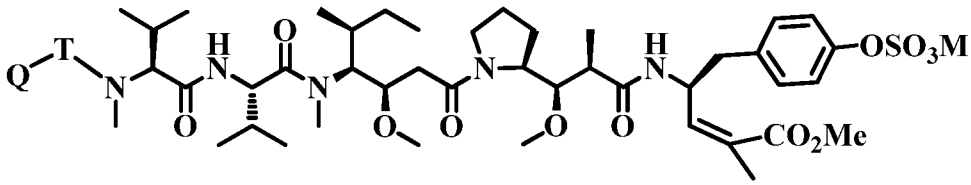

IIIr

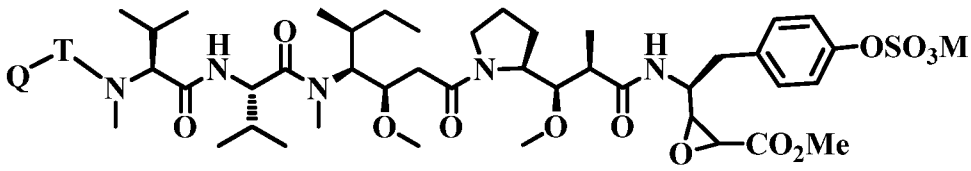

IIIs

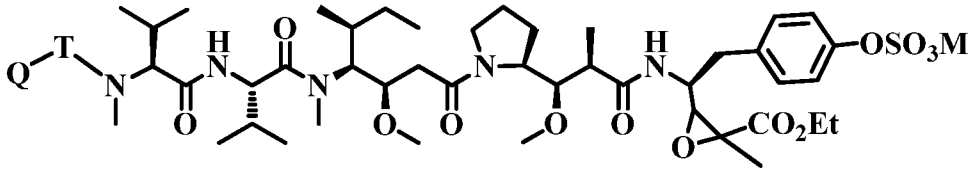

IIIt

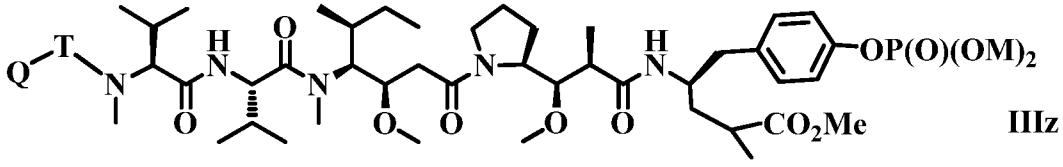

IIIu

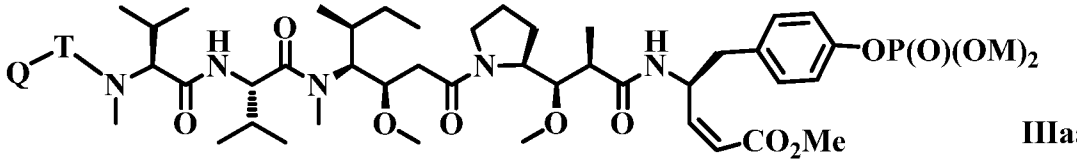

IIIv

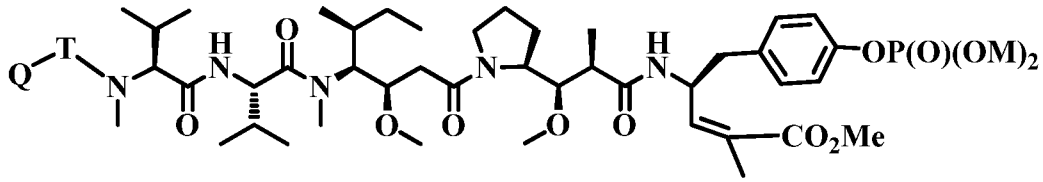

IIIw


IIIx

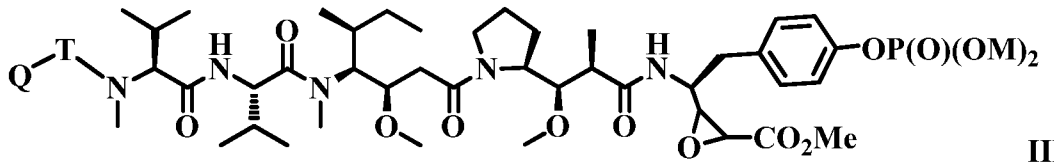

IIIy


IIIz

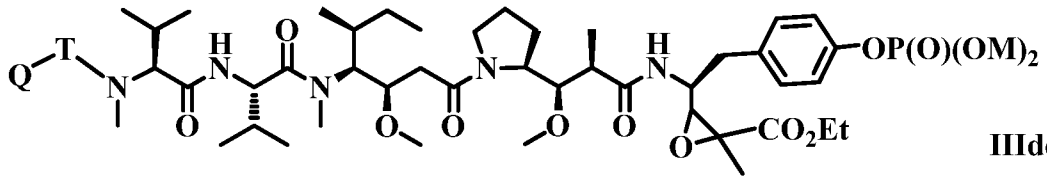

IIIaa



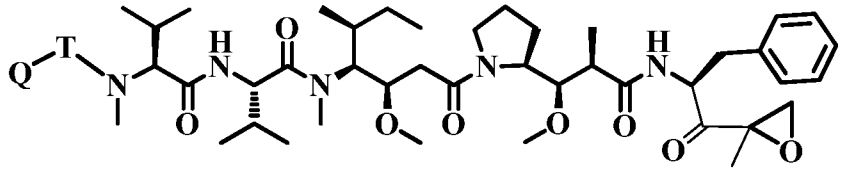
IIIbb



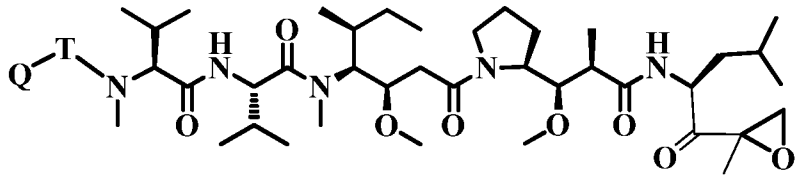
IIIcc



IIIdd



IIIee



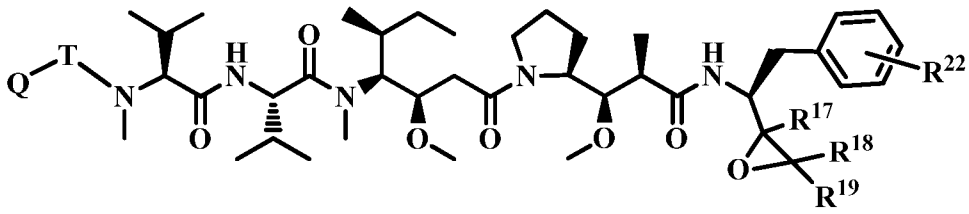
IIIff

Wherein M is H, or Na, or K, or $N^+R^1R^2R^3$ or a pharmaceutical salt; R^1, R^2 and R^3 are described above in claim 1;

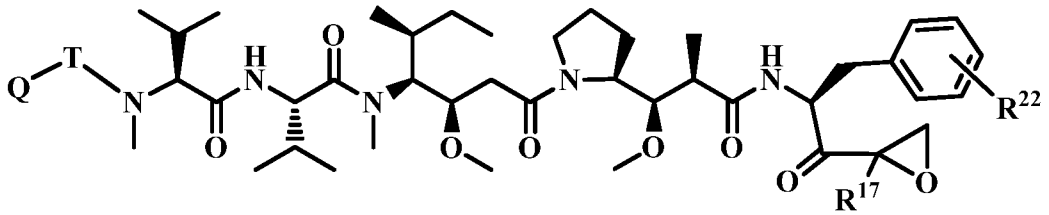
T is independently L, R^1, -O-, -S-, -SS-, -NH-, =N-, =NNH-, -NH(R^1), -OR^1, -C(=O)-, -C(=O)R^1, -COOR^1-, -C(O)NH-, -C(=O)NHR^1-, -SR^1-, -S(=O)R^1-, -P(=O)(OR^1)$_2$-, -P(=O)(NHR^1)$_2$-, -CH_2OP(=O)(OR^1)$_2$-, -CH_2NHP(=O)(NHR^1)$_2$-, -CH_2NHP(=O)(NHR^1)$_2$-, -CH_2NHP(=O)(NHR^1)(NHR^2)-, -SO_2R^1-, $(CH_2CH_2O)_r$-, -$(CH(CH_3)CH_2O)_r$-, C_1-C_8 of alkyl, heteroalkyl; C_2-C_8 of alkenyl, alkynyl, heterocyclic, carbocyclic; C_3-C_8 of aryl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroaralkyl, heteroalkylcycloalkyl, alkylcarbonyl, one to ten amino acid units; r is an integer ranging from 0 to 12; L, R^1 and R^2 are defined above in Claim 1.

Q is a cell-binding agent; or a function group readily link to a cell binding molecule. The function group is selected from, disulfide substituent, a maleimido, haloacetyl group, hydrazide, aldehyde, ketone, azide, amine, alkoxylamino, or an *N*-hydroxysuccinimide ester.

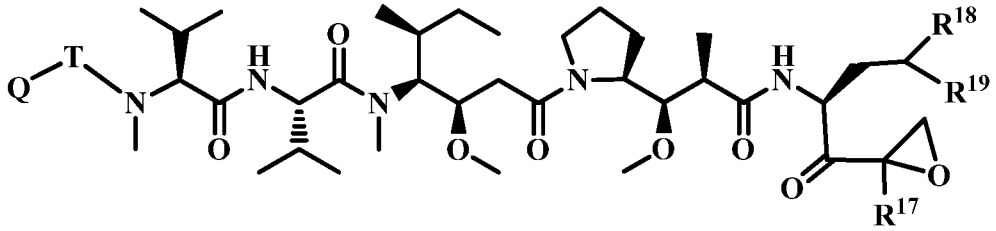
4. A conjugate of a cell binding molecule with the cytotoxic agents of the invention according to claim **1** having the following preferred structures:



IIIgg



IIIhh

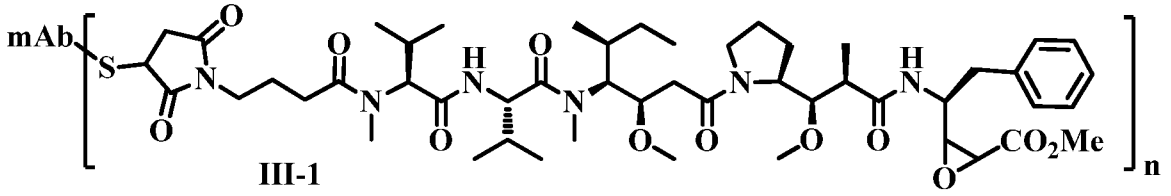


IIIii

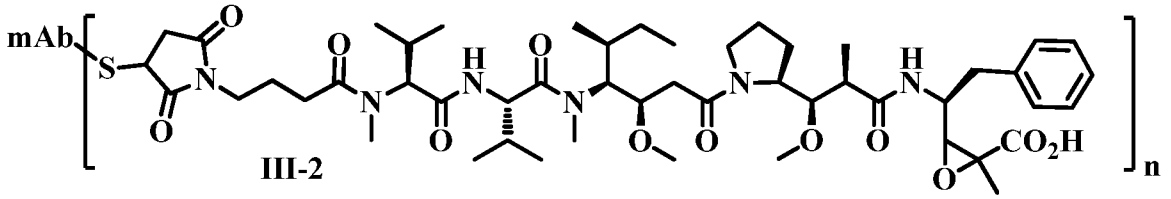
and pharmaceutical acceptable salts and solvates thereof,

Wherein Q, T, R^1, R^2 and R^3 are defined the same in claims **1** and **3**; R^{22} is H, OPO_3M_2, OSO_3M, OCH_2PO_3M, OH, NO_2, F, Cl, Br, I, SR^{13}, SSR^{13}, NH_2, NHR^{13}, $NR^{13}R^{14}$, or OR^{13}; M is H, Na, K, Ca, Mg, Zn or $N^+R^1R^2R^3$; R^{13}, R^{14}, R^{15} , R^{17}, R^{18}, and R^{19} are defined in Claim 1. In addition, R^{18} and R^{19} can jointly form a cycloalkyl, heterocycloalkyl, carbocyclic, heterocyclic ring system, aromatic, or heteroaromatic group;

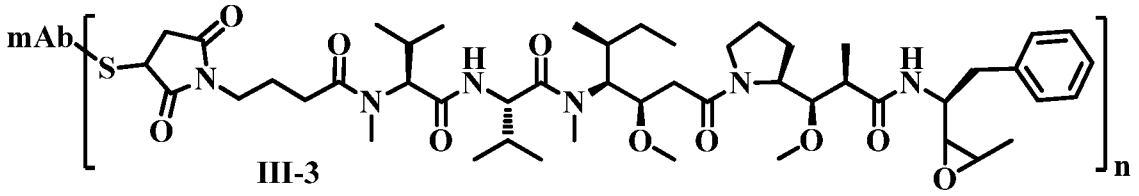
5. A conjugate compound according to claim **2, 3** or **4** bearing Formula (III) having the following structures:



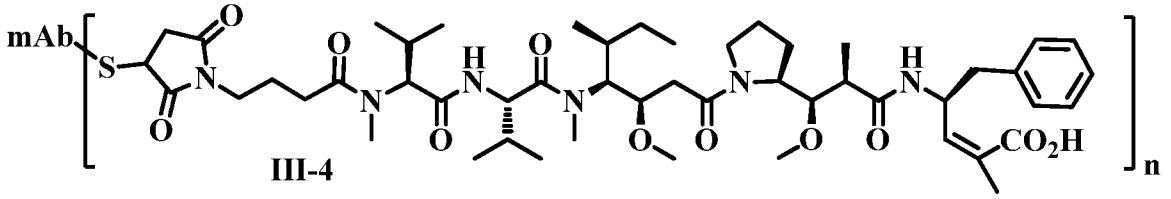
III-1



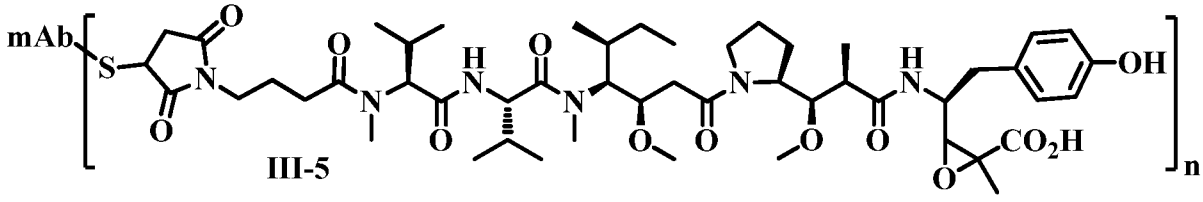
III-2



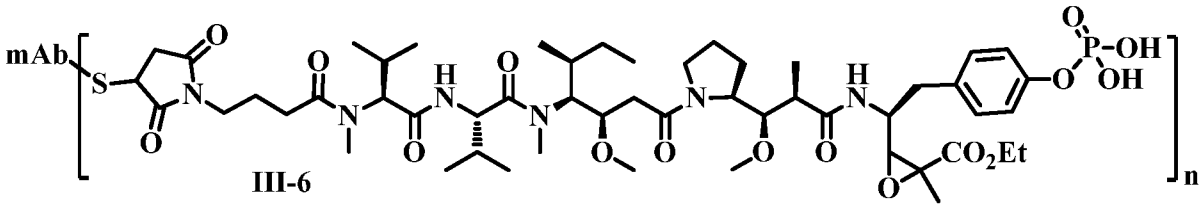
III-3



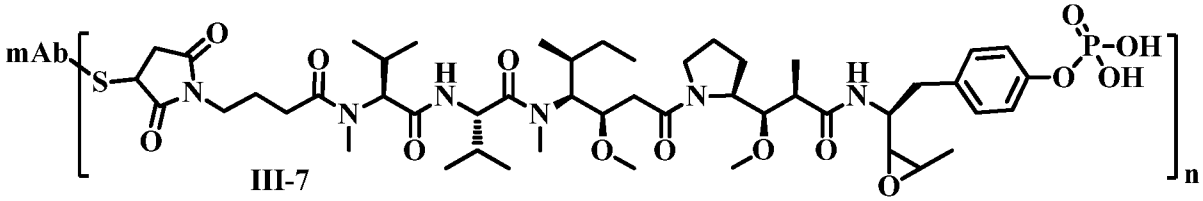
III-4



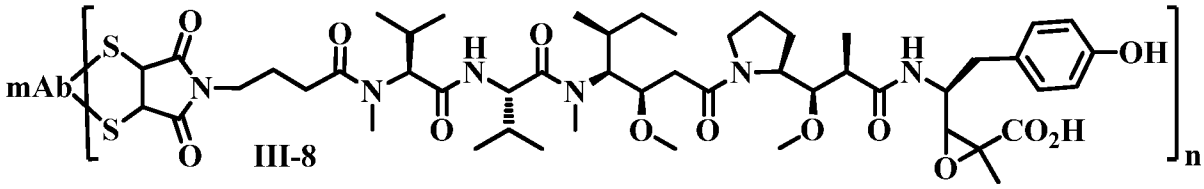
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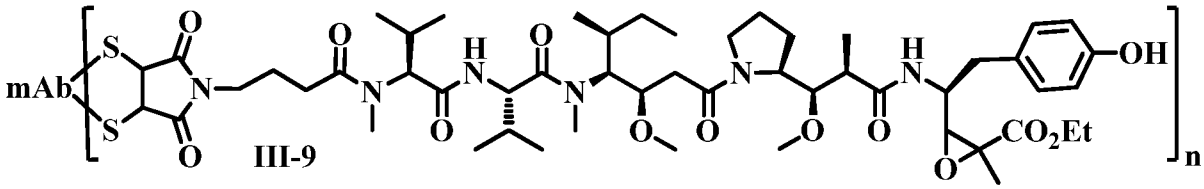
III-6



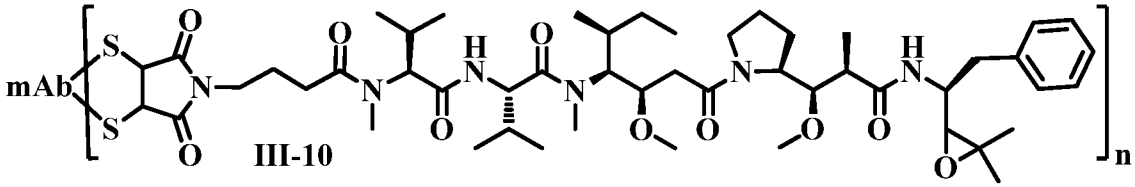
III-7



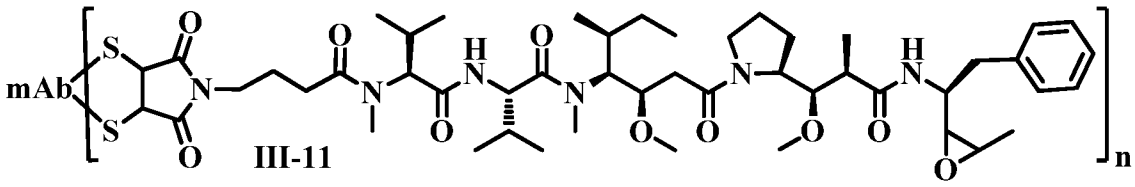
III-8



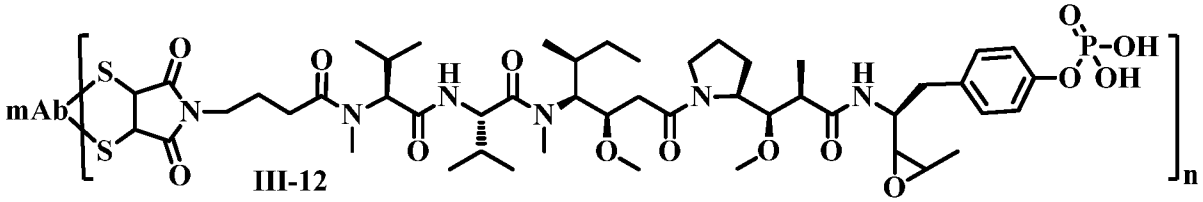
III-9



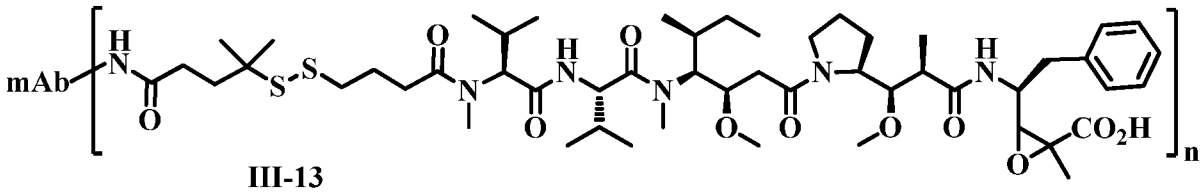
III-10



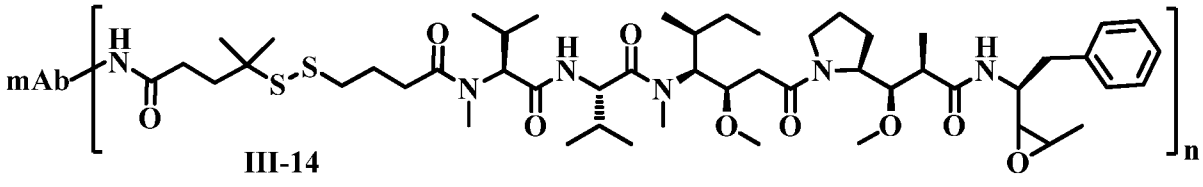
III-11



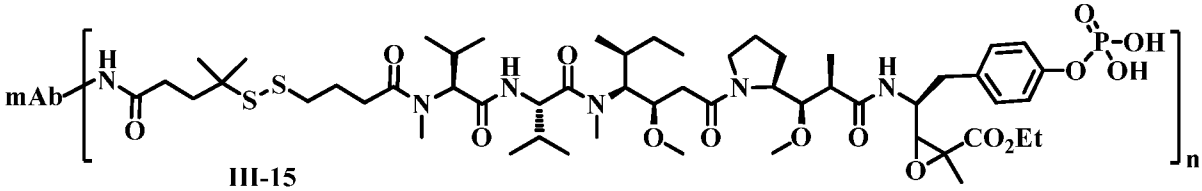
III-12



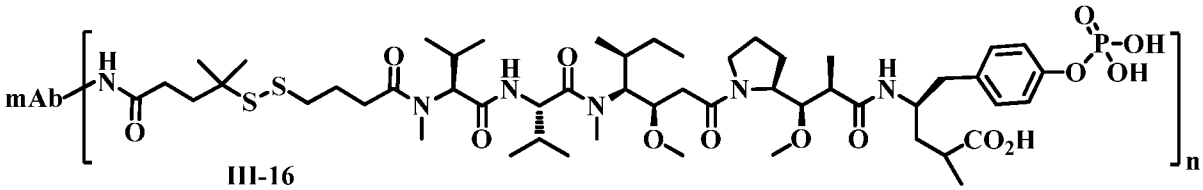
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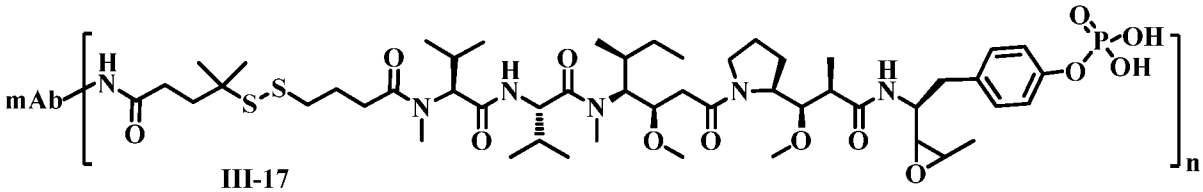
III-14



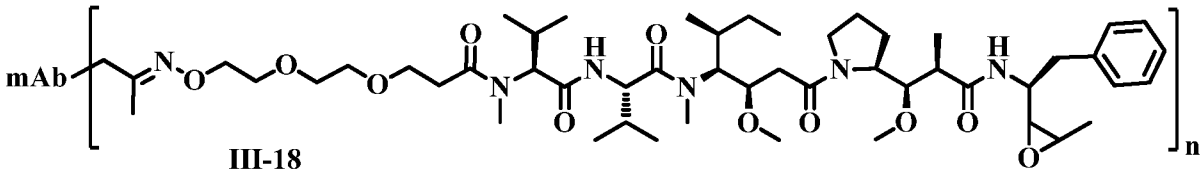
III-15



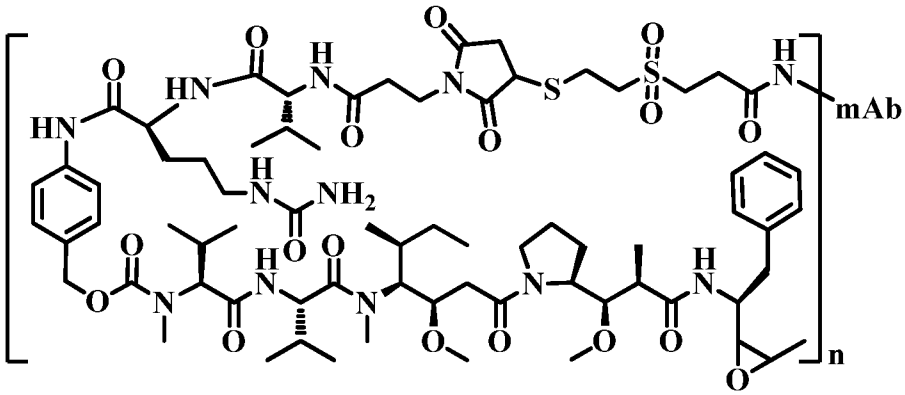
III-16



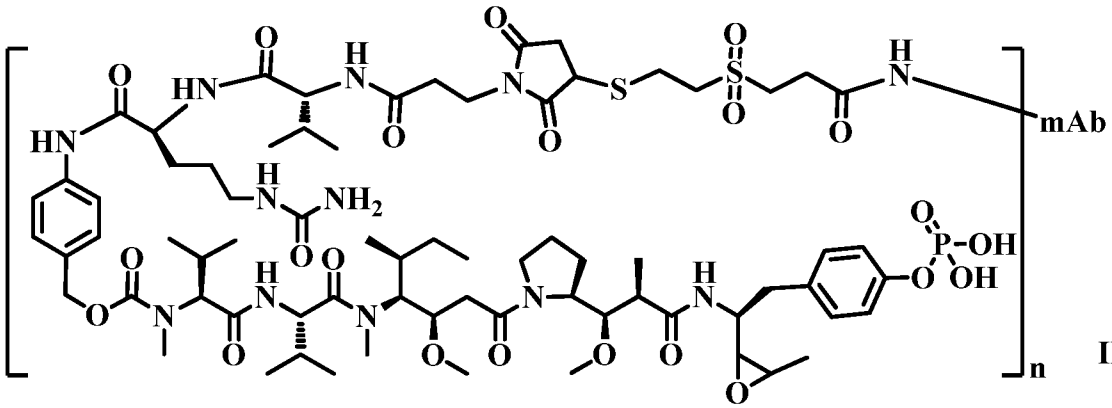
III-17



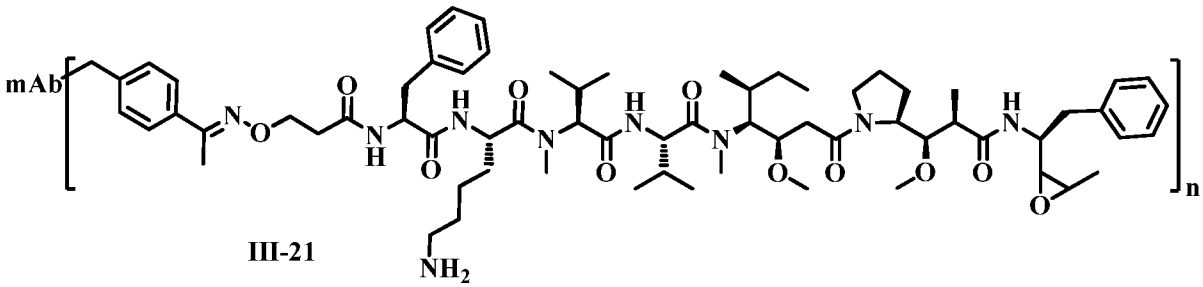
III-18



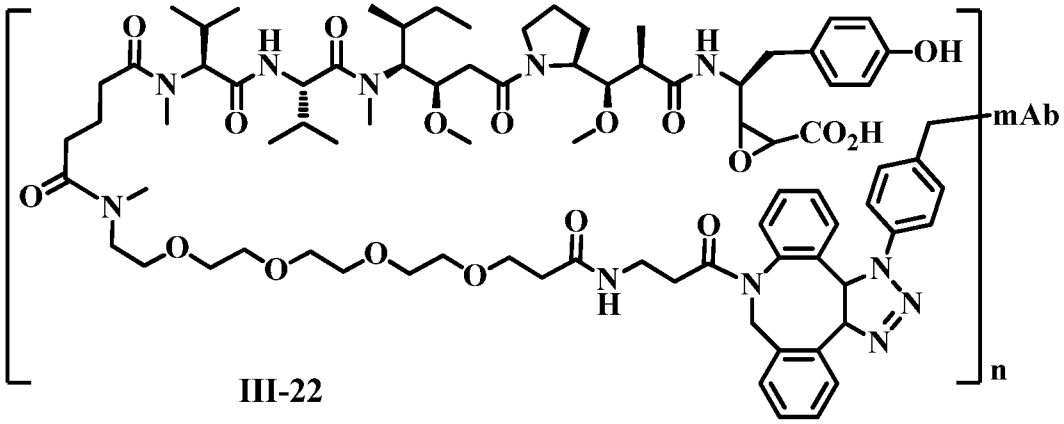
III-19



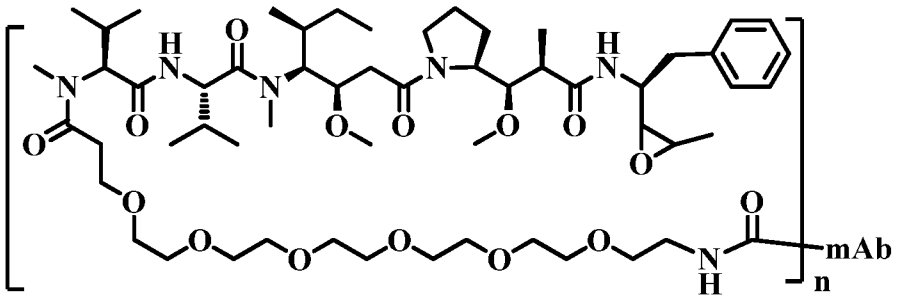
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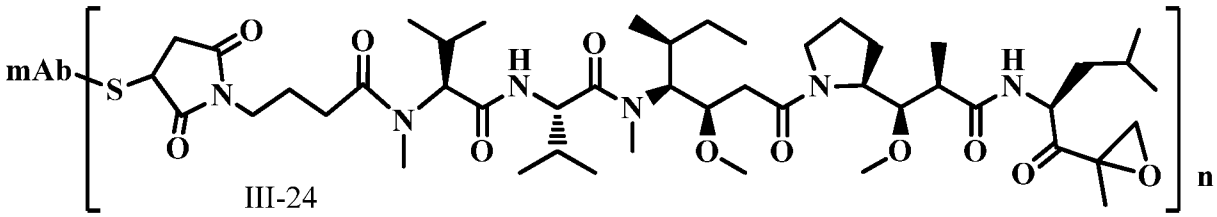
III-21



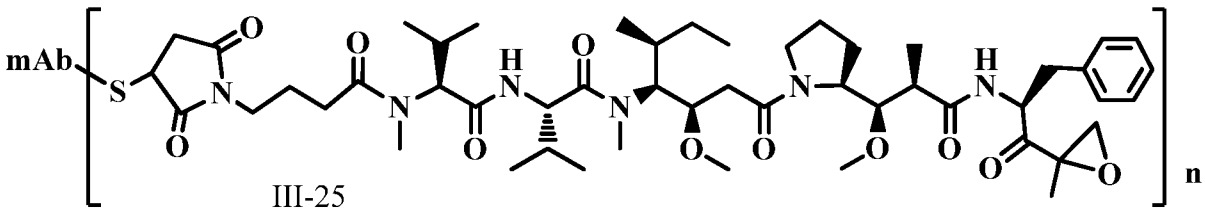
III-22



III-23



III-24

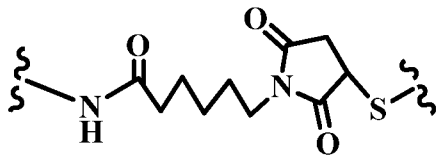


III-25

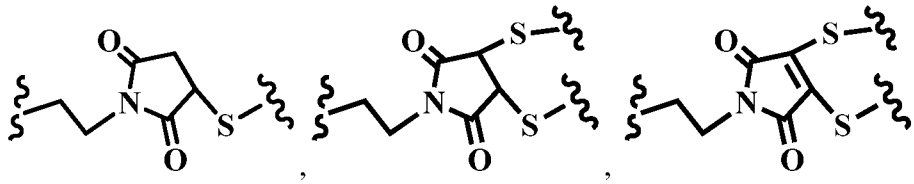
and pharmaceutical acceptable salts and solvates thereof.

Wherein mAb is an antibody and n=1~30.

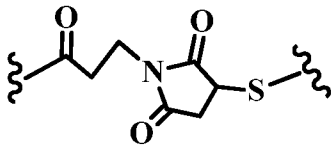
6. The linker L according to claim 1 can be composed of one or more linker components of 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe" or "af"), *p*-aminobenzyloxycarbonyl ("PAB"), 4-thiopentanoate ("SPP"), 4-(*N*-maleimidomethyl)cyclohexane-1 carboxylate ("MCC"), (4-acetyl)aminobenzoate ("SIAB"), 4-thio-butyrate (SPDB), 4-thio-2-hydroxysulfonyl-butyrate (2-Sulfo-SPDB), ethyleneoxy $-CH_2CH_2O-$ as one or more repeating units ("EO" or "PEO"), Oxime (aldoxime, ketoxime), 1,2,3-triazoles (formed by a click chemistry), acetal (formed by a sugar or its analog), which have a structure of:



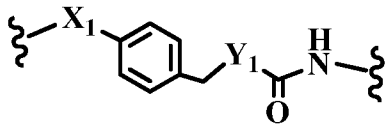
(MC, 6-maleimidocaproyl containing)



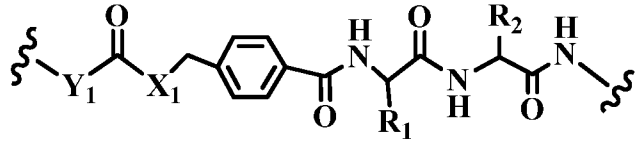
(ME, maleimidoethyl containing).



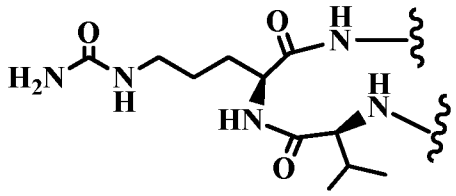
(MP, maleimidopropanoyl containing)



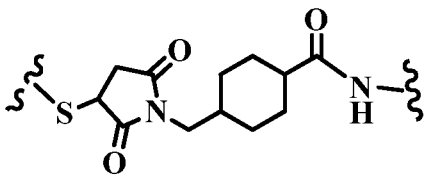
, (PAB, p-aminobenzyloxycarbonyl analog, X_1 and Y_1 are independently O, S, NH, CH_2)



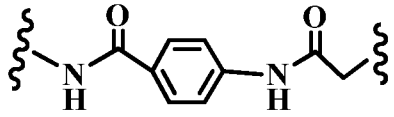
(carbonylbenzylpeptidyl analog, X_1 and Y_1 are independently O, S, NH, CH_2, R_1 and R_2 are defined above).



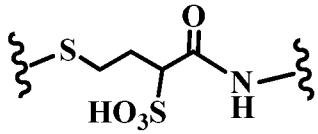
, (valine-citrulline containing)



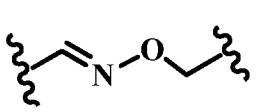
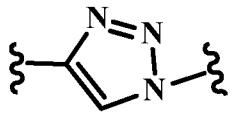
, (MCC, 4-(N-maleimidomethyl)cyclohexane-1 carboxylate)

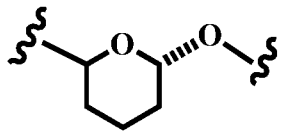
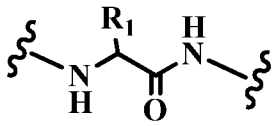


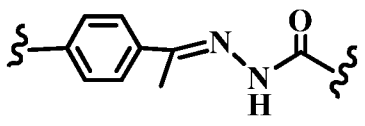
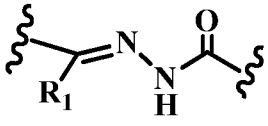
, ((4-acetyl)aminobenzoate containing)

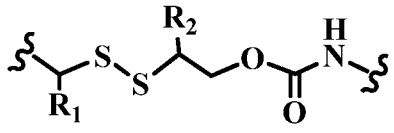


, (4-thio-2-hydroxysulfonyl-butyrate, 2-sulfo-SPDB)

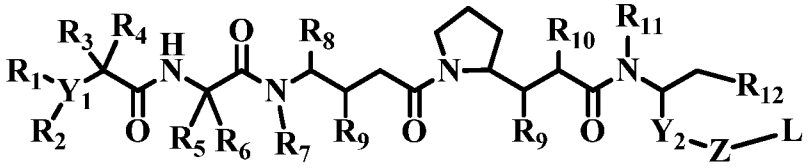

, (Oxime);

, (1,2,3-triazoles)


(Acetal);

(Stable peptide)


,

(Hydrazone)


((2-disulfanyl) ethylcarbamate)

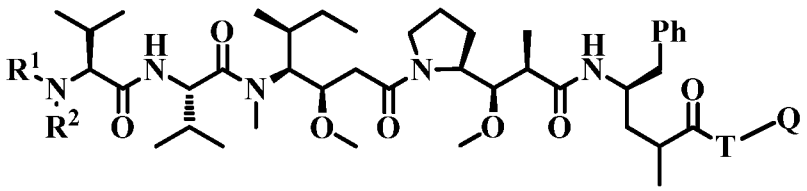
7. A linker-cytotoxic agent (L_m-D_n) for the conjugate according to claim **1** having Formula (IV):

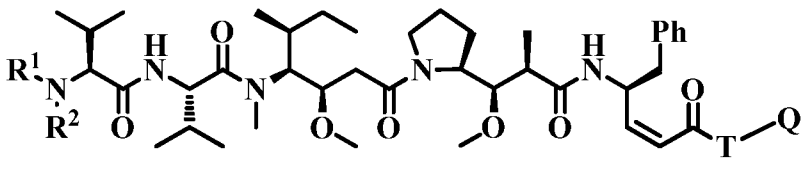

(IV)

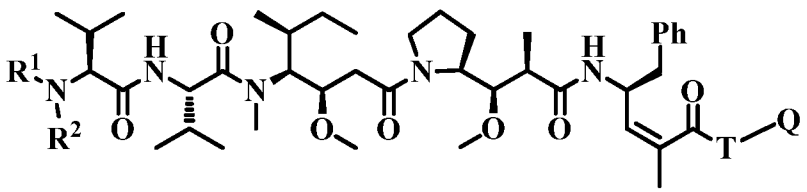
and pharmaceutical acceptable salts and solvates thereof.

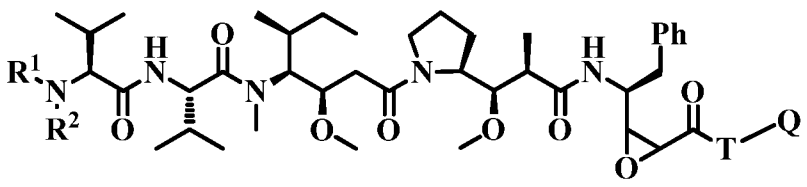
Wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, Y^1, Y^2, Z and L are described previously the same as in claim **2**.

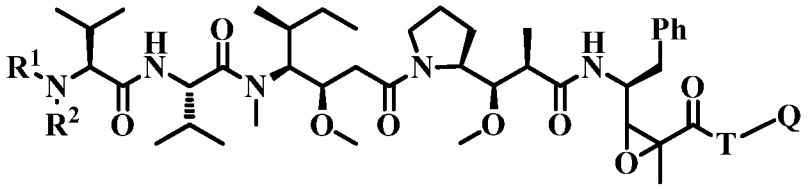
8. A conjugate of a cell binding molecule according to claim **1** bearing a linker-cytotoxic agent of Formula (IV) of claim **7** having the following structures:


IVa

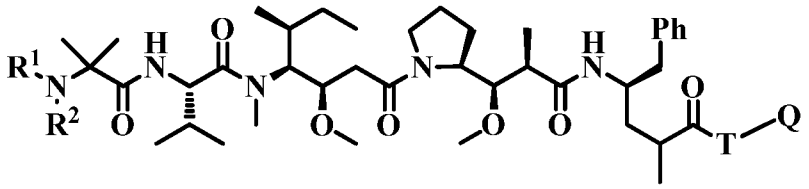

IVb


IVc

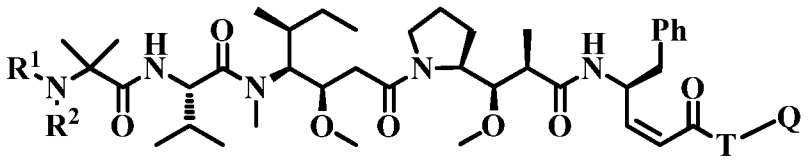

IVd



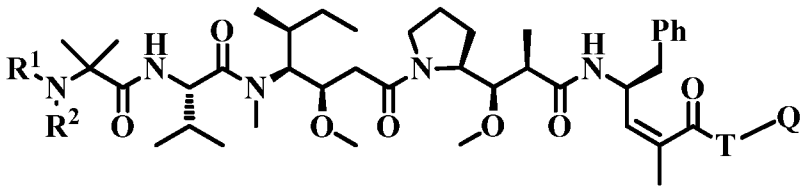
IVe



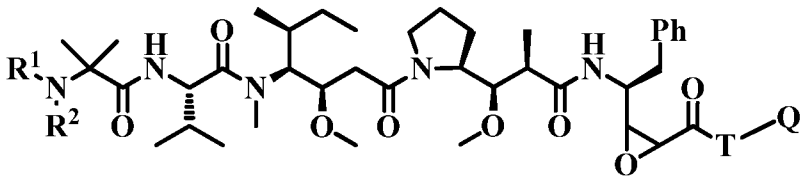
IVf



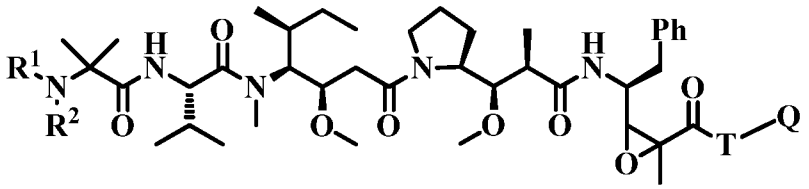
IVg



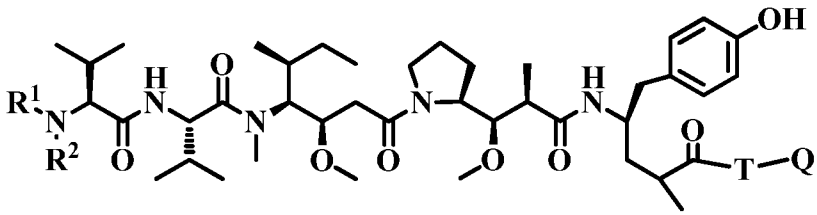
IVh



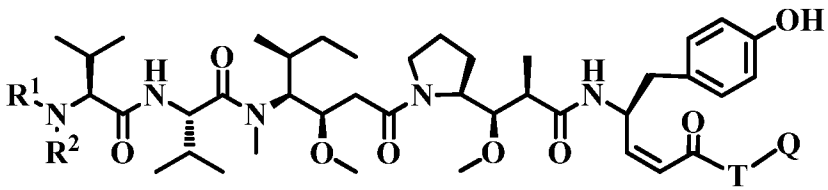
IVi



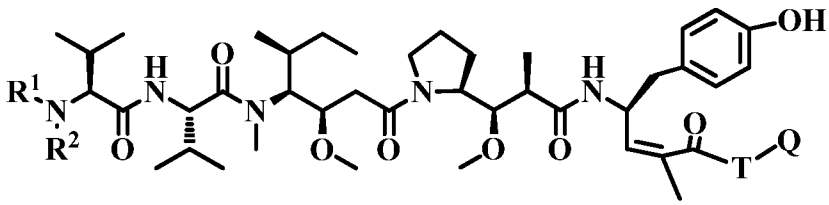
IVj



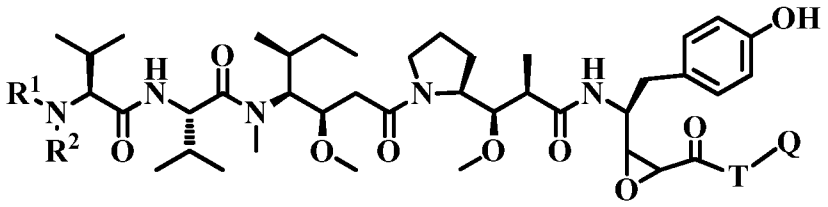
IVk



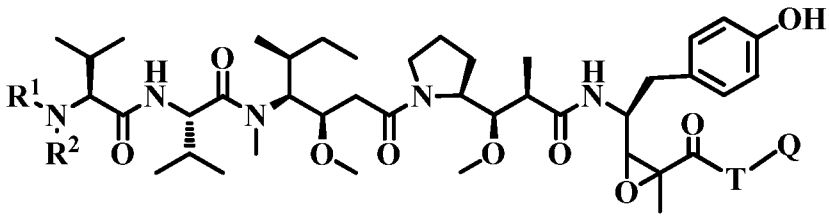
IVl



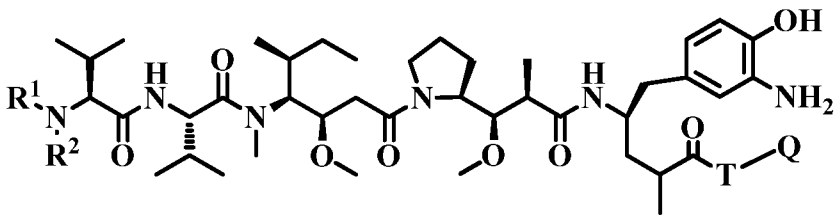
IVm



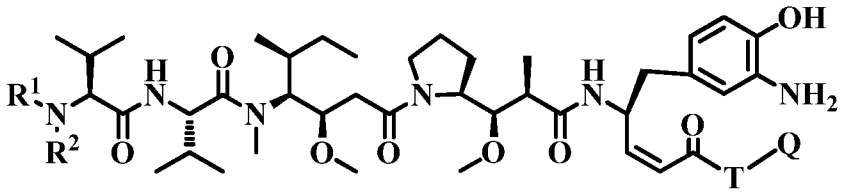
IVn



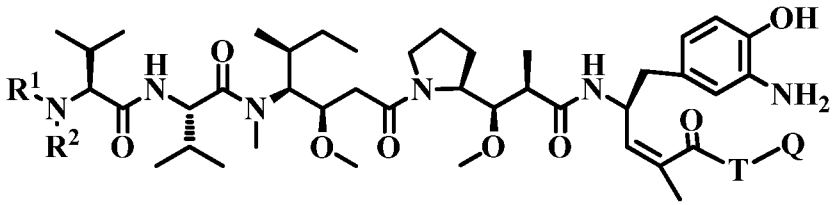
IVo



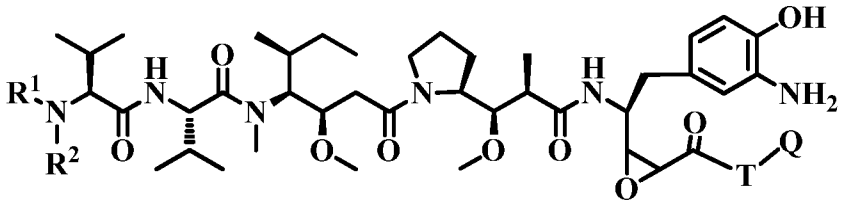
IVp



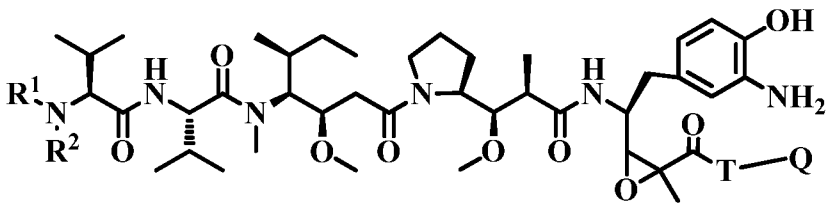
IVq



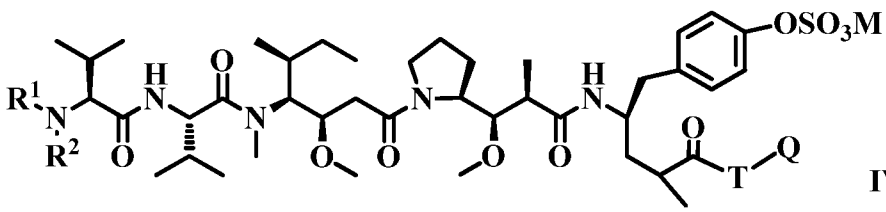
IVr



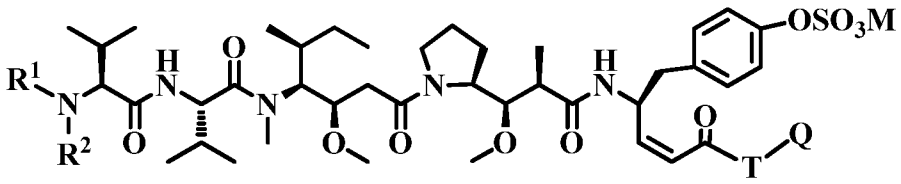
IVs

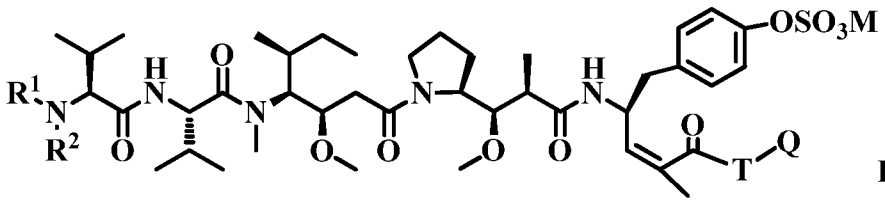


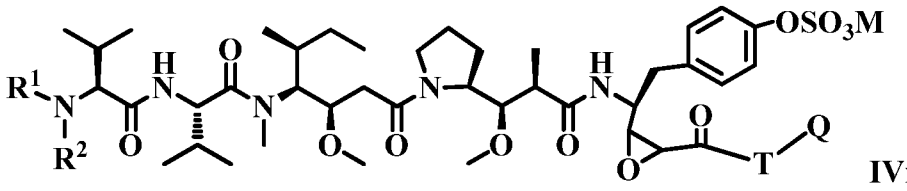
IVt

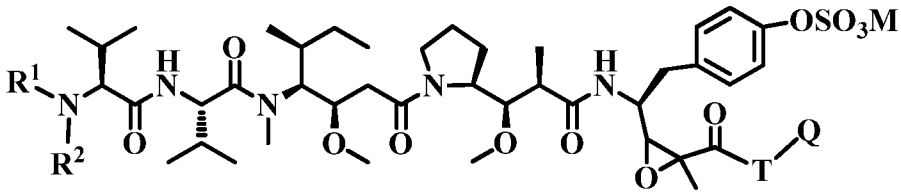


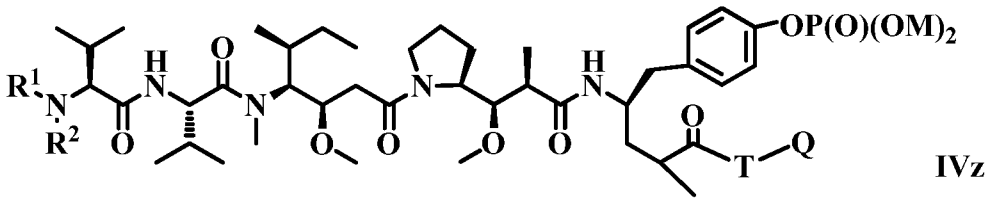
IVu

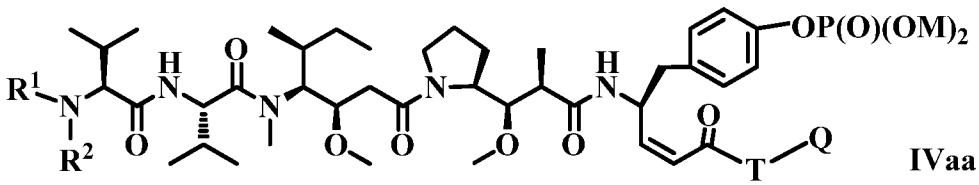

IVv

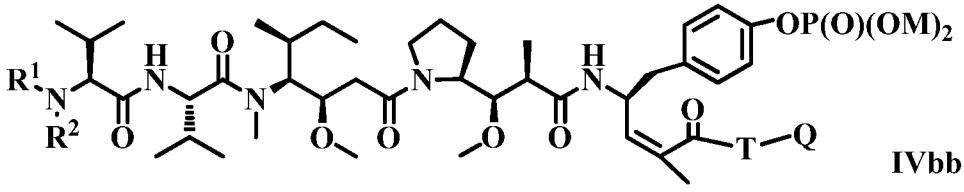

IVw

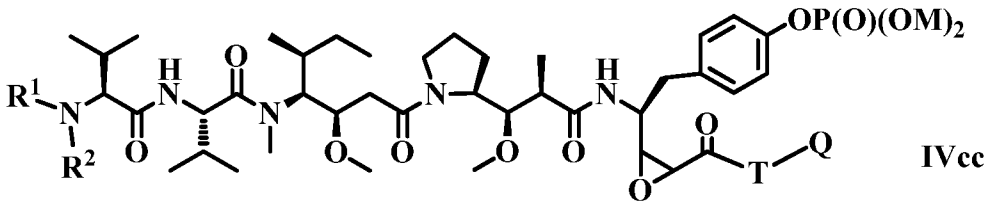

IVx

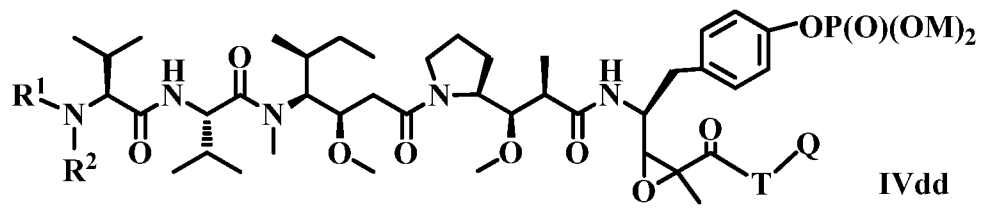

IVy


IVz


IVaa


IVbb

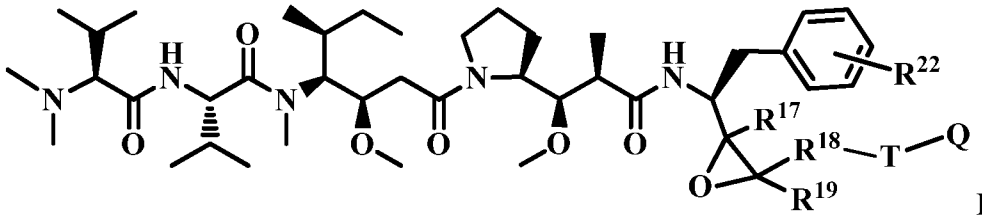

IVcc


IVdd

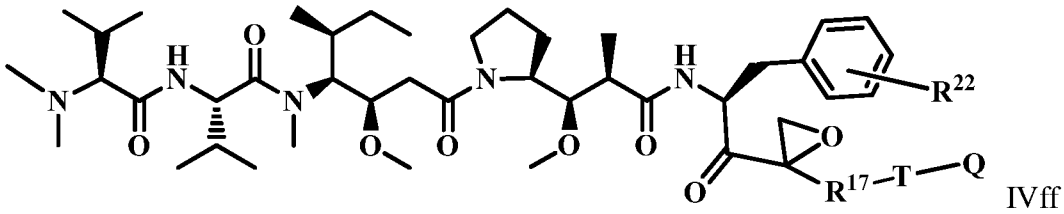
and pharmaceutical acceptable salts and solvates thereof.

Wherein M is H, or Na, or K, or $N^+R^1R^2R^3$ or a pharmaceutical salt. R^1, R^2, R^3, T, and Q are described the same in claims 2 and 3 above.

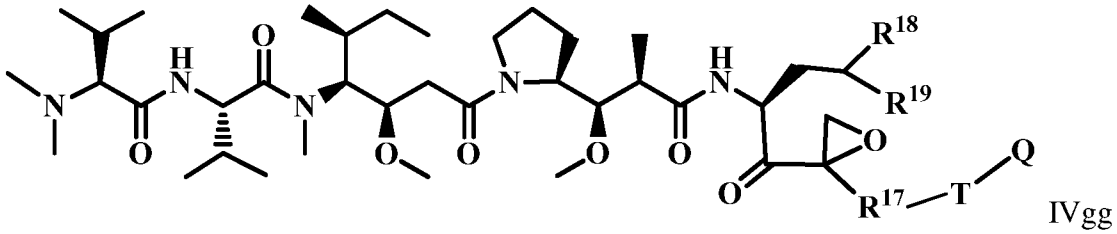
9. A conjugate of a cell binding molecule according to claim **1** bearing a linker-cytotoxic agent of Formula (IV) of claim **7** having the following preferred structures:



IVee



IVff

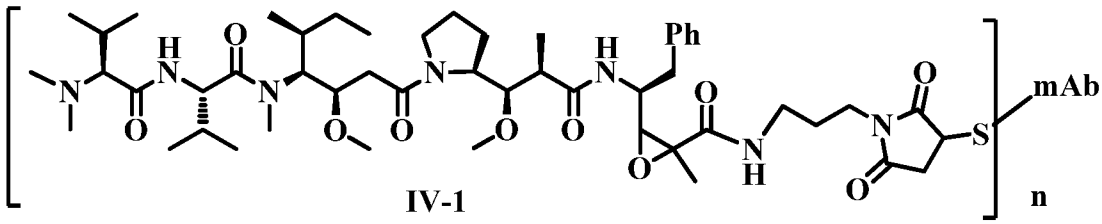


IVgg

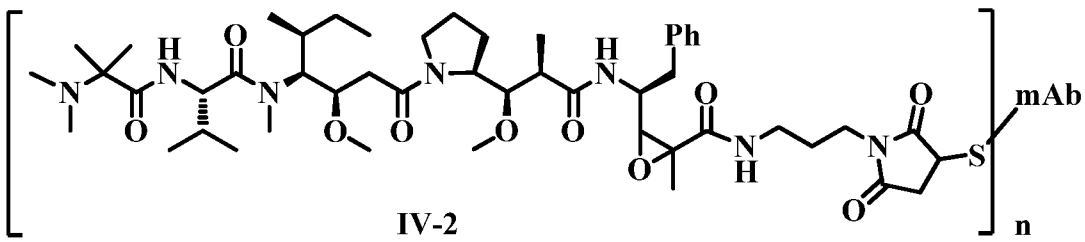
and pharmaceutical acceptable salts and solvates thereof.

Wherein Q, T, R^1, R^2, R^3, R^{17}, R^{18}, R^{19} and R^{22}, are defined the same in claims **1**, **3** and **4.** In addition, R^{17} and R^{18} can be absent.

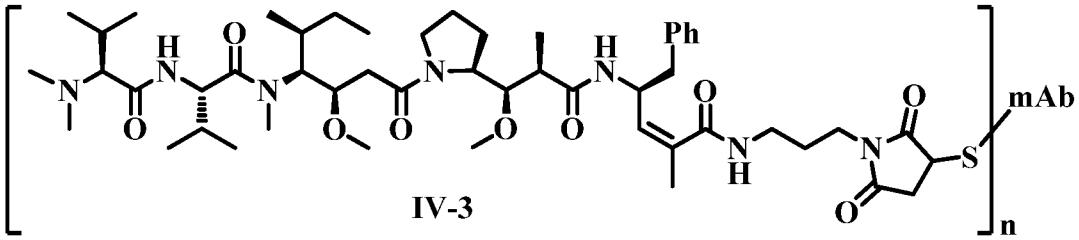
10. The conjugate according to claims **1** and **8** bearing Formula (IV) having the following structures:



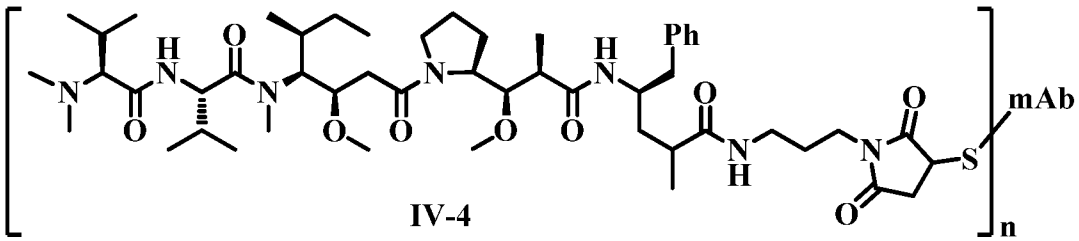
IV-1



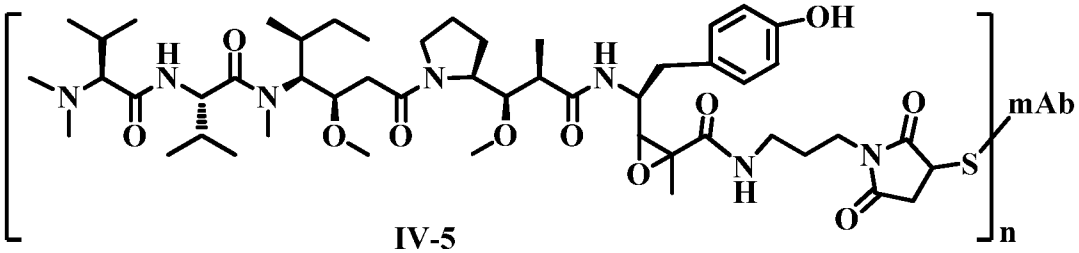
IV-2



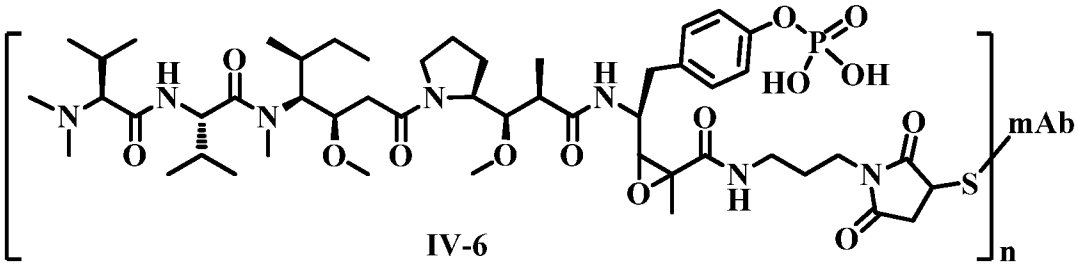
IV-3



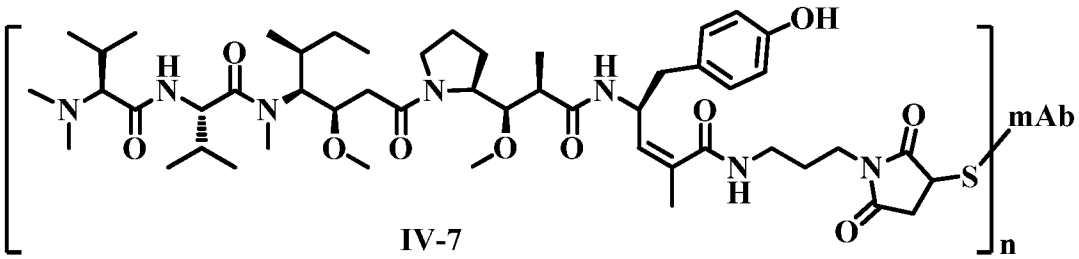
IV-4



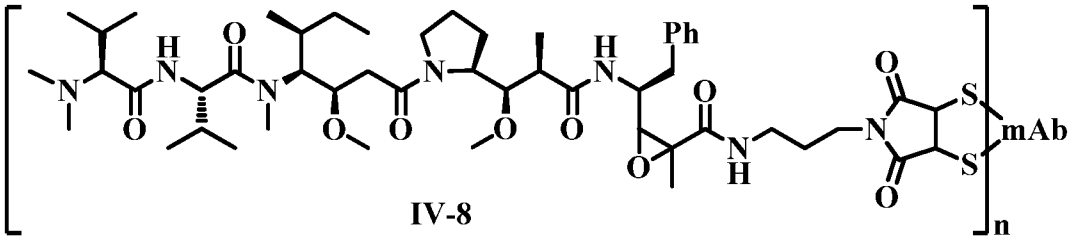
IV-5



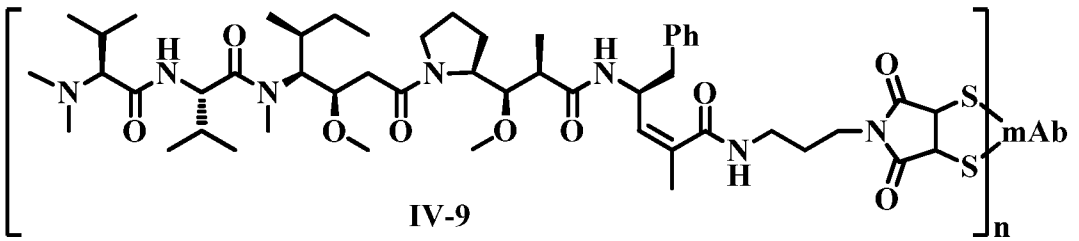
IV-6



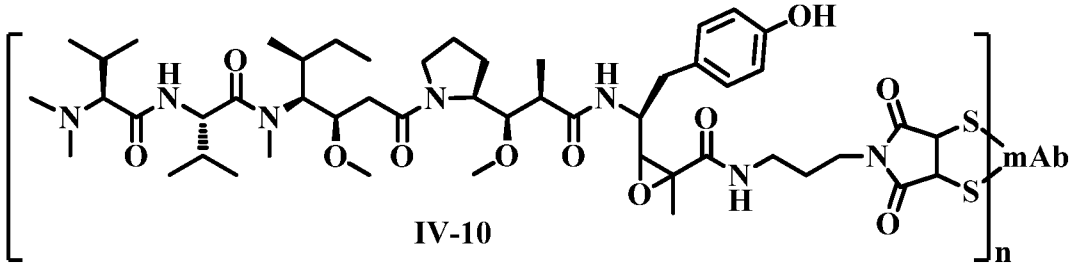
IV-7



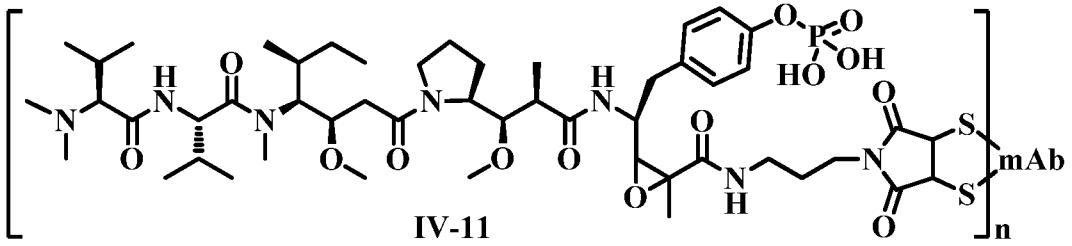
IV-8



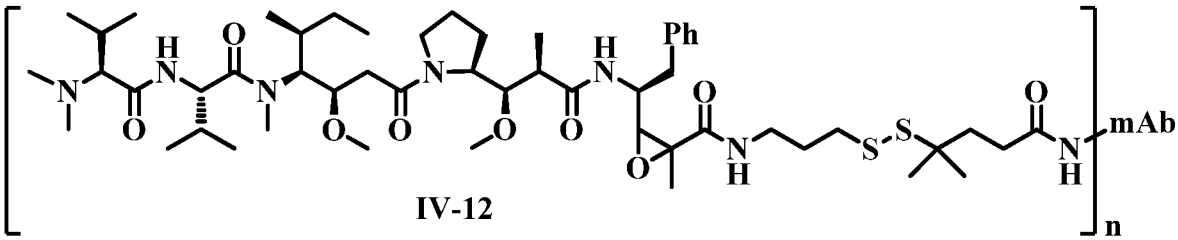
IV-9



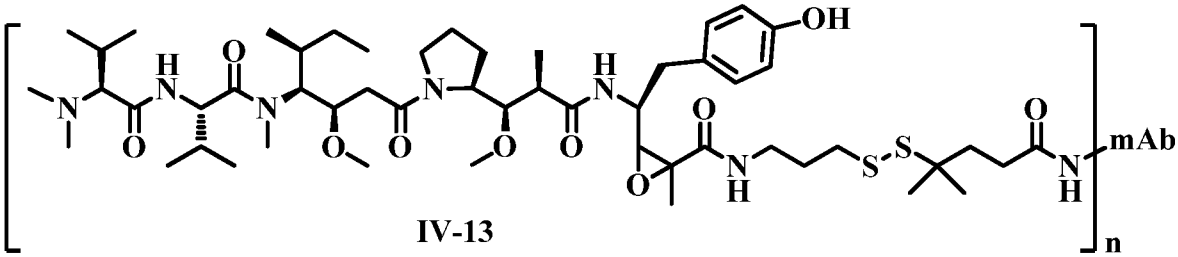
IV-10



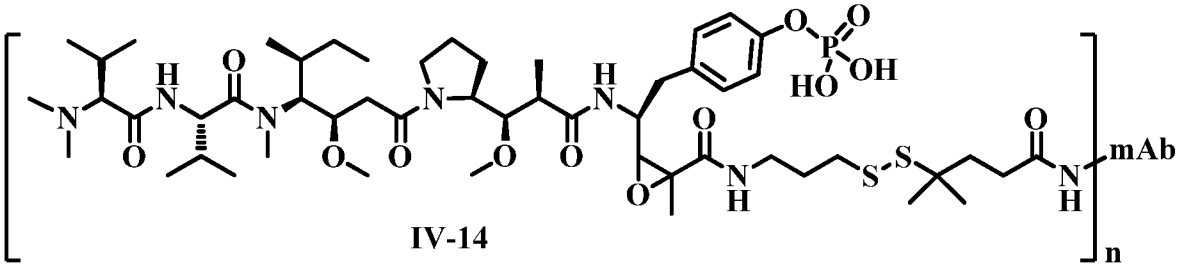
IV-11



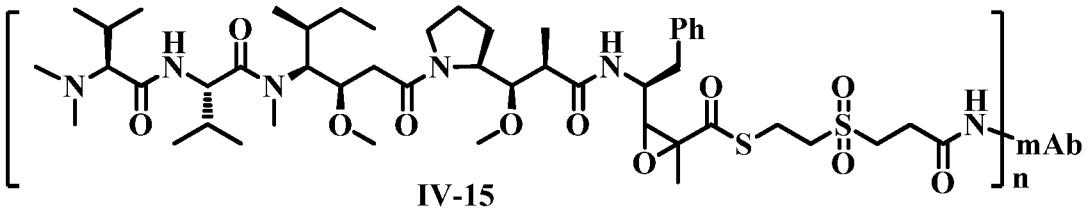
IV-12



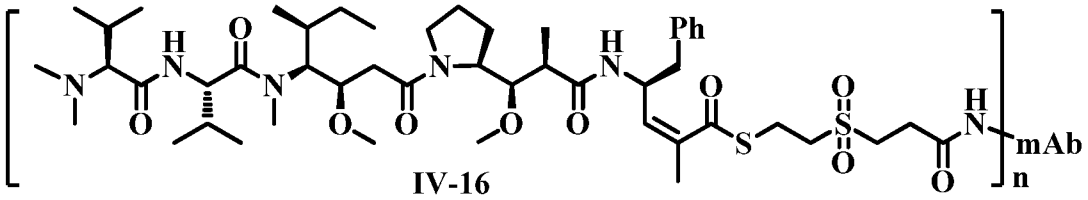
IV-13



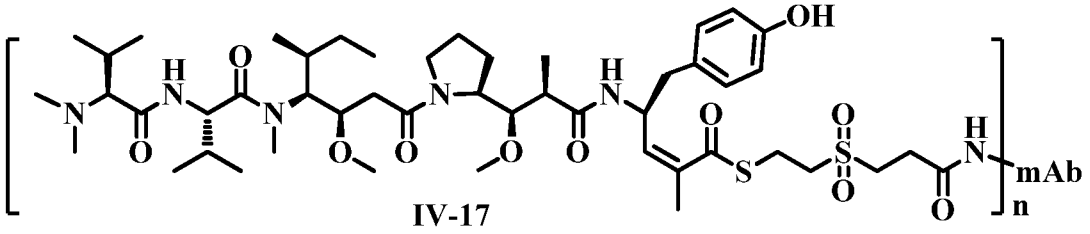
IV-14



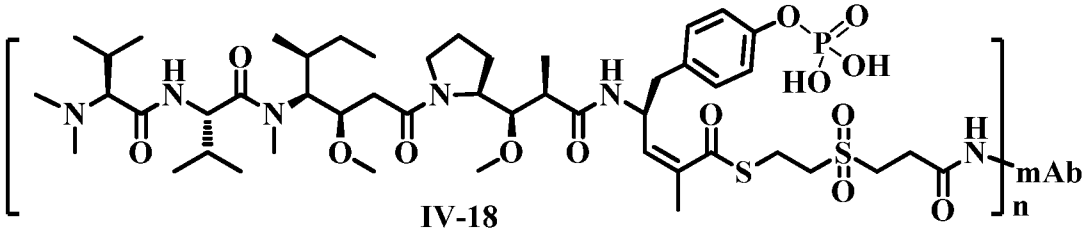
IV-15



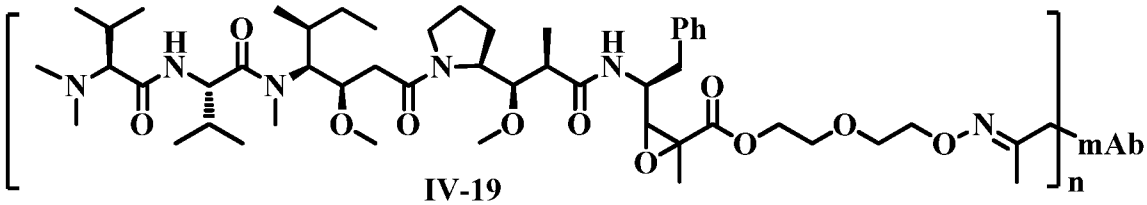
IV-16



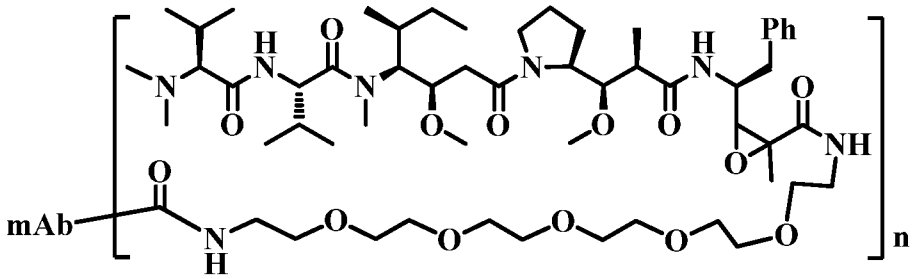
IV-17



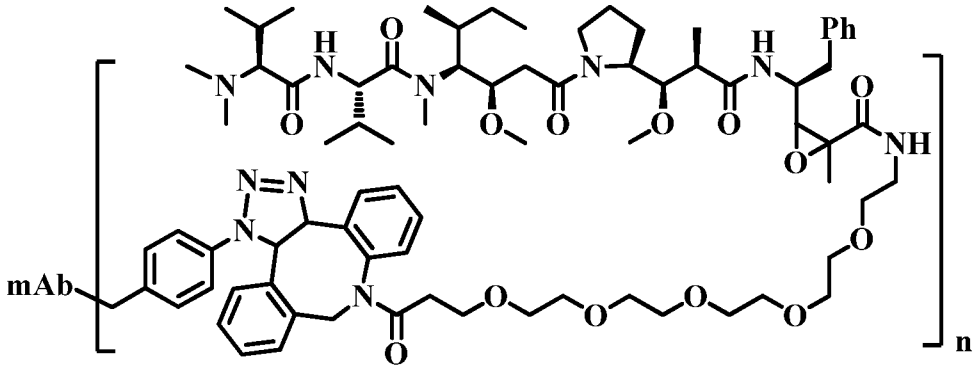
IV-18



IV-19



IV-20

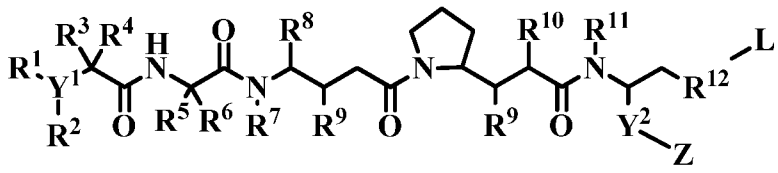


IV-21

and pharmaceutical acceptable salts and solvates thereof,

wherein mAb is an antibody and n=1~30.

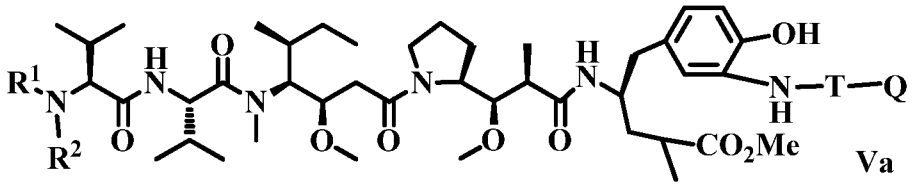
11. A linker-cytotoxic agent (L_m-D_n) according to claim **1** having Formula (V):



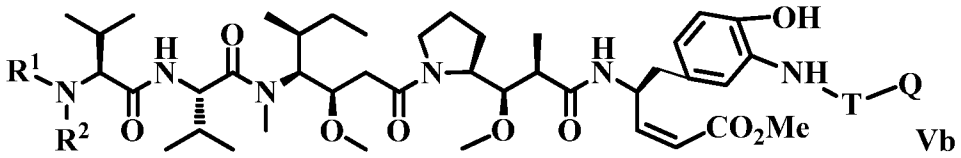
(V)

and pharmaceutical acceptable salts and solvates thereof, wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, Y^1, Y^2, Z and L are the same as described previously in claim **2**.

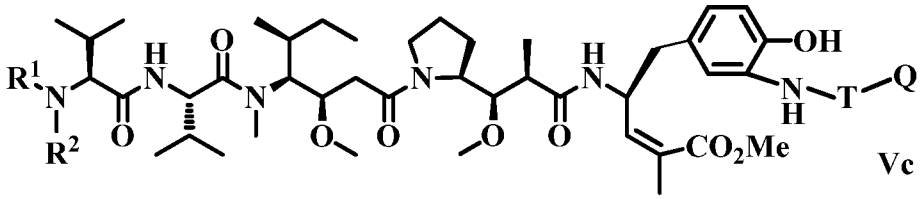
12. A conjugate of a cell binding molecule with the cytotoxic agent of the invention according to claim **11** having the following structures:



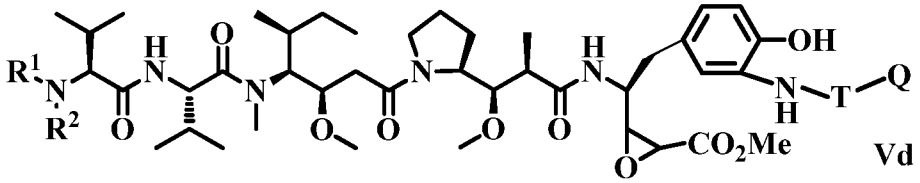
Va



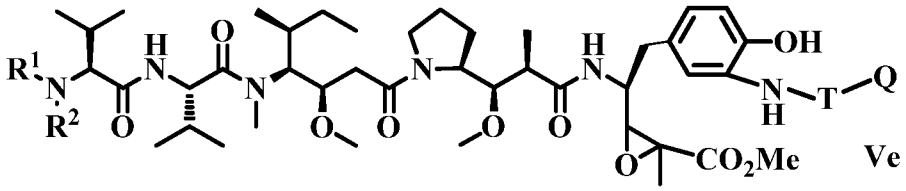
Vb



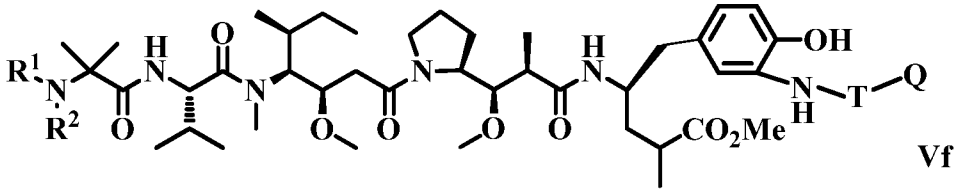
Vc



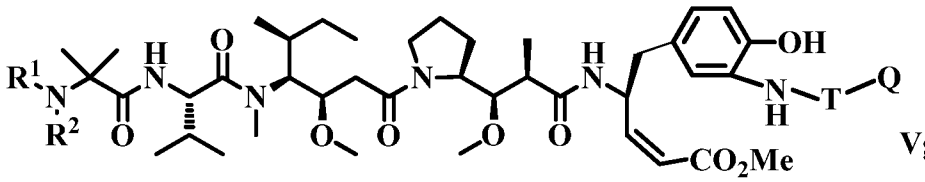
Vd



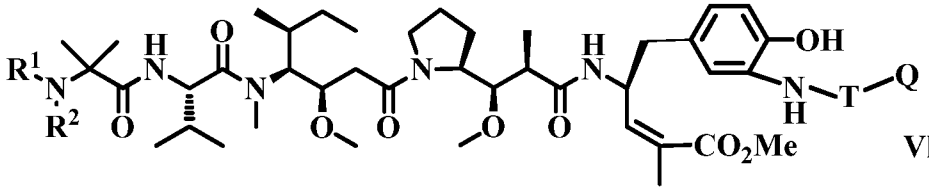
Ve



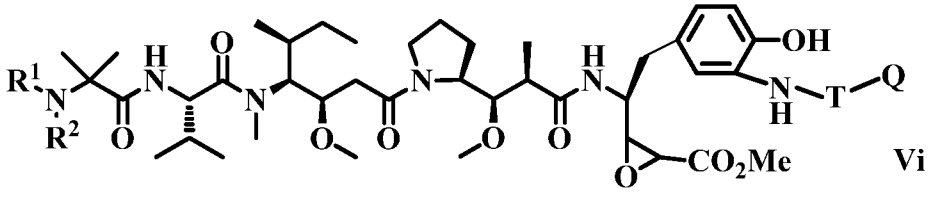
Vf



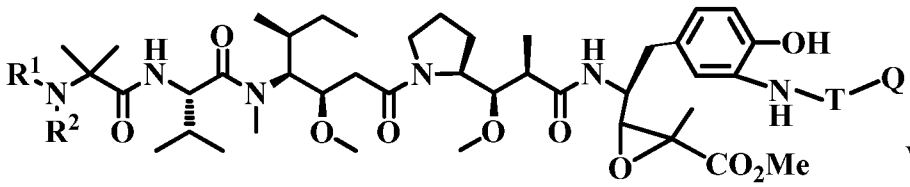
Vg



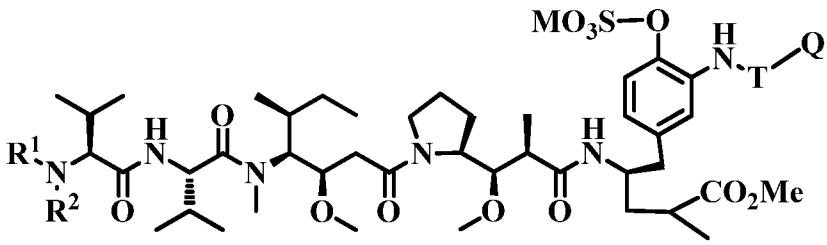
Vh



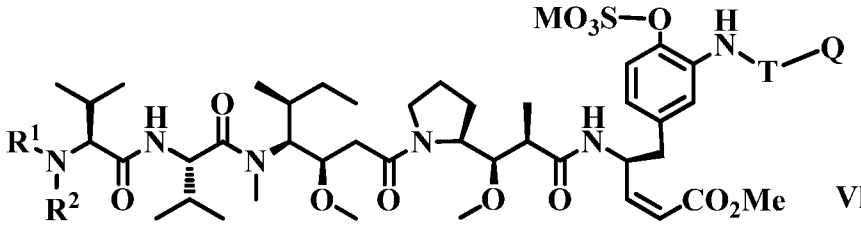
Vi



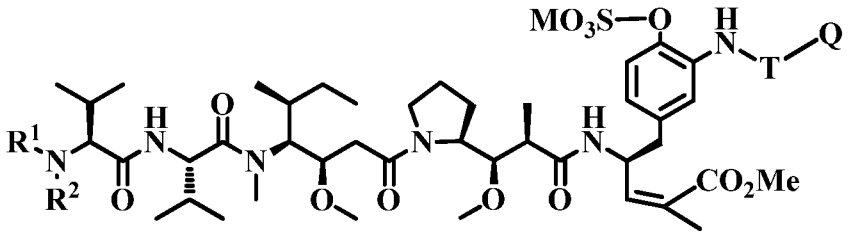
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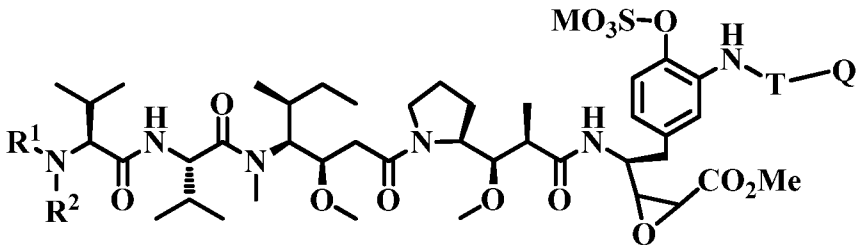
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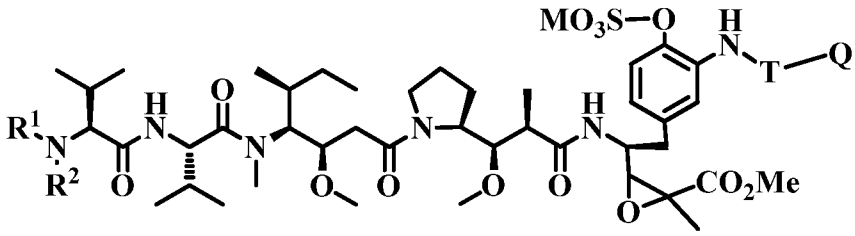
Vl



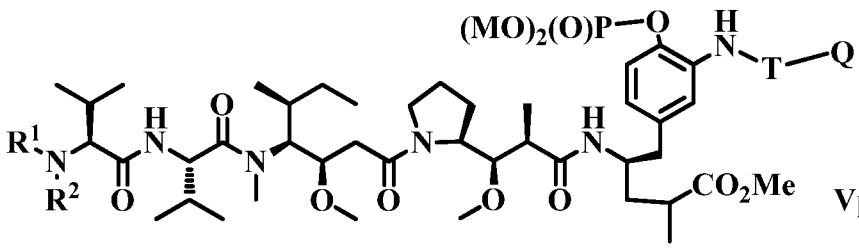
Vm



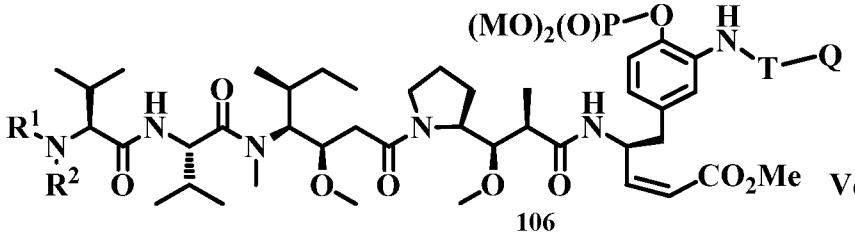
Vn



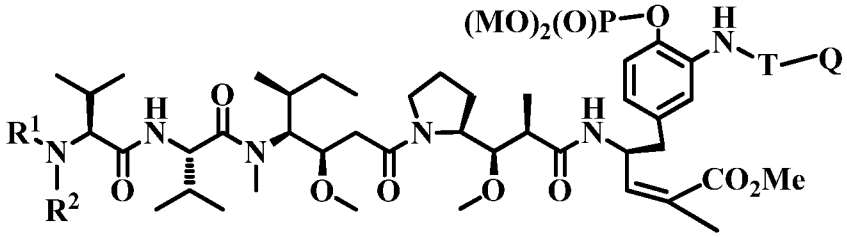
Vo



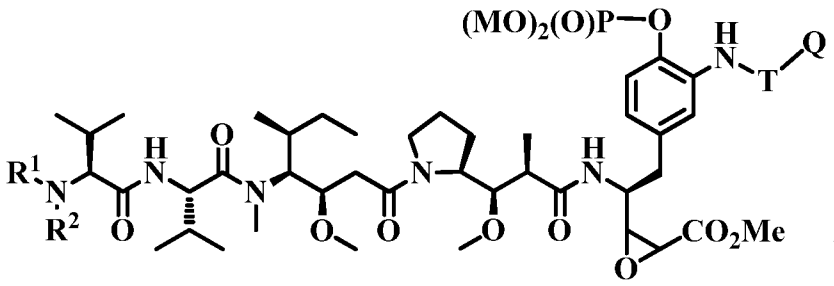
Vp



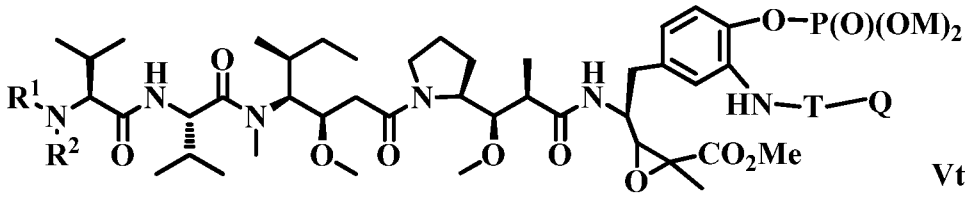
Vq



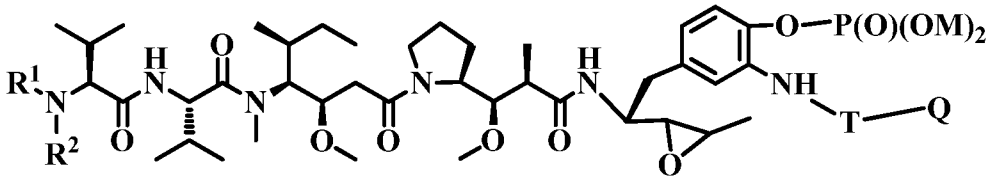
Vr



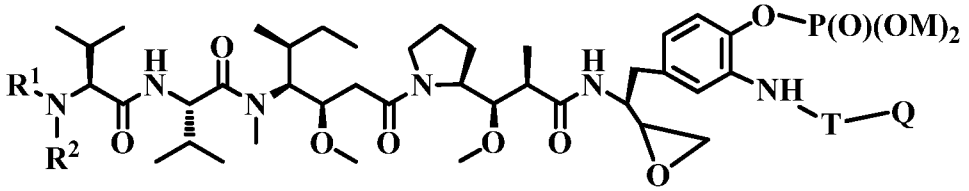
Vs



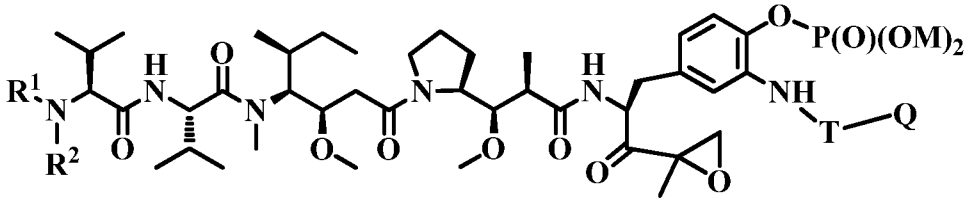
Vt



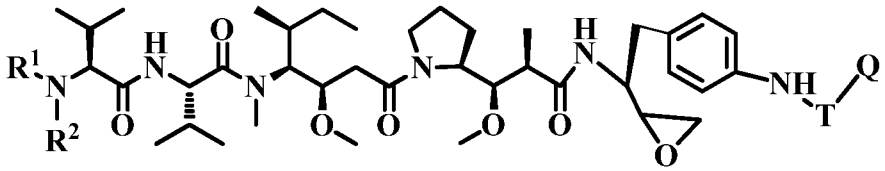
Vu



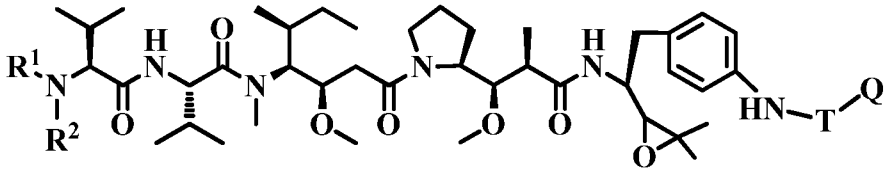
Vw



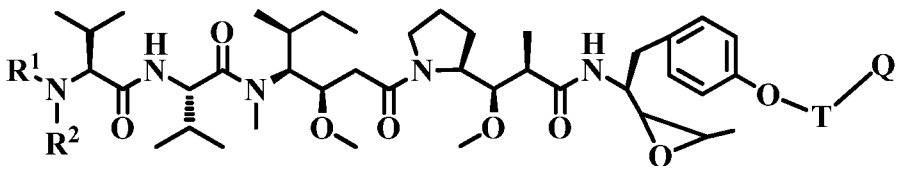
Vx



Vy



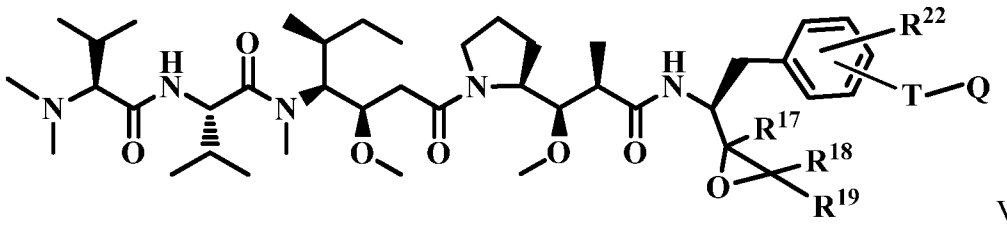
Vz



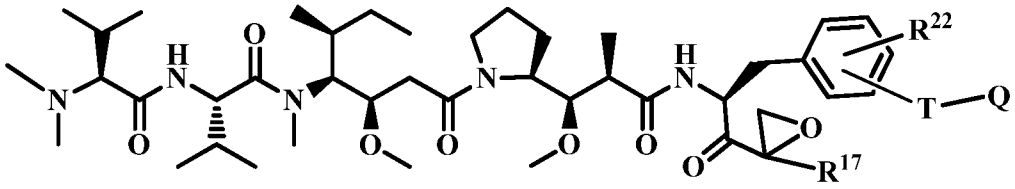
Vaa

and pharmaceutical acceptable salts and solvates thereof, wherein M is H, or Na, or K, or $N^+R^1R^2R^3$ or a pharmaceutical salt. R_1, R_2, R_3 T, Q and M are defined the same as in claim **1** and **3**.

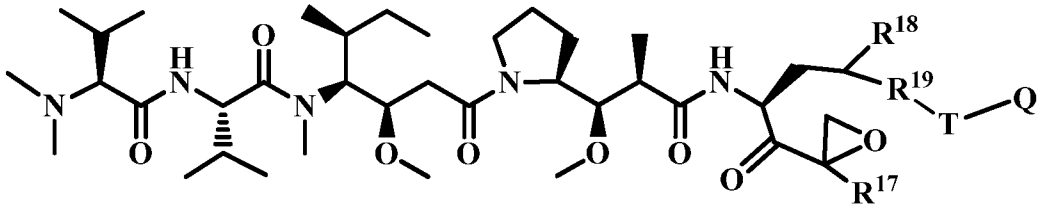
13. A conjugate of a cell binding molecule according to claim **1** bearing a linker-cytotoxic agent of Formula (V) of claim **11** having the following preferred structures:



Vbb



Vcc

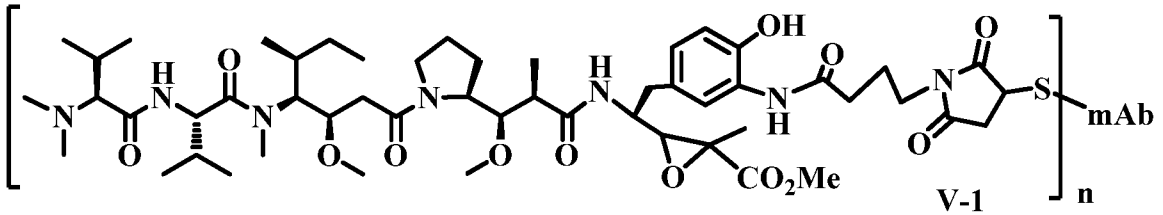


Vdd

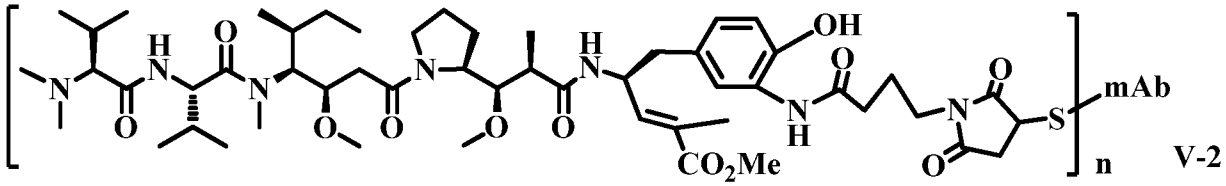
and pharmaceutical acceptable salts and solvates thereof,

wherein Q, T, R^1, R^2, R^3, R^{17}, R^{18}, R^{19} and $R^{22}$are defined the same in claims **1**, **3** and **4.** In addition, R^{19} can be absent.

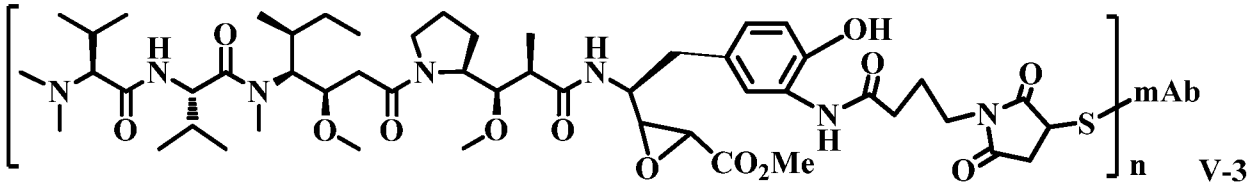
14. The conjugate according to claims **1** and **11** bearing formula (V) having the following structures:



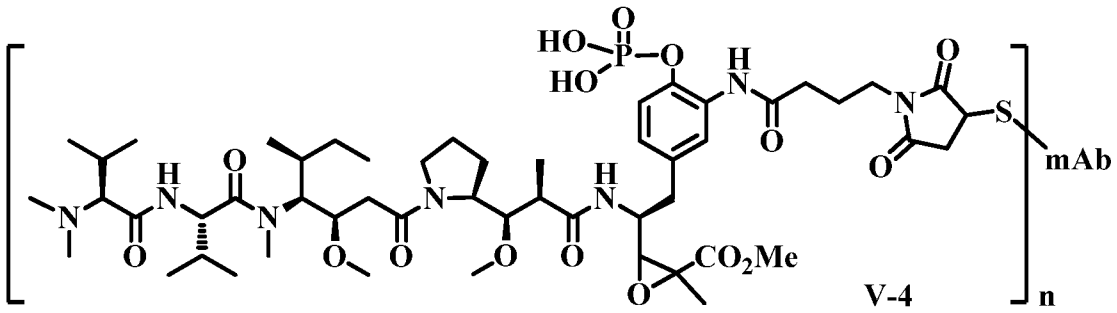
V-1



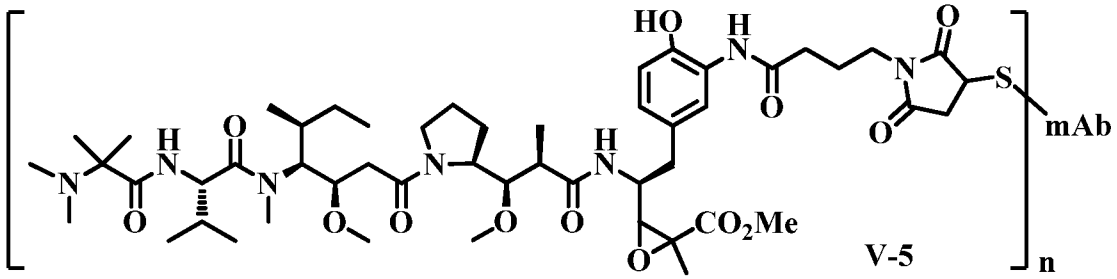
V-2



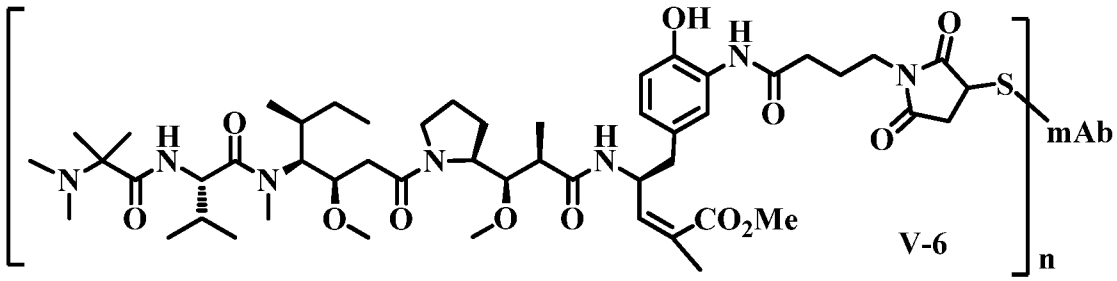
V-3



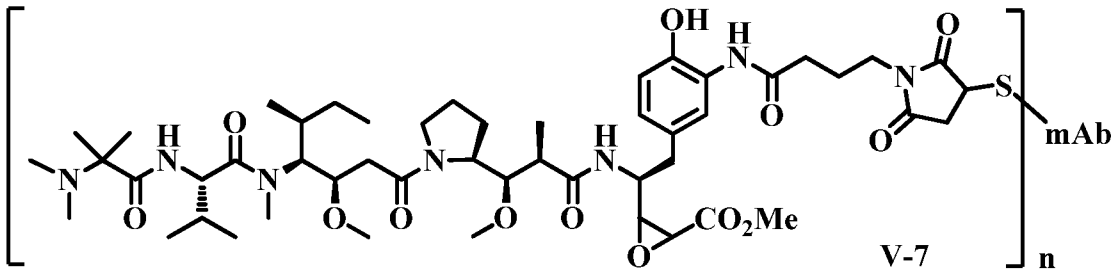
V-4



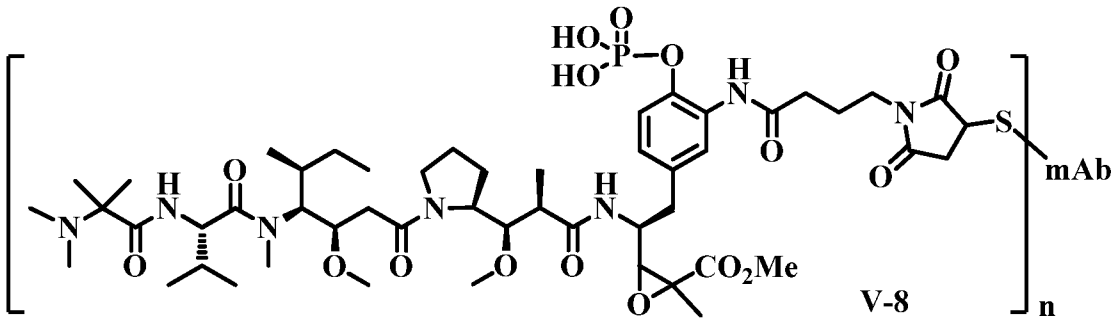
V-5



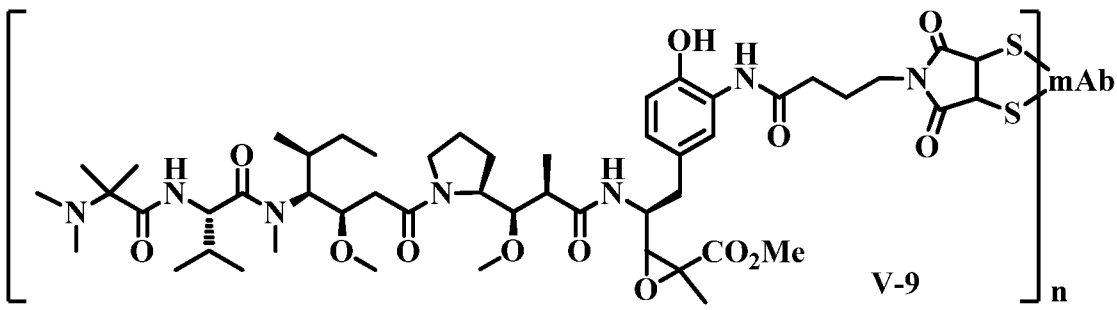
V-6



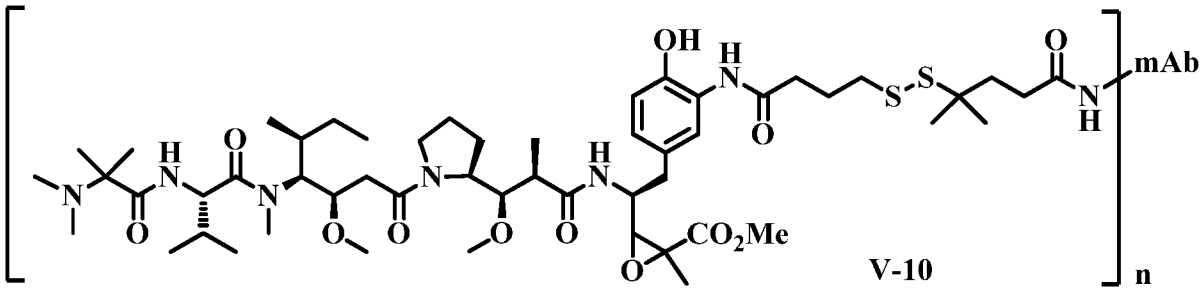
V-7



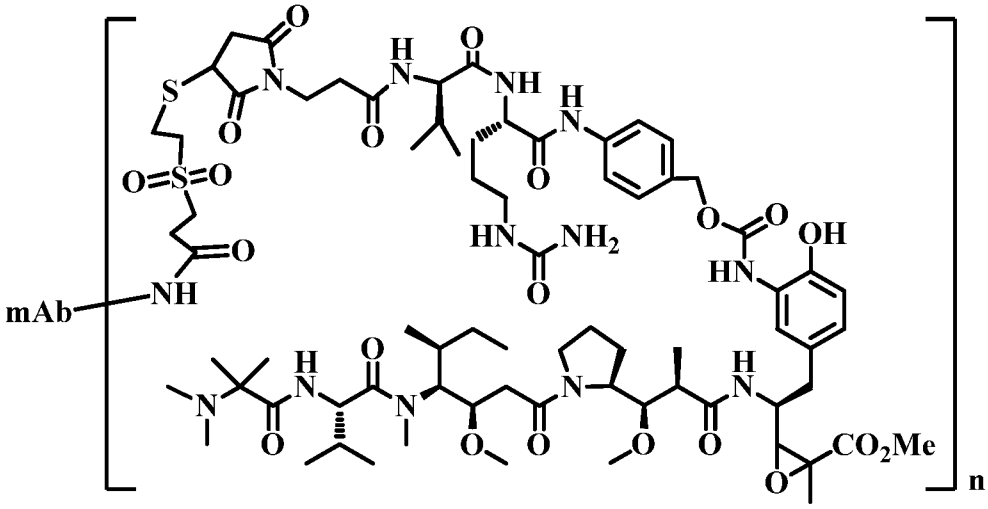
V-8



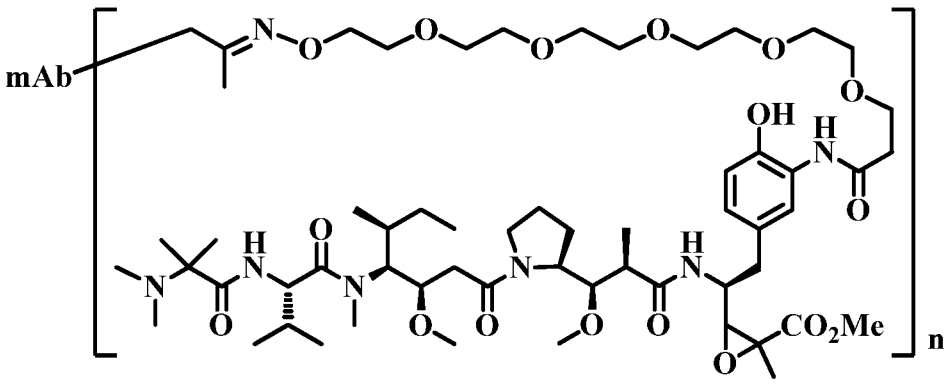
V-9



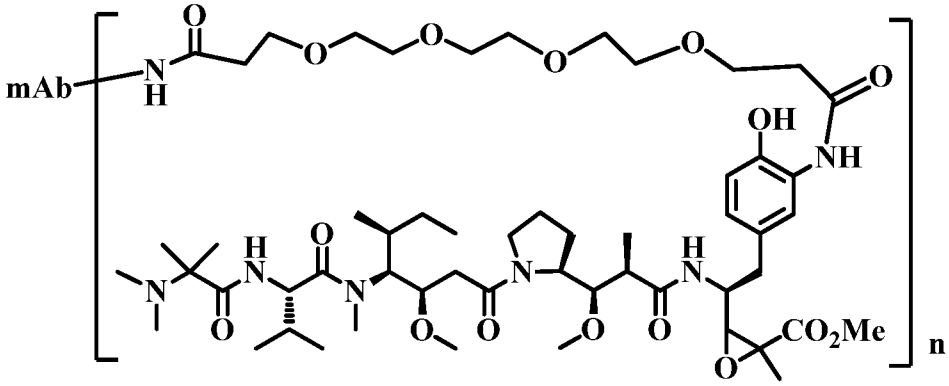
V-10



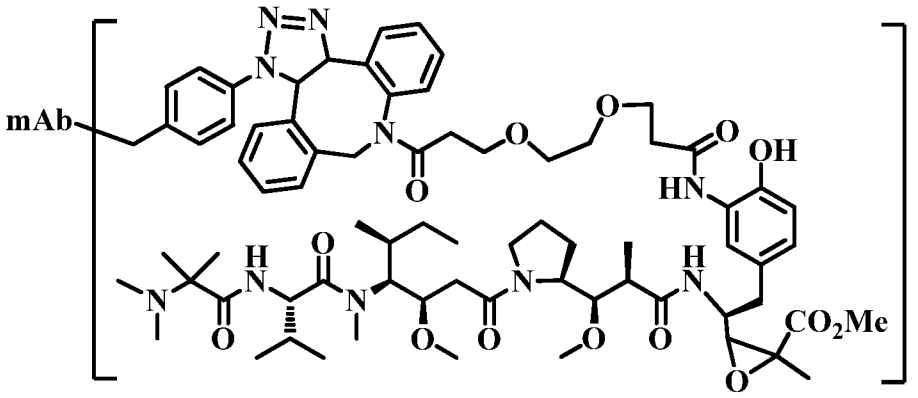
V-11



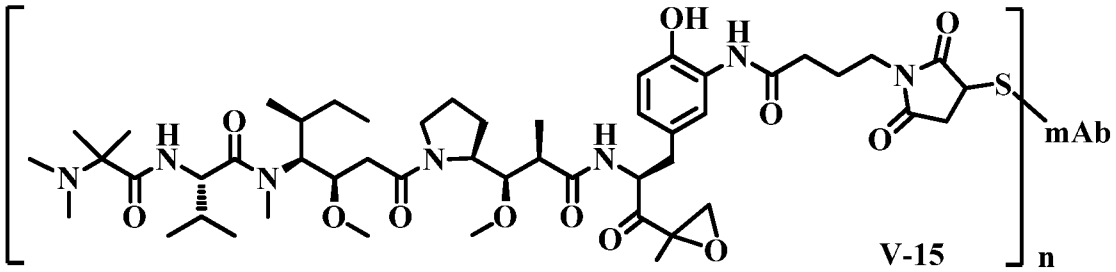
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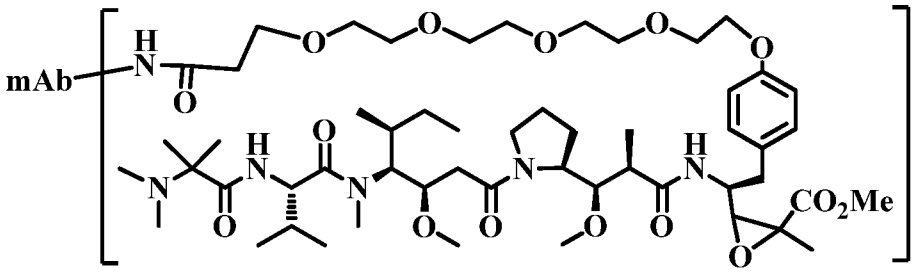
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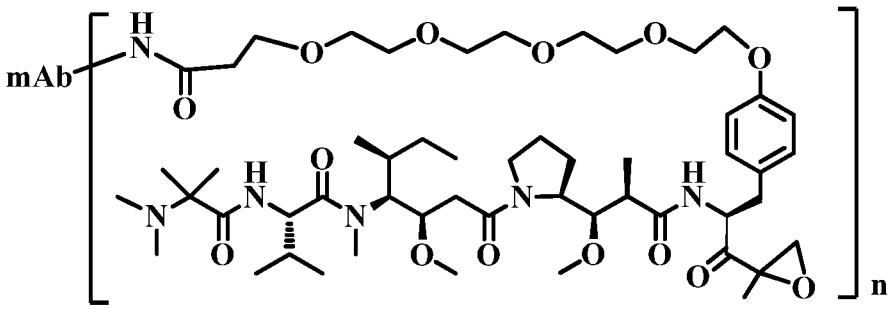
V-14



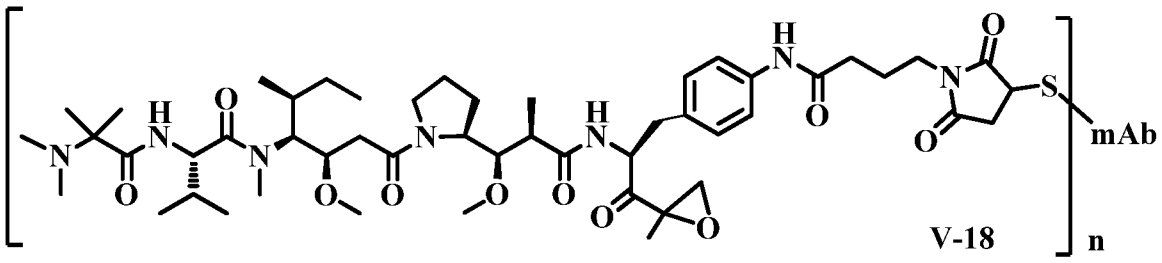
V-15



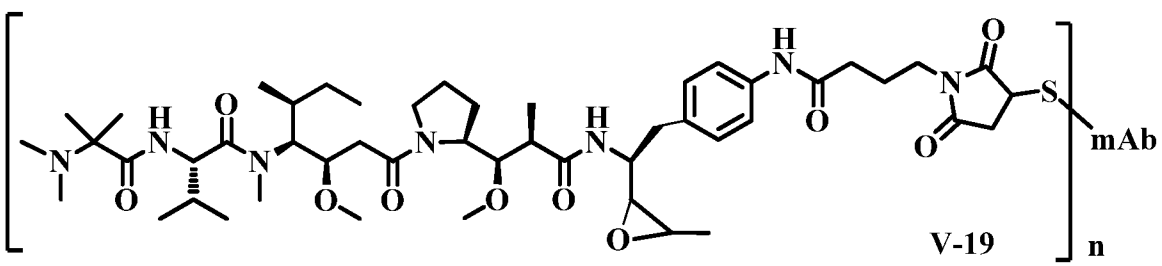
V-16



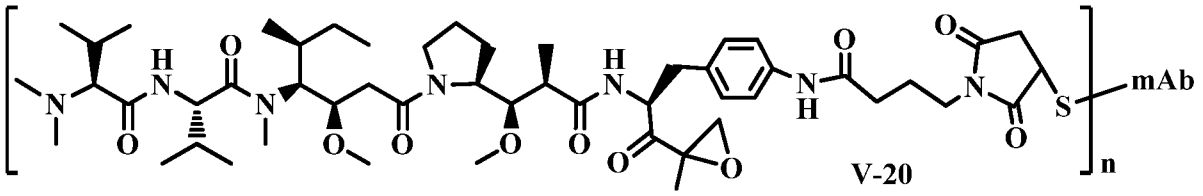
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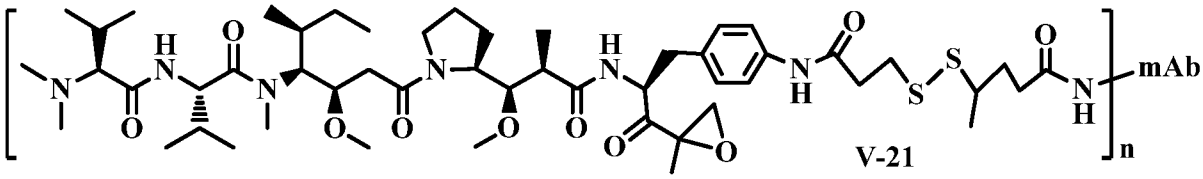
V-18



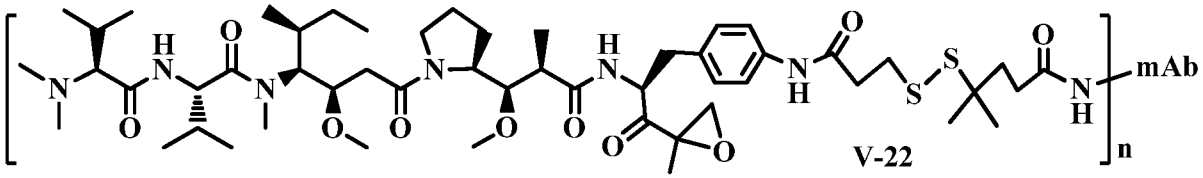
V-19



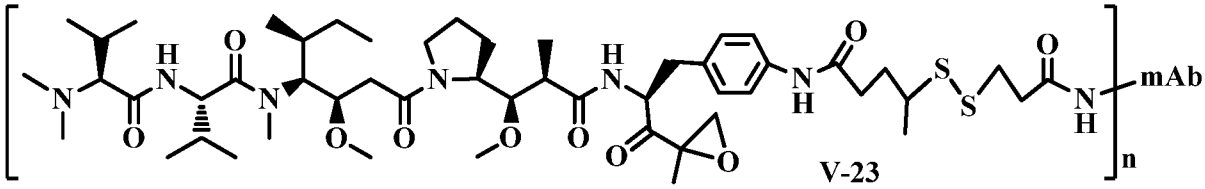
V-20



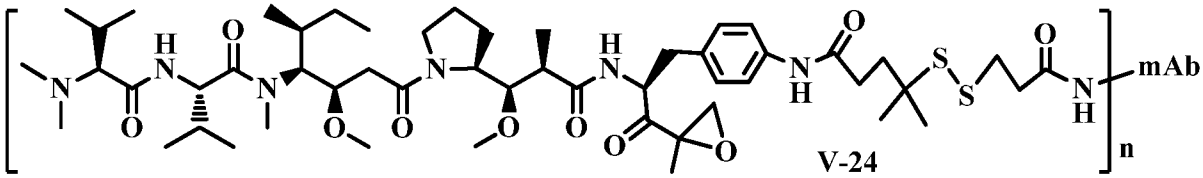
V-21



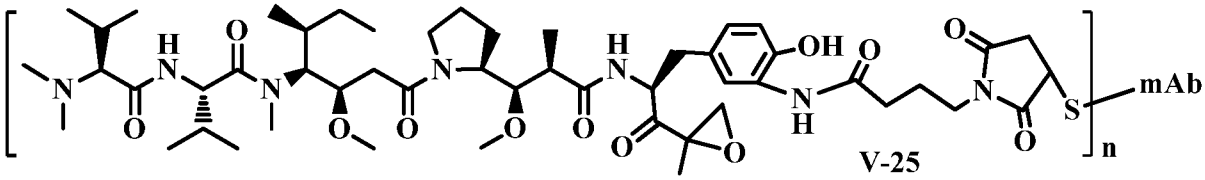
V-22



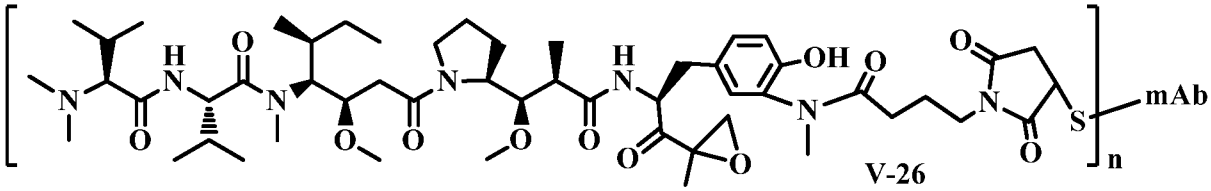
V-23



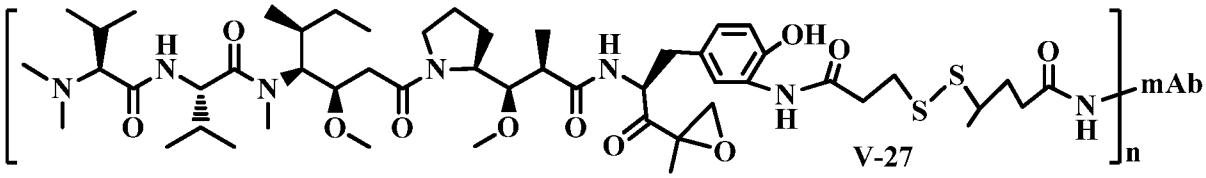
V-24



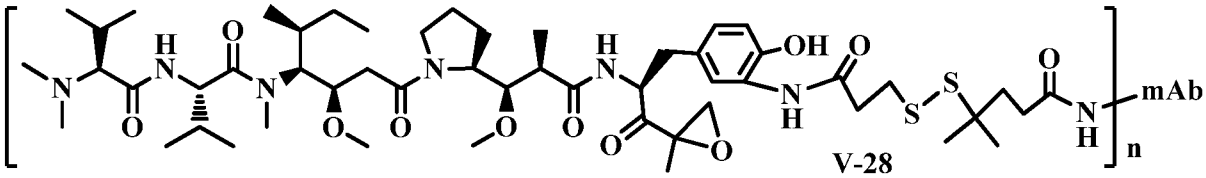
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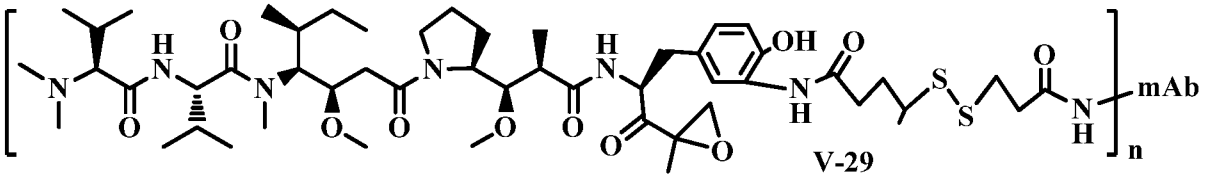
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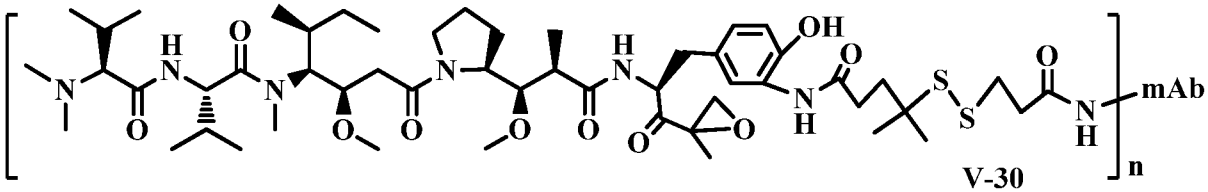
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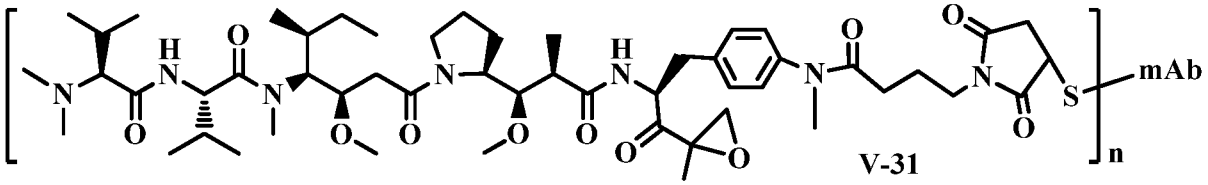
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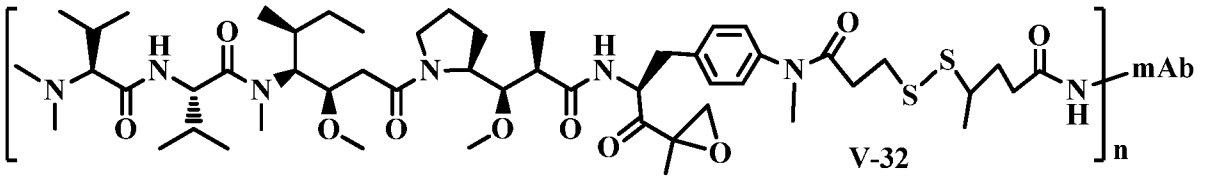
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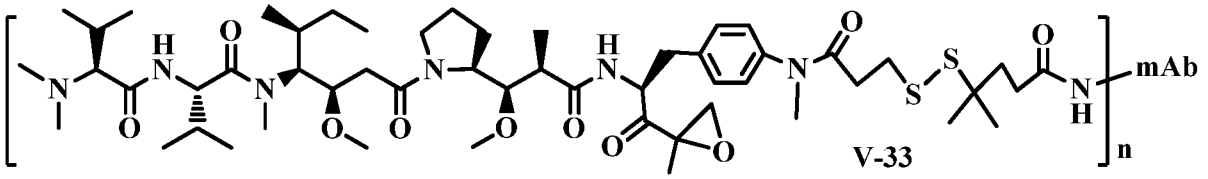
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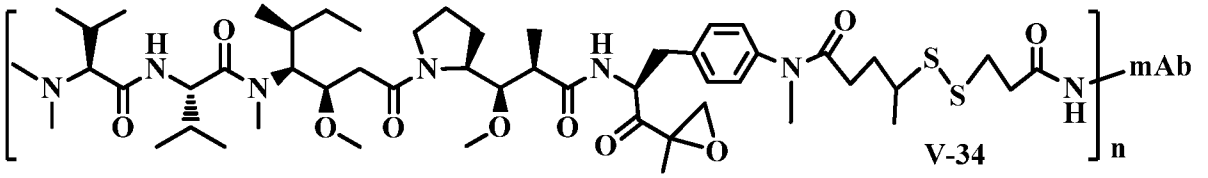
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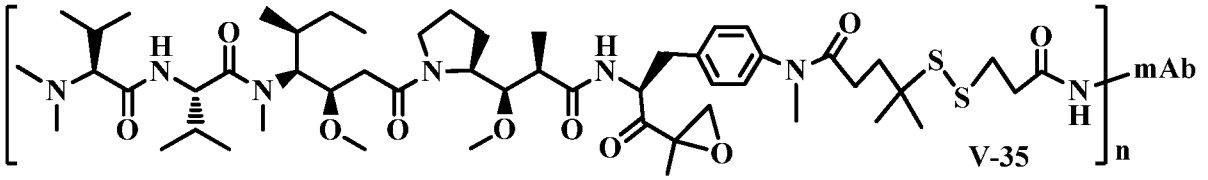
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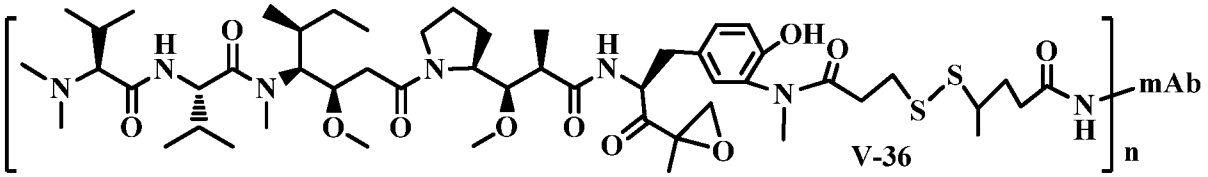
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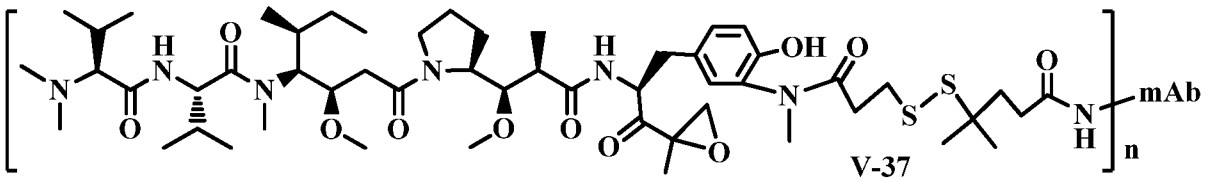
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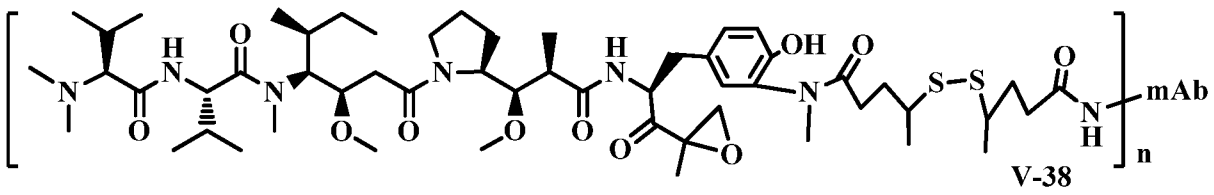
V-35



V-36



V-37



V-38

and pharmaceutical acceptable salts and solvates thereof.

Wherein mAb is an antibody and n = 1~30.

15. The cell binding molecule according to claims **1, 3, 4, 8, 9, 12** and **13** can be any agent/molecule that is able to against any one of the following antigens or receptors: CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD14, CD15, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD46, CD47, CD48, CD49b, CD49c, CD51, CD52, CD53, CD54, CD55, CD56, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD66, CD68, CD69, CD70, CD72, CD74, CD79, CD79a, CD79b, CD80, CD81, CD82, CD83, CD86, CD87, CD88, CD89, CD90, CD91, CD95, CD96, CD98, CD100, CD103, CD105, CD106, CD109, CD117, CD120, CD125, CD126, CD127, CD133, CD134, CD135, CD138, CD141, CD142, CD143, CD144, CD147, CD151, CD147, CD152, CD154, CD156, CD158, CD163, CD166, .CD168, CD174, CD180, CD184, CDw186, CD194, CD195, CD200, CD200a, CD200b, CD209, CD221, CD227, CD235a, CD240, CD262, CD271, CD274, CD276 (B7-H3), CD303, CD304, CD309, CD326, 4-1BB, 5AC, 5T4 (Trophoblast glycoprotein, TPBG, 5T4, Wnt-Activated Inhibitory Factor 1 or WAIF1), Adenocarcinoma antigen, AGS-5, AGS-22M6, Activin receptor-like kinase 1, AFP, AKAP-4, ALK, Alpha intergrin, Alpha v beta6, Amino-peptidase N, Amyloid beta, Androgen receptor, Angiopoietin 2, Angiopoietin 3, Annexin A1, Anthrax toxin protective antigen, Anti-transferrin receptor, AOC3 (VAP-1), B7-H3, Bacillus anthracis anthrax, BAFF (B-cell activating factor), B-lymphoma cell, bcr-abl, Bombesin, BORIS, C5, C242 antigen, CA125 (carbohydrate antigen 125, MUC16), CA-IX (or CAIX, carbonic anhydrase 9), CALLA, CanAg, Canis lupus familiaris IL31, Carbonic anhydrase IX, Cardiac myosin, CCL11(C-C motif chemokine 11), CCR4 (C-C chemokine receptor type 4, CD194), CCR5, CD3E (epsilon), CEA (Carcinoembryonic antigen), CEACAM3, CEACAM5 (carcinoembryonic antigen), CFD (Factor D), Ch4D5, Cholecystokinin 2 (CCK2R), CLDN18 (Claudin-18), Clumping factor A, CRIPTO, FCSF1R (Colony stimulating factor 1 receptor, CD115), CSF2 (colony stimulating factor 2, Granulocyte-macrophage colony-stimulating factor (GM-CSF)), CTLA4 (cytotoxic T-lymphocyte-associated protein 4), CTAA16.88 tumor antigen, CXCR4 (CD184), C-X-C chemokine receptor type 4, cyclic ADP ribose hydrolase, Cyclin B1, CYP1B1, Cytomegalovirus, Cytomegalovirus glycoprotein B, Dabigatran, DLL4 (delta-like-ligand 4), DPP4 (Dipeptidyl-

peptidase 4), DR5 (Death receptor 5), E. coli shiga toxin type-1, E. coli shiga toxin type-2, ED-B, EGFL7 (EGF-like domain-containing protein 7), EGFR, EGFRII, EGFRvIII, Endoglin (CD105), Endothelin B receptor, Endotoxin, EpCAM (epithelial cell adhesion molecule), EphA2, Episialin, ERBB2 (Epidermal Growth Factor Receptor 2), ERBB3, ERG (TMPRSS2 ETS fusion gene), Escherichia coli, ETV6-AML, FAP (Fibroblast activation protein alpha), FCGR1, alpha-Fetoprotein, Fibrin II, beta chain, Fibronectin extra domain-B, FOLR (folate receptor), Folate receptor alpha, Folate hydrolase, Fos-related antigen 1.F protein of respiratory syncytial virus, Frizzled receptor, Fucosyl GM1, GD2 ganglioside, G-28 (a cell surface antigen glyvolipid), GD3 idiotype, GloboH, Glypican 3, N-glycolylneuraminic acid, GM3, GMCSF receptor α-chain, Growth differentiation factor 8, GP100, GPNMB (Transmembrane glycoprotein NMB), GUCY2C (Guanylate cyclase 2C, guanylyl cyclase C(GC-C), intestinal Guanylate cyclase, Guanylate cyclase-C receptor, Heat-stable enterotoxin receptor (hSTAR)), Heat shock proteins, Hemagglutinin, Hepatitis B surface antigen, Hepatitis B virus, HER1 (human epidermal growth factor receptor 1), HER2, HER2/neu, HER3 (ERBB-3), IgG4, HGF/SF (Hepatocyte growth factor/scatter factor), HHGFR, HIV-1, Histone complex, HLA-DR (human leukocyte antigen), HLA-DR10, HLA-DRB , HMWMAA, Human chorionic gonadotropin, HNGF, Human scatter factor receptor kinase, HPV E6/E7, Hsp90, hTERT, ICAM-1 (Intercellular Adhesion Molecule 1), Idiotype, IGF1R (IGF-1, insulin-like growth factor 1 receptor), IGHE, IFN-γ, Influenza hemagglutinin, IgE, Fc region, IGHE, IL-1, IL-2 receptor (interleukin 2 receptor), IL-4, IL-5, IL-6, IL-6R (interleukin 6 receptor), IL-9, IL-10, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL31RA, ILGF2 (Insulin-like growth factor 2), Integrins (α4, $\alpha_{IIb}\beta_3$, αvβ3, $\alpha_4\beta_7$, α5β1, α6β4, α7β7, αIIβ3, α5β5, αvβ5), Interferon gamma-induced protein, ITGA2, ITGB2, KIR2D, LCK, Le, Legumain, Lewis-Y antigen, LFA-1(Lymphocyte function-associated antigen 1, CD11a), LHRH, LINGO-1, Lipoteichoic acid, LIV1A, LMP2, LTA, MAD-CT-1, MAD-CT-2, MAGE-1, MAGE-2, MAGE-3, MAGE A1, MAGE A3, MAGE 4, MART1, MCP-1, MIF (Macrophage migration inhibitory factor, or glycosylation-inhibiting factor (GIF)), MS4A1 (membrane-spanning 4-domains subfamily A member 1), MSLN (mesothelin), MUC1(Mucin 1, cell surface associated (MUC1) or polymorphic epithelial mucin (PEM)), MUC1-KLH, MUC16 (CA125), MCP1(monocyte chemotactic protein 1), MelanA/MART1, ML-IAP, MPG, MS4A1 (membrane-spanning 4-domains subfamily A), MYCN, Myelin-associated glycoprotein, Myostatin, NA17, NARP-1, NCA-90 (granulocyte antigen), Nectin-4 (ASG-22ME), NGF, Neural apoptosis-regulated proteinase 1, NOGO-A, Notch receptor, Nucleolin, Neu oncogene product, NY-BR-1, NY-ESO-1, OX-40, OxLDL (Oxidized low-density lipoprotein), OY-TES1, P21, p53 nonmutant, P97, Page4, PAP, Paratope of anti-(N-glycolylneuraminic acid), PAX3, PAX5, PCSK9, PDCD1 (PD-1, Programmed cell death protein 1,CD279), PDGF-Rα (Alpha-

type platelet-derived growth factor receptor), PDGFR-β, PDL-1, PLAC1, PLAP-like testicular alkaline phosphatase, Platelet-derived growth factor receptor beta, Phosphate-sodium co-transporter, PMEL 17, Polysialic acid, Proteinase3 (PR1), Prostatic carcinoma, PS (Phosphatidylserine), Prostatic carcinoma cells, Pseudomonas aeruginosa, PSMA, PSA, PSCA, Rabies virus glycoprotein, RHD (Rh polypeptide 1 (RhPI), CD240), Rhesus factor, RANKL, RhoC, Ras mutant, RGS5, ROBO4, Respiratory syncytial virus, RON, Sarcoma translocation breakpoints, SART3, Sclerostin, SLAMF7 (SLAM family member 7), Selectin P, SDC1 (Syndecan 1), sLe(a), Somatomedin C, SIP (Sphingosine-1-phosphate), Somatostatin, Sperm protein 17, SSX2, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), STEAP2, STn, TAG-72 (tumor associated glycoprotein 72), Survivin, T-cell receptor, T cell transmembrane protein, TEM1 (Tumor endothelial marker 1), TENB2, Tenascin C (TN-C), TGF-α, TGF-β (Transforming growth factor beta), TGF-β1, TGF-β2 (Transforming growth factor-beta 2), Tie (CD202b), Tie2, TIM-1 (CDX-014), Tn, TNF, TNF-α, TNFRSF8, TNFRSF10B (tumor necrosis factor receptor superfamily member 10B), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B), TPBG (trophoblast glycoprotein), TRAIL-R1 (Tumor necrosis apoprosis Inducing ligand Receptor 1), TRAILR2 (Death receptor 5 (DR5)), tumor-associated calcium signal transducer 2, tumor specific glycosylation of MUC1, TWEAK receptor, TYRP1(glycoprotein 75), TRP-2, Tyrosinase, VCAM-1 (CD106), VEGF, VEGF-A, VEGF-2 (CD309), VEGFR-1, VEGFR2, or vimentin, WT1, XAGE 1, or cells expressing any insulin growth factor receptors, or any epidermal growth factor receptors.

16. The cell binding molecule according to claims **1, 3, 4, 8, 9, 12** and **13** is preferred a full-length antibody (polyclonal and monoclonal antibody); a single chain antibody; diabody, tribody, an antibody fragment (e.g. Fab, Fab', $F(ab')_2$, F_v, a fragment produced by a Fab expression library) that binds to a target cell; a monoclonal antibody; a single chain monoclonal antibody; or a monoclonal antibody fragment that binds the target cell; a chimeric antibody; a chimeric antibody fragment that binds to the target cell; a domain antibody; a domain antibody fragment that binds to the target cell; a resurfaced antibody; a resurfaced single chain antibody; or a resurfaced antibody fragment that binds to the target cell; a humanized antibody or a resurfaced antibody; a humanized single chain antibody; a humanized antibody fragment that binds to the target cell; an anti-idiotypic (anti-Id) antibody; a lymphokine (e.g. IL-2, IL-3, IL-4, IL-5, IL-6, IL-10); a hormone [e.g. insulin, TRH (thyrotropin releasing hormone), MSH (melanocyte-stimulating hormone), a steroid hormone, e.g. androgens and estrogens, melanocyte-stimulating hormone (MSH)]; an interferon (e.g, IFN-γ); a vitamin (e.g. folate); a growth factor and a colony stimulating factor [e.g. an epidermal growth factors (EGF), a granulocyte-macrophage colony-stimulating factor (GM-CSF), a transforming growth factors (TGF), e.g.

TGFα, TGFβ; a insulin and insulin like growth factor (IGF-I, IGF-II) G-CSF, M-CSF and GM-CSF; a vaccinia growth factor (VGF), a fibroblast growth factor (FGFs), a platelet-derived growth factor]; a nutrient-transport molecule; an interleukin and a cytokine [e.g. interleukin-2 (IL-2), interleukin-6 (IL-6)]; peptides and peptide hormones (e.g. bombesin, gastrin, gastrin-releasing peptide); an apoprotein and a lycoproteis (e.g.a transferrin); a sugar-binding protein or a lipoproteins (e.g. a lectin); a cell nutrient-transport molecule (e.g. transferrin); a small molecular inhibitor [e.g. a prostate-specific membrane antigen (PSMA)]; small molecular tyrosine kinase inhibitors (TKI); a conjugated proteins that are able to bind targeted cells; a cell binding molecule attached on a polymer, a dendrimer, a nanoparticle, a liposome, or a viral capside; or a combination of above compounds thereof.

17. The conjugate of the cytotoxic agents according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 is used in a pharmaceutical composition to target abnormal cell, in particular, tumor cells; virus infected cells; microorganism infected cells; parasite infected cells; autoimmune cells; activated cells; myeloid cells; activated T-cells; B cells; melanocytes; proliferative disorders; benign or malignant tumors; leukemia and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, angiogenic and immunologic disorders; inflammatory; autoimmune disorders; destructive disorders; bone disorders; infectious diseases; viral diseases; fibrotic diseases; neurodegenerative disorders; and/or pancreatitis or kidney diseases in a mammal.

18. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate of the cytotoxic agents according to claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 or its pharmaceutically acceptable salt, and carrier, diluent, or excipient thereof, or a combination of them thereof, for the treatment or prevention of cancer, autoimmune disease, or infectious disease.

19. A pharmaceutical composition comprising a therapeutically effective amount of the conjugate of the cytotoxic agents according to claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 13 and/or 14 administered concurrently with the other therapeutic agents such as chemotherapeutic agent, radiation therapy, immunotherapy agents, anti-autoimmune disorder agents, anti-infectious agents or other antibody-drug conjugates for synergistically treatment or prevention of a cancer, an autoimmune disease, or an infectious disease.

20. The tumor cell according to claim **17** is selected from lymphoma cells, myeloma cells, renal cells, breast cancer cells, prostate cancer cells, ovarian cancer cells, colorectal cancer cells, gastric cancer cells, squamous cancer cells, small-cell lung cancer cells, none small-cell lung cancer cells, testicular cancer cells, or any cells that grow and divide at an unregulated, quickened pace to cause cancers.

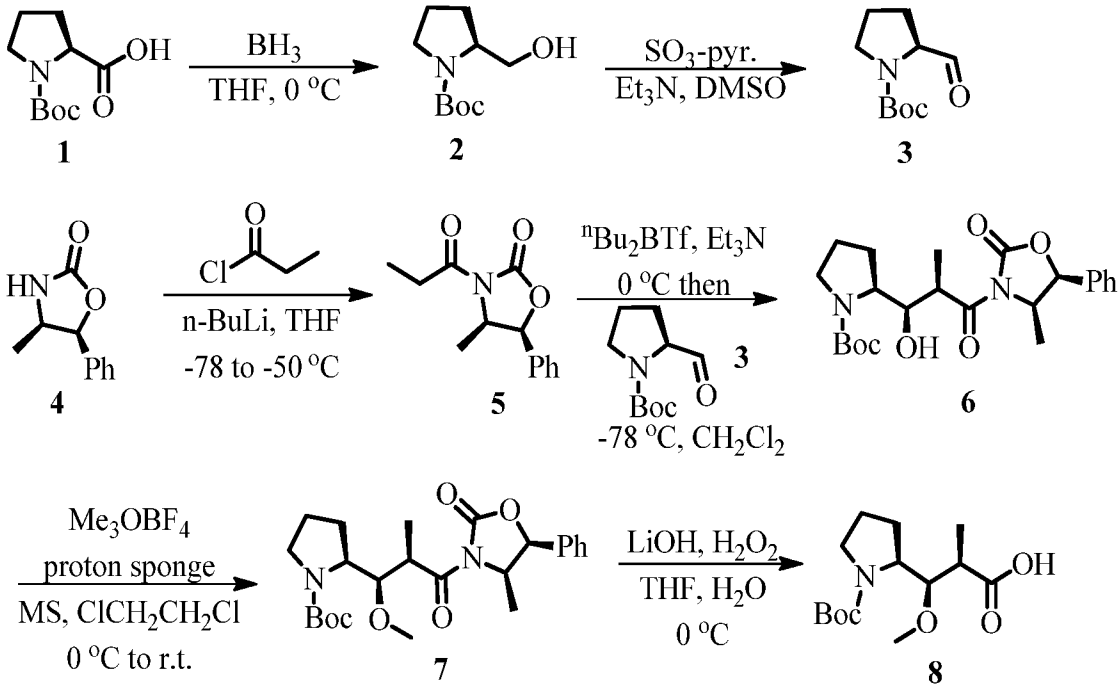
21. The conjugate of claims **1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13** and **14**, having in vitro, in vivo or ex vivo cell killing activity.

22. The linkage components L and T according to claims **1**, **2**, **3, 4, 7, 8, 9, 11, 12** and **13** can comprise either a peptides of 1~20 units of natural or unnatural amino acids, or a p-aminobenzyl unit, or a 6-maleimidocaproyl unit, or a disulfide unit, or a thioether unit, or a hydrozone unit, a triazole unit, or an alkoxime unit, and can be conditionally cleavable by a protease.

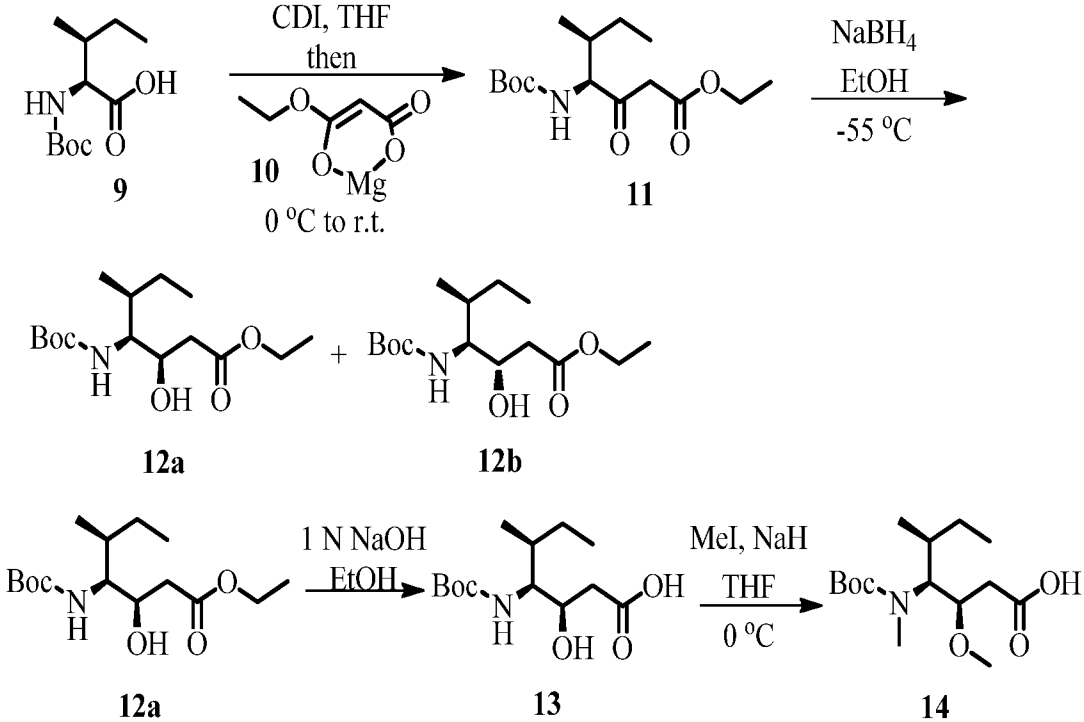
23. The synergistic agents according to claim **19** are preferably selected from one or several of the following drugs: Abatacept (Orencia), Abiraterone acetate (Zytiga®), Acetaminophen /hydrocodone, Adalimumab, afatinib dimaleate (Gilotrif®), alemtuzumab (Campath®), Alitretinoin (Panretin®), ado-trastuzumab emtansine (Kadcyla™), Amphetamine mixed salts (Amphetamine/dextroamphetamine, or Adderall XR), anastrozole (Arimidex®), Aripiprazole, Atazanavir, Atezolizumab (MPDL3280A), Atorvastatin, axitinib (Inlyta®), belinostat (Beleodaq™), Bevacizumab (Avastin®), Cabazitaxel (Jevtana®), Cabozantinib (Cometriq™), bexarotene (Targretin®), blinatumomab (Blincyto™), Bortezomib (Velcade®), bosutinib (Bosulif®), brentuximab vedotin (Adcetris®), Budesonide, Budesonide/formoterol, Buprenorphine, Capecitabine, carfilzomib (Kyprolis®), Celecoxib, ceritinib (LDK378/Zykadia), Cetuximab (Erbitux®), Ciclosporin, Cinacalcet, crizotinib (Xalkori®), Dabigatran, dabrafenib (Tafinlar®), Darbepoetin alfa, Darunavir, imatinib mesylate (Gleevec®), dasatinib (Sprycel®), denileukin diftitox (Ontak®), Denosumab (Xgeva®), Depakote, Dexlansoprazole, Dexmethylphenidate, Dinutuximab (Unituxin™), Doxycycline, Duloxetine, Emtricitabine/Rilpivirine/Tenofovir disoproxil fumarate, Emtricitabine/tenofovir/efavirenz, Enoxaparin, Enzalutamide (Xtandi®), Epoetin alfa, erlotinib (Tarceva®), Esomeprazole, Eszopiclone, Etanercept, Everolimus (Afinitor®), exemestane (Aromasin®), everolimus (Afinitor®), Ezetimibe, Ezetimibe/simvastatin, Fenofibrate, Filgrastim, fingolimod, Fluticasone propionate, Fluticasone/salmeterol, fulvestrant (Faslodex®), gefitinib (Iressa®), Glatiramer, Goserelin acetate (Zoladex), Imatinib (Gleevec), Ibritumomab tiuxetan (Zevalin®), ibrutinib (Imbruvica™), idelalisib (Zydelig®), Infliximab, Insulin aspart, Insulin detemir, Insulin glargine, Insulin lispro, Interferon beta 1a, Interferon beta 1b, lapatinib (Tykerb®), Ipilimumab (Yervoy®), Ipratropium bromide/salbutamol, Lanreotide acetate (Somatuline® Depot), lenaliomide (Revlimid®), lenvatinib mesylate (Lenvima™), letrozole (Femara®), Levothyroxine, Levothyroxine, Lidocaine, Linezolid, Liraglutide, Lisdexamfetamine, MEDI4736 (AstraZeneca, Celgene), Memantine, Methylphenidate, Metoprolol, Modafinil, Mometasone, Nilotinib (Tasigna®), Nivolumab (Opdivo®), ofatumumab (Arzerra®), obinutuzumab (Gazyva™), olaparib (Lynparza™), Olmesartan, Olmesartan/hydrochlorothiazide, Omalizumab, Omega-3 fatty acid ethyl esters, Oseltamivir, Oxycodone, palbociclib (Ibrance®),

Palivizumab, panitumumab (Vectibix®), panobinostat (Farydak®), pazopanib (Votrient®), pembrolizumab (Keytruda®), Pemetrexed (Alimta), pertuzumab (Perjeta™), Pneumococcal conjugate vaccine, pomalidomide (Pomalyst®), Pregabalin, Quetiapine, Rabeprazole, radium 223 chloride (Xofigo®), Raloxifene, Raltegravir, ramucirumab (Cyramza®), Ranibizumab, regorafenib (Stivarga®), Rituximab (Rituxan®), Rivaroxaban, romidepsin (Istodax®), Rosuvastatin, ruxolitinib phosphate (Jakafi™), Salbutamol, Sevelamer, Sildenafil, siltuximab (Sylvant™), Sitagliptin, Sitagliptin/metformin, Solifenacin, Sorafenib (Nexavar®), Sunitinib (Sutent®),Tadalafil, tamoxifen, Telaprevir, temsirolimus (Torisel®), Tenofovir/emtricitabine, Testosterone gel, Thalidomide (Immunoprin, Talidex), Tiotropium bromide, toremifene (Fareston®), trametinib (Mekinist®), Trastuzumab, Tretinoin (Vesanoid®), Ustekinumab, Valsartan, vandetanib (Caprelsa®), vemurafenib (Zelboraf®), vorinostat (Zolinza®), ziv-aflibercept (Zaltrap®), Zostavax., and their analogs, derivatives, pharmaceutically acceptable salts, carriers, diluents, or excipients therefore, or a combination above thereof.

Schemes

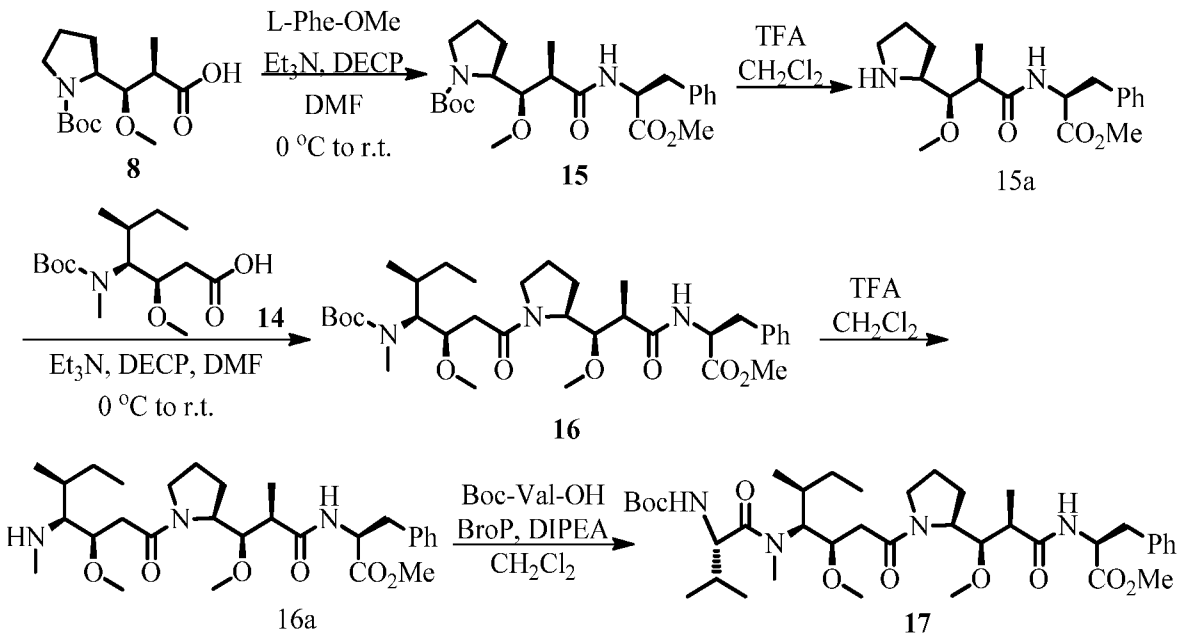


Scheme 1. The synthesis of Dap unit ((2*R*,3*R*,4*S*)-dolaproine) of MMAF.

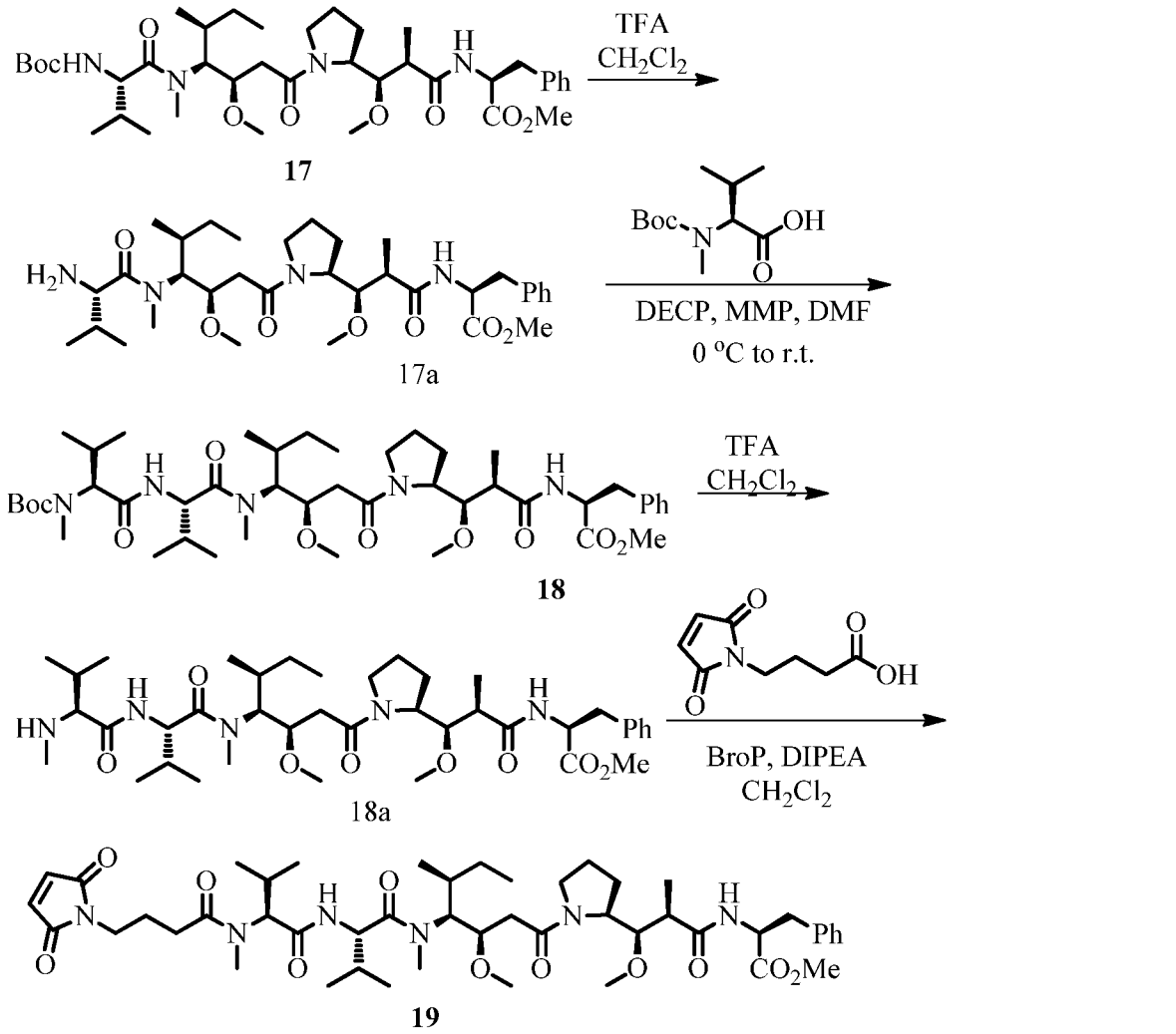


Scheme 2. The synthesis of Dil unit ((3*R*,4*S*,5*S*)-dolaisoleucine) of MMAF.

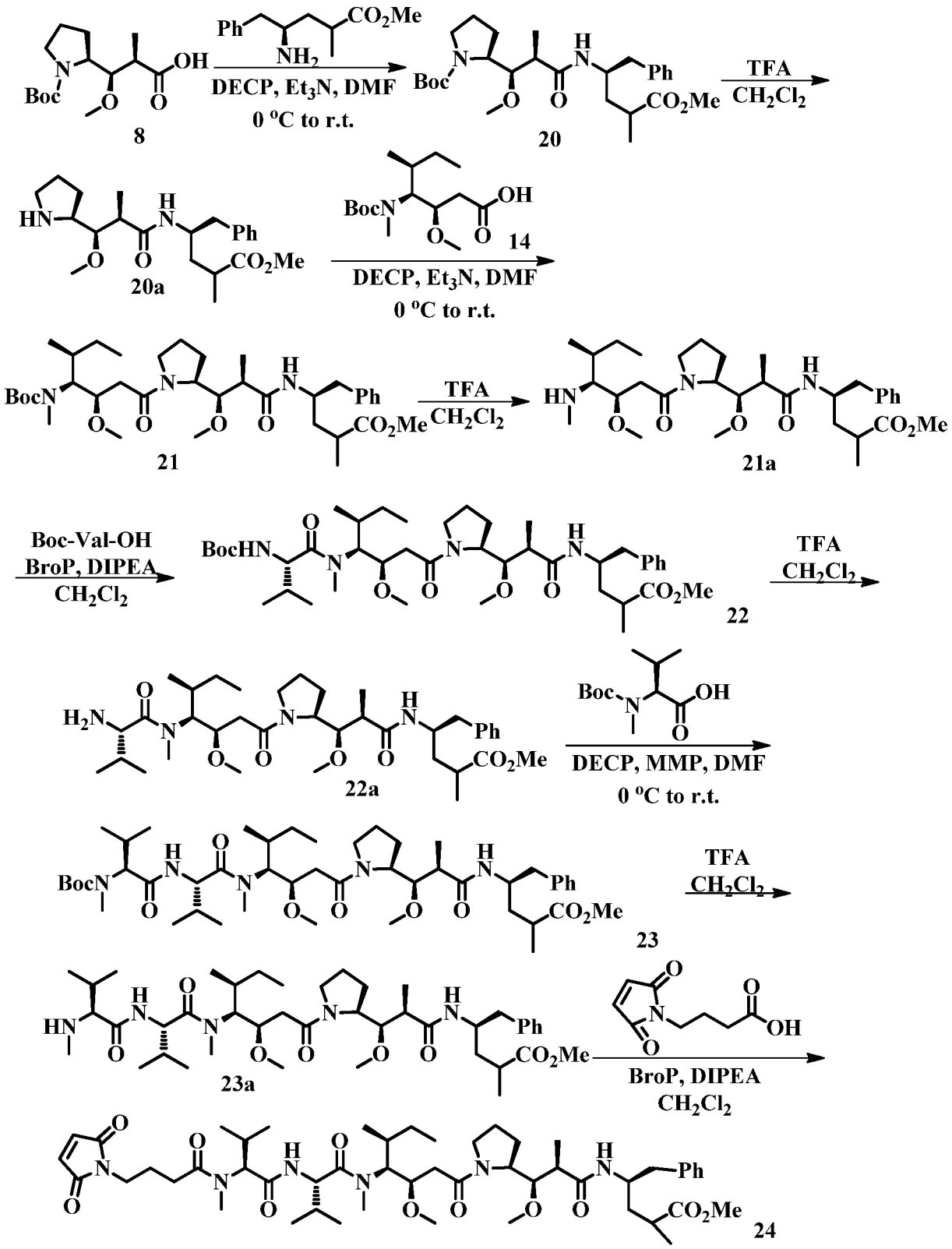
Scheme 3a



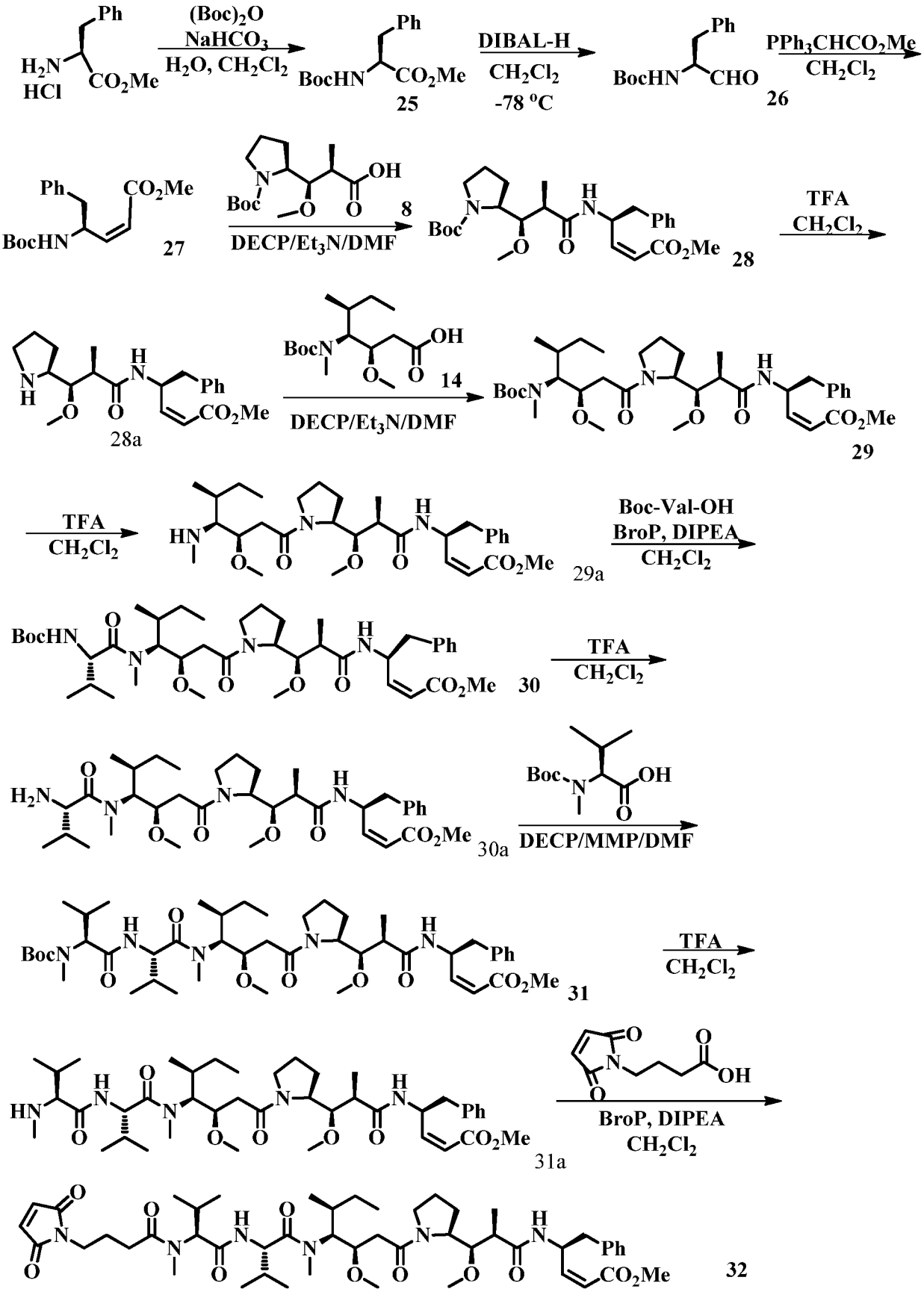
Scheme 3b



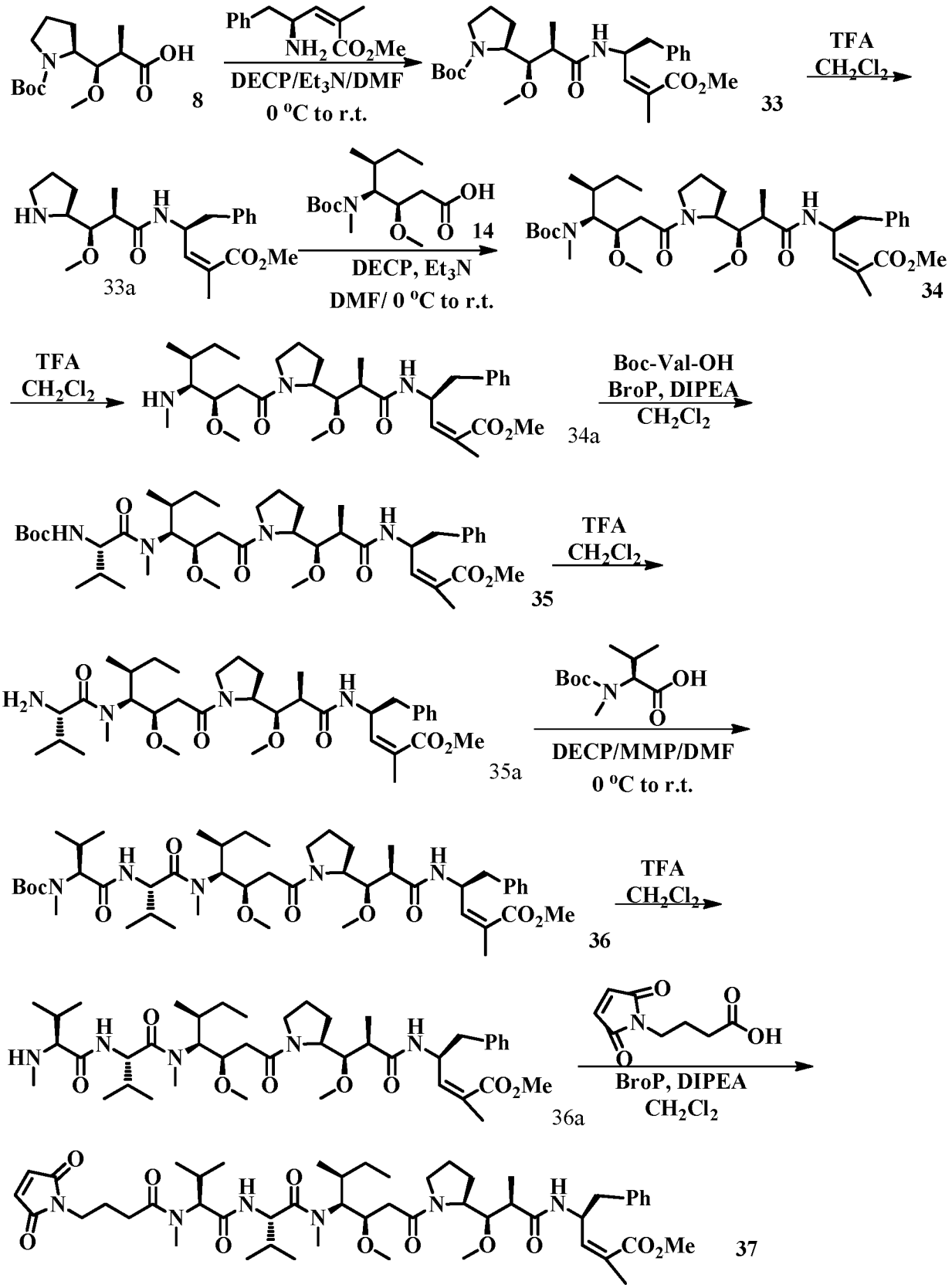
Scheme 3. The synthesis of a Drug-Linker compound that contains a maleimide unit, linking to MMAF-OMe.



Scheme 4. The synthesis of a Drug-Linker compound that contains a maleimide unit, linking to MMAF-OMe.

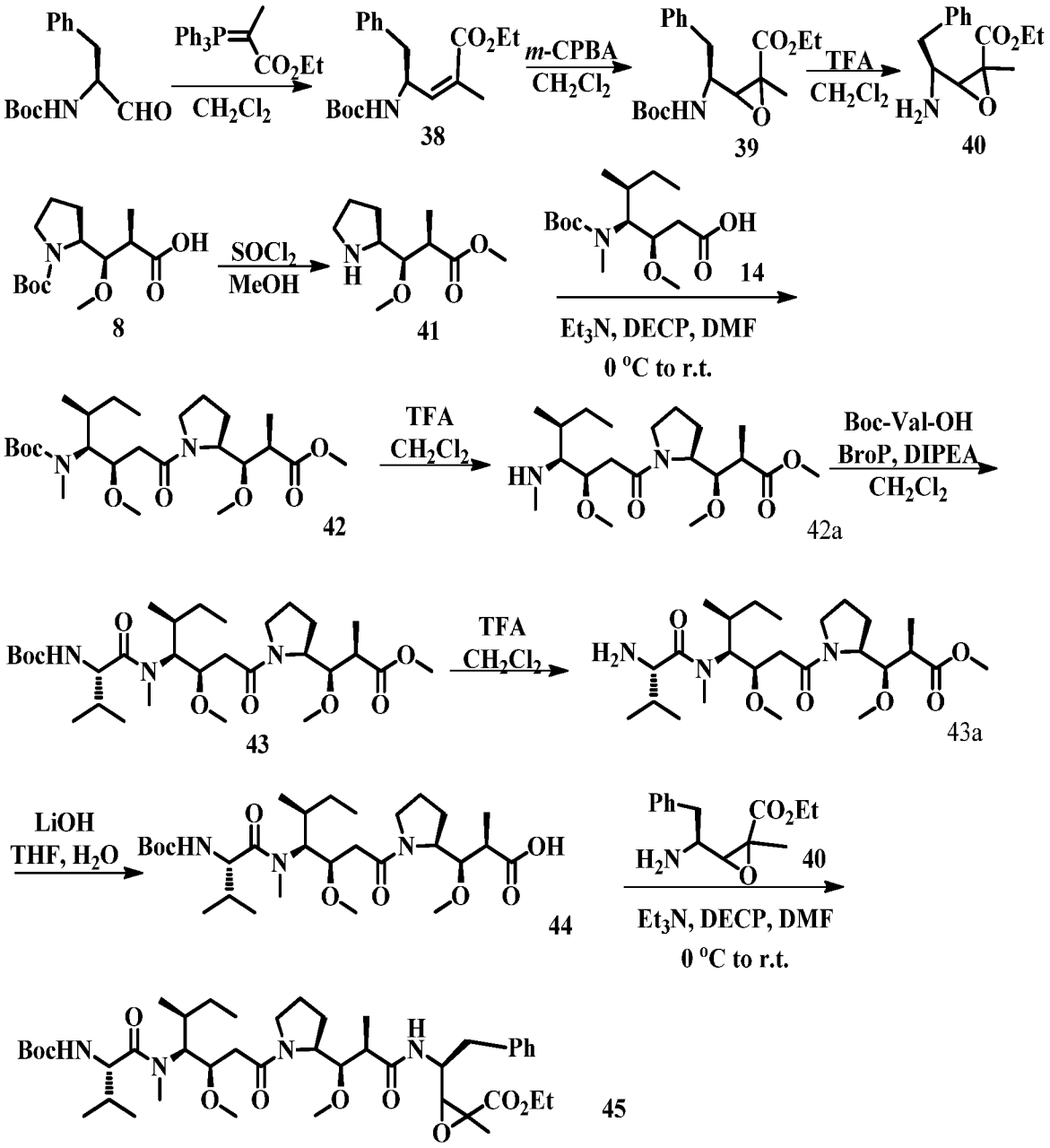


Scheme 5. The synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

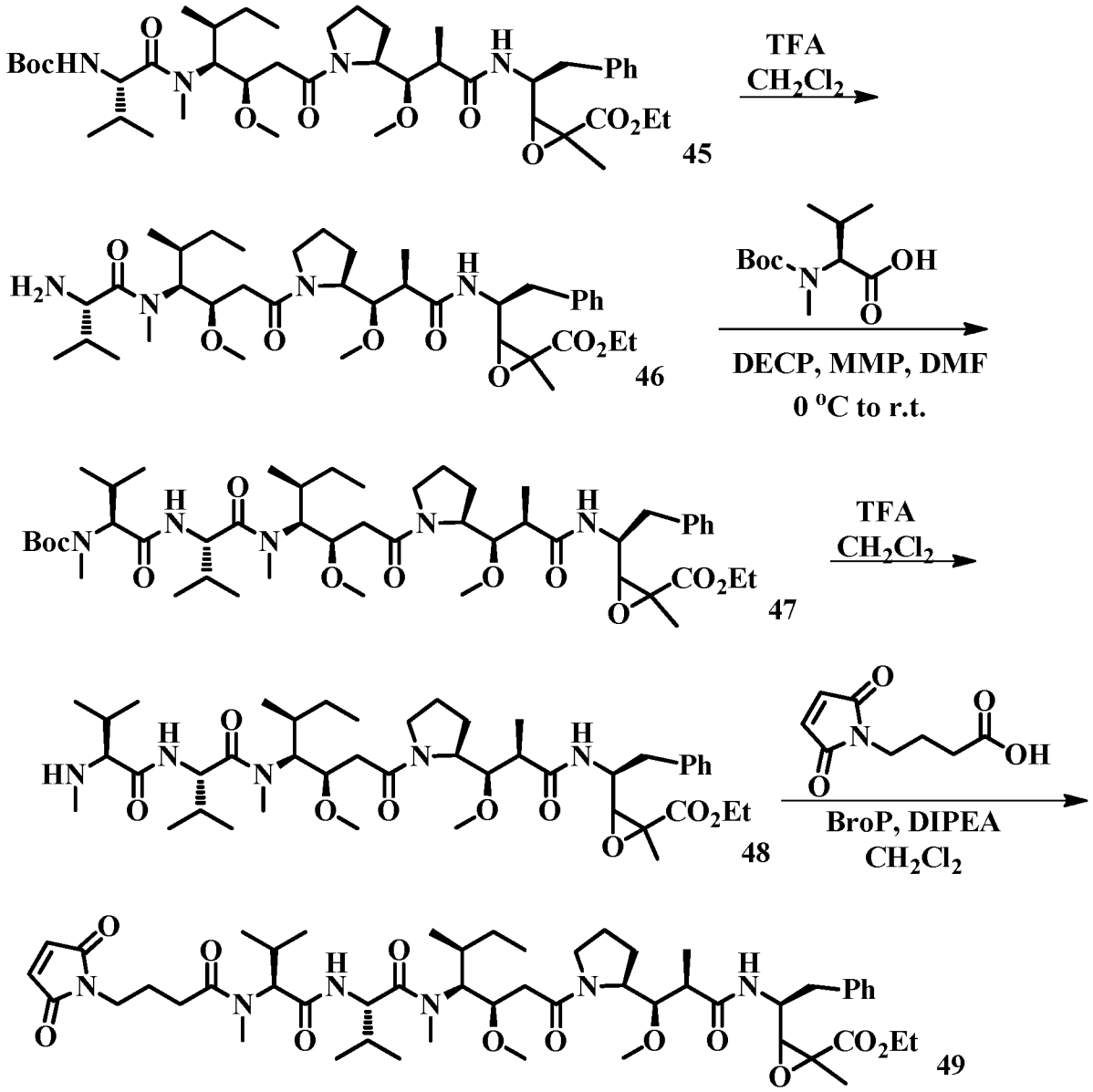


Scheme 6. The synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.

Scheme 7a


Ph
BocHN
CHO
Ph3P=
CO2Et
CH2Cl2
CO2Et
38
m-CPBA
CH2Cl2
39
TFA
CH2Cl2
H2N
40
Boc
OH
SOCl2
MeOH
8
41
14
Et3N, DECP, DMF
0 °C to r.t.
42
TFA
CH2Cl2
42a
Boc-Val-OH
BroP, DIPEA
CH2Cl2
43
43a
LiOH
THF, H2O
44
Et3N, DECP, DMF
0 °C to r.t.
45

Scheme 7b



Scheme 7 (7a & 7b). The synthesis of a Drug-Linker compound that contains a maleimide unit, linking to a MMAF-OMe analogue.