



- (51) **International Patent Classification:**
A61K 47/48 (2006.01)
- (21) **International Application Number:**
PCT/US2014/039919
- (22) **International Filing Date:**
29 May 2014 (29.05.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/829,893 31 May 2013 (31.05.2013) US
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- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

[Continued on next page]

(54) **Title:** CONJUGATES COMPRISING CELL-BINDING AGENTS AND CYTOTOXIC AGENTS

(57) **Abstract:** The invention provides a branched linker compound with reactive moieties for forming covalent bonds with multiple cytotoxic agents (drugs) and/or a cell-binding agent (CBA); a cytotoxic compound comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates; a modified cell-binding agent comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates; and conjugates so formed. Such conjugates and/or cytotoxic compounds may be effective for treating a range of diseases, such as cancer, with a relatively high activity at a relatively low, non-toxic dose.

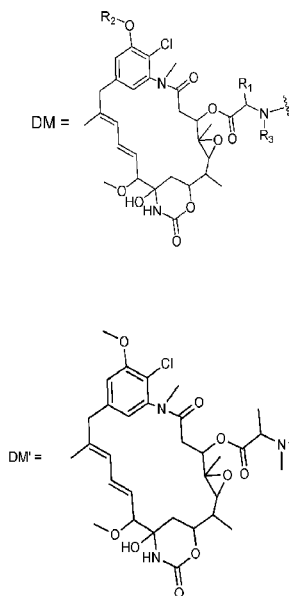


Figure 1



MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

— *as to the applicant's entitlement to claim the priority of
the earlier application (Rule 4.17(iii))*

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted
a patent (Rule 4.17(ii))*

Published:

— *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*

CONJUGATES COMPRISING CELL-BINDING AGENTS AND CYTOTOXIC AGENTS

REFERENCE TO RELATED APPLICATIONS

[01] This application claims priority to and the benefit of the filing date of U.S. provisional application No. 61/829,893, filed on May 31, 2013, the entire content of which is incorporated herein by reference.

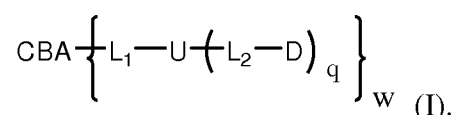
BACKGROUND OF THE INVENTION

[02] Antibody-drug conjugates (ADC) and cell binding agent-drug conjugates are emerging as a powerful class of anti-tumor agents with efficacy across a range of cancers. The cell binding agent-drug conjugates (such as ADCs) are commonly composed of three distinct elements: a cell-binding agent (e.g., antibody); a linking component; and a cytotoxic moiety. The linking component of ADC is an important element in developing targeted anti-cancer agents that possess an optimal therapeutic window, *i.e.*, therapeutic activity at a low, non-toxic dose.

[03] Therefore, there is a need for targeted therapies such as ADCs and other cell binding agent-drug conjugates having a new class of linking components.

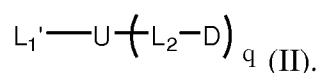
SUMMARY OF THE INVENTION

[04] A first embodiment of the invention features a conjugate represented by the following formula, or a pharmaceutically acceptable salt thereof:



[05] In Formula (I) above, CBA is a cell binding agent; L_1 is a spacer; U is a branched scaffold; L_2 , for each occurrence, is independently a spacer; D, for each occurrence, is independently a cytotoxic drug moiety; q is an integer from 2 to 5; and w is an integer between 1 and 10.

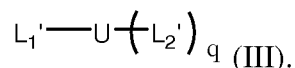
[06] A second embodiment of the invention features a cytotoxic compound represented by the following formula, or a salt (*e.g.*, a pharmaceutically acceptable salt) thereof:



[07] In Formula (II) above, L_1' is a spacer attached to a reactive functional group that can form a covalent bond with a cell-binding agent; U is a branched scaffold; L_2 , for each

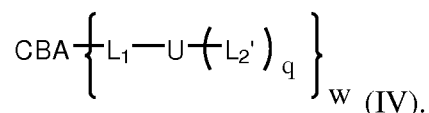
occurrence, is independently a spacer; D, for each occurrence, is independently a cytotoxic drug moiety; and q is an integer from 2 to 5.

[08] A third embodiment of the invention features a linker compound represented by the following formula, or a salt (*e.g.*, a pharmaceutically acceptable salt) thereof:



[09] In Formula (III) above, L_1' is a spacer attached to a reactive functional group that can form a covalent bond with a cell-binding agent; U is a branched scaffold; L_2' , for each occurrence, is independently a spacer attached to a reactive functional group that can form a covalent bond with a cytotoxic agent; and q is an integer from 2 to 5.

[10] A fourth embodiment of the invention features a modified cell binding agent represented by the following formula, or a salt (*e.g.*, a pharmaceutically acceptable salt) thereof:



[11] In Formula (IV), CBA is a cell binding agent; L_1 is a spacer; U is a branched scaffold; L_2' , for each occurrence, is independently a spacer attached to a reactive functional group that can form a covalent bond with a cytotoxic agent; q is an integer from 2 to 5; and w is an integer between 1 and 10.

[12] Also within the scope of this invention is a composition (*e.g.*, a pharmaceutical composition) comprising a conjugate represented by Formula (I), a cytotoxic compound represented by Formula (II), or a salt (*e.g.*, a pharmaceutical acceptable salt) thereof. The composition may also include a carrier (*e.g.*, a pharmaceutically acceptable carrier). The composition can further include a second therapeutic agent.

[13] The present invention also includes a method of inhibiting abnormal cell growth or treating a proliferative disorder, a destructive bone disorder, an autoimmune disorder, a graft versus host disease, a transplant rejection, an immune deficiency, an inflammatory diseases, an infectious disease, a viral disease, a fibrotic disease, a neurodegenerative disorder, pancreatitis, or a kidney disease in a mammal (*e.g.*, human), comprising administering to said mammal a therapeutically effective amount of a conjugate represented by Formula (I), a cytotoxic compound represented by Formula (II), or a salt (*e.g.*, a pharmaceutical acceptable salt) thereof.

[14] In a related embodiment, the method described above further comprises administering to said mammal simultaneously, sequentially, or consecutively a second therapeutic (*e.g.*, chemotherapeutic) agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[15] Figure 1 depicts the structures of certain maytansinoids.

[16] Figures 2-6 depict the synthetic schemes for preparing cytotoxic compounds each comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates.

[17] Figures 7 and 8 depict the synthetic schemes for preparing branched linker compounds each with reactive moieties for forming covalent bonds with multiple cytotoxic agents (drugs).

[18] Figures 9 and 10 depict the synthetic schemes for preparing cytotoxic compounds each comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates.

[19] Figure 11 depicts the structure of a cytotoxic compound comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates.

[20] Figure 12 depicts the synthetic scheme for preparing a branched linker compound with reactive moieties for forming covalent bonds with multiple cytotoxic agents (drugs).

[21] Figures 13-16 depict the synthetic schemes for preparing cytotoxic compounds each comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugates.

[22] Figures 17-23 depict the synthetic schemes for preparing branched linker compounds containing branched scaffolds.

[23] Figures 24-25 show cell-binding properties of conjugates having branched linkers (*i.e.*, Ab-linker2-(DM4)₂ or Ab-linker3-(MayNMA)₂ in comparison with naked antibody.

[24] Figures 26-28 show the cytotoxicity effects of conjugates having branched linkers (Ab-linker2-(DM4)₂ and Ab-linker3-(MayNMA)₂ in comparison with those having a non-branched linker (Ab-SPDB-DM4) on COLO205 cells, HCT-15 cells, and RPMI8226. The CBA used in the conjugates is chB38.1. In the plots SPDB-DM4 4.03D/A, Ab-linker2-(DM4)₂ 3.1D/A, and Ab-linker3-(MayNMA)₂ 4.26D/A, the cytotoxicity assay was performed in the absence of the competing non-conjugated antibody. In the plots SPDB-DM4 4.03D/A with blocking, Ab-linker2-(DM4)₂ 3.1D/A with blocking, and Ab-linker3-

(MayNMA)2 4.26D/A with blocking, the cytotoxicity assay was performed in the presence of the competing non-conjugated antibody.

[25] Figure 29 depicts the synthetic scheme for preparing a cytotoxic compound comprising a branched linker moiety with a reactive group for forming a CBA-drug conjugate.

[26] Figure 30 depicts the synthetic scheme for preparing a branched linker compound containing branched scaffolds.

DETAILED DESCRIPTION OF THE INVENTION

[27] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents which may be included within the scope of the present invention as defined by the claims. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention.

[28] It should be understood that any of the embodiments described herein, including those described under different aspects of the invention (*e.g.*, compounds, conjugates, compositions, methods of making and using) and different parts of the specification (including embodiments described only in the Examples) can be combined with one or more other embodiments of the invention, unless explicitly disclaimed or improper. Combination of embodiments are not limited to those specific combinations claimed via the multiple dependent claims.

DEFINITIONS

[29] “**Alkyl**” as used herein refers to a saturated linear or branched-chain monovalent hydrocarbon radical of one to twenty carbon atoms. “Monovalent” means that alkyl has one point of attachment to the remainder of the molecule. Examples of alkyl groups include, but are not limited to, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl, -CH₂CH(CH₃)₂, 2-butyl, 2-methyl-2-propyl, 1-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, 3,3-dimethyl-2-butyl, 1-heptyl, 1-octyl, and the like. Specifically, the alkyl group has one to ten carbon atoms. More specifically, the alkyl group has one to four carbon

atoms.

[30] “**Alkylene**” as used herein refers to a saturated linear or branched-chain divalent hydrocarbon radical of one to twenty carbon atoms, examples of which include, but are not limited to, those having the same core structures of the alkyl groups as exemplified above. “Divalent” means that the alkylene has two points of attachment to the remainder of the molecule. Specifically, the alkylene group has one to ten carbon atoms. More specifically, the alkylene group has one to four carbon atoms.

[31] The terms “**carbocycle**,” “**carbocyclyl**,” **carbocyclic** and “**carbocyclic ring**” refer to a monovalent non-aromatic, saturated or partially unsaturated ring having 3 to 12 carbon atoms as a monocyclic ring or 7 to 12 carbon atoms as a bicyclic ring. Bicyclic carbocycles having 7 to 12 atoms can be arranged, for example, as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, and bicyclic carbocycles having 9 or 10 ring atoms can be arranged as a bicyclo [5,6] or [6,6] system, or as bridged systems such as bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane and bicyclo[3.2.2]nonane. Examples of monocyclic carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, cyclododecyl, and the like.

[32] The term “**cycloalkylalkyl**” refers to a cycloalkyl group that is connected to another group by an alkylene group. Examples of cycloalkylalkyls include, but are not limited to, cyclohexylmethyl, cyclohexylethyl, cyclopentylmethyl, cyclopentylethyl, and the like. Specifically, cycloalkylalkyl is cyclohexylmethyl.

[33] As used herein, an integer “**between**” x and y includes integers x and y unless otherwise specified to the contrary. For example, “an integer between 1 and 5” can be 1, 2, 3, 4, or 5.

[34] The terms “**cyclic alkyl**” and “**cycloalkyl**” can be used interchangeably. They refer to a monovalent saturated carbocyclic ring radical. “Monovalent” means that cycloalkyl has one point of **attachment** to the remainder of the molecule. The saturated carbocyclic ring can be monocyclic or bicyclic (fused, bridged, or spiro bicyclic). Specifically, the cycloalkyl is 3 to 7 membered monocyclic ring radical. Bicyclic cycloalkyl having 7 to 12 atoms can be arranged, for example, as a bicyclo [4,5], [5,5], [5,6], or [6,6] system, and bicyclic cycloalkyl having 9 or 10 ring atoms can be arranged as a bicyclo [5,6] or [6,6] system, or as bridged systems such as bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane and bicyclo[3.2.2]nonane. Examples of monocyclic cycloalkyl groups include, but are not limited to, cyclopropyl,

cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, cyclododecyl, and the like. More specifically, the cycloalkyl is cyclohexyl.

[35] “**Cycloalkylene**” as used herein refers to a divalent saturated carbocyclic ring radical having 3 to 12 carbon atoms as a monocyclic ring, or 7 to 12 carbon atoms as a bicyclic ring. “Divalent” means that the cycloalkylene has two points of attachment to the remainder of the molecule. Specifically, the cycloalkylene is a 3- to 7-membered monocyclic. Examples of cyclic alkylene groups include, but not limited to, those having the same core structures of the cycloalkyl groups as exemplified above. More specifically, the cycloalkylene group is cyclohexylene.

[36] “**Alkenyl**” as used herein refers to linear or branched-chain monovalent hydrocarbon radical of two to twenty carbon atoms with at least one carbon-carbon double bond, wherein the alkenyl radical includes radicals having “*cis*” and “*trans*” orientations, or by an alternative nomenclature, “E” and “Z” orientations. “Monovalent” means that alkenyl has one point of attachment to the remainder of the molecule. Examples include, but are not limited to, ethylenyl or vinyl ($-\text{CH}=\text{CH}_2$), allyl ($-\text{CH}_2\text{CH}=\text{CH}_2$), and the like. Specifically, the alkenyl has two to ten carbon atoms, also referred to as “C₂₋₁₀ alkenyl.” More specifically, the alkenyl has two to four carbon atoms, also referred to as “C₂₋₄ alkenyl.”

[37] “**Alkenylene**” as used herein refers to linear or branched-chain divalent hydrocarbon radical of two to twenty carbon atoms with at least one carbon-carbon double bond, wherein the alkenyl radical includes radicals having “*cis*” and “*trans*” orientations, or by an alternative nomenclature, “E” and “Z” orientations. “Divalent” means that the alkenylene has two points of attachment to the remainder of the molecule. Examples include, but are not limited to, ethylenylene or vinylenylene ($-\text{CH}=\text{CH}-$), allylene ($-\text{CH}_2\text{CH}=\text{CH}-$), and the like. Specifically, the alkenylene has two to ten carbon atoms, also referred to as “C₂₋₁₀ alkenylene.” More specifically, the alkenylene has two to four carbon atoms, also referred to as “C₂₋₄ alkenylene.”

[38] The terms “**cyclic alkenyl**” and “**cycloalkenyl**” can be used interchangeably. They refer to a monovalent carbocyclic ring radical with at least one carbon-carbon double bond, having 3 to 12 carbon atoms as a monocyclic ring, or 7 to 12 carbon atoms as a bicyclic ring (fused, bridged, or spiro bicyclic). “Monovalent” means that cycloalkenyl has one point of **attachment** to the remainder of the molecule. Specifically, the cycloalkenyl is 3 to 7 membered monocyclic ring radical. Bicyclic cycloalkenyl having 7 to 12 atoms can be arranged, for example, as a bicyclo [4,5], [5,5], [5,6], or [6,6] system, and bicyclic cycloalkenyl having 9 or 10 ring atoms can be arranged as a bicyclo [5,6] or [6,6] system, or

as bridged systems such as bicyclo[2.2.1]heptene, bicyclo[2.2.2]octene and bicyclo[3.2.2]nonene. Examples of the monocyclic alkenyl include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, cyclodecenyl, cycloundecenyl, cyclododecenyl, and the like. More specifically, the cycloalkenyl is cyclohexenyl.

[39] “**Cycloalkenylene**” as used herein refers to a divalent unsaturated carbocyclic ring radical having 3 to 12 carbon atoms as a monocyclic ring, or 7 to 12 carbon atoms as a bicyclic ring. “Divalent” means that the cycloalkenylene has two points of attachment to the remainder of the molecule. Specifically, the cycloalkenylene is a 3- to 7-membered monocyclic. Examples of cyclic alkenylene include, but not limited to, those having the same core structures of the cycloalkyl groups as exemplified above. More specifically, the cycloalkenylene group is cyclohexenylene.

[40] “**Alkynyl**” as used herein refers to linear or branched-chain monovalent hydrocarbon radical of two to twenty carbon atoms with at least one carbon-carbon triple bond.

“Monovalent” means that alkynyl has one point of attachment to the remainder of the molecule. Examples include, but are not limited to ethynyl, propynyl, 1-butylnyl, 2-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, hexynyl, and the like. Specifically, the alkynyl has two to ten carbon atoms, also referred to as “C₂₋₁₀ alkynyl.” More specifically, the alkynyl has two to four carbon atoms, also referred to as “C₂₋₄ alkynyl.”

[41] “**Alkynylene**” as used herein refers to an linear or branched-chain divalent hydrocarbon radical of two to twenty carbon atoms with at least one carbon-carbon triple bond, examples of which include, but are not limited to, those having the same core structures of the alkynyl groups as exemplified above. “Divalent” means that the alkynylene has two points of attachment to the remainder of the molecule. Specifically, the alkynylene group has one to ten carbon atoms. More specifically, the alkynylene group has one to four carbon atoms.

[42] The terms “**cyclic alkynyl**” and “**cycloalkynyl**” can be used interchangeably. They refer to a monovalent carbocyclic ring radical with at least one carbon-carbon triple bond, having 8 to 12 carbon atoms as a monocyclic ring, or 11 to 17 carbon atoms as a bicyclic ring (fused, bridged, or spiro bicyclic). “Monovalent” means that cycloalkynyl has one point of **attachment** to the remainder of the molecule. Specifically, the cycloalkynyl is 8-membered monocyclic ring radical.

[43] “**Cycloalkyne**” as used herein refers carbocyclic ring having one or more triple bonds. It can be monocyclic, bicyclic or tricyclic; bicyclic and tricyclic can be bridged or

fused. The carbocyclic ring optionally contains one or more double bonds and/or is optionally fused with one or more aromatic (*e.g.*, phenyl ring) or heteroaromatic rings. Examples of cycloalkyne include, but are not limited to, those described in *J. Am. Chem. Soc.* (2012) 134:9199-9208; WO 2011/136645, US 2009/0068738, Lang K. *et al.*, *J. Am. Chem. Soc.* (2012) 134:10317-10320, for example, cyclooctyne, monofluorocyclooctyne, difluorooctyne, DIFO, DIFO₂, DIFO₃, bicyclo[6.1.0]non-4-yne, benzocyclooctyne, difluorobenzocyclooctyne, dibenzocyclooctyne, DIBO, and those described in Debets, M. F. *et al.*, *Acc. Chem. Res.*, (2011) 44(9):805–815; and Gold B. *et al.*, *J. Am. Chem. Soc.* (2013) 135(4):1558–1569. Specifically, cycloalkyne is cyclooctyne.

[44] **“Cycloalkynylene”** as used herein refers to a divalent carbocyclic ring radical having at least one carbon-carbon triple bond. “Divalent” means that the cycloalkynylene has two points of attachment to the remainder of the molecule. Specifically, the cycloalkynylene is a 8 to 14-membered monocyclic.

[45] **“Heterocycloalkyne”** as used herein refers to a heterocyclic ring having one or more triple bonds. Examples of heterocycloalkyne include, but are not limited to, dibenzoazacyclooctyne (DIBAC), biarylazacyclooctynone (BARAC), thiacyclooctyne, thiabenzocyclooctyne, thiacycloheptyne and tetramethylthiacycloheptyne.

[46] **“Strained cycloalkene”** as used herein refers to carbocyclic ring having one trans double bond and 7 to 14 ring atoms. Examples of strained cycloalkene include, but are not limited to, cyclooctene, norbornene and other cycloalkenes described in Debets, M. F. *et al.*, *Acc. Chem. Res.* (2011) 44(9):805–815.

[47] **“Strained heterocycloalkene”** as used herein refers to a heterocyclic ring having one trans double bonds and 7 to 14 ring atoms selected from carbon and at least one (typically 1 to 4, more typically 1 or 2) heteroatom (*e.g.*, oxygen, nitrogen or sulfur).

[48] The term **“aryl group”** means an aromatic hydrocarbon ring system having six to fourteen carbon ring atoms. The term “aryl” may be used interchangeably with the terms “aryl ring” “aromatic ring,” “aryl group” and “aromatic group.” “Aryl group” also includes an aromatic hydrocarbon ring system fused to a non-aromatic carbocyclic ring system, such as a cycloalkyl group. Examples includes phenyl, naphthyl, anthracenyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indanyl, indenyl and the like. An aryl group is monovalent, *i.e.*, has one point of attachment to the remainder of the molecule. A “substituted aryl group” is substituted at any one or more substitutable ring atom, which is a ring carbon atom bonded to a hydrogen.”

[49] “**Arylene**” as used herein refers to a divalent aryl group, *i.e.*, an aryl group having two points of attachment to the remainder of the molecule. “Divalent” means that the arylene has two points of attachment to the remainder of the molecule. Both aryl and arylene groups are sometime represented herein by “Ar.” Arylene is specifically phenylene.

[50] “**Heteroaryl**” (used interchangeably with “heteroaromatic,” “heteroaryl ring,” “heteroaryl group,” “heteroaromatic ring,” and “heteroaromatic group”) refers to aromatic ring systems having five to fourteen ring atoms selected from carbon and at least one (typically 1 to 4, more typically 1 or 2) heteroatoms (*e.g.*, oxygen, nitrogen or sulfur). “Heteroaryl” includes monocyclic rings and polycyclic rings (*e.g.*, bicyclic) in which a monocyclic heteroaromatic ring is fused to one or more other aromatic or heteroaromatic rings. As such, “5-14 membered heteroaryl” includes monocyclic, bicyclic or tricyclic ring systems. Heteroaryls are monovalent, meaning that there is one point of attachment to the remainder of the molecule.

[51] “Monocyclic 5-6 membered heteroaryl” means a monocyclic aromatic ring system having five or six ring atoms selected from carbon and at least one (typically 1 to 3, more typically 1 or 2) heteroatoms (*e.g.*, oxygen, nitrogen or sulfur). Examples of monocyclic 5-6 membered heteroaryl groups include furanyl (*e.g.*, 2-furanyl, 3-furanyl), imidazolyl (*e.g.*, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), isoxazolyl (*e.g.*, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl), oxadiazolyl (*e.g.*, 2-oxadiazolyl, 5-oxadiazolyl), oxazolyl (*e.g.*, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl), pyrazolyl (*e.g.*, 3-pyrazolyl, 4-pyrazolyl), pyrrolyl (*e.g.*, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl), pyridyl (*e.g.*, 2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (*e.g.*, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl), pyridazinyl (*e.g.*, 3-pyridazinyl), thiazolyl (*e.g.*, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl), isothiazolyl, triazolyl (*e.g.*, 2-triazolyl, 5-triazolyl), tetrazolyl (*e.g.*, tetrazolyl), and thienyl (*e.g.*, 2-thienyl, 3-thienyl). Examples of polycyclic aromatic heteroaryl groups include carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, isobenzofuranyl, indolyl, benzotriazolyl, benzothiazolyl, benzoxazolyl, quinolinyl, isoquinolinyl, indazolyl, isoindolyl, acridinyl, or benzisoxazolyl. A “substituted heteroaryl group” is substituted at any one or more substitutable ring atom, which is a ring carbon or ring nitrogen atom bonded to a hydrogen.

[52] “**Heteroarylene**” as used herein refers to a divalent heteroaryl, *i.e.*, a heteroaryl with two points of attachment to the remainder of the molecule.

[53] The terms “**heterocycle**,” “**heterocyclyl**,” “**heterocyclic**” and “**heterocyclic ring**” are used interchangeably herein and refer to a saturated or unsaturated non-aromatic 3-12 membered ring radical optionally containing one or more double bonds. It can be

monocyclic, bicyclic, or tricyclic; bicyclic and tricyclic can be bridged or fused. The heterocycle contains 1 to 4 heteroatoms, which may be the same or different, selected from N, O or S. The heterocycle optionally contains one or more double bonds and/or is optionally fused with one or more aromatic (*e.g.*, phenyl ring) or heteroaromatic rings. “3-7 membered monocyclic heterocycle” means a radical having from 3-7 atoms (including 1-3 heteroatoms) arranged in a monocyclic ring. The term “heterocycle” is intended to include all the possible isomeric forms. A heterocycle may be a monocycle having 3 to 7 ring members (*e.g.*, 2 to 6 carbon atoms and 1 to 4 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (*e.g.*, 4 to 9 carbon atoms and 1 to 6 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system. Heterocycles are described in Paquette, Leo A., *Principles of Modern Heterocyclic Chemistry* (W. A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; *The Chemistry of Heterocyclic Compounds, A Series of Monographs* (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.

[54] Examples of heterocyclic rings include, but are not limited to, aziridinyl, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, tetrahydropyrrolyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, homopiperazinyl, azetidiny, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, isoindolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyco[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, and azabicyclo[2.2.2]hexanyl. Spiro moieties are also included within the scope of this definition. Examples of a heterocyclic group wherein ring atoms are substituted with oxo (=O) moieties are pyrimidinonyl and 1,1-dioxo-thiomorpholinyl.

[55] The heterocycle, heteroaryl, or heteroarylene groups may be carbon (carbon-linked) or nitrogen (nitrogen-linked) attached where such is possible. By way of example and not limitation, carbon bonded heterocycle, heteroaryl or heteroarylene groups are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline.

[56] By way of example and not limitation, nitrogen bonded heterocycle, heteroaryl, or heteroarylene groups are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or O-carboline.

[57] The heteroatoms present in heteroaryl, heteroarylene, or heterocyclcyl can include the oxidized forms such as NO, SO, and SO₂.

[58] “**Halogen**” refers to F, Cl, Br or I.

[59] If a group is described as being “**optionally substituted**,” the group may be either (1) not substituted, or (2) substituted. If a carbon of a group is described as being optionally substituted with one or more of a list of substituents, one or more of the hydrogen atoms on the carbon (to the extent there are any) may separately and/or together be replaced with an independently selected optional substituent.

[60] Suitable substituents for an alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocycle, alkylene, alkenylene, alkynylene, cycloalkene, heterocycloalkene, cycloalkyne, heterocycloalkyne, cycloalkylene arylene, and heterarylene are those which do not significantly adversely affect the biological activity of the conjugate. Unless otherwise specified, exemplary substituents for these groups include linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, aryl, heteroaryl, heterocyclcyl, halogen, guanidinium [-NH(C=NH)NH₂], -OR₁₀₀, NR₁₀₁R₁₀₂, -NO₂, -NR₁₀₁COR₁₀₂, -SR₁₀₀, a sulfoxide represented by -SOR₁₀₁, a sulfone represented by -SO₂R₁₀₁, a sulfonate -SO₃M, a sulfate -OSO₃M, a sulfonamide represented by -SO₂NR₁₀₁R₁₀₂, cyano, an azido, -COR₁₀₁, -OCOR₁₀₁, -OCONR₁₀₁R₁₀₂ and a polyethylene glycol unit (-OCH₂CH₂)_nR₁₀₁ wherein M is H or a cation (such as Na⁺ or K⁺); R₁₀₁, R₁₀₂ and R₁₀₃ are each independently selected from H, linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit (-OCH₂CH₂)_n-R₁₀₄, wherein n is an integer from 1 to 24, an aryl having from 6 to 10 carbon atoms, a heterocyclic ring having from 3 to 10 carbon atoms and a heteroaryl having 5 to 10 carbon atoms; and R₁₀₄ is H or a linear or branched alkyl having 1 to 4 carbon atoms, wherein the alkyl, alkenyl, alkynyl, aryl, heteroaryl and heterocyclcyl in the groups represented by R₁₀₀, R₁₀₁, R₁₀₂, R₁₀₃ and R₁₀₄ are optionally substituted with one or more (e.g., 2, 3, 4, 5, 6 or more) substituents independently selected from halogen, -OH, -CN, -NO₂, and unsubstituted linear or branched alkyl having 1 to 4 carbon atoms. Specifically, the substituent for the optionally substituted alkyl, alkylene, cycloalkylene, arylene, and

heteroarylene described above is selected from the group consisting of halogen, -CN, -NR₁₀₁R₁₀₂, -CF₃, -OR₁₀₀, aryl, heteroaryl, heterocyclyl, -SR₁₀₁, -SOR₁₀₁, -SO₂R₁₀₁, and -SO₃M. Alternatively, the suitable substituent is selected from the group consisting of -halogen, -OH, -NO₂, -CN, C₁₋₄ alkyl, -OR₁₀₀, NR₁₀₁R₁₀₂, -NR₁₀₁COR₁₀₂, -SR₁₀₀, -SO₂R₁₀₁, -SO₂NR₁₀₁R₁₀₂, -COR₁₀₁, -OCOR₁₀₁, and -OCONR₁₀₁R₁₀₂, wherein R₁₀₀, R₁₀₁, and R₁₀₂ are each independently -H or C₁₋₄ alkyl.

[61] The term “**ionizable group**” refers to a functional group that can be converted to a charged group by protonation with an acid or deprotonation with a base. Examples of the ionizable groups include -SO₃H, -Z'-SO₃H, -OPO₃H₂, -Z'-OPO₃H₂, -PO₃H₂, -Z'-PO₃H₂, -CO₂H, -Z'CO₂H, -NR₁₁R₁₂, or -Z'-NR₁₁R₁₂, R₁₁ and R₁₂, for each occurrence, are independently H or an optionally substituted alkyl; and Z' includes an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene. In certain embodiments, Z' is alkylene.

[62] The term “**charged substituent**” refers to a substituent that is either positively or negatively charged. The charge in such a substituent is not removable by treatment with a base or an acid and thus permanent. Examples of the charged substituents include, but not limited to, -N⁺R₁₃R₁₄R₁₅ and -Z'-N⁺R₁₃R₁₄R₁₅, in which R₁₃ to R₁₅ are each independently an optionally substituted alkyl; and Z' includes an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene. In certain embodiments, Z' is alkylene.

[63] Charged substituents may contain a counter ion. For positive charged substituents, the counter ion is negative and can be represented by “X⁻,” *e.g.*, as -N⁺R₁₃R₁₄R₁₅X⁻ and -Z'-N⁺R₁₃R₁₄R₁₅X⁻. The counter ions for the positively charged substituents are anions (specifically pharmaceutically acceptable anions), which include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, bromide, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glyceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, pamoate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, and triethiodide. Specifically, the counter ions for the positively charged substituents are chloride, bromide, sulfate, and phosphate.

[64] The counter ions for the negatively charged substituents include, but are not limited to, an alkali metal ion (*e.g.*, sodium and potassium), an alkaline earth metal ion (*e.g.*, calcium

and magnesium), aluminum ion, ammonium, protonated trialkyl amines (*e.g.*, trimethylamine and triethylamine), a tetraalkyl ammonium (*e.g.*, tetra methyl ammonium, and tetrabutyl ammonium), and a protonated heteroaromatic group (*e.g.*, pyridine, pyrimidine, triazines, tetrazines). Specifically, the counter ions for the negatively charged substituents are sodium, potassium, lithium, protonated triethyl amine, protonated pyridine. Most specifically, the counter ions for the negatively charged substituents are sodium and potassium. The counter ions for both the negatively and positively charged substituents may be removed or replaced in subsequent purification steps.

[65] “**Amino acid**” as used herein, including the residue represented by variable “XX” described above, refers to naturally occurring amino acids, unnatural amino acids, amino acid analogs, or amino acid mimetics that function in a manner similar to the naturally occurring amino acids.

[66] The term “**naturally occurring amino acids**” as used herein refers to those twenty L-amino acids encoded by the universal genetic codes and appearing in proteins or peptides, as well as selenocysteine and pyrrolysine that are incorporated into proteins by distinctive biosynthetic mechanisms. They include histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, selenocysteine and pyrrolysine. The term “**naturally occurring amino acids**” also refers to those produced by the body, but are not encoded by the universal genetic codes, such as β -alanine, ornithine, and citrulline. The term “**naturally occurring amino acids**” further includes those naturally occurring L-amino acids that are later modified (*e.g.*, via post-translational modification by enzymes) in the body, such as hydroxyproline, γ -carboxyglutamate, and O-phosphoserine.

[67] The term “**unnatural amino acids**” as used herein is intended to include the “D” stereochemical form of the naturally occurring amino acids described above. It is further understood that the term “**unnatural amino acids**” includes homologues of the natural L-amino acids or their D isomers, and synthetically modified forms of the natural L-amino acids or their D isomers. The synthetically modified forms include, but are not limited to, amino acids having side chains shortened or lengthened by up to two carbon atoms, amino acids comprising optionally substituted aryl groups, and amino acids comprised halogenated groups, specifically halogenated alkyl and aryl groups and also N substituted amino acids *e.g.* N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-

threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-proline, N-methyl-serine, N-methyl-tyrosine, N-methyl-selenocysteine, and N-methyl-pyrrolysine, each including an L or D isomer. In one embodiment, the “**unnatural amino acids**” includes, for example, glutamic acid 5-methyl ester (Glu(OMe)), including an L or D isomer.

[68] The term “**amino acid analogs**” as used herein refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group. They include 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, homoserine, norleucine, cysteine sulfonic acid, cysteine sulfinic acid, methionine sulfoxide, and methionine methyl sulfonium. Such analogs may have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid analogs also include D isomers of the above-referenced L-isomers.

[69] The term “**amino acid mimetics**” as used herein refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but functions in a manner similar to a naturally occurring amino acid.

[70] [XX]₂₋₁₀ denotes a peptide of 2 to 10 residues. Peptides are short polymers of amino acid monomers linked by peptide bonds, the covalent chemical bonds formed between two amino acid monomers when the carboxyl group of N-terminal monomer reacts with the amino group of the C-terminal monomer. A preferred peptide length is about two to ten amino acids as described above, although a peptide of longer length may also be used.

[71] Any one of the peptides described herein can be connected to the rest of the molecules in either direction. For example, when AA1, AA2, and AA3 each represent an amino acid (naturally-occurring, or unnatural), “AA1-AA2-AA3 in either direction” refers to the tripeptide N-AA1-AA2-AA3-C), and the tripeptide N-AA3-AA2-AA1-C). Unless otherwise specified, peptide sequences are represented conventionally, with the N-terminus to the left and the C-terminus to the right.

[72] In certain embodiments, [XX]₁₋₁₀ is an amino acid represented by [XX]₁ or a peptide represented by [XX]₂₋₁₀, in which each XX is the residue of an independently selected amino acid selected from the group consisting of: histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, selenocysteine,

and pyrrolysine, N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-proline, N-methyl-serine, N-methyl-tyrosine, N-methyl-selenocysteine, N-methyl-pyrrolysine, hydroxyproline, γ -carboxyglutamate, selenocysteine, O-phosphoserine, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, citrulline, ornithine, cysteine sulfonic acid, cysteine sulfinic acid, 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, and β -alanine, each independently as an L or D isomer. Each [XX] can additionally be glutamic acid 5-methyl ester (Glu(OMe)), including an L or D isomer. In another alternative, each XX is the residue of an independently selected amino acid selected from glycine or alanine, each independently as an L or D isomer.

[73] In certain embodiments, [XX]₁₋₁₀ is a peptide represented by [XX]₂₋₅ (a peptide of 2 to 5 residues).

[74] In certain embodiments, the peptide represented by [XX]₂₋₁₀ is cleavable by a protease. In one embodiment, the protease is a protease expressed in tumor tissue. In another embodiment, the protease is a lysosomal protease.

[75] The cleavable peptide refers to peptides containing a cleavage recognition sequence of a protease. A cleavage recognition sequence for a protease is an amino acid sequence recognized by the protease during proteolytic cleavage. Many protease cleavage sites are known in the art, and these and other cleavage sites can be included a linker, a spacer, or a linker moiety. See, *e.g.*, Matayoshi *et al.*, *Science* 247:954 (1990); Dunn *et al.*, *Meth. Enzymol.* 241:254 (1994); Seidah *et al.*, *Meth. Enzymol.* 244:175 (1994); Thornberry, *Meth. Enzymol.* 244:615 (1994); Weber *et al.*, *Meth. Enzymol.* 244:595 (1994); Smith *et al.*, *Meth. Enzymol.* 244:412 (1994); Bouvier *et al.*, *Meth. Enzymol.* 248: 614(1995), Hardy *et al.*, in *AMYLOID PROTEIN PRECURSOR IN DEVELOPMENT, AGING, AND ALZHEIMER'S DISEASE*, Ed. Masters *et al.*, pp. 190-198 (1994).

[76] In one embodiment, the peptide sequence is chosen based on its ability to be cleaved by a tumor-associated protease, *e.g.*, a protease that is found on the surface of a cancerous cell or extracellularly in the vicinity of tumor cells. The examples of such proteases include thimet oligopeptidase (TOP), CD10 (neprilysin), a matrix metalloprotease (such as MMP2 or MMP9), a type II transmembrane serine protease (such as Hepsin, testisin, TMPRSS4 or

matriptase/MT-SP1), legumain and enzymes described in the following reference: *Current Topics in Developmental Biology: Cell Surface Proteases*, vol. 54 Zucker S. 2003, Boston, MA. The ability of a peptide to be cleaved by tumor-associated protease can be tested using *in vitro* protease cleavage assays known in the art.

[77] In another embodiment, the peptide sequence is chosen based on its ability to be cleaved by a lysosomal protease, which include cathepsins B, C, D, H, L and S, and furin. Specifically, the peptide sequence is capable of being cleaved by an appropriate isolated protease *in vitro*, which can be tested using *in vitro* protease cleavage assays known in the art.

[78] In certain embodiments, the peptide is selected from the group consisting of Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Leu, Ala-Leu-Ala-Leu, β -Ala-Leu-Ala-Leu, Gly-Phe-Leu-Gly, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, and Ala-Val-Ala. In another alternative, the peptide is selected from the group consisting of Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, and Ala-Val-Ala. Alternatively, the peptide is Gly-Gly-Ala, Val-Ala, Glu-Ala, or Glu(OMe)-Ala. In a related embodiment, any of the peptide sequences herein above may be in either direction, as defined above.

[79] An amino acid or peptide can be attached to or be present in a linker or in a spacer, or be attached to a cell binding agent through the terminal amine or terminal carboxylic acid of the amino acid or peptide. The amino acid can also be attached to a linker or a spacer, or a cell-binding agent through a side chain reactive group, including, but not restricted to, the thiol group of cysteine, the epsilon amine of lysine, and the side chain hydroxyls of serine or threonine.

[80] The term “**spacer**” as used herein includes a chemical moiety interposed between any two chemical groups. For example, in some embodiments, one end of the spacer is linked to a cell-bind agent (e.g., an antibody, such as a human or humanized monoclonal antibody, or an antigen-binding portion or fragment thereof), or a reactive functional group that can form a covalent bond with a cell-binding agent. In other embodiments, one end of the spacer is linked to a cytotoxic drug (e.g., a maytansinoid, such as DM1 or DM4), or a reactive functional group that can form a covalent bond with a cytotoxic drug. In some embodiments, one end of the spacer is linked to a branched scaffold. In some embodiments, the spacer is

interposed between (1) a cell-binding agent, or a reactive functional group that can form a covalent bond with a cell-binding agent; and (2) a branched scaffold. In some embodiments, the spacer is interposed between (1) a cytotoxic drug, or a reactive functional group that can form a covalent bond with a cytotoxic drug; and (2) a branched scaffold. A spacer may be attached to a reactive functional group at one end to form a linker moiety that can further react with a cell-binding agent or a cytotoxic drug.

[81] In certain embodiments, the spacer creates a desired distance between the two chemical groups to, for example, avoid stereo hindrance or to promote molecular flexibility. In certain embodiments, the presence of the spacer does not hinder, inhibit, or otherwise negatively affect the function of the flanking chemical groups (e.g., the ability of the cell-binding agent to bind a target molecule on a cell, or the cytotoxicity of the cytotoxic drug). In certain embodiments, the spacer confers additional beneficial characteristics, such as enhanced potency, solubility, serum stability, and/or efficacy, to the immunoconjugate or linker compound comprising the spacer. In certain embodiments, the spacer may comprise one or more amino acid residues (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more residues), which may or may not be resistant to protease or peptidases (such as intracellular / lysosomal peptidase) cleavage. In certain embodiments, the spacer may comprise one or more repeats of polyethylene glycol (PEG) units $-(CH_2-CH_2-O)-$, such as 1-1000 PEG units, 1-500 PEG units, 1-24 PEG units, or 2-8 PEG units (2, 4, 6, or 8 PEG units). Exemplary spacers L_1 and L_2 are described in more details below.

[82] The term “**branched scaffold**” as used herein includes a chemical moiety linked to three or more spacers. A branched scaffold allows two or more drugs to be attached to the cell binding agent. Exemplary branched scaffolds may be derived from an amino acid with a side chain comprising an amino group (such as Lys) or a carboxyl group (such as a Glu or an Asp), or a peptide comprising two or more such amino acids (e.g., Lys-Lys dimer etc.). Each amino acid may be independently D- or L-amino acids. Chemically modified amino acids or analogs thereof having similar structures may also give rise to branches scaffolds. In certain embodiments, one cell-binding agent and two or more cytotoxic drugs (same or different) may be linked to the branched scaffold, each independently through a spacer (same or different).

[83] The term “**residue of a functional group**” is a moiety remaining after a reaction with another functional group. For example, a residue of thiol group can form disulfide bond after reacting with another thiol group or thioether after reacting with an electrophilic group, such as maleimide.

[84] The term “**reactive ester**” as used herein refers to an ester group having a leaving group that is readily displaced by an amine group. Examples of a reactive ester, include, but are not limited to, N-hydroxysuccinimide ester, N-hydroxysulfosuccinimide ester, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl) ester, dinitrophenyl (*e.g.*, 2,4-dinitrophenyl) ester, sulfo-tetrafluorophenyl (*e.g.*, 4-sulfo-2,3,5,6-tetrafluorophenyl) ester and pentafluorophenyl ester.

[85] As used herein, the terms “DARPin” and “(designed) ankyrin repeat protein” are used interchangeably to refer to certain genetically engineered antibody mimetic proteins typically exhibiting preferential (sometimes specific) target binding. The target may be protein, carbohydrate, or other chemical entities, and the binding affinity can be quite high. The DARPins may be derived from natural ankyrin repeat-containing proteins, and specifically consist of at least three, usually four or five ankyrin repeat motifs (typically about 33 residues in each ankyrin repeat motif) of these proteins. In certain embodiments, a DARPin contains about four- or five-repeats, and may have a molecular mass of about 14 or 18 kDa, respectively. Libraries of DARPins with randomized potential target interaction residues with diversities of over 10^{12} variants can be generated at the DNA level, for use in selecting DARPins that bind desired targets (*e.g.*, acting as receptor agonists or antagonists, inverse agonists, enzyme inhibitors, or simple target protein binders) with picomolar affinity and specificity, using a variety of technologies such as ribosome display or signal recognition particle (SRP) phage display. See, for example, U.S. Patent Publication Nos. 2004/0132028, 2009/0082274, 2011/0118146, and 2011/0224100, WO 02/20565 and WO 06/083275 (the entire teachings of which are incorporated herein by reference), and also see C. Zahnd *et al.*, *Cancer Res.* (2010) 70:1595-1605; Zahnd *et al.*, *J. Biol. Chem.* (2006) 281(46):35167-35175; and Binz, H.K., Amstutz, P. & Pluckthun, A., *Nature Biotechnology* (2005) 23:1257-1268 (all incorporated herein by reference). Also see U.S. Patent Publication No. 2007/0238667; U.S. Patent No. 7,101,675; WO 2007/147213; and WO 2007/062466 (the entire teachings of which are incorporated herein by reference), for the related ankyrin-like repeats protein or synthetic peptide. As used herein, “AVIBODYTM” cell binding agents (or “AVIBODY CBA” in short) include a family of proteins as cell binding agents that specifically bind desired targets. As is well known, antibodies bind such desired targets through “Target Binding Regions” (TBRs) or Fv domains. AVIBODYTM CBA typically contains two, three, or four TBRs more commonly known as Dia-, Tria- and Tetra-bodies. These TBRs / Fv domains are linked together by fusing the Fv V-domains together in a “head to tail” orientation, forming stable, specific, and highly customizable multimeric antibody-like

proteins as AVIBODY™ CBA. See, for example, U.S. Publication Nos. 2008/0152586 and 2012/0171115 for details, the entire teachings of which are incorporated herein by reference.

[86] The term “**cell binding agent**” as used herein refers to a compound that can bind a cell (*e.g.*, on a cell-surface ligand) or bind a ligand associated with or proximate to the cell, either in a specific or non-specific manner. In certain embodiments, binding to the cell or a ligand on or near the cell is specific. The cell-binding agent may be of any kind presently known, or that become known and includes peptides and non-peptides.

[87] In certain embodiments, the cell-binding agents are proteins or polypeptides, or compounds comprising proteins or polypeptides, including antibody and non-antibody proteins or polypeptides. Specifically, the cell-binding agents (*e.g.*, proteins or polypeptides) comprise one or more Cys residues. The side chain -SH group of the Cys residues may be intact, or may be in a disulfide bond that can be reduced. Specifically, reduction of the disulfide bond(s) does not significantly negatively impact the cell-binding function of the proteins or polypeptides (*e.g.*, in the case of antibody or antigen-binding portion thereof, reduction of the disulfide bonds does not substantially increase the dissociation of light chains / heavy chains). Alternatively or in addition, a cell-binding agent (*e.g.*, a protein such as an antibody, or polypeptide) may be a part of a modified cell-binding agent, which is linked to a spacer connected to a branched scaffold, which branched scaffold is capable of being connected to two or more cytotoxic drugs, each through a reactive functional group attached to a spacer linked to the branched scaffold. An exemplary such modified cell-binding agent is represented in Formula (IV). The reactive functional group can be -SH, -C(=O)-, -NHNH₂, -N₃, -alkyne, tetrazine, strained cycloalkene or strained heterocycloalkene, dithioester, or diene (see, for example, Vu Hong *et al.*, *Bioconjugate Chem.* (2010) 21(10):1912–1916; Glassner, M. *et al.*, *J. Am. Chem. Soc.* (2012) 134:7274–7277; Hansell, C. F. *et al.*, *J. Am. Chem. Soc.* (2011) 133:13828–13831; Neal K. Devaraj, *Synlett* (2012) 23(15):2147–2152; Chenoweth, K. *et al.*, *Organic & Biomolecular Chemistry* (2009) (24):5255; Jewett, J.C. *et al.*, *J. Am. Chem. Soc.* (2010) 132(11):3688–3690; Seitchik J.L. *et al.*, *J. Am. Chem. Soc.* (2012) 134(6):2898–2901; and Sletten E.M. *et al.*, *Angew Chem. Int. Ed. Engl.* (2009) 48(38):6974–6998). The reactive functional group can be introduced into the modified cell-binding agent through any chemical or enzymatic method known in the art. See, for example, Davis L.K. *et al.*, *J. Am. Chem. Soc.* (2012) 134:10317–10320; Boeggeman E, *et al.*, *Bioconjug Chem.* (2009 Jun 20) (6):1228–36; Stan AC, *et al.*, *Cancer Res.* (1999 Jan 1) 59(1):115–21; Mahal, L. K. *et al.*, *Science* (1997) 276:1125–1128; Saxon, E., and Bertozzi, C. R., *Science* (2000) 287:2007–2010; Hang, H. C. *et al.*, *Proc. Natl.*

Acad. Sci. USA (2003) 100:14846–14851; Vocadlo, D. J. *et al.*, *Proc. Natl. Acad. Sci. USA* (2003) 100:9116–9121; Prescher, J. A. *et al.*, *Nature* (2004) 430:873–877; Dube, D. H. *et al.*, *Proc. Natl. Acad. Sci. USA* (2006) 103:4819–4824; Jeger S, *et al.*, *Angew Chem Int Ed Engl.* (2010 Dec 17) 49(51):9995-9997; and Lang K. *et al.*, *J. Am. Chem. Soc.* (2012) 134:10317-10320.

[88] Cell-binding agent can also be peptides derived from phage display (see, for example, Wang *et al.*, *Proc. Natl. Acad. Sci. USA* (2011) 108(17):6909-6914) or peptide library techniques (see, for example, Dane *et al.*, *Mol. Cancer. Ther.* (2009) 8(5):1312-1318).

[89] In one embodiment, one or more reactive functional groups that are capable of reacting with the cytotoxic drug can be introduced into the modified cell-binding agent (e.g., Formula (IV)) by any methods known in the art. For example, a terminal amine group on the modified cell-binding agent can be converted to a carbonyl group through transamination reaction (see, for example, US 2010/0099649; *Angew. Chem. Int. Ed.*, 45(32):5307 (2006); *Chem. Biol.*, 2(4):247 (2007); *J. Am. Chem. Soc.*, 130(35):11762, 2008).

[90] The cell-binding agent may be linked to a spacer through a free Cys residue, which can be engineered into the cell-binding agent if necessary (*i.e.*, cysteine residues having a free -SH group that can react with a reactive functional group attached to a spacer) according to any methods known in the art (see, for example, US 7,521,541). In another alternative, thiol groups (-SH) can be generated by controlled reduction of interchain disulfides of antibodies, followed by treatment with a maleimido group as the reactive functional group, as described in US patent 7,659,241. Thiol groups can also be introduced into the cell-binding agent (*e.g.*, antibodies) by reaction with a crosslinking agent such as 2-iminothiolane (see for example Goff and Carroll, *Bioconjugate Chem.* (1990) 1(6):381–386) followed by reaction with a maleimido group to form a modified cell-binding agent. All these methods for introducing reactive functional groups are applicable of being used for cell-binding agents that are not antibodies, which, for example, include Centyrin, DARPin, Avibody, adnectin or antibody fragment, such as minibodies, diabodies, tribodies, tetrabodies, nanobodies, probodies, domain bodies or unibodies.

[91] In one embodiment, when the cell-binding agent is a Centyrin, one or more reactive functional groups (*e.g.*, a cysteine having a free thiol group) can be introduced according to methods described in US 2010/0255056, US 2010/0216708 and US 2011/0274623.

[92] In another embodiment, the cell-binding agent is a DARPin and it can be prepared according to methods described in US Publication Nos. 2004/0132028, 2009/0082274, 2011/0118146, and 2011/0224100, WO 02/20565 and WO 06/083275. Specifically, DARPin

comprises one or more cysteine residues at specific positions that do not interfere with antigen binding. Such cysteine residue can react with the reactive functional groups attached to a spacer.

[93] In yet another embodiment, Avibodies having one or more cysteine residues can be prepared according to methods described in US 2008/0139791 and US 2012/0171115.

[94] The Cys side chain -SH groups may react with a reactive functional group described above covalently linked to a spacer connected to the branched scaffold, which can in turn be linked to two or more cytotoxic compounds through additional reactive functional groups attached to spacers, thus conjugating the cell-binding agents to the cytotoxic compounds to yield the conjugates of the invention (*e.g.*, conjugates of Formula (I)). Each protein-based cell-binding agents may contain multiple Cys side chain -SH groups, each available for linking the cell-binding agent to a spacer.

[95] Examples of the cell binding agents include an antibody, a single chain antibody, an antibody fragment that specifically binds to the target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that specifically binds to a target cell, a chimeric antibody, a chimeric antibody fragment that specifically binds to the target cell, a bispecific antibody, a domain antibody, a domain antibody fragment that specifically binds to the target cell, an interferon, a lymphokine (*e.g.*, IL-2, IL-3, IL-4, and IL-6), a hormone (*e.g.*, insulin, thyrotropin releasing hormone, melanocyte-stimulating hormone, and a steroid hormone (*e.g.*, androgen and estrogen)), a vitamin (*e.g.*, folate), a growth factor (*e.g.*, EGF, TGF- α , FGF, VEGF), a colony stimulating factor, a nutrient-transport molecule (*e.g.*, transferrin; see O'Keefe *et al.*, *J. Biol. Chem.* (1985) 260:932-937, incorporated herein by reference), a Centyrin (a protein scaffold based on a consensus sequence of fibronectin type III (FN3) repeats; see U.S. Patent Publication Nos. 2010/0255056, 2010/0216708 and 2011/0274623 incorporated herein by reference), an Ankyrin Repeat Protein (*e.g.*, a designed ankyrin repeat protein, known as DARPin; see U.S. Patent Publication Nos. 2004/0132028, 2009/0082274, 2011/0118146, and 2011/0224100, incorporated herein by reference, and also see C. Zahnd *et al.*, *Cancer Res.* (2010) 70:1595-1605; Zahnd *et al.*, *J. Biol. Chem.* (2006) 281(46):35167-35175; and Binz, H.K., Amstutz, P. & Pluckthun, A., *Nature Biotechnology* (2005) 23:1257-1268, incorporated herein by reference), an ankyrin-like repeats protein or synthetic peptide (see *e.g.*, U.S. Patent Publication No. 2007/0238667; U.S. Patent No. 7,101,675; WO 2007/147213; and WO 2007/062466, incorporated herein by reference), an Adnectin (a fibronectin domain scaffold protein; see US Patent Publication Nos. 2007/0082365; 2008/0139791, incorporated

herein by reference), Avibody (including diabodies, triabodies, and tetrabodies; see U.S. Publication Nos. 2008/0152586 and 2012/0171115), and other cell-binding molecules or substances.

[96] In certain embodiments, the cell-binding agent is an antibody, a single chain antibody, an antibody fragment that specifically binds to the target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that specifically binds to a target cell, a chimeric antibody, a chimeric antibody fragment that specifically binds to the target cell, a domain antibody, a domain antibody fragment that specifically binds to the target cell, a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, or a nutrient-transport molecule. Alternatively, the cell-binding agent is a monoclonal antibody, a single chain monoclonal antibody, or a monoclonal antibody fragment that specifically binds to a target cell.

[97] In certain embodiments, the cell-binding agent is a bispecific antibody, an ankryrin repeat protein, a Centyrin, or an Avibody.

[98] **“Antibody fragment”** and **“antigen-binding portion or fragment”** are used interchangeably here to refer to Fab, Fab', and F(ab')₂, Fv, minibodies, diabodies, tribodies, tetrabodies, nanobodies, probodies, domain bodies, unibodies, and the like (Parham, *J. Immunol.* 131:2895-2902 (1983); Spring *et al.*, *J. Immunol.* 113:470-478 (1974); Nisonoff *et al.*, *Arch. Biochem. Biophys.* 89:230-244 (1960), Kim *et al.*, *Mol. Cancer Ther.*, 7:2486-2497 (2008), Carter, *Nature Revs.*, 6:343-357 (2006), R. Kontermann & S. Dubel, 2001 *Antibody Engineering*, Springer-Verlag, Heidelberg-New York).

[99] In certain embodiments, the cell-binding agent is a minibody, a diabody, a tribody, a tetrabody, a nanobody, a probody, a domain body, or an unibody.

[100] Monoclonal antibody techniques allow for the production of extremely specific cell-binding agents in the form of specific monoclonal antibodies. Particularly well known in the art are techniques for creating monoclonal antibodies 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 such as viral coat proteins. Sensitized human cells can also be used. Another method of creating monoclonal antibodies is the use of phage libraries of scFv (single chain variable region), specifically human scFv (*see e.g.*, Griffiths *et al.*, U.S. Patent Nos. 5,885,793 and 5,969,108; McCafferty *et al.*, WO 92/01047; Liming *et al.*, WO 99/06587). In addition, resurfaced antibodies disclosed in U.S. Patent No. 5,639,641 may also be used, as may chimeric antibodies and humanized antibodies.

[101] Selection of the appropriate cell-binding agent is a matter of choice that depends upon the particular cell population that is to be targeted, but in general human monoclonal antibodies are preferred if an appropriate one is available. For example, the monoclonal antibody MY9 is a murine IgG₁ antibody that binds specifically to the CD33 Antigen (J.D. Griffin *et al.*, *Leukemia Res.* (1984) 8:521) and can be used if the target cells express CD33 as in the disease of acute myelogenous leukemia (AML).

[102] In one embodiment, the cell-binding agent is a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment.

[103] In another embodiment, the cell-binding agent is a humanized antibody, a humanized single chain antibody, or a humanized antibody fragment. In a specific embodiment, the humanized antibody is huMy9-6 or another related antibody, which is described in U.S. Pat. Nos. 7,342,110 and 7,557,189. In another specific embodiment, the humanized antibody is an anti-folate receptor antibody described in U.S. Provisional Application Nos. 61/307,797, 61/346,595, and 61/413,172 and U.S. Application No. 13/033,723 (published as US 2012/0009181 A1). The teachings of all these applications are incorporated herein by reference in its entirety.

[104] In certain embodiments, the cell-binding agent is an antigen-binding portion of a monoclonal antibody, sharing sequences critical for antigen-binding with an antibody disclosed herein, such as huMy9-6 or its related antibodies described in U.S. Pat. Nos. 7,342,110 and 7,557,189, incorporated herein by reference. These derivative antibodies may have substantially the same or identical (1) light chain and/or heavy chain CDR3 regions; (2) light chain and/or heavy chain CDR1, CDR2, and CDR3 regions; or (3) light chain and/or heavy chain regions, compared to an antibody described herein. Sequences within these regions may contain conservative amino acid substitutions, including substitutions within the CDR regions. Specifically, there is no more than 1, 2, 3, 4, or 5 conservative substitutions. In an alternative, the derivative antibodies have a light chain region and/or a heavy chain region that is at least about 90%, 95%, 99% or 100% identical to an antibody described herein. These derivative antibodies may have substantially the same binding specificity and/or affinity to the target antigen compared to an antibody described herein. Specifically, the K_d and/or k_{off} values of the derivative antibodies are within 10-fold (either higher or lower), 5-fold (either higher or lower), 3-fold (either higher or lower), or 2-fold (either higher or lower) of an antibody described herein. These derivative antibodies may be fully human antibodies, or humanized antibodies, or chimeric antibodies. The derivative antibodies may be produced according to any art-recognized methods.

[105] Specific exemplary antigens or ligands include renin; a growth hormone (*e.g.*, human growth hormone and bovine growth hormone); a growth hormone releasing factor; a parathyroid hormone; a thyroid stimulating hormone; a lipoprotein; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; a follicle stimulating hormone; calcitonin; a luteinizing hormone; glucagon; a clotting factor (*e.g.*, factor vmc, factor IX, tissue factor, and von Willebrands factor); an anti-clotting factor (*e.g.*, Protein C); an atrial natriuretic factor; a lung surfactant; a plasminogen activator (*e.g.*, a urokinase, a human urine or tissue-type plasminogen activator); bombesin; a thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; an enkephalinase; RANTES (*i.e.*, the regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein-1-alpha; a serum albumin (human serum albumin); Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; a mouse gonadotropin-associated peptide; a microbial protein (beta-lactamase); DNase; IgE; a cytotoxic T-lymphocyte associated antigen (*e.g.*, CTLA-4); inhibin; activin; a vascular endothelial growth factor; protein A or D; a rheumatoid factor; a neurotrophic factor (*e.g.*, bone-derived neurotrophic factor, neurotrophin-3, -4, -5, or -6), a nerve growth factor (*e.g.*, NGF- β); a platelet-derived growth factor; a fibroblast growth factor (*e.g.*, aFGF and bFGF); fibroblast growth factor receptor 2; an epidermal growth factor; a transforming growth factor (*e.g.*, TGF-alpha, TGF- β 1, TGF- β 2, TGF- β 3, TGF- β 4, and TGF- β 5); insulin-like growth factor-I and -II; des(1-3)-IGF-I (brain IGF-I); an insulin-like growth factor binding protein; melanotransferrin; EpCAM; GD3; FLT3; PSMA; PSCA; MUC1; MUC16; STEAP; CEA; TENB2; an EphA receptor; an EphB receptor; a folate receptor; FOLR1; mesothelin; cripto; an $\alpha_v\beta_6$; integrins; VEGF; VEGFR; EGFR; transferrin receptor; IRTA1; IRTA2; IRTA3; IRTA4; IRTA5; CD proteins (*e.g.*, CD2, CD3, CD4, CD5, CD6, CD8, CD11, CD14, CD19, CD20, CD21, CD22, CD25, CD26, CD28, CD30, CD33, CD36, CD37, CD38, CD40, CD44, CD52, CD55, CD56, CD59, CD70, CD79, CD80, CD81, CD103, CD105, CD134, CD137, CD138, and CD152), one or more tumor-associated antigens or cell-surface receptors (see US Publication No. 20080171040 or US Publication No. 20080305044, incorporated in their entirety by reference); erythropoietin; an osteoinductive factor; an immunotoxin; a bone morphogenetic protein; an interferon (*e.g.*, interferon-alpha, -beta, and -gamma); a colony stimulating factor (*e.g.*, M-CSF, GM-CSF, and G-CSF); interleukins (*e.g.*, IL-1 to IL-10); a superoxide dismutase; a T-cell receptor; a surface membrane protein; a decay accelerating factor; a viral antigen (*e.g.*, a portion of the HIV envelope); a transport protein, a homing receptor; an addressin; a regulatory protein; an integrin (*e.g.*, CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4, and VCAM); a tumor

associated antigen (*e.g.*, HER2, HER3 and HER4 receptor); endoglin; c-Met; c-kit; IGF1R; PSGR; NGEF; PSMA; PSCA; TMEFF2; LGR5; B7H4; and fragments of any of the above-listed polypeptides.

[106] For example, GM-CSF, a ligand / growth factor which binds to myeloid cells can be used as a cell-binding agent to diseased cells from acute myelogenous leukemia. IL-2 which binds to activated T-cells can be used for prevention of transplant graft rejection, for therapy and prevention of graft-versus-host disease, and for treatment of acute T-cell leukemia.

MSH, which binds to melanocytes, can be used for the treatment of melanoma, as can antibodies directed towards melanomas. Folic acid can be used to target the folate receptor expressed on ovarian and other tumors. Epidermal growth factor can be used to target squamous cancers, such as lung and head and neck. Somatostatin can be used to target neuroblastomas and other tumor types. Estrogen (or estrogen analogues) can be used to target breast cancer. Androgen (or androgen analogues) can be used to target testes.

[107] The term “**salt**” as used herein refers to organic or inorganic salts of a compound of the invention. Specifically, a salt is a pharmaceutically acceptable salt. Other non-pharmaceutically acceptable salts are also included in the present invention. The salts include salts, formed by reacting a compound of the invention, which comprises a basic group, with an inorganic acid or organic acid (such as a carboxylic acid), and salts, formed by reacting a compound of the invention, which comprises an acidic group, with an inorganic base or organic base (such as an amine). Exemplary salts include those pharmaceutically acceptable salts described immediately below.

[108] The term “**pharmaceutically acceptable salt**” as used herein refers to pharmaceutically acceptable organic or inorganic salts of a compound of the invention. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate “mesylate,” ethanesulfonate, benzenesulfonate, p-toluenesulfonate, pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts, alkali metal (*e.g.*, sodium and potassium) salts, alkaline earth metal (*e.g.*, magnesium) salts, and ammonium salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counter ion. The counter ion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure.

Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counter ion.

[109] If the compound of the invention contains one or more basic moieties, desired salts (*e.g.*, pharmaceutically acceptable salts) may be prepared by any suitable method available in the art, for example, treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, methanesulfonic acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, a pyranosidyl acid, such as glucuronic acid or galacturonic acid, an alpha hydroxy acid, such as citric acid or tartaric acid, an amino acid, such as aspartic acid or glutamic acid, an aromatic acid, such as benzoic acid or cinnamic acid, a sulfonic acid, such as p-toluenesulfonic acid or ethanesulfonic acid, or the like.

[110] If the compound of the invention contains one or more acidic moieties, desired salts (*e.g.*, pharmaceutically acceptable salts) may be prepared by any suitable method, for example, treatment of the free acid with an inorganic, such as an alkali metal hydroxide or alkaline earth metal hydroxide, organic base, such as an amine (primary, secondary or tertiary), or the like. Illustrative examples of suitable salts include, but are not limited to, organic salts derived from amino acids, such as glycine and arginine, ammonia, primary, secondary, and tertiary amines, and cyclic amines, such as piperidine, morpholine and piperazine, and inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum and lithium.

[111] The terms “**abnormal cell growth**” and “**proliferative disorder**” are used interchangeably in this application. “**Abnormal cell growth**,” as used herein, unless otherwise indicated, refers to cell growth that is independent of normal regulatory mechanisms (*e.g.*, loss of contact inhibition). This includes, for example, the abnormal growth of: (1) tumor cells (tumors) that proliferate by expressing a mutated tyrosine kinase or overexpression of a receptor tyrosine kinase; (2) benign and malignant cells of other proliferative diseases in which aberrant tyrosine kinase activation occurs; (3) any tumors that proliferate by receptor tyrosine kinases; (4) any tumors that proliferate by aberrant serine/threonine kinase activation; and (5) benign and malignant cells of other proliferative diseases in which aberrant serine/threonine kinase activation occurs.

[112] The terms “**cancer**” and “**cancerous**” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. A “**tumor**” comprises

one or more cancerous cells, and/or benign or pre-cancerous cells. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include skin cancer (*e.g.*, melanoma), Merkel cell carcinoma, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (*e.g.*, gastrointestinal cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, testicular cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, acute leukemia, head and neck cancer, brain cancer (*e.g.*, glioblastoma and neuroblastoma), cancers of lymphatic organs and hematological malignancy including Leukemia (Acute lymphoblastic leukemia (ALL), Acute myelogenous leukemia (AML), Chronic lymphocytic leukemia (CLL), Chronic myelogenous leukemia (CML), Acute monocytic leukemia (AMOL), Hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), Large granular lymphocytic leukemia, Adult T-cell leukemia), Lymphoma (small lymphocytic lymphoma (SLL), Hodgkin's lymphomas (Nodular sclerosis, Mixed cellularity, Lymphocyte-rich, Lymphocyte depleted or not depleted, and Nodular lymphocyte-predominant Hodgkin lymphoma), Non-Hodgkin's lymphomas (all subtypes), Chronic lymphocytic leukemia/Small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma (such as Waldenström macroglobulinemia), Splenic marginal zone lymphoma, Plasma cell neoplasms (Plasma cell myeloma, Plasmacytoma, Monoclonal immunoglobulin deposition diseases, Heavy chain diseases), Extranodal marginal zone B cell lymphoma (MALT lymphoma), Nodal marginal zone B cell lymphoma (NMZL), Follicular lymphoma, Mantle cell lymphoma, Diffuse large B cell lymphoma, Mediastinal (thymic) large B cell lymphoma, Intravascular large B cell lymphoma, Primary effusion lymphoma, Burkitt lymphoma/leukemia, T cell prolymphocytic leukemia, T cell large granular lymphocytic leukemia, Aggressive NK cell leukemia, Adult T cell leukemia/lymphoma, Extranodal NK/T cell lymphoma (nasal type), Enteropathy-type T cell lymphoma, Hepatosplenic T cell lymphoma, Blastic NK cell lymphoma, Mycosis fungoides / Sezary syndrome, Primary cutaneous CD30-positive T cell lymphoproliferative disorders, Primary cutaneous anaplastic large cell lymphoma, Lymphomatoid papulosis, Angioimmunoblastic T cell lymphoma, Peripheral T cell lymphoma (unspecified), Anaplastic large cell lymphoma), multiple myeloma (plasma cell myeloma or Kahler's disease).

[113] The term “**therapeutic agent**” encompasses both a biological agent such as an antibody, a peptide, a protein, an enzyme or a chemotherapeutic agent.

[114] The “**cytotoxic compound**” is referred to as “D” or “DM” interchangeably.

[115] The term “**chemotherapeutic agent**” or “**cytotoxic drug**” includes is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Erlotinib (TARCEVA®, Genentech/OSI Pharm.), Bortezomib (VELCADE®, Millennium Pharm.), Fulvestrant (FASLODEX®, AstraZeneca), Sutent (SU11248, Pfizer), Letrozole (FEMARA®, Novartis), Imatinib mesylate (GLEEVEC®, Novartis), PTK787/ZK 222584 (Novartis), Oxaliplatin (Eloxatin®, Sanofi), 5-FU (5-fluorouracil), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafermib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs), and Gefitinib (IRESSA®, AstraZeneca), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylmelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratiastatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammall and calicheamicin omegall (*Angew Chem. Intl. Ed. Engl.* (1994) 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid,

nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiaminiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®] polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL[®] (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE[®] (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERE[®] (doxetaxel; Rhone-Poulenc Rorer, Antony, France); chloranmbucil; GEMZAR[®] (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE[®] (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA[®]); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[116] Also included in the definition of "**chemotherapeutic agent**" or "**cytotoxic drug**" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX[®]; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON[®]

(toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE[®] (megestrol acetate), AROMASIN[®] (exemestane; Pfizer), formestane, fadrozole, RIVISOR[®] (vorozole), FEMARA[®] (letrozole; Novartis), and ARIMIDEX[®] (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC- α , Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (*e.g.*, ANGIOZYME[®]) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN[®], LEUVECTIN[®], and VAXID[®]; PROLEUKIN[®] rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN[®]; ABARELIX[®] rmRH; (ix) anti-angiogenic agents such as bevacizumab (AVASTIN[®], Genentech); and (x) pharmaceutically acceptable salts, acids and derivatives of any of the above. Other anti-angiogenic agents include MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, COX-II (cyclooxygenase II) inhibitors, and VEGF receptor tyrosine kinase inhibitors. Examples of such useful matrix metalloproteinase inhibitors that can be used in combination with the present compounds/compositions are described in WO 96/33172, WO 96/27583, EP 818442, EP 1004578, WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, EP 606,046, EP 931,788, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, WO 99/07675, EP 945864, U.S. Pat. No. 5,863,949, U.S. Pat. No. 5,861,510, and EP 780,386, all of which are incorporated herein in their entireties by reference. Examples of VEGF receptor tyrosine kinase inhibitors include 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474; Example 2 within WO 01/32651), 4-(4-fluoro-2-methylindol-5-yl)oxy-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)-quinazoline (AZD2171; Example 240 within WO 00/47212), vatalanib (PTK787; WO 98/35985) and SU11248 (sunitinib; WO 01/60814), and compounds such as those disclosed in PCT Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856, and WO 98/13354).

[117] Other examples of chemotherapeutic agents / cytotoxic drugs that can be used in combination with the present compounds include inhibitors of PI3K (phosphoinositide-3 kinase), such as those reported in Yaguchi *et al* (2006) Jour. of the Nat. Cancer Inst. 98(8):545-556; U.S. Pat. No. 7,173,029; U.S. Pat. No. 7,037,915; U.S. Pat. No. 6,608,056; U.S. Pat. No. 6,608,053; U.S. Pat. No. 6,838,457; U.S. Pat. No. 6,770,641; U.S. Pat. No.

6,653,320; U.S. Pat. No. 6,403,588; WO 2006/046031; WO 2006/046035; WO 2006/046040; WO 2007/042806; WO 2007/042810; WO 2004/017950; US 2004/092561; WO 2004/007491; WO 2004/006916; WO 2003/037886; US 2003/149074; WO 2003/035618; WO 2003/034997; US 2003/158212; EP 1417976; US 2004/053946; JP 2001247477; JP 08175990; JP 08176070; U.S. Pat. No. 6,703,414; and WO 97/15658, all of which are incorporated herein in their entireties by reference. Specific examples of such PI3K inhibitors include SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis, Inc.).

[118] Chemotherapeutic agents / cytotoxic drugs may also include any of the generic drugs or biosimilars of the brand-name drugs referenced herein, or improvements thereof, including improved formulations, delivery means (sustained release, bioadhesive coating, targeted delivery *etc.*), and dosage forms.

[119] The term “**viral infection**” refers to the invasion of a host organism’s bodily tissues by disease-causing viruses. Examples of the viral infections include CMV infection, HIV infection and AIDS.

[120] The term “**parasite infection**” refers to the invasion of a host organism’s bodily tissues by disease-causing parasites. Examples of the parasite infections include giardiasis, amoebiasis, and schistosomiasis.

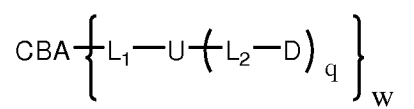
[121] The phrase “**pharmaceutically acceptable**” indicates that the substance or composition must be compatible chemically and/or toxicologically, with the other ingredients comprising a formulation, and/or the mammal being treated therewith.

[122] The term “**therapeutically effective amount**” means that amount of active compound or conjugate that elicits the desired biological response in a subject. Such response includes alleviation of the symptoms of the disease or disorder being treated, prevention, inhibition or a delay in the recurrence of symptom of the disease or of the disease itself, an increase in the longevity of the subject compared with the absence of the treatment, or prevention, inhibition or delay in the progression of symptom of the disease or of the disease itself. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Toxicity and therapeutic efficacy of compound I can be determined by standard pharmaceutical procedures in cell cultures and in experimental animals. The effective amount of compound or conjugate of the present invention or other therapeutic agent to be administered to a subject will depend on the stage, category and status of the multiple myeloma and characteristics of the subject, such as general health, age, sex, body weight and drug tolerance. The effective amount of compound

or conjugate of the present invention or other therapeutic agent to be administered will also depend on administration route and dosage form. Dosage amount and interval can be adjusted individually to provide plasma levels of the active compound that are sufficient to maintain desired therapeutic effects.

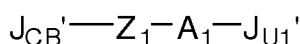
CELL BINDING AGENT-DRUG MOIETY CONJUGATES

[123] In the first embodiment, the present invention provides a conjugate represented by Formula (I) below:



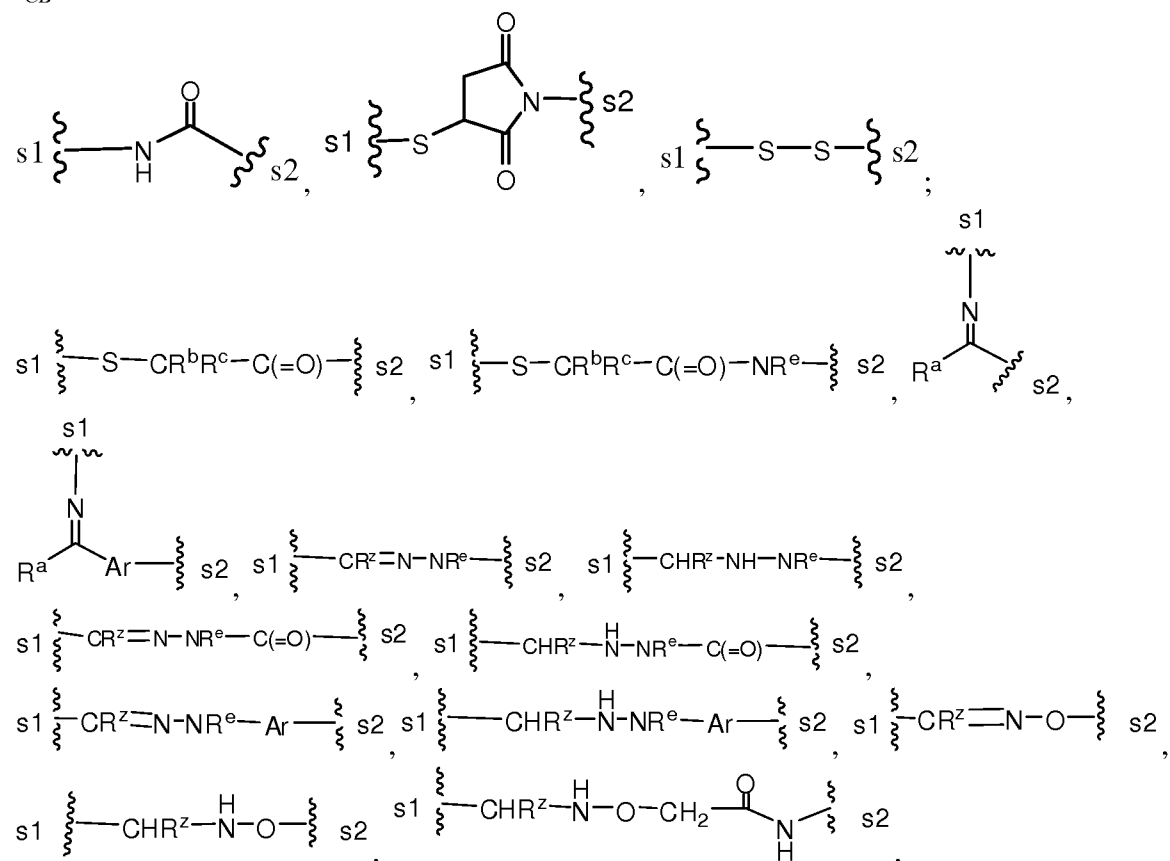
In addition, nineteen specific embodiments for the conjugate are further described below in this conjugate section.

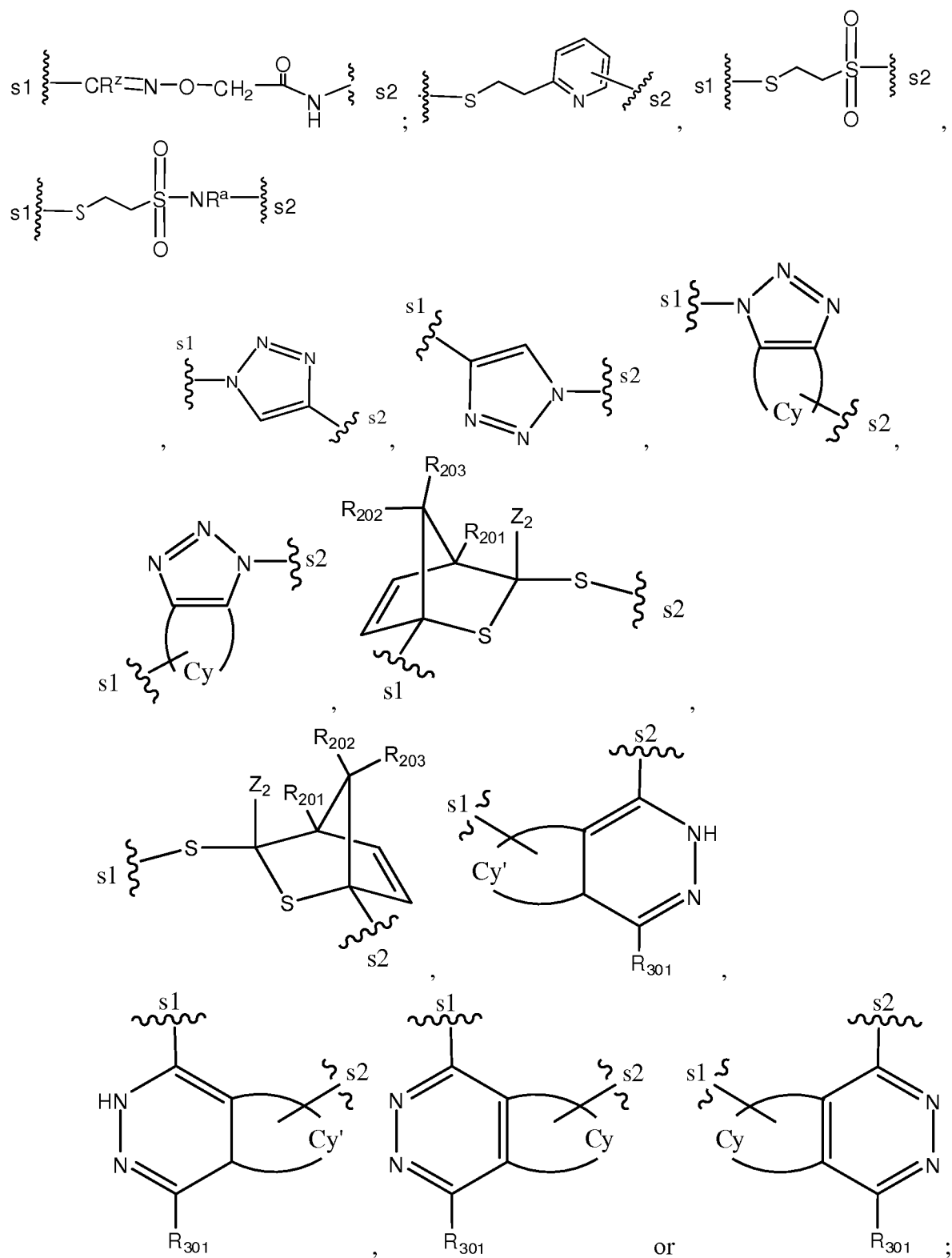
[124] In a first specific embodiment, L_1 is represented by the following formula:



in which:

$\text{J}_{\text{CB}'}$ is





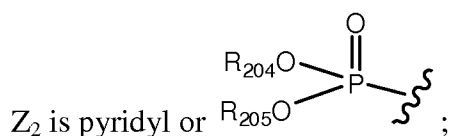
s1 is the site covalently linked to the CBA;

s2 is the site covalently linked to the group U;

Ar is an optionally substituted arylene or an optionally substituted heteroarylene;

Cy is an optionally substituted carbocyclic or heterocyclic ring;

R₂₀₁, R₂₀₂ and R₂₀₃ each are independently H or an optionally substituted alkyl;



R_{204} and R_{205} are each independently optionally substituted alkyl;

R_{301} is H or optionally substituted alkyl;

Cy' is an optionally substituted carbocyclic or heterocyclic ring;

Z_1 is $-NR_9-C(=O)-$ or $-C(=O)-NR_9-$ or absent,

A_1 is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, $-(CH_2-CH_2-O)_{p1}-(CR^bR^c)_{p1}-$ or $-(CR^bR^c)_{p1}-(O-CH_2-CH_2)_{p1}-$,

J_{U1}' is absent, $-NR_9-$ or $-C(=O)-$

$p1$ is an integer from 1 to 1000 (specifically, 1 to 24, more specifically 1 to 8 (e.g., 2, 4, 6 or 8))

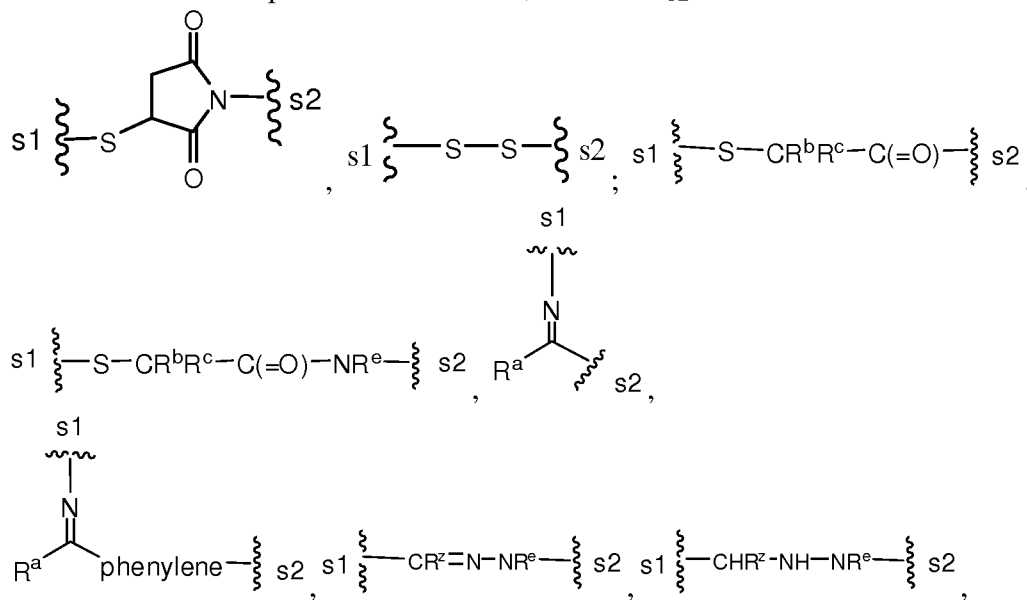
$p1'$ is an integer from 0 to 10;

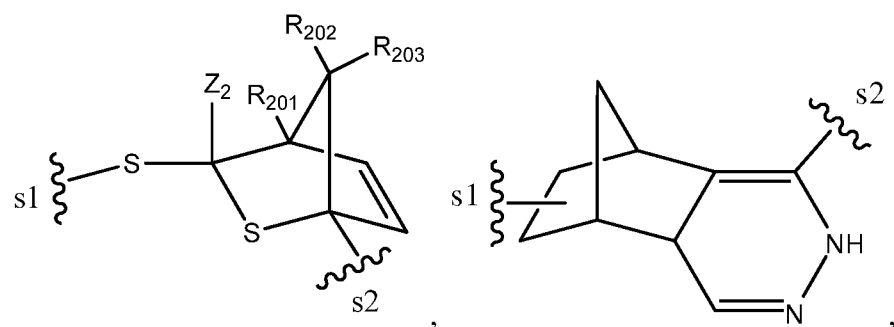
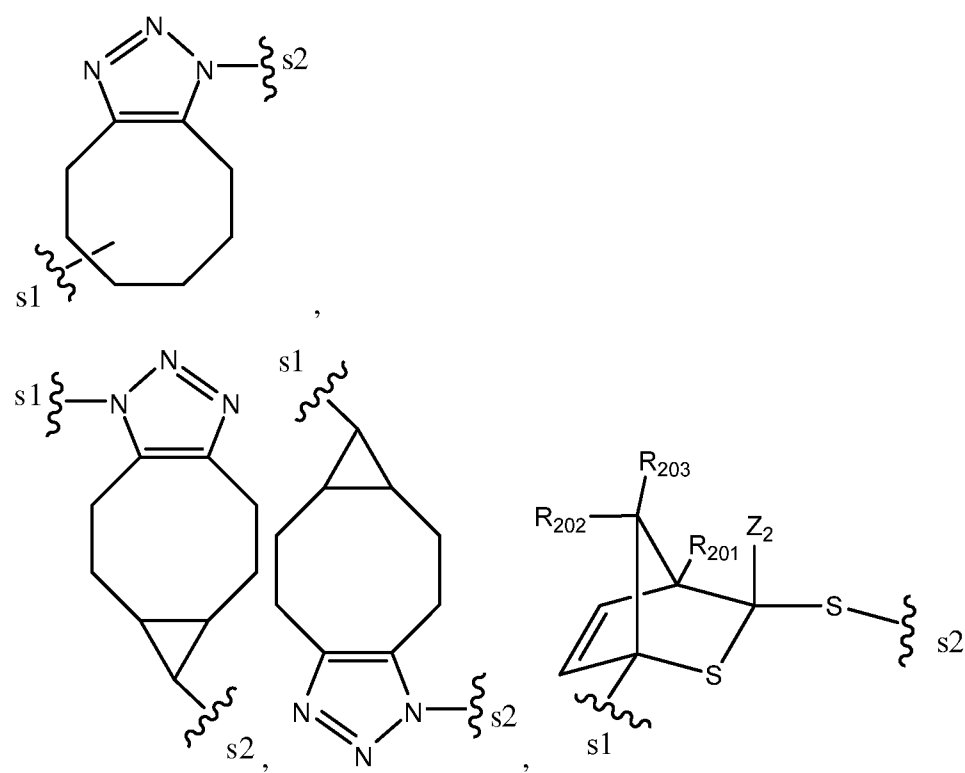
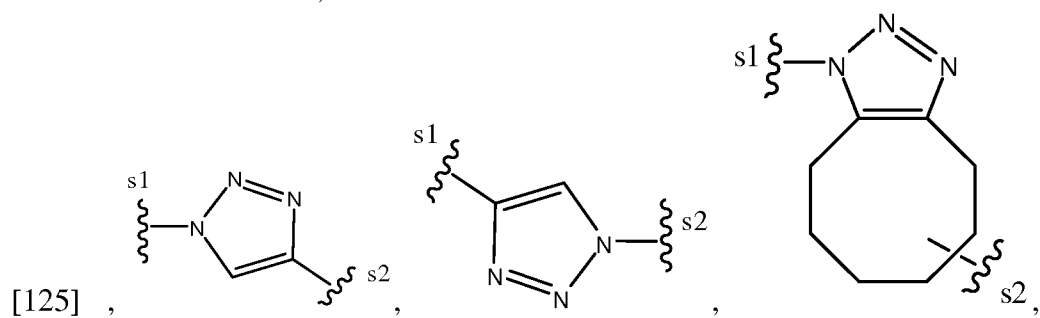
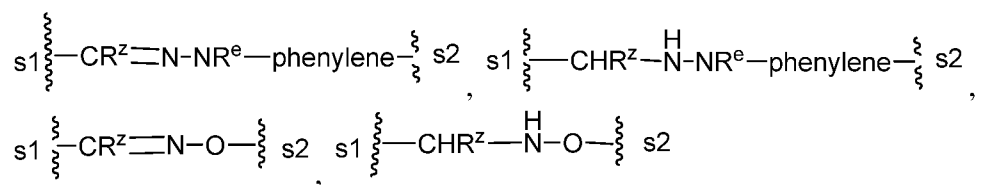
R_9 is H or an optionally substituted alkyl; and

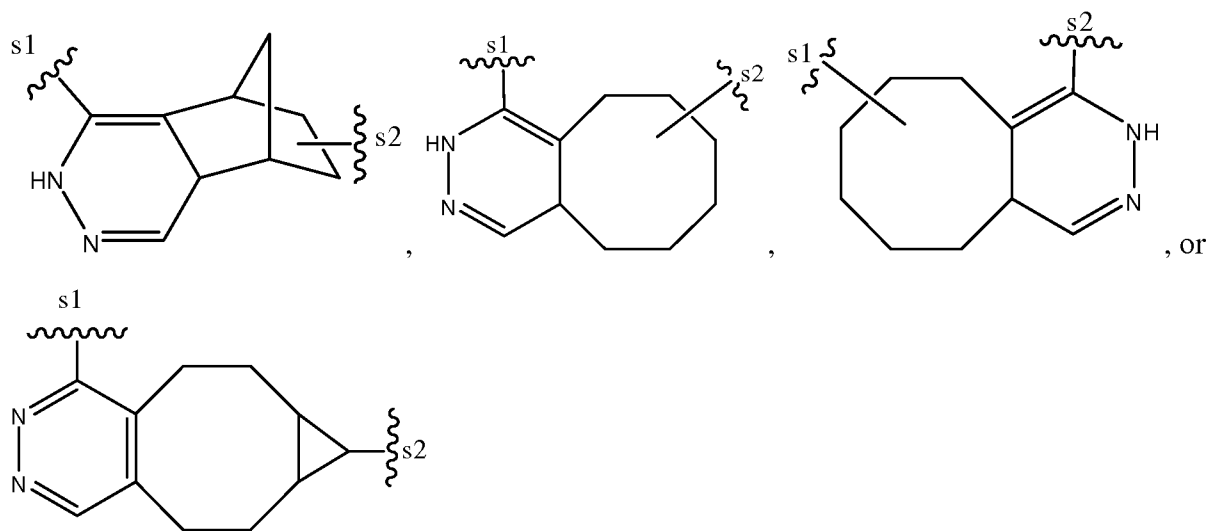
R^a , R^b , R^c and R^e , for each occurrence, are independently H or an optionally substituted alkyl.

Alternatively, $p1$ is 1 to 24. In another alternative, $p1$ is 1 to 8. In yet another alternative, $p1$ is 2, 4, 6, or 8. The remainder of the variables in Formula (I) are as described in the first embodiment.

In a second specific embodiment, variable J_{CB}' is

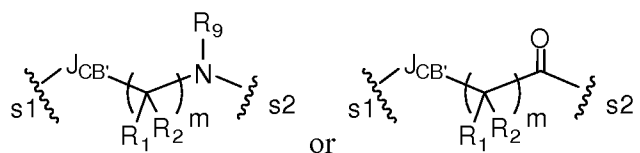






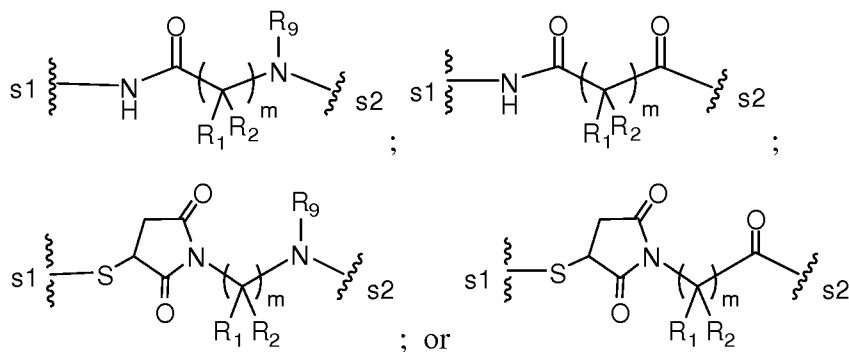
The remainder of the variables in Formula (I) or the formula designated in the first specific embodiment are as defined in the first embodiment or its first specific embodiment.

[126] In a third specific embodiment, L_1 is represented by the following formula:



in which R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10. The remainder of the variables in Formula (I) or each of the above formulas representing L_1 are as defined in the first embodiment or its first or second specific embodiment.

[127] In a fourth specific embodiment, L_1 is represented by the following formula:



in which:

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

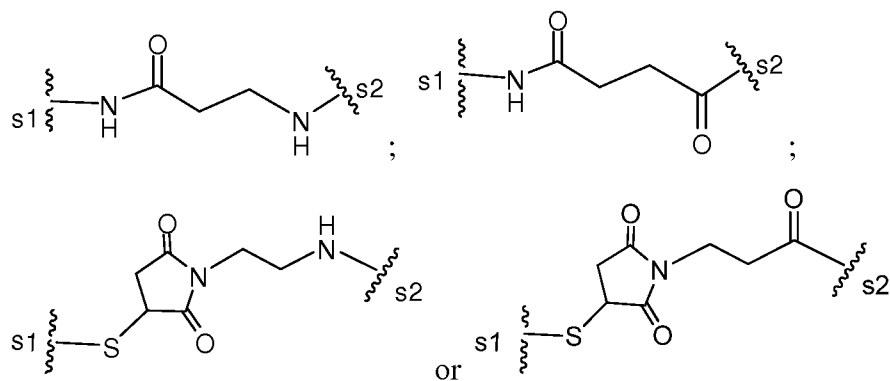
m is an integer from 1 to 5; and

R^9 is H or an alkyl.

The remainder of the variables in Formula (I) or each of the formulas in this specific embodiment are as defined in the first embodiment or its third specific embodiment.

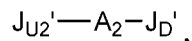
[128] In a fifth specific embodiment, R_1 and R_2 are both H; and R^9 is H. The remainder of the variables in Formula (I) or each of the formulas designated in the third or fourth specific embodiment are as defined in the first embodiment or its third or fourth specific embodiment.

[129] In a sixth specific embodiment, L_1 is represented by the following formula:



. The remainder of the variables in Formula (I) or each of the formulas designated in this specific embodiment are as defined in the first embodiment or its fifth specific embodiment.

[130] In a seventh specific embodiment, L_2 is represented by the following formula:

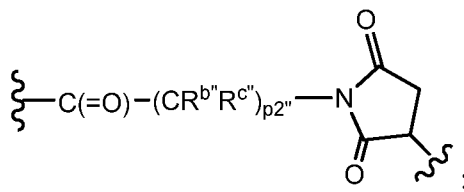


in which:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_{D}' is $-C(=O)-$, $-C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-$, $-C(=O)-(CH_2-CH_2-O)_{p2'}-(CR^{b'}R^{c'})_{p2'}-S-$, -



$S-$, absent, $-C(=O)-(CR^{b''}R^{c''})_{p2''}-S-$ or

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl (more specifically H);

$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H, an optionally substituted alkyl or a charged substituent or an ionizable group;

p_2 is an integer from 1 to 1000 (specifically 1 to 14, 1 to 8);

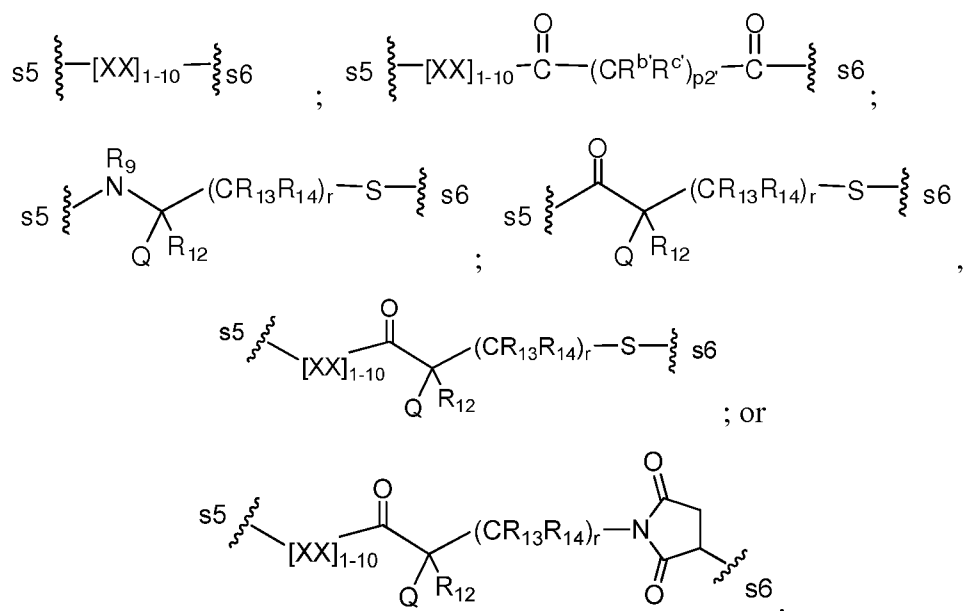
$p_{2'}$ is an integer from 1 to 10 (specifically 1 to 5); and

$p_{2''}$ is an integer from 1 to 10 (specifically 1 to 5).

Alternatively, $R^{b'}$ and $R^{c'}$ are H; p_2 is 1-14; and $p_{2'}$ is 1 to 5; and in another alternative, $R^{b'}$ and $R^{c'}$ are H; p_2 is 1-8; and $p_{2'}$ is 1 to 5. The remainder of the variables in

Formula (I) are as defined in the first embodiment or its first, second, third, fourth, fifth, or sixth specific embodiment.

[131] In an eighth specific embodiment, L_2 is represented by the following formula:



in which:

R_{12} , R_{13} , and R_{14} , for each occurrence, are each independently H, an optionally substituted alkyl;

Q is H, a charged substituent or an ionizable group;

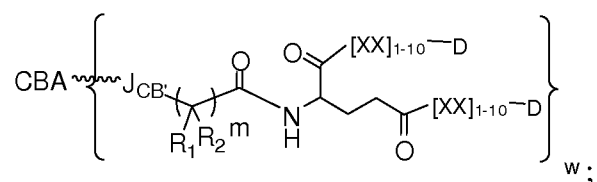
r is an integer from 0 to 10;

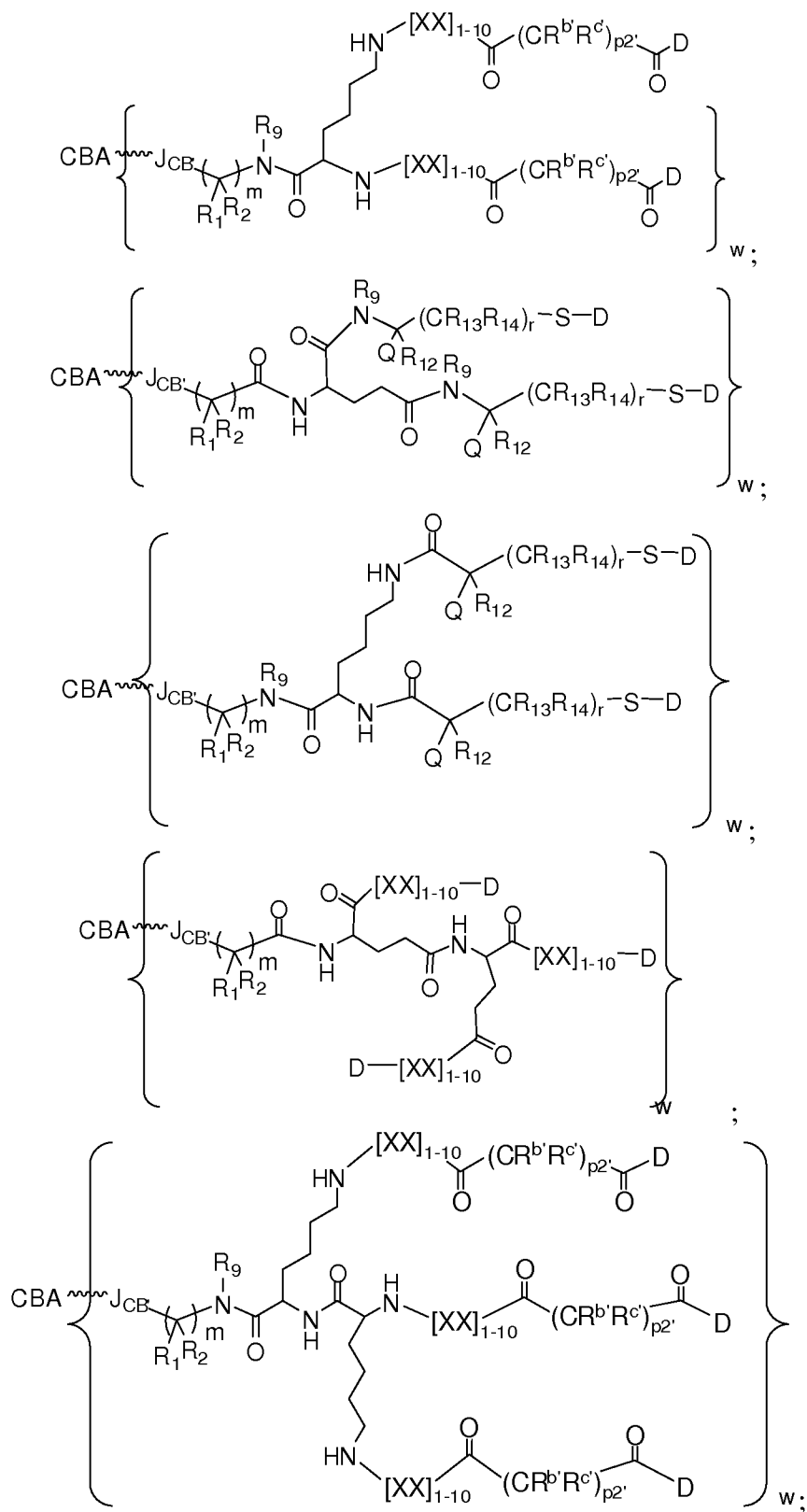
s5 is the site covalently linked to the group U; and

s6 is the site covalently linked to the group D.

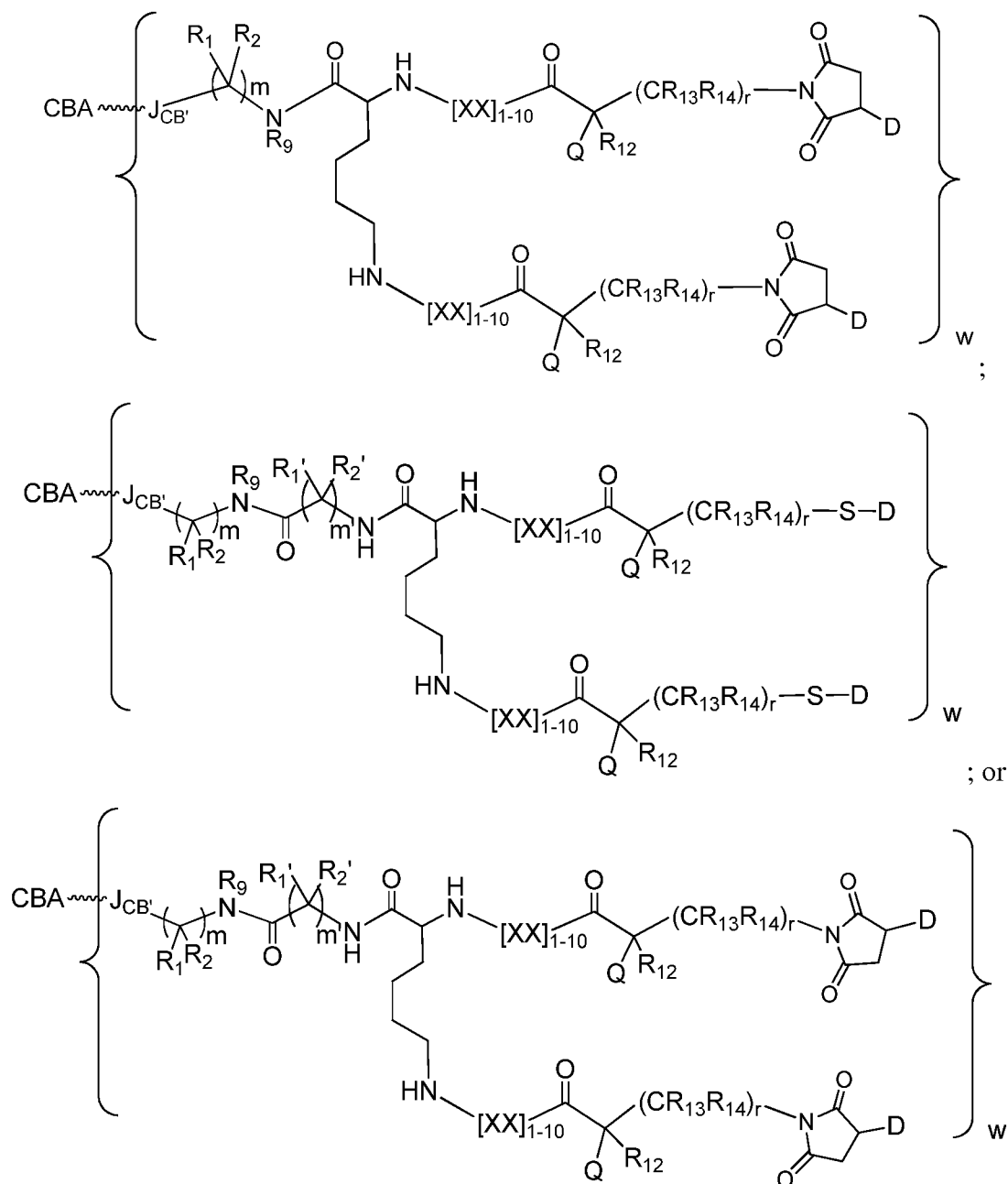
The remainder of the variables in Formula (I) or each of the formulas designated in this specific embodiment are as defined in the first embodiment or its seventh specific embodiment.

[132] In a ninth specific embodiment, the conjugate is represented by the following formula, or a pharmaceutically acceptable salt thereof:









The variables in each of the formulas designated in this specific embodiment are as defined in the first embodiment or its seventh or eighth specific embodiment.

[133] In a tenth specific embodiment, Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion. The remainder of the variables in Formula (I) or each

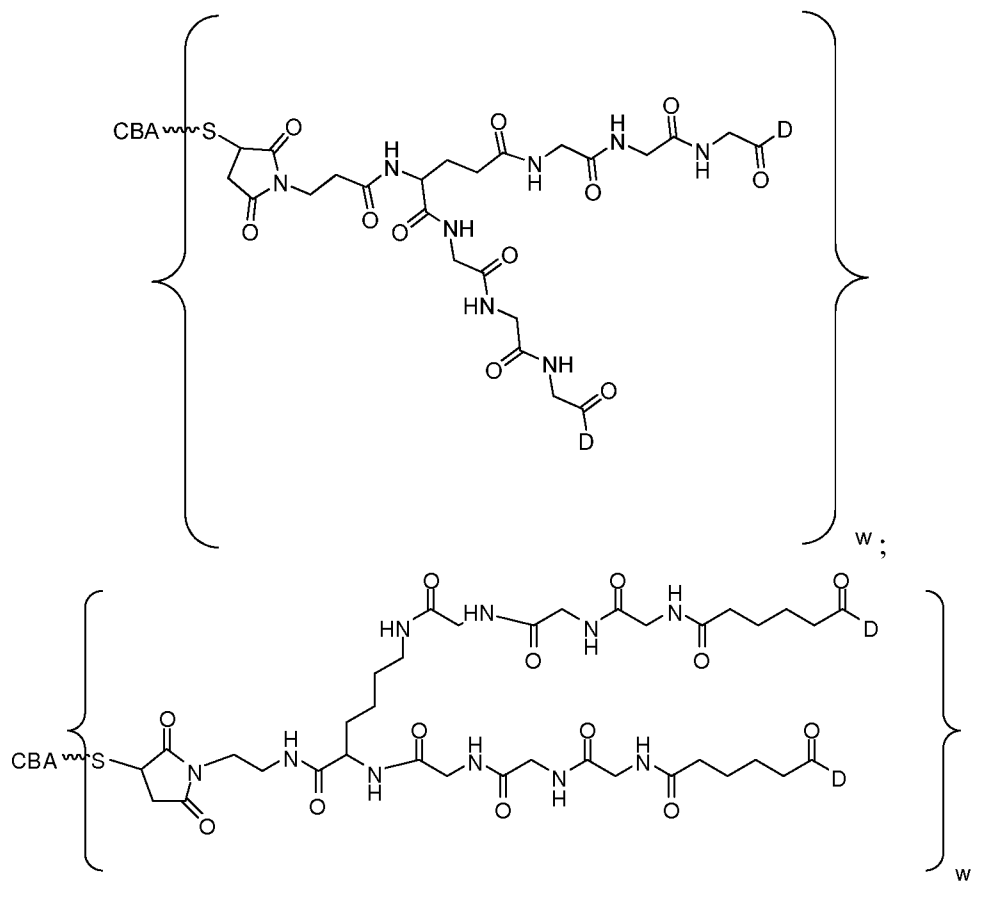
of the formulas designated in the eighth or ninth embodiment above are as defined in the first embodiment or its eighth or ninth specific embodiment.

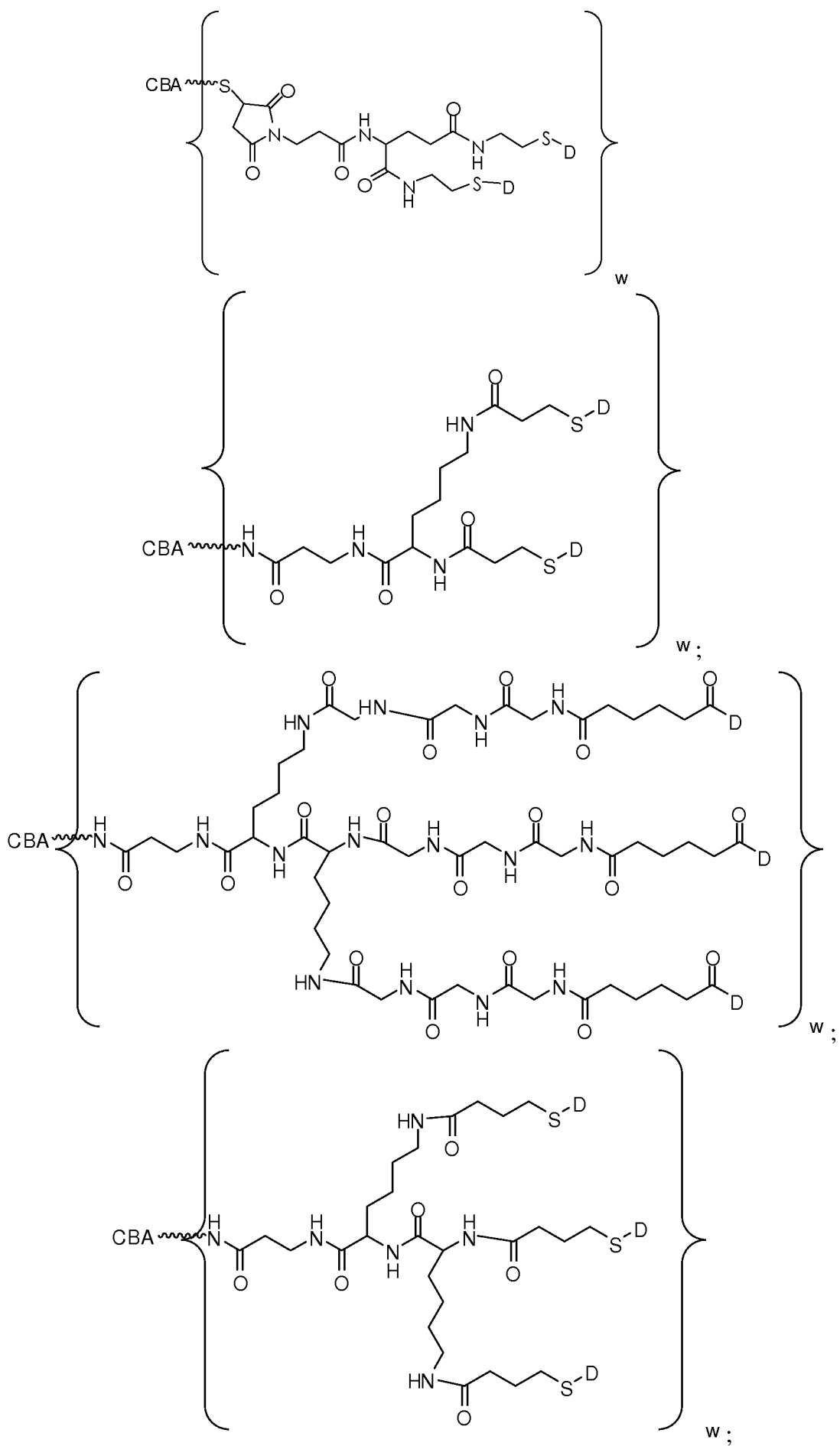
[134] In an eleventh specific embodiment, R_{12} is H; and Q is H, SO_3H or a pharmaceutically acceptable salt thereof. The remainder of the variables in Formula (I) or each of the formulas designated in the eighth or ninth embodiment above are as defined in the first embodiment or its eighth, ninth or tenth specific embodiment.

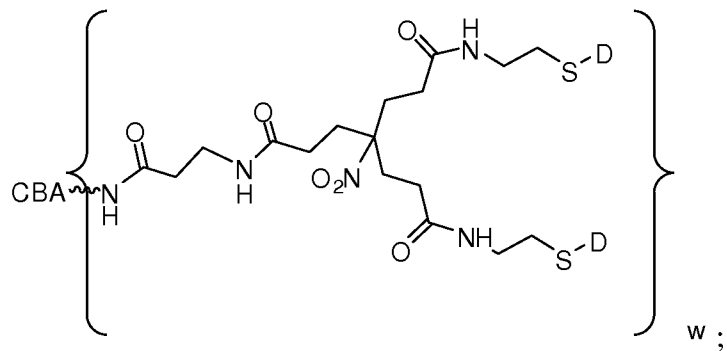
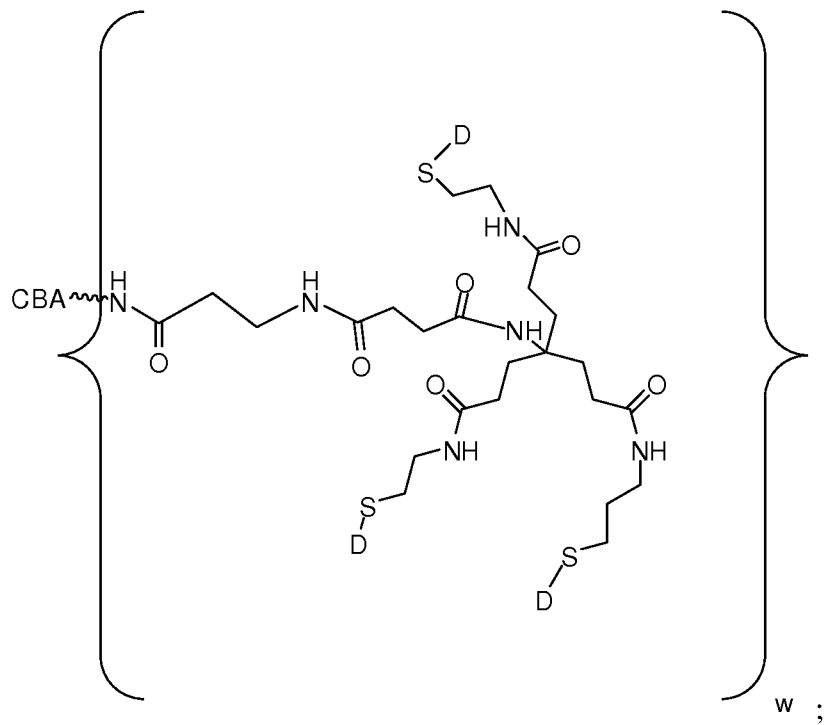
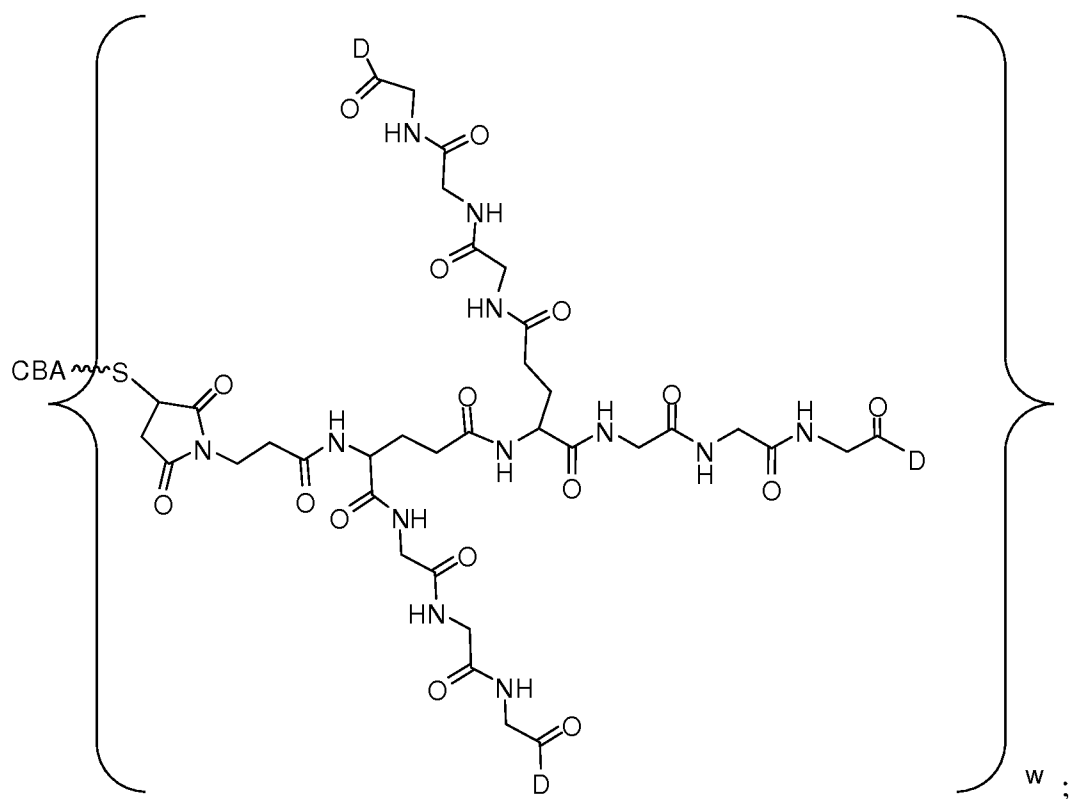
[135] In a twelfth specific embodiment, $R^{b'}$ and $R^{c'}$ are both H; and p_2' is an integer from 1 to 5. The remainder of the variables in Formula (I) or each of the formulas designated in the seventh, eighth, or ninth specific embodiment are as defined in the first embodiment or its seventh, eighth, ninth, tenth, or eleventh specific embodiment.

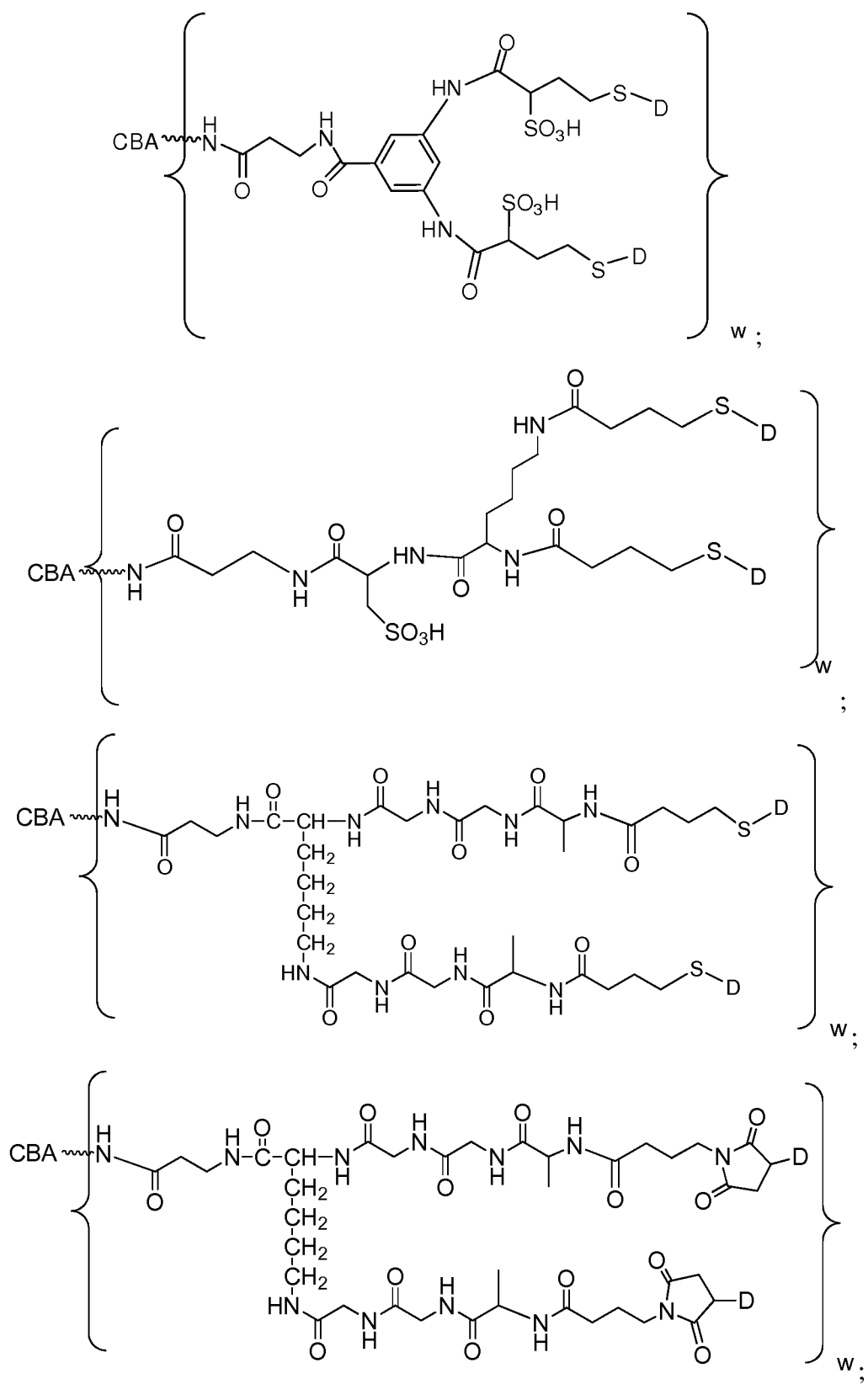
[136] In a thirteenth specific embodiment, R_{13} and R_{14} are both H; and r is 1 to 5. The remainder of the variables in Formula (I) or each of the formulas designated in the eighth, or ninth embodiment are as defined in the first embodiment or its eighth, ninth, tenth, eleventh, or twelfth specific embodiment.

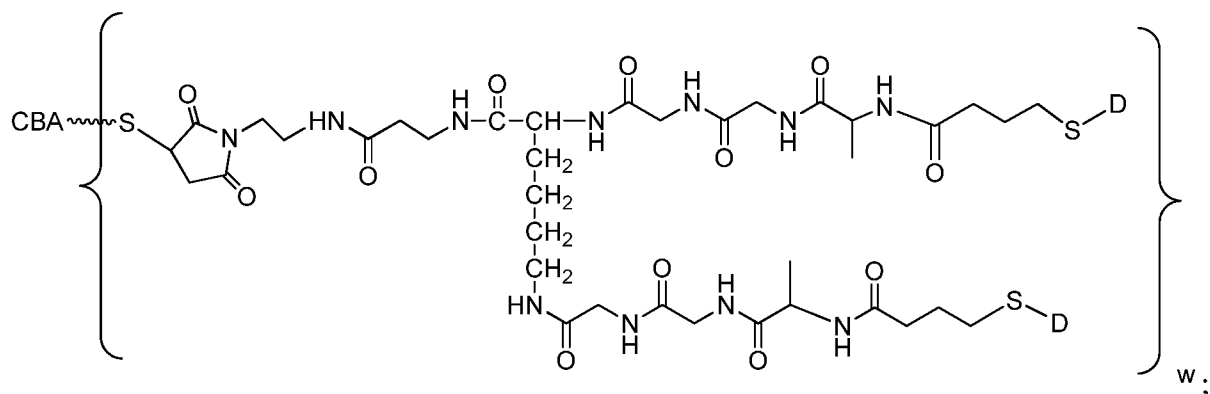
[137] In a fourteenth specific embodiment, the conjugate is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:



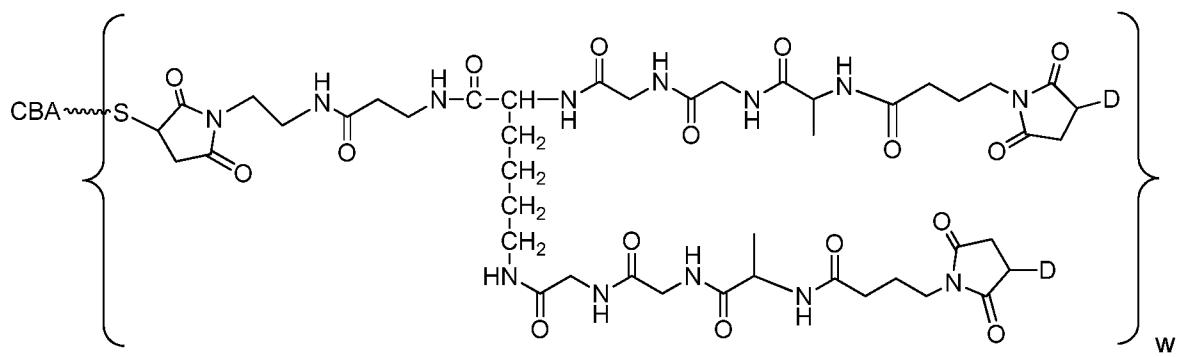








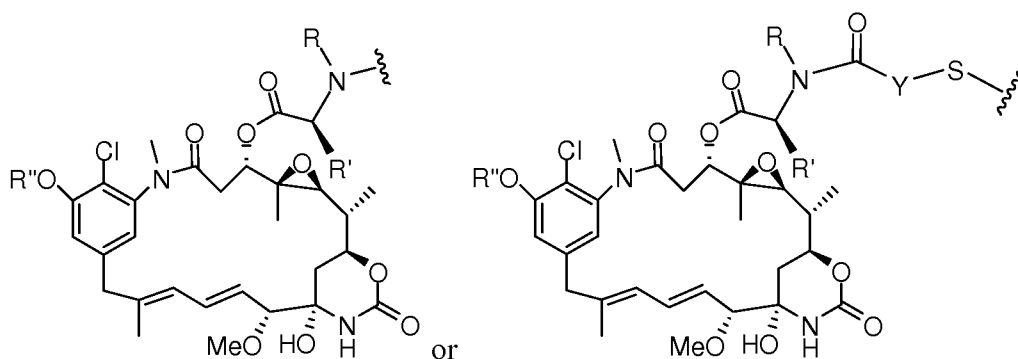
or



The variables in each of the formulas designated in this specific embodiment are as defined in the first embodiment.

[138] In a fifteenth specific embodiment, D is a maytansinoid in the conjugate in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, or fourteenth specific embodiment.

[139] In a sixteenth specific embodiment, D in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, or fourteenth specific embodiment is represented by:



in which:

R, R' and R'', for each occurrence, are independently H or an optionally substituted alkyl; and

Y is an optionally substituted alkylene, an optionally substituted alkenylene, or an optionally substituted alkynylene.

[140] In a seventeenth specific embodiment, Y in the structural formulas designated in the sixteenth embodiment is represented by $-(\text{CR}_{15}\text{R}_{16})_r\text{CR}_{17}\text{R}_{18}-$,

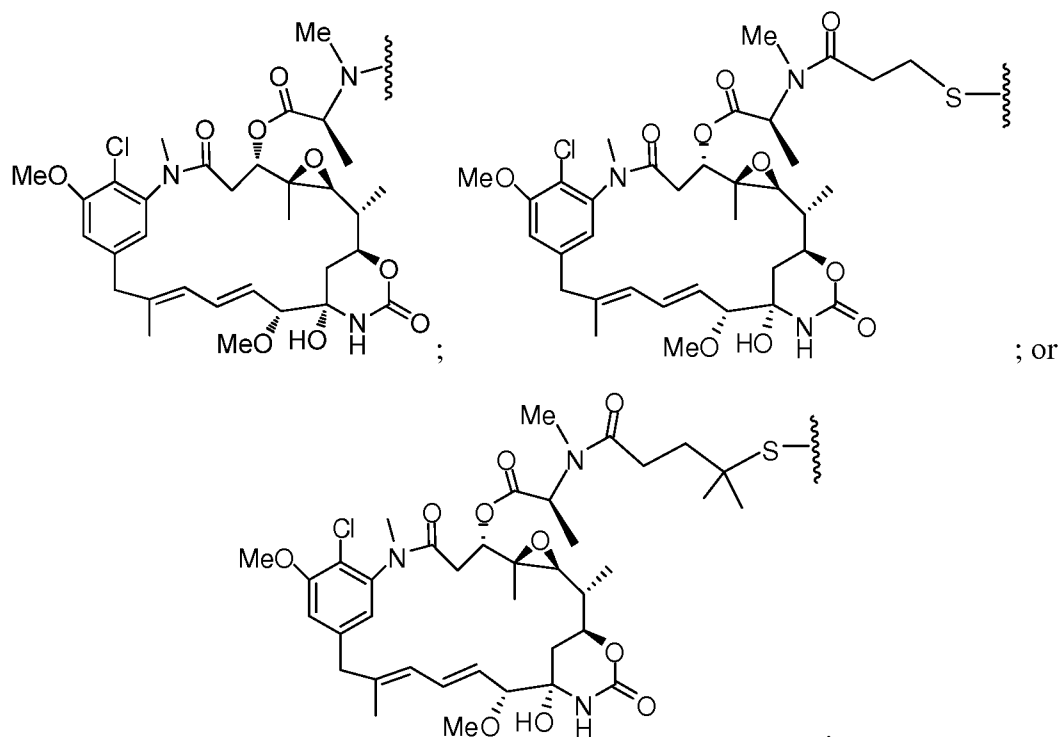
in which:

R₁₅ to R₁₈, for each occurrence, are independently H or an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted cycloalkyl, an optionally substituted heterocyclyl, an optionally substituted aryl or an optionally substituted heteroaryl; and

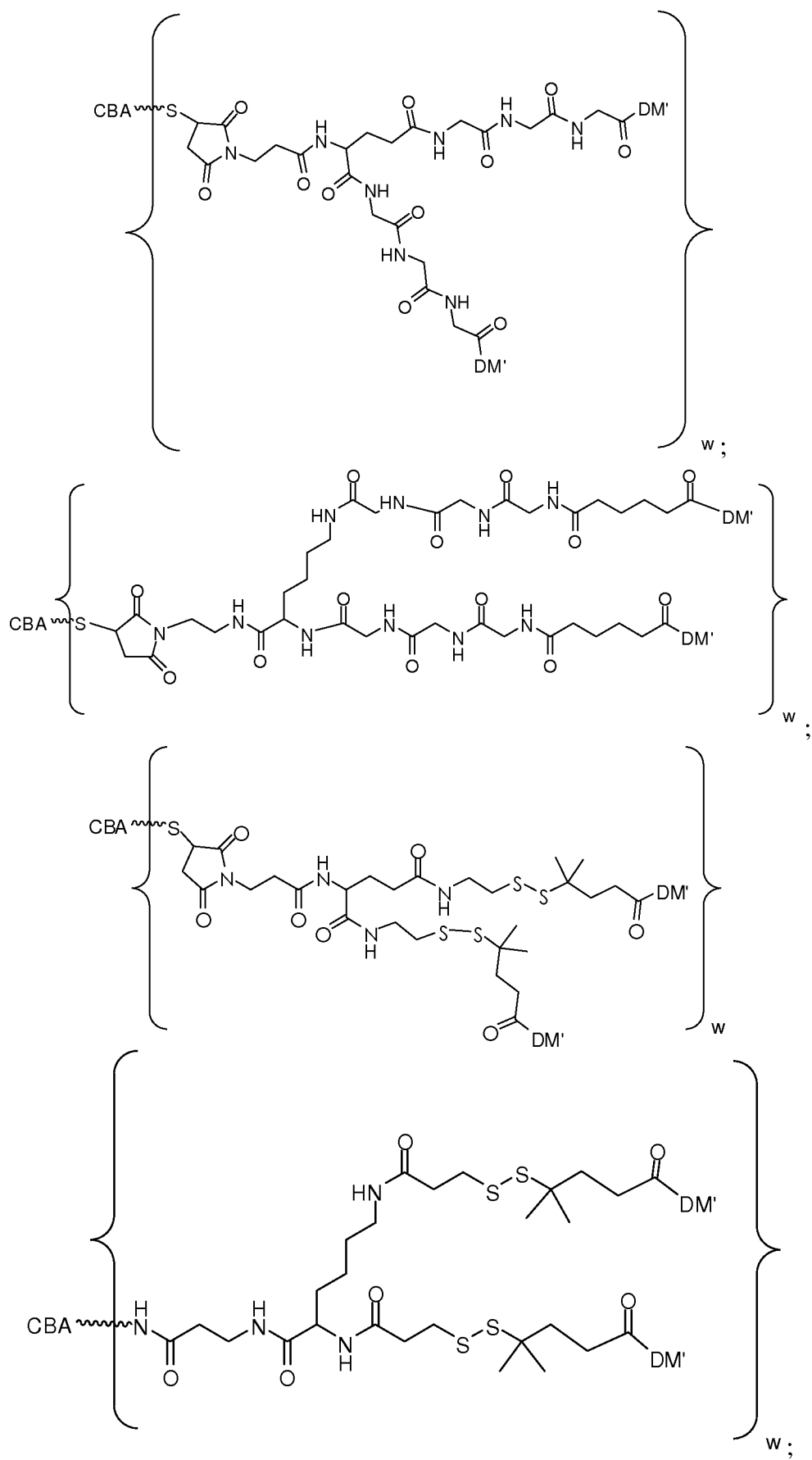
r is an integer between 0 to 15.

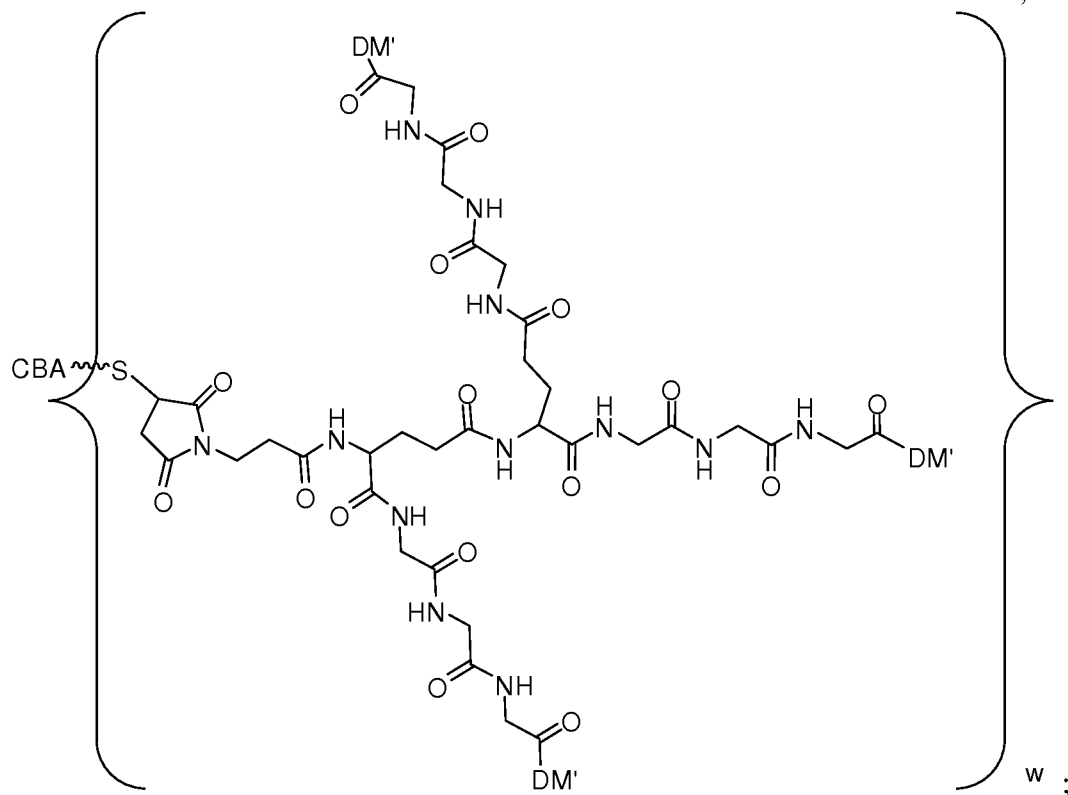
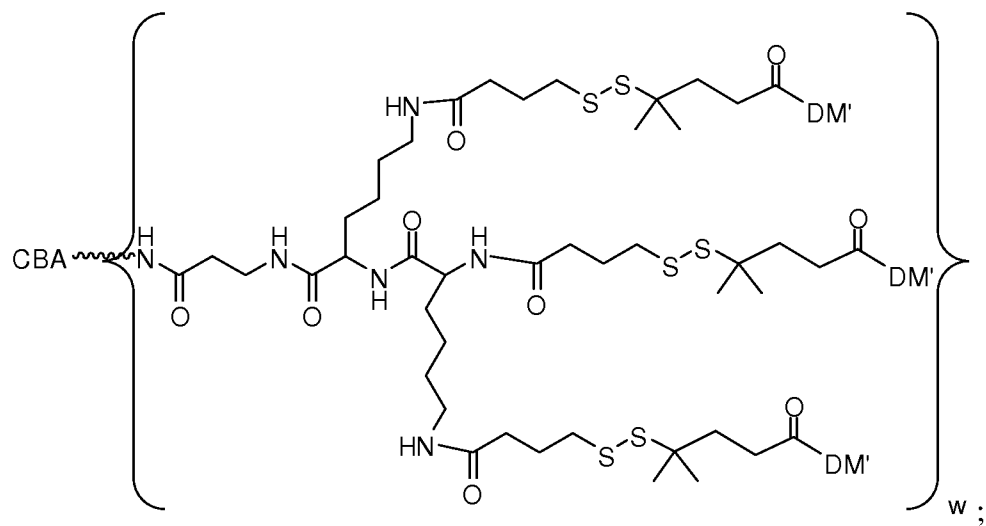
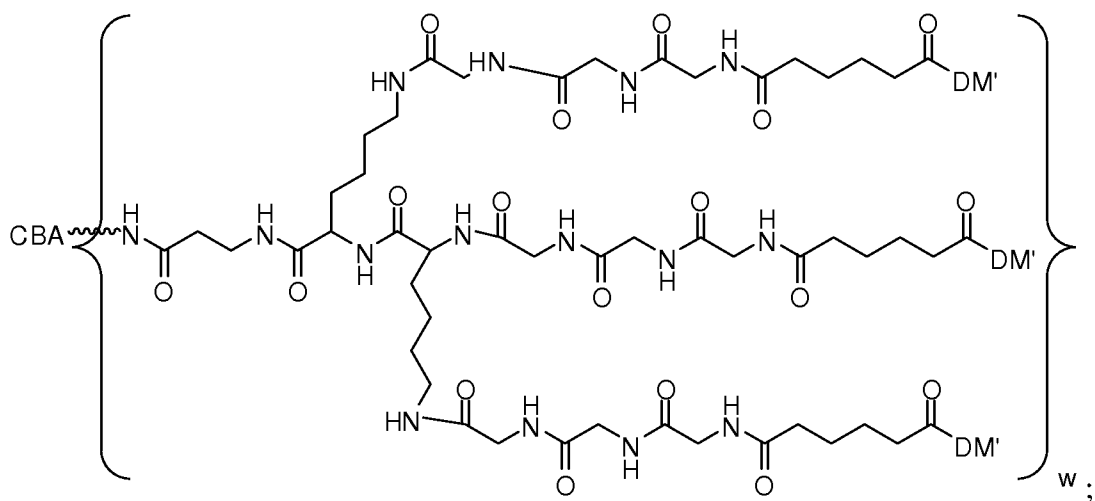
Alternatively, R₁₅ to R₁₈, for each occurrence, are independently H or an optionally substituted alkyl. In another alternative, R₁₅ to R₁₈ are all H and r is 1. In yet another alternative, R₁₅ and R₁₆ are H, r is 2, and R₁₇ and R₁₈ are both methyl. .

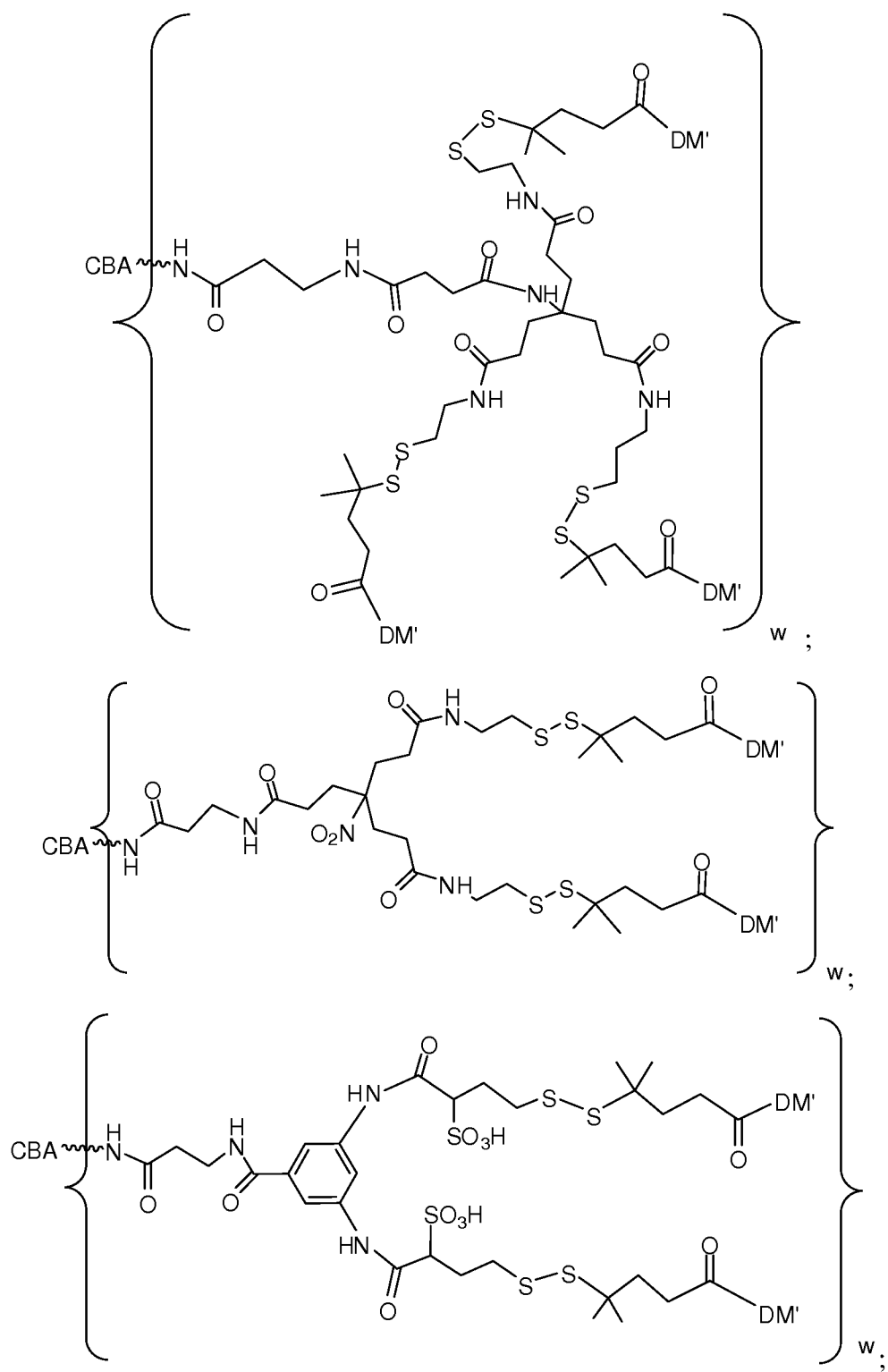
[141] In an eighteenth specific embodiment, D in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, or seventeenth specific embodiment is represented by the following formula:

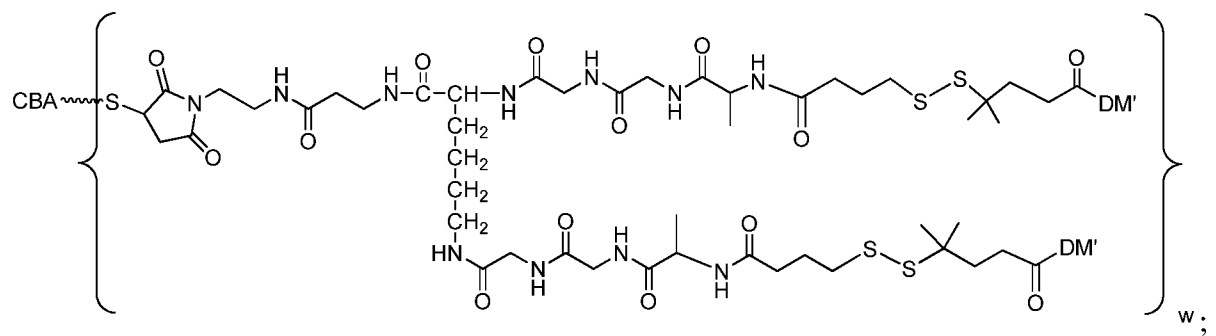
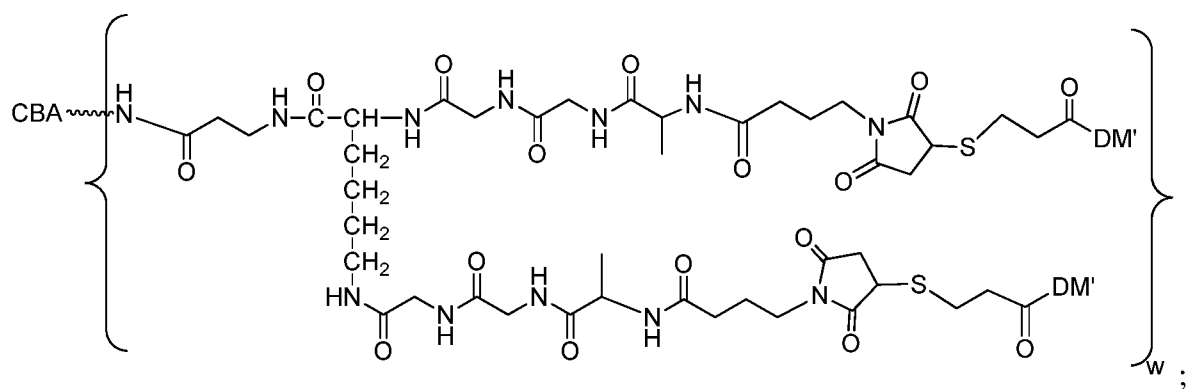
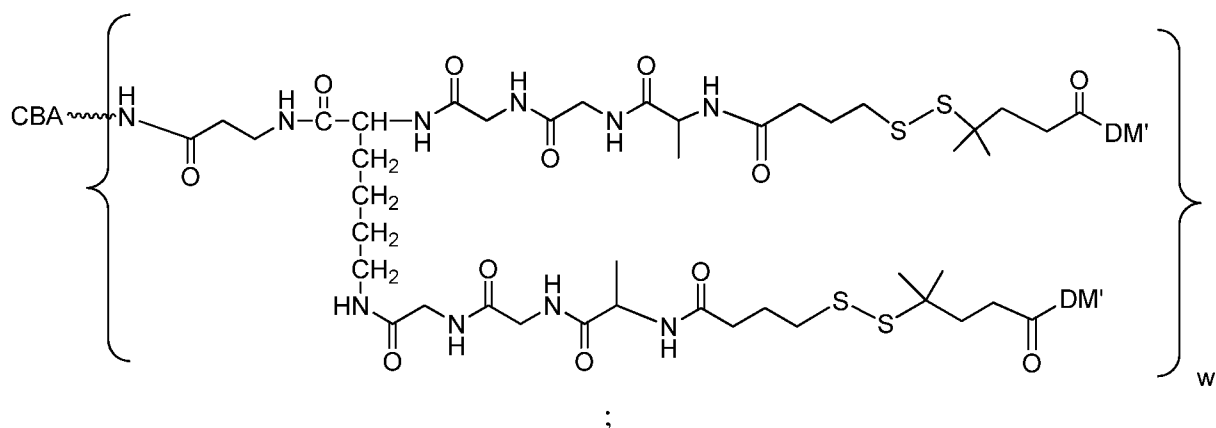
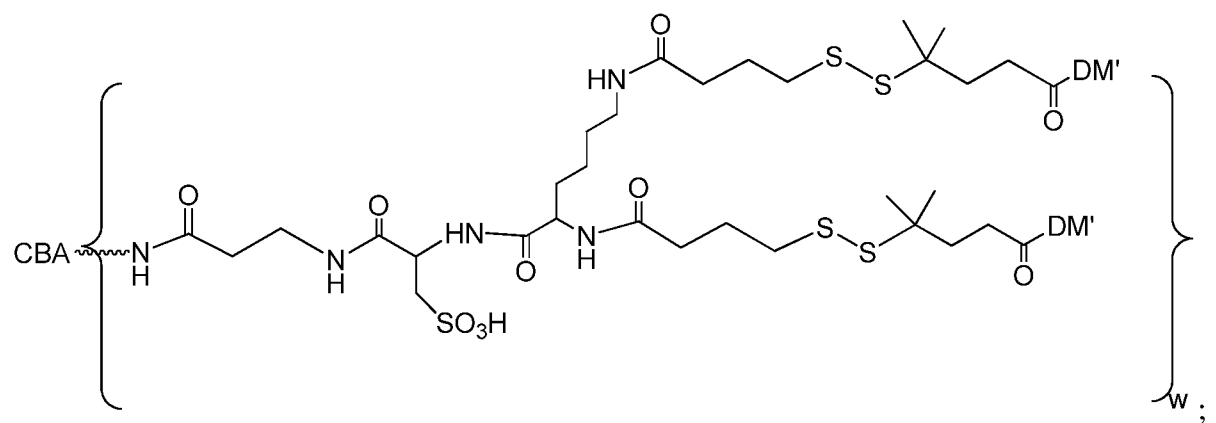


[142] In a nineteenth specific embodiment, the conjugate is represented by the following formula, or a pharmaceutically acceptable salt thereof:

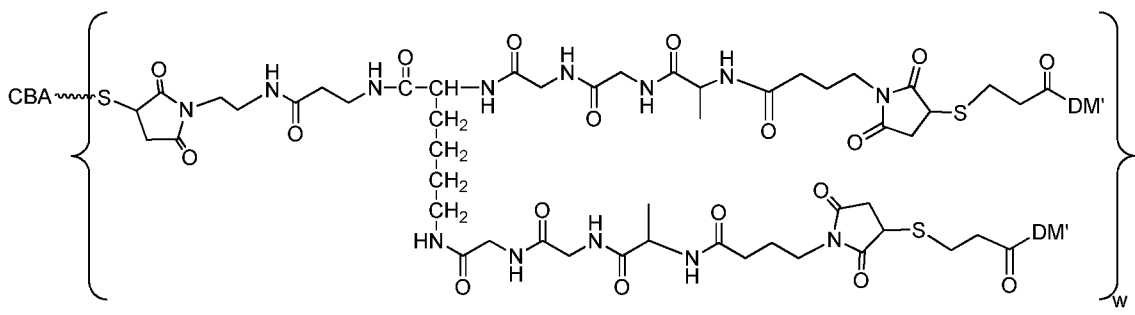




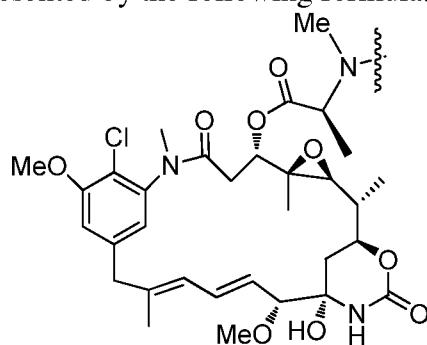




or



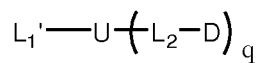
in which: DM' is represented by the following formula:



[143] In the first embodiment or each of its nineteen specific embodiments described in this section above, w is specifically an integer between 1 and 6 or alternatively, between 1 and 4.

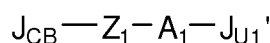
CYTOTOXIC COMPOUND

[144] In a second embodiment, the present invention also provides a cytotoxic compound represented by Formula (II) below:

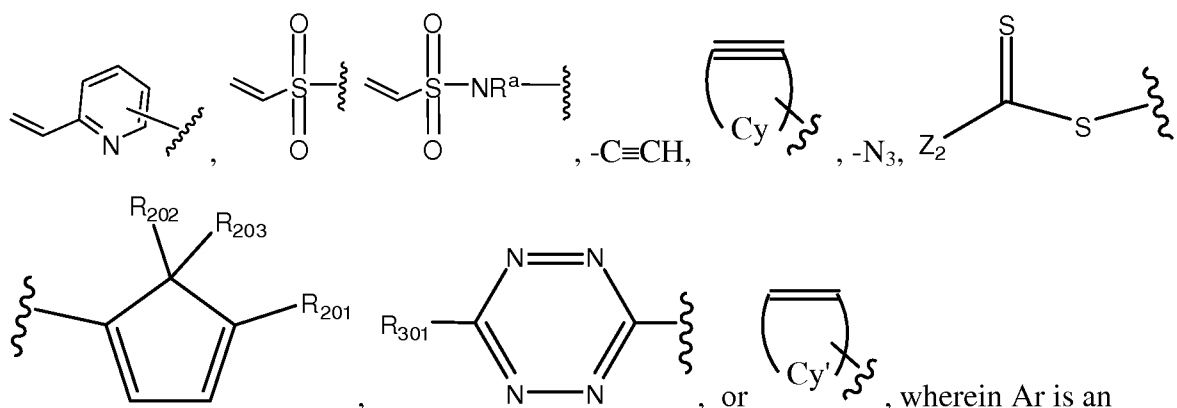



In addition, nineteen specific embodiments for the cytotoxic compound are further described below in this cytotoxic compound section.

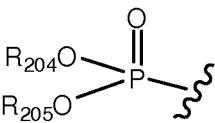
[145] In a first specific embodiment, L_1 is represented by the following formula:

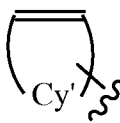


in which: J_{CB} is -COX'', maleimide, -SZ, X'-CR^bR^c-C(=O)-, X'-CR^bR^c-C(=O)-NR^e-, R^a-C(=O)-, R^a-C(=O)-Ar-, NH₂-NR^e-, NH₂-NR^e-C(=O)-, NH₂-NR^e-Ar-, NH₂-O-,



optionally substituted arylene or an optionally substituted heteroarylene,  is an optionally substituted cycloalkyne or an optionally substituted heterocycloalkyne, R_{201} , R_{202} and R_{203} each are independently H or an optionally substituted alkyl; Z_2 is pyridyl or

, wherein R_{204} and R_{205} are each independently optionally substituted alkyl,

and  is an optionally substituted strained cycloalkene or an optionally substituted strained heterocycloalkene;

X' is a halogen;

X'' is $-OH$ or a carboxylic acid activating group (specifically COX'' is a reactive ester);

Z is H or $-SR^d$;

R^a , R^b , R^c and R^e , for each occurrence, are independently H or an optionally substituted alkyl;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

Z_1 is $-NR_9-C(=O)-$ or $-C(=O)-NR_9-$ or absent;

A_1 is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, $-(CH_2-CH_2-O)_{p1}-(CR^bR^c)_{p1}-$ or $-(CR^bR^c)_{p1}-(O-CH_2-CH_2)_{p1}-$;

J_{U1}' is absent, $-NR_9-$ or $-C(=O)-$;

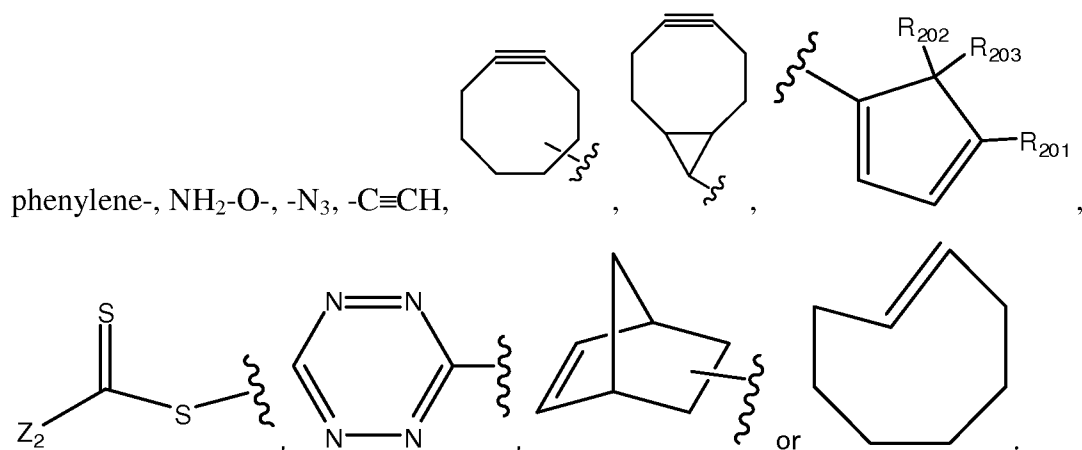
$p1$ is an integer from 1 to 1000 (specifically, 1 to 24, more specifically 1 to 8 (e.g., 2, 4, 6 or 8));

$p1'$ is an integer from 0 to 10; and

R_9 is H or an optionally substituted alkyl.

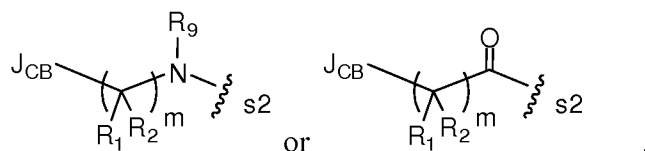
Alternatively, COX'' is a reactive ester and $p1$ is 1 to 24. In another alternative, COX'' is a reactive ester and $p1$ is 1 to 8. In yet another alternative, COX'' is a reactive ester and $p1$ is 2, 4, 6, or 8. Alternatively, The remainder of the variables in Formula (II) are as described in the second embodiment.

[146] In a second specific embodiment, variable J_{CB} is $-COX''$, maleimide, $-SZ$, $X'-CR^bR^c-C(=O)-$, $X'-CR^bR^c-C(=O)-NR^e-$, $R^a-C(=O)-$, $R^a-C(=O)-phenylene-$, NH_2-NR^e- , NH_2-NR^e-



The remainder of the variables in Formula (II) or the formula designated in the first specific embodiment are as defined in the second embodiment, each of its alternative embodiments, or its first specific embodiment.

[147] In a third specific embodiment, L_1' is represented by the following formula:

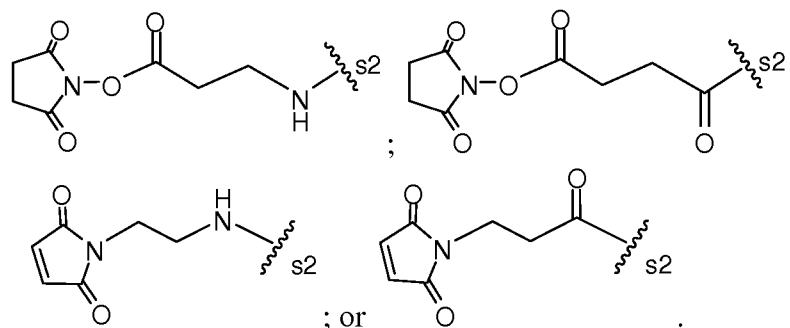


in which R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10. The remainder of the variables in Formula (II) are as defined in the second embodiment or its first or second specific embodiment.

[148] In a fourth specific embodiment, J_{CB} is -COX'' or a maleimide; R_1 and R_2 , for each occurrence, are each independently H or an alkyl; m is an integer from 1 to 5; and R^9 is H or an alkyl. The remainder of the variables in Formula (II) or each of the above formulas representing L_1' are as defined in the second embodiment or its third specific embodiment.

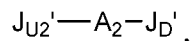
[149] In a fifth specific embodiment, R_1 and R_2 are both H; and R^9 is H. The remainder of the variables in Formula (II) or each of the above formulas representing L_1' are as defined in the second embodiment or its third or fourth specific embodiment.

[150] In a sixth specific embodiment, L_1' is represented by the following formula:



The remainder of the variables in Formula (II) or each of the formulas designated in this specific embodiment are as defined in the second embodiment or its fifth specific embodiment.

[151] In a seventh specific embodiment, L_2 is represented by the following formula:

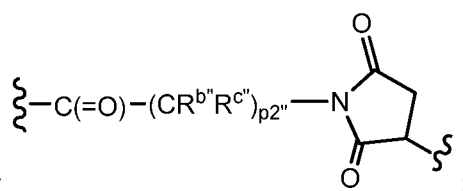


in which:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_{D}' is $-C(=O)-$, $-C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-$, $-C(=O)-(CH_2-CH_2-O)_{p2'}-(CR^{b'}R^{c'})_{p2'}-S-$,



$-S-$ absent, $-C(=O)-(CR^{b''}R^{c''})_{p2''}-S-$ or

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl (Specifically H);

$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H, an optionally substituted alkyl or a charged substituent or an ionizable group;

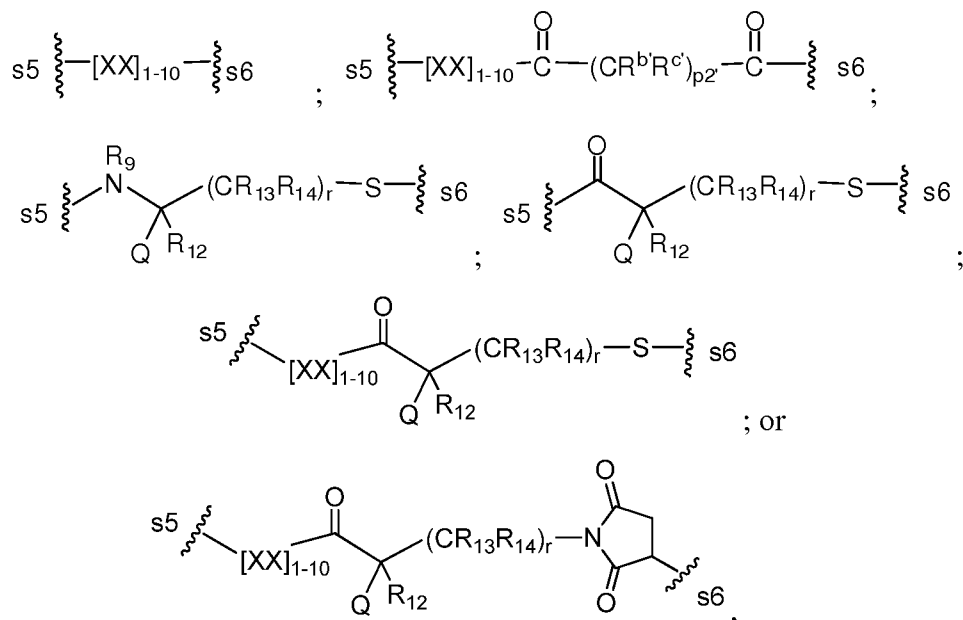
p_2 is an integer from 1 to 1000 (specifically 1 to 14, 1 to 8);

$p_{2'}$ is an integer from 1 to 10 (specifically 1 to 5); and

$p_{2''}$ is an integer from 1 to 10 (specifically 1 to 5).

Alternatively, $R^{b'}$ and $R^{c'}$ are H; p_2 is 1-14; and $p_{2'}$ is 1 to 5; and in another alternative, $R^{b'}$ and $R^{c'}$ are H; p_2 is 1-8; and $p_{2'}$ is 1 to 5. The remainder of the variables in Formula (II) are as defined in the second embodiment or its first, second, third, fourth, fifth, or sixth specific embodiment.

[152] In an eighth specific embodiment, L_2 is represented by the following formula:



in which:

R_{12} , R_{13} , and R_{14} , for each occurrence, are each independently H, an optionally substituted alkyl;

Q is H, a charged substituent or an ionizable group;

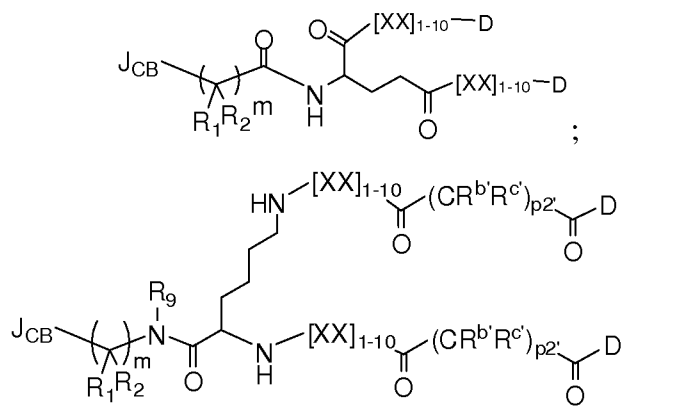
r is an integer from 0 to 10;

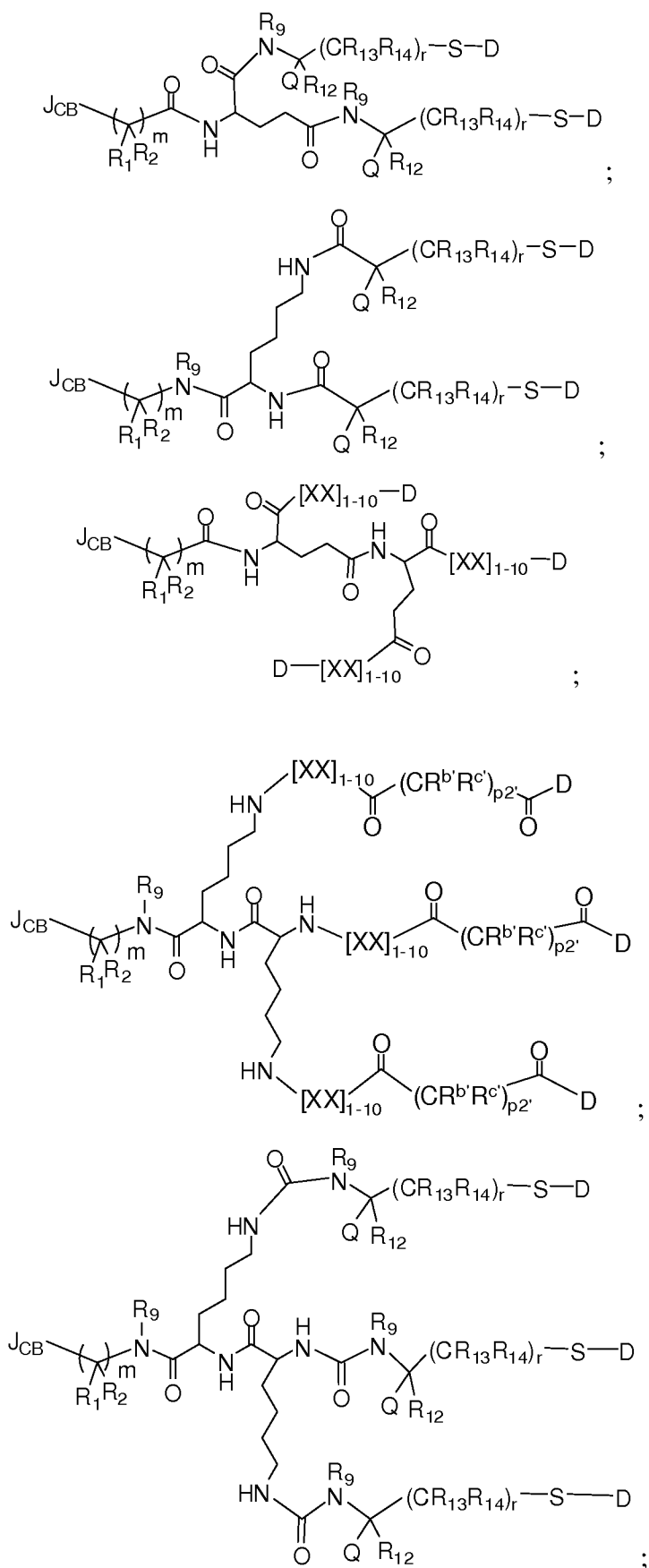
s5 is the site covalently linked to the group U; and

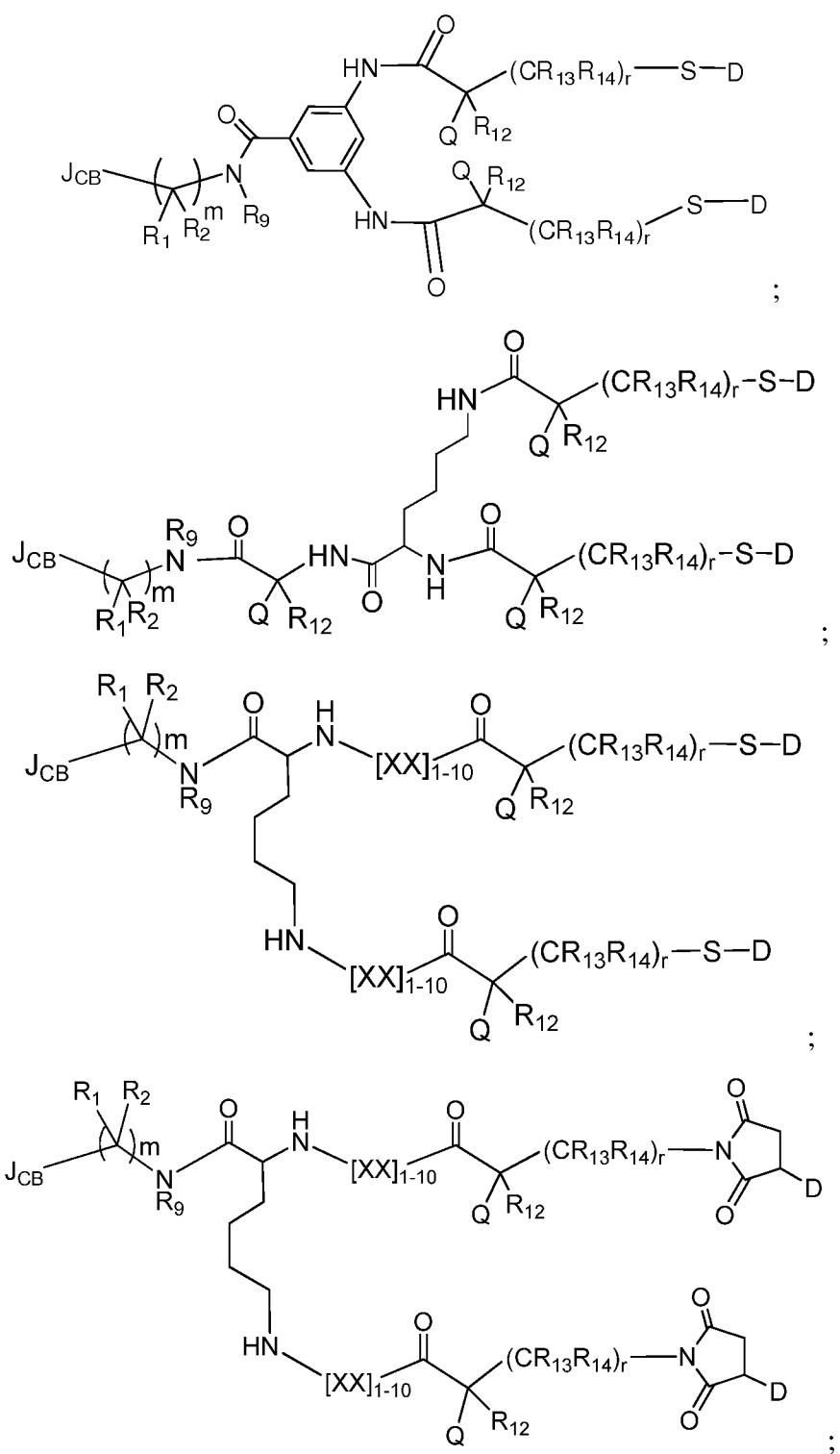
s6 is the site covalently linked to the group D.

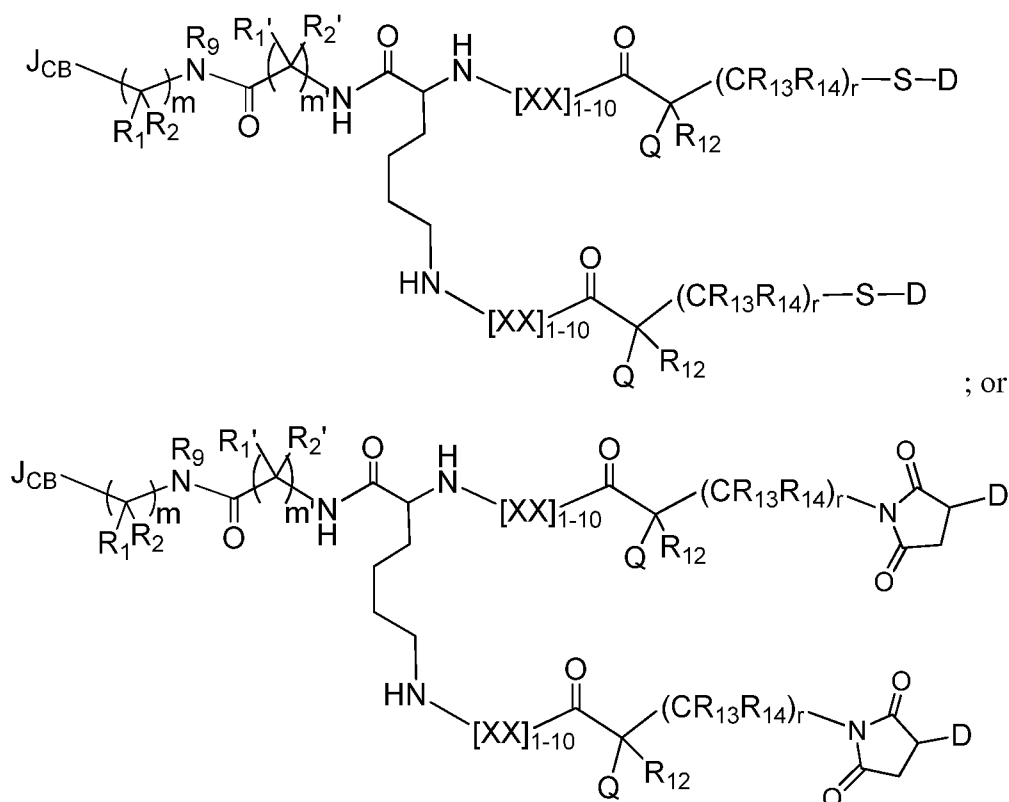
The remainder of the variables in Formula (II) or each of the formulas designated in this specific embodiment are as defined in the second embodiment or its seventh specific embodiment.

[153] In a ninth specific embodiment, the cytotoxic compound is represented by the following formula, or a salt thereof:









The variables in each of the formulas designated in this specific embodiment are as defined in the second embodiment or its seventh or eighth specific embodiment.

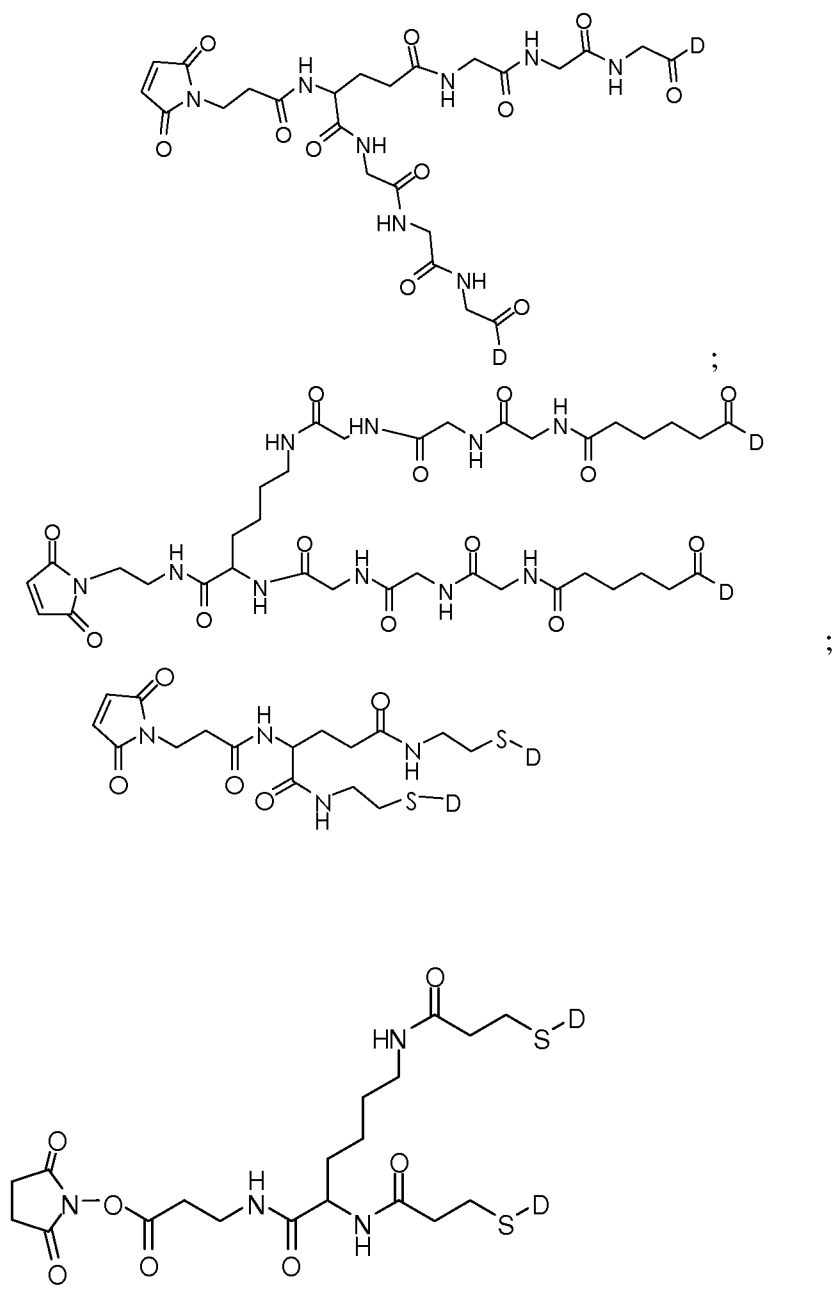
[154] In a tenth specific embodiment, Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion. The remainder of the variables in Formula (II) or each of the formulas designated in the eighth or ninth embodiment above are as defined in the second embodiment or its eighth or ninth specific embodiment.

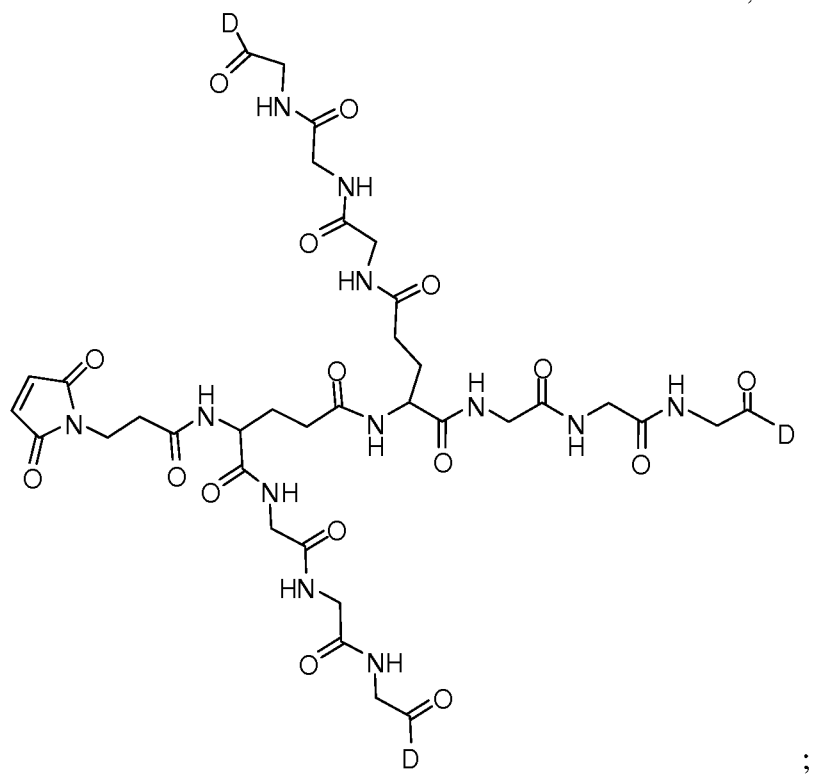
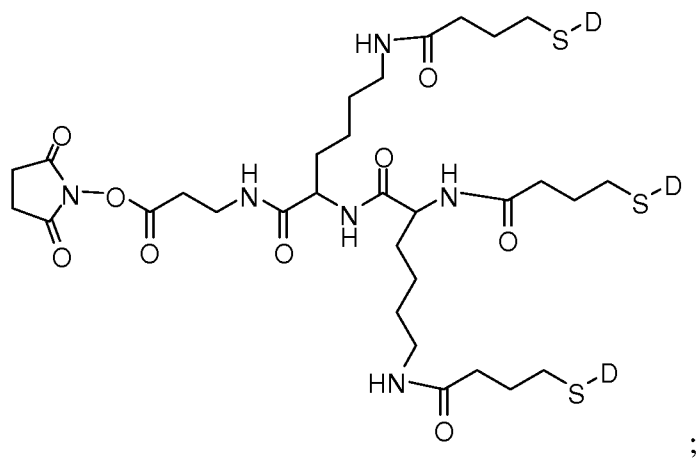
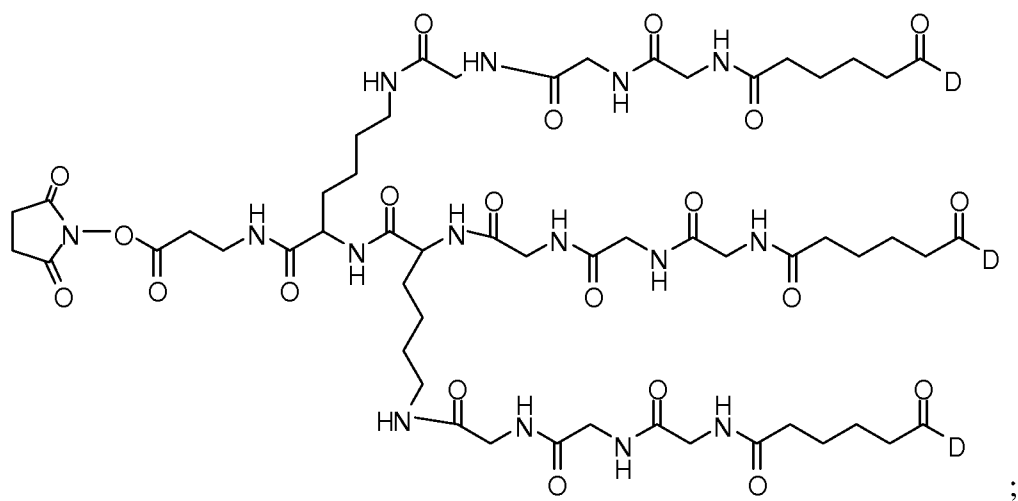
[155] In an eleventh specific embodiment, R_{12} is H; and Q is H, SO_3H or a salt (e.g., pharmaceutically acceptable salt) thereof. The remainder of the variables in Formula (II) or each of the formulas designated in the eighth or ninth embodiment above are as defined in the second embodiment or its eighth, ninth, or tenth specific embodiment.

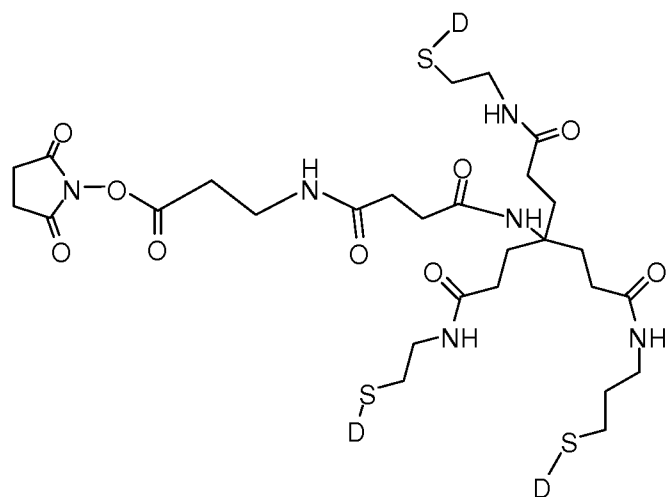
[156] In a twelfth specific embodiment, $\text{R}^{b'}$ and $\text{R}^{c'}$ are both H; and p_2' is an integer from 1 to 5. The remainder of the variables in Formula (II) or each of the formulas designated in the seventh, eighth, or ninth specific embodiment are as defined in the second embodiment or its seventh, eighth, ninth, tenth, or eleventh specific embodiment.

[157] In a thirteenth specific embodiment, R_{13} and R_{14} are both H; and r is 1 to 5. The remainder of the variables in Formula (II) or each of the formulas designated in the eighth, or ninth embodiment are as defined in the second embodiment or its eighth, ninth, tenth, eleventh, or twelfth specific embodiment.

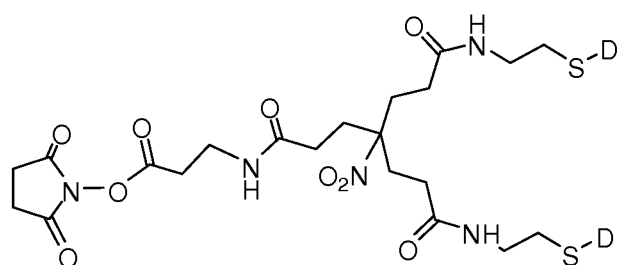
[158] In a fourteenth specific embodiment, the cytotoxic compound is represented by one of the following formulas, or a salt thereof:



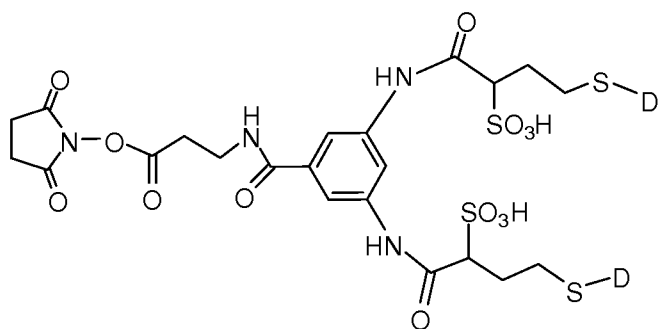




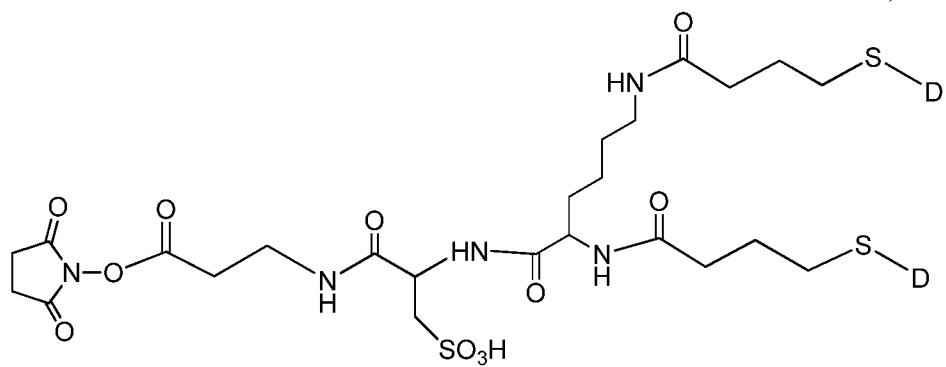
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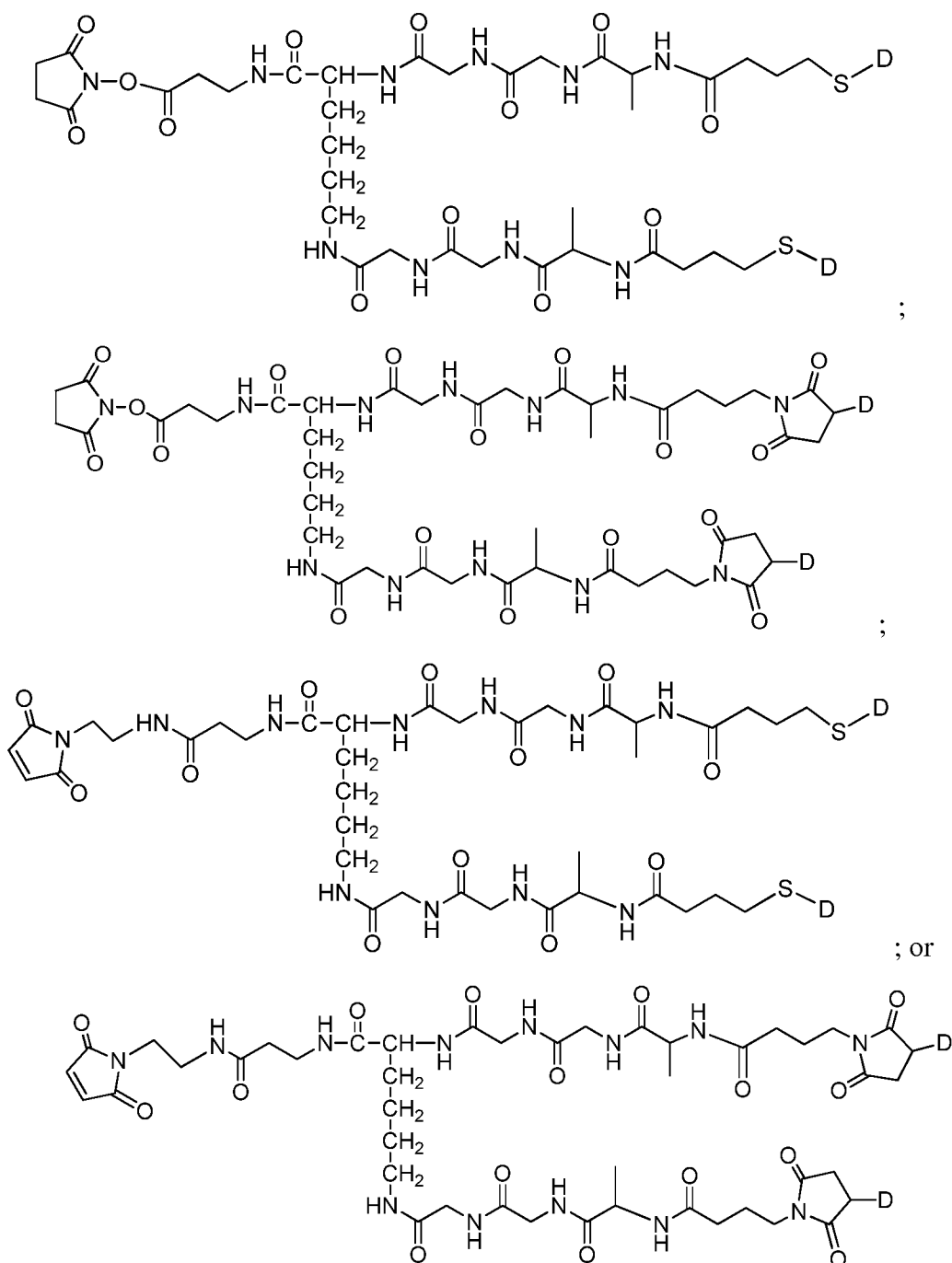
;



;



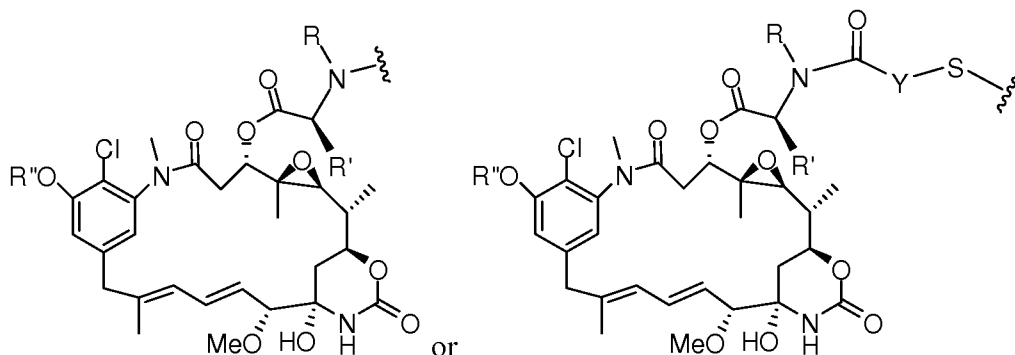
;



The variables in each of the formulas designated in this specific embodiment are as defined in the second embodiment.

[159] In a fifteenth specific embodiment, D is a maytansinoid in the cytotoxic compound in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, or fourteenth specific embodiment.

[160] In a sixteenth specific embodiment, D in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, or fourteenth specific embodiment is represented by:



in which:

R, R' and R'', for each occurrence, are independently H or an optionally substituted alkyl; and

Y is an optionally substituted alkylene, an optionally substituted alkenylene, or an optionally substituted alkynylene.

[161] In a seventeenth specific embodiment, Y in the structural formulas designated in the sixteenth embodiment is represented by $-(CR_{15}R_{16})_rCR_{17}R_{18}-$,

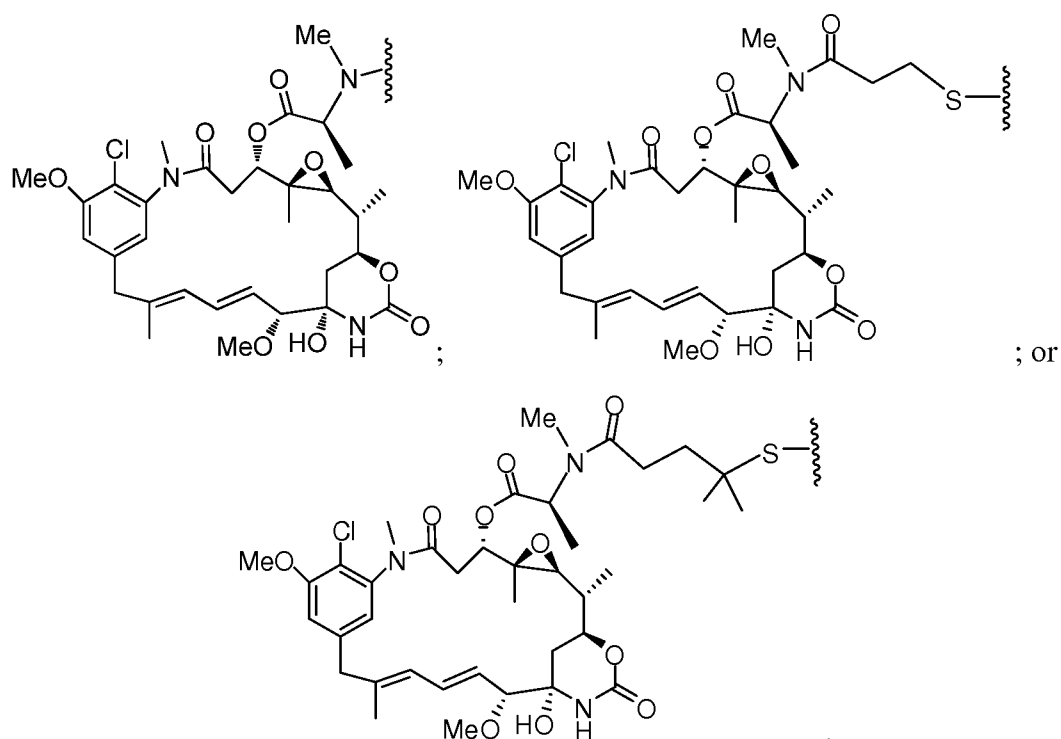
in which:

R₁₅ to R₁₈, for each occurrence, are independently H or an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted cycloalkyl, an optionally substituted heterocyclyl, an optionally substituted aryl or an optionally substituted heteroaryl; and

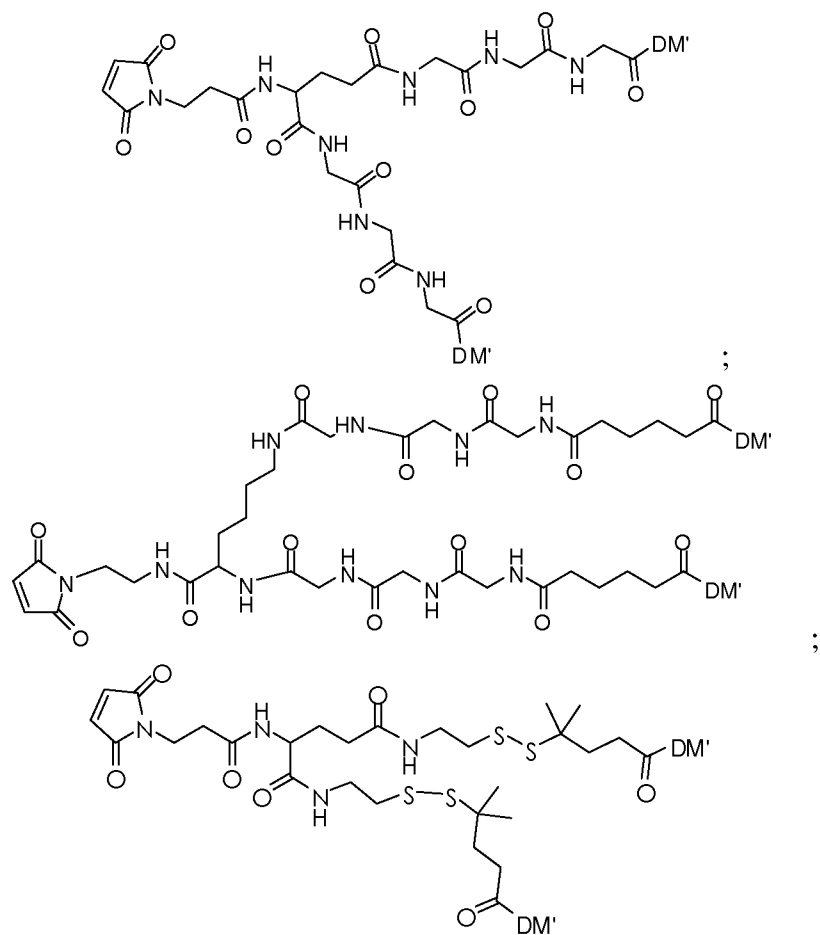
r is an integer between 0 to 15.

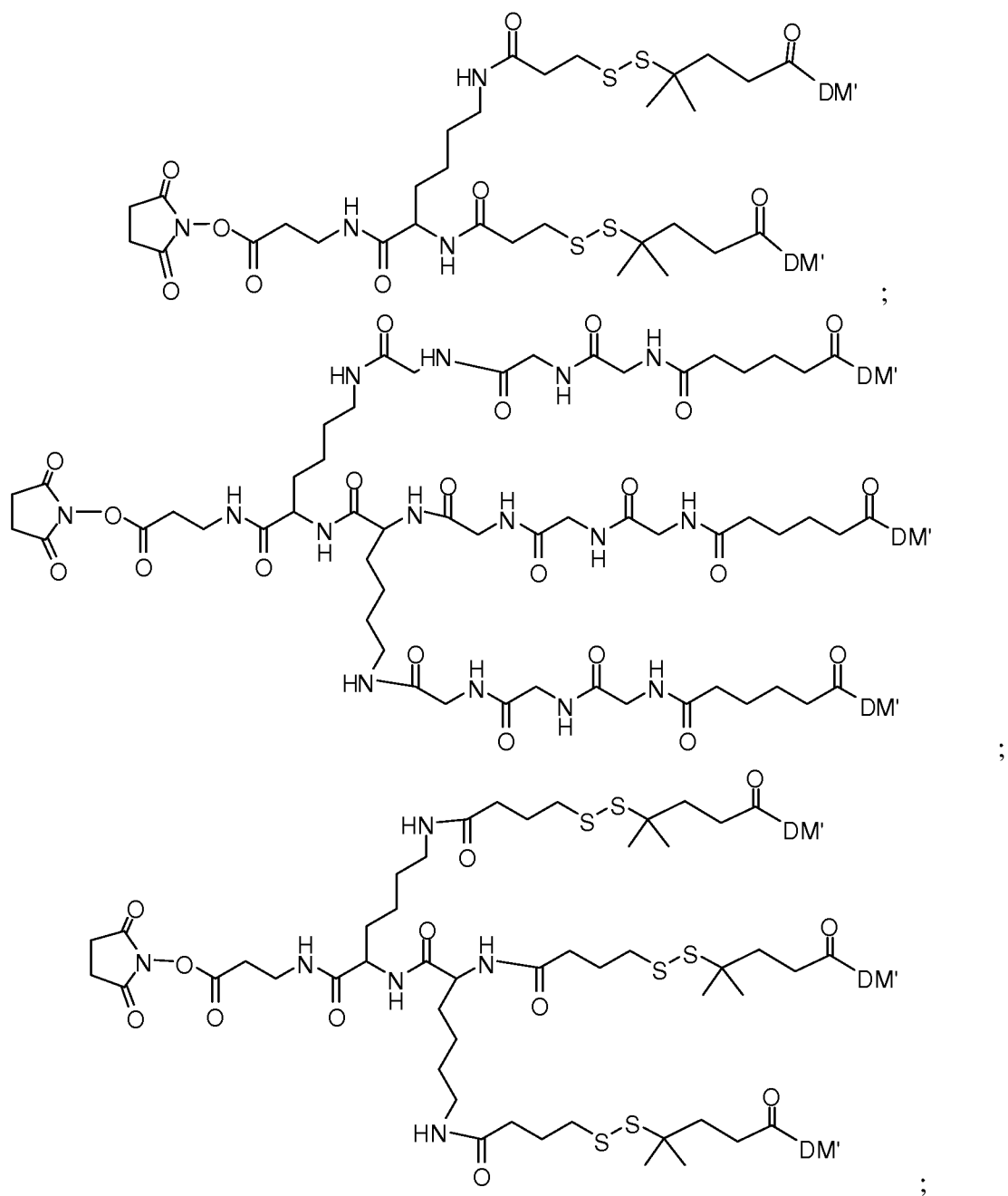
Alternatively, R₁₅ to R₁₈, for each occurrence, are independently H or an optionally substituted alkyl. In another alternative, R₁₅ to R₁₈ are all H and r is 1. In yet another alternative, R₁₅ and R₁₆ are H, r is 2, and R₁₇ and R₁₈ are both methyl.

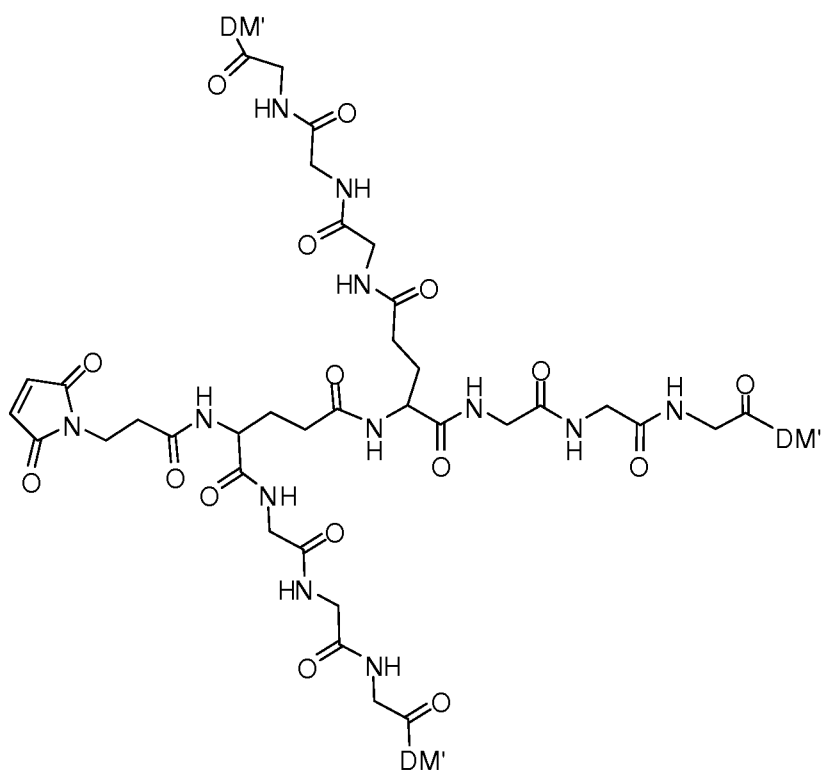
[162] In an eighteenth specific embodiment, D in the first embodiment or its first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, or seventeenth specific embodiment is represented by the following formula:



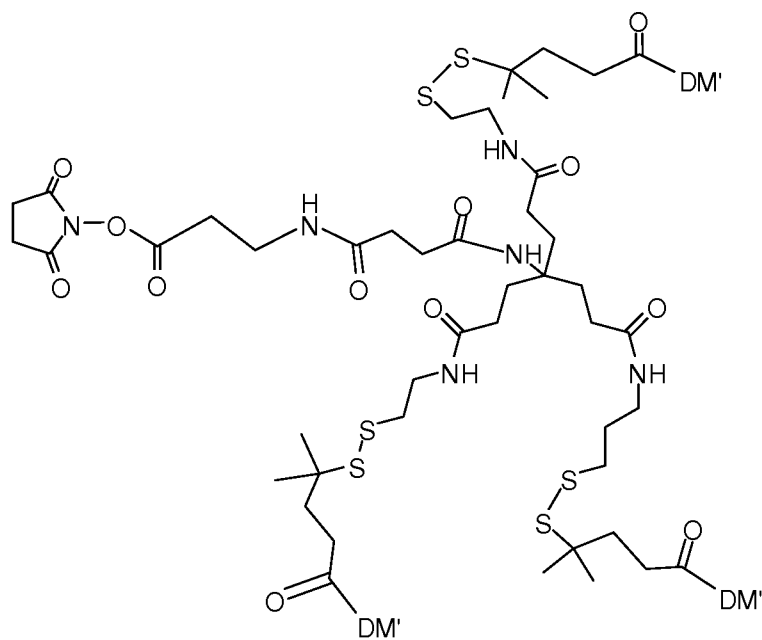
[163] In a nineteenth specific embodiment, the cytotoxic compound is represented by the following formula, or a salt thereof:



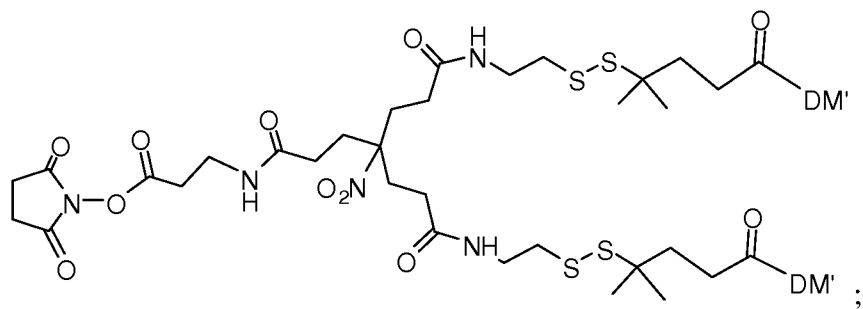




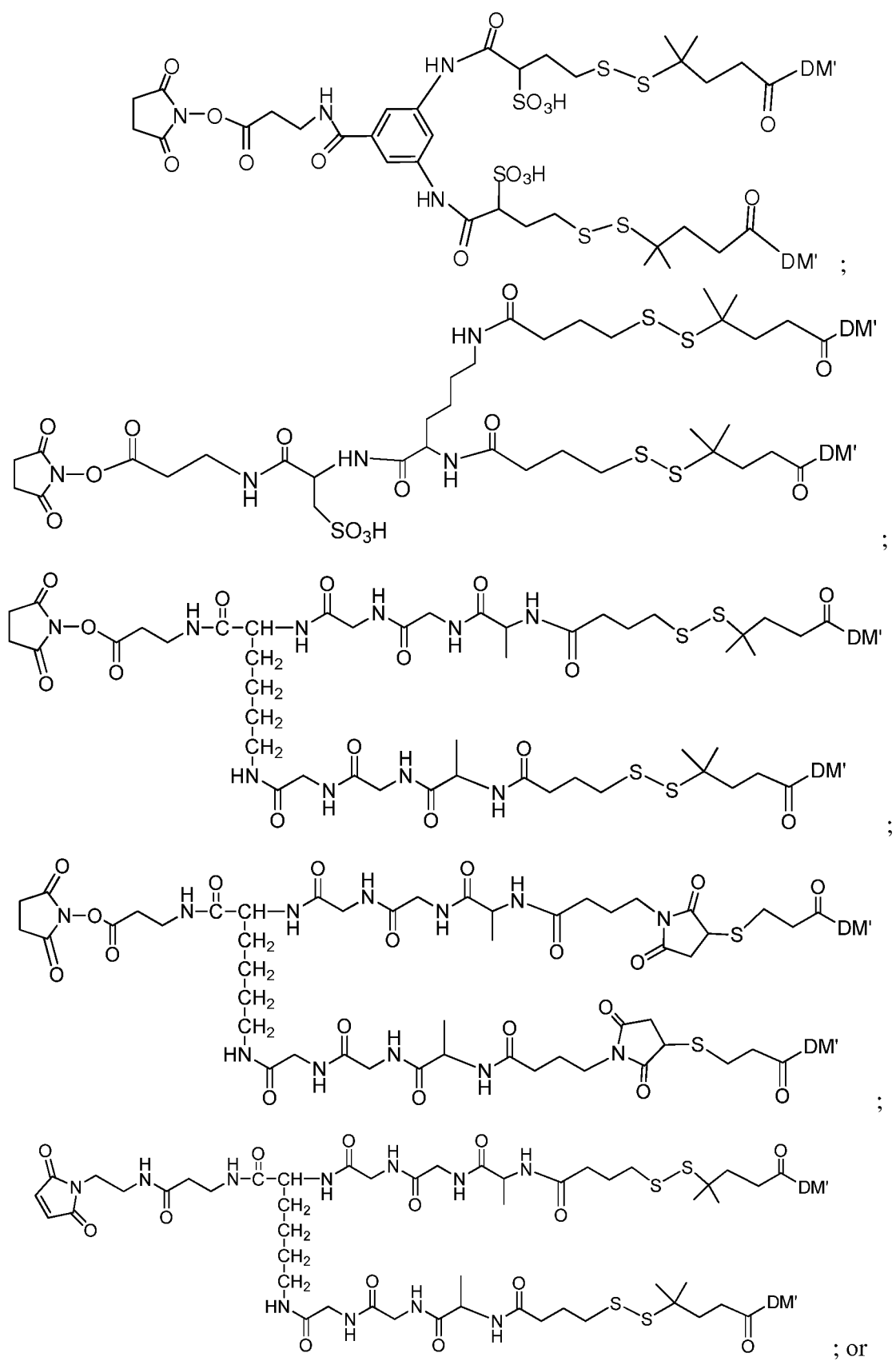
;

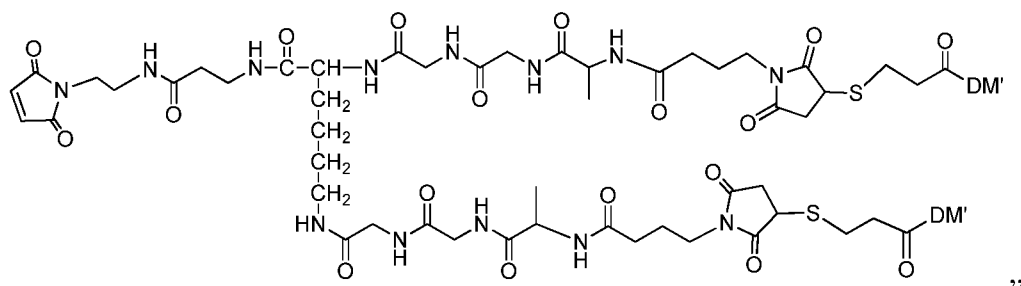


;

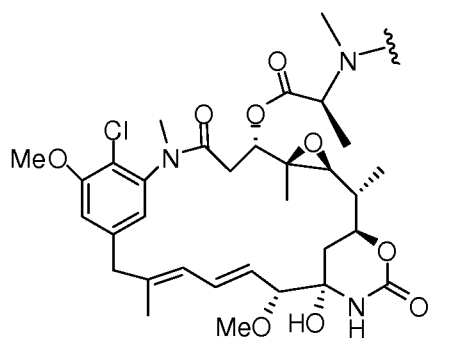


;



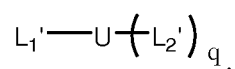


in which: DM' is represented by the following formula:



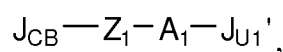
LINKER COMPOUNDS

[164] In the third embodiment, the present invention further provides a linker compound represented by Formula (III) below:



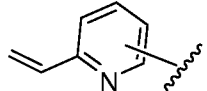
In addition, sixteen specific embodiments for the linker compound are further described below in this linker compound section.

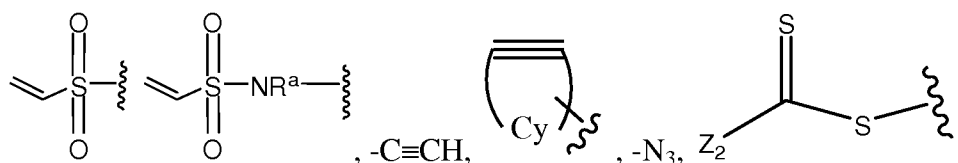
[165] In a first specific embodiment, L_1' is represented by the following formula:

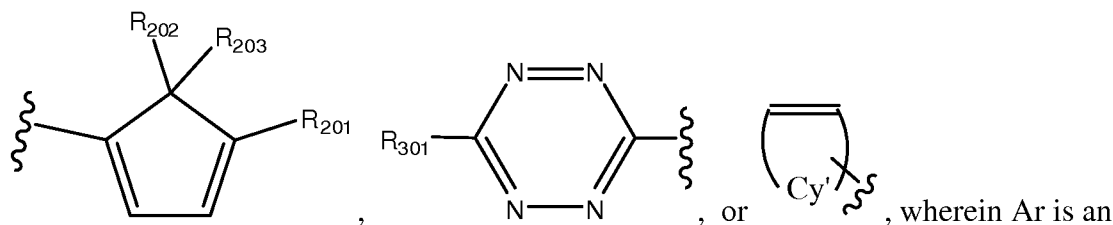



wherein:

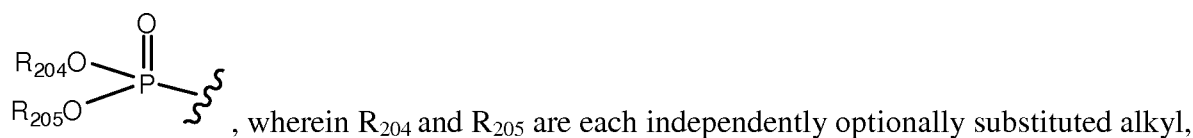
J_{CB} is -COX'', maleimide, -SZ, $X' - CR^bR^c - C(=O)-$, $X' - CR^bR^c - C(=O) - NR^e-$, $R^a - C(=O)-$

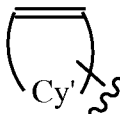
, $R^a - C(=O) - Ar-$, $NH_2 - NR^e-$, $NH_2 - NR^e - C(=O)-$, $NH_2 - NR^e - Ar-$, $NH_2 - O-$, ,





optionally substituted arylene or an optionally substituted heteroarylene,  is an optionally substituted cycloalkyne or an optionally substituted heterocycloalkyne, R₂₀₁, R₂₀₂ and R₂₀₃ each are independently H or an optionally substituted alkyl; Z₂ is pyridyl or



and  is an optionally substituted strained cycloalkene or an optionally substituted strained heterocycloalkene;

X' is a halogen;

X'' is -OH or a carboxylic acid activating group (specifically COX'' is a reactive ester);

Z is H or -SR^d;

R^a, R^b, R^c and R^e, for each occurrence, are independently H or an optionally substituted alkyl;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

Z₁ is -NR₉-C(=O)- or -C(=O)-NR₉- or absent;

A₁ is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, -(CH₂-CH₂-O)_{p1}-(CR^bR^c)_{p1'}- or -(CR^bR^c)_{p1'}-(O-CH₂-CH₂)_{p1}-;

J_{U1}' is absent, -NR₉- or -C(=O)-;

p₁ is an integer from 1 to 1000 (specifically, 1 to 24, more specifically 1 to 8 (e.g., 2, 4, 6 or 8));

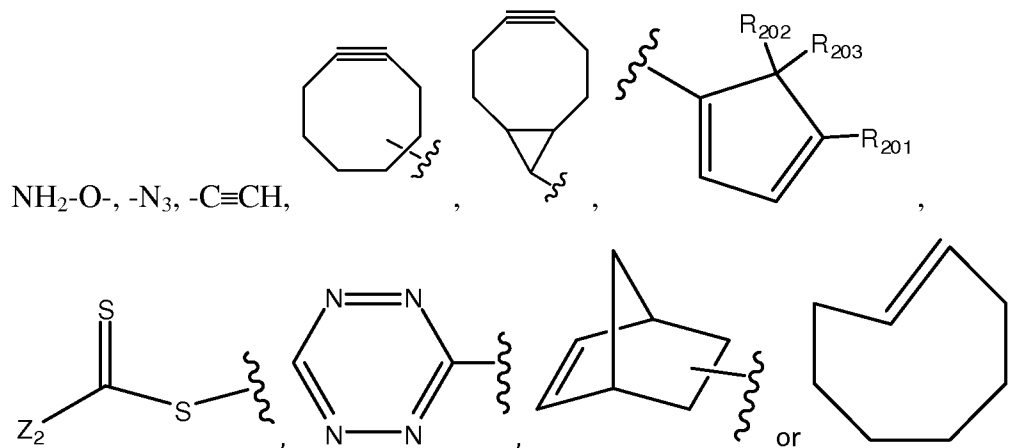
p₁' is an integer from 0 to 10; and

R₉ is H or an optionally substituted alkyl.

Alternatively, COX'' is a reactive ester and p₁ is 1 to 24. In another alternative, COX'' is a reactive ester and p₁ is 1 to 8. In yet another alternative, COX'' is a reactive ester and p₁ is 2,

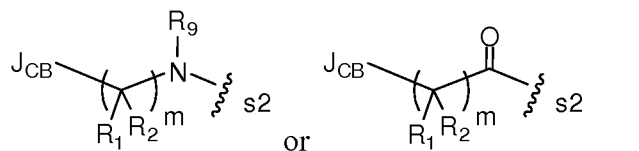
4, 6, or 8. The remainder of the variables in Formula (III) are as described in the third embodiment.

[166] In a second specific embodiment, J_{CB} is $-\text{COX}''$, maleimide, $-\text{SZ}$, $\text{X}'-\text{CR}^b\text{R}^c-\text{C}(=\text{O})-$, $\text{X}'-\text{CR}^b\text{R}^c-\text{C}(=\text{O})-\text{NR}^e-$, $\text{R}^a-\text{C}(=\text{O})-$, $\text{R}^a-\text{C}(=\text{O})$ -phenylene-, NH_2-NR^e- , NH_2-NR^e -phenylene-,



The remainder of the variables in Formula (III) or the formula designated in the first specific embodiment are as defined in the third embodiment, each of its alternative embodiments, or its first specific embodiment.

[167] In a third specific embodiment, L_1' is represented by the following formula:

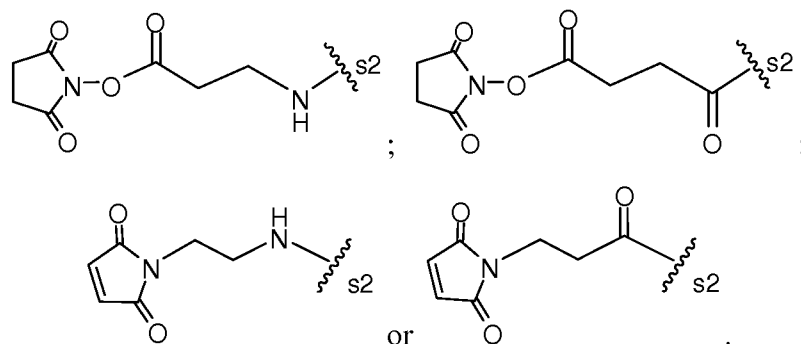


in which, R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10. The remainder of the variables in Formula (III) or each of the formulas described in this specific embodiment are as defined in the third embodiment or its first or second specific embodiment.

[168] In a fourth specific embodiment, J_{CB} is $-\text{COX}''$ or a maleimide; R_1 and R_2 , for each occurrence, are each independently H or an alkyl; R_9 is H or an alkyl; and m is an integer from 1 to 5. The remainder of the variables in Formula (III) or each of the formulas designated in the third specific embodiment are as defined in the third embodiment or its first, second, or third specific embodiment.

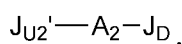
[169] In a fifth specific embodiment, R_1 and R_2 are both H; and R_9 is H. The remainder of the variables in Formula (III) or each of the above formulas representing L_1 are as defined in the third embodiment or its third or fourth specific embodiment.

[170] In a sixth specific embodiment, L_1' is represented by the following formula:



The remainder of the variables in Formula (III) or each of the formulas designated in this specific embodiment are as defined in the third embodiment or its fifth specific embodiment.

[171] In a seventh specific embodiment, L_2' is represented by the following formula:

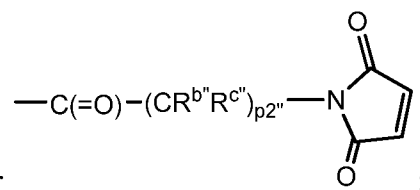


wherein:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_D is $-C(=O)-X''$, $-C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-X''$, $-C(=O)-(CH_2-CH_2-$



$O)_{p2}-(CR^{b'}R^{c'})_{p2'}-SZ$, $-SZ$, absent, $-C(=O)-(CR^{b''}R^{c''})_{p2''}-SZ$ or

X'' is $-OH$ or a carboxylic acid activating group;

Z is H or $-SR^d$;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl (specifically H);

$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H , an optionally substituted alkyl or a charged substituent or an ionizable group;

p_2 is an integer from 1 to 1000 (specifically 1 to 14, 1 to 8);

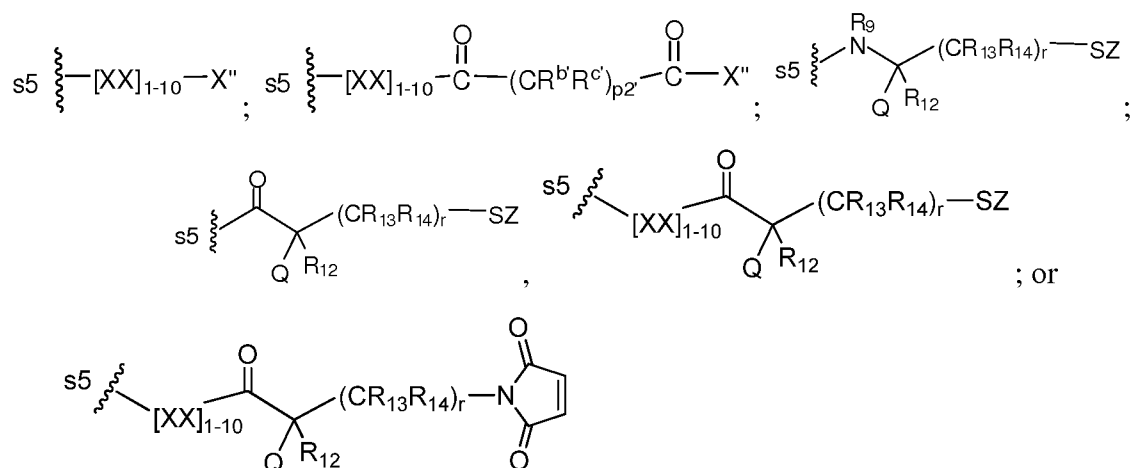
p_2' is an integer from 1 to 10 (specifically 1 to 5);

p_2'' is an integer from 1 to 10 (specifically 1 to 5).

Alternatively, $R^{b'}$ and $R^{c'}$ are H ; p_2 is 1-14; and p_2' is 1 to 5; and in another alternative, $R^{b'}$ and $R^{c'}$ are H ; p_2 is 1-8; and p_2' is 1 to 5. The remainder of the variables in Formula (III) are

as defined in the third embodiment or its first, second, third, fourth, fifth, or sixth specific embodiment.

[172] In an eighth specific embodiment, L_2' is represented by the following formula:



wherein:

R₁₂, R₁₃, and R₁₄, for each occurrence, are each independently H, an optionally substituted alkyl;

Q is H, a charged substituent or an ionizable group;

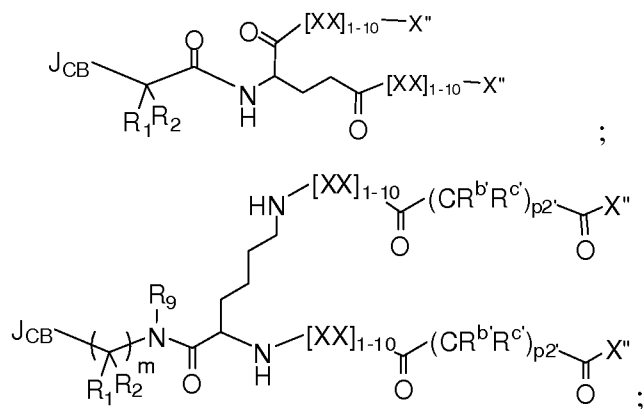
r is an integer from 0 to 10; and

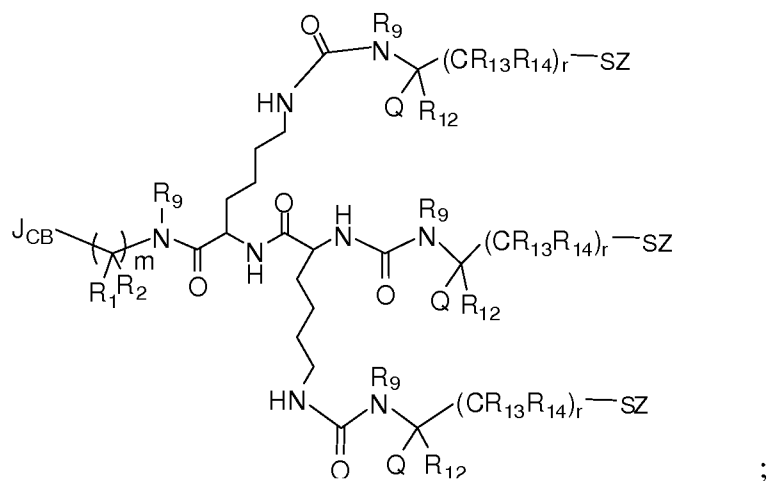
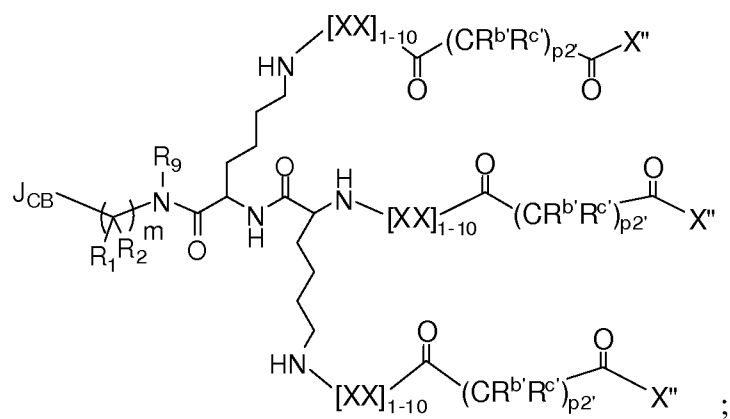
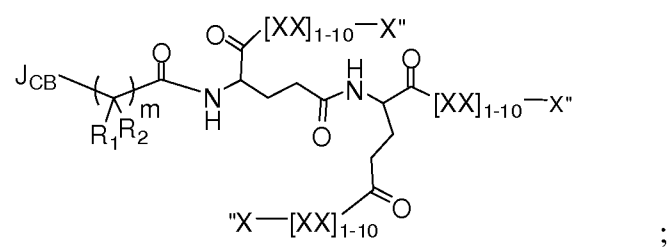
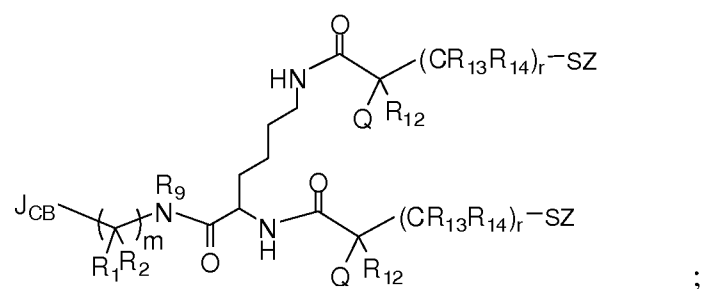
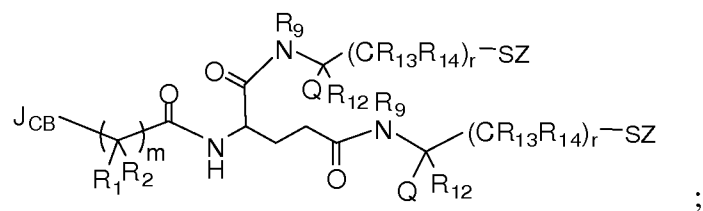
s5 is the site covalently linked to the group U.

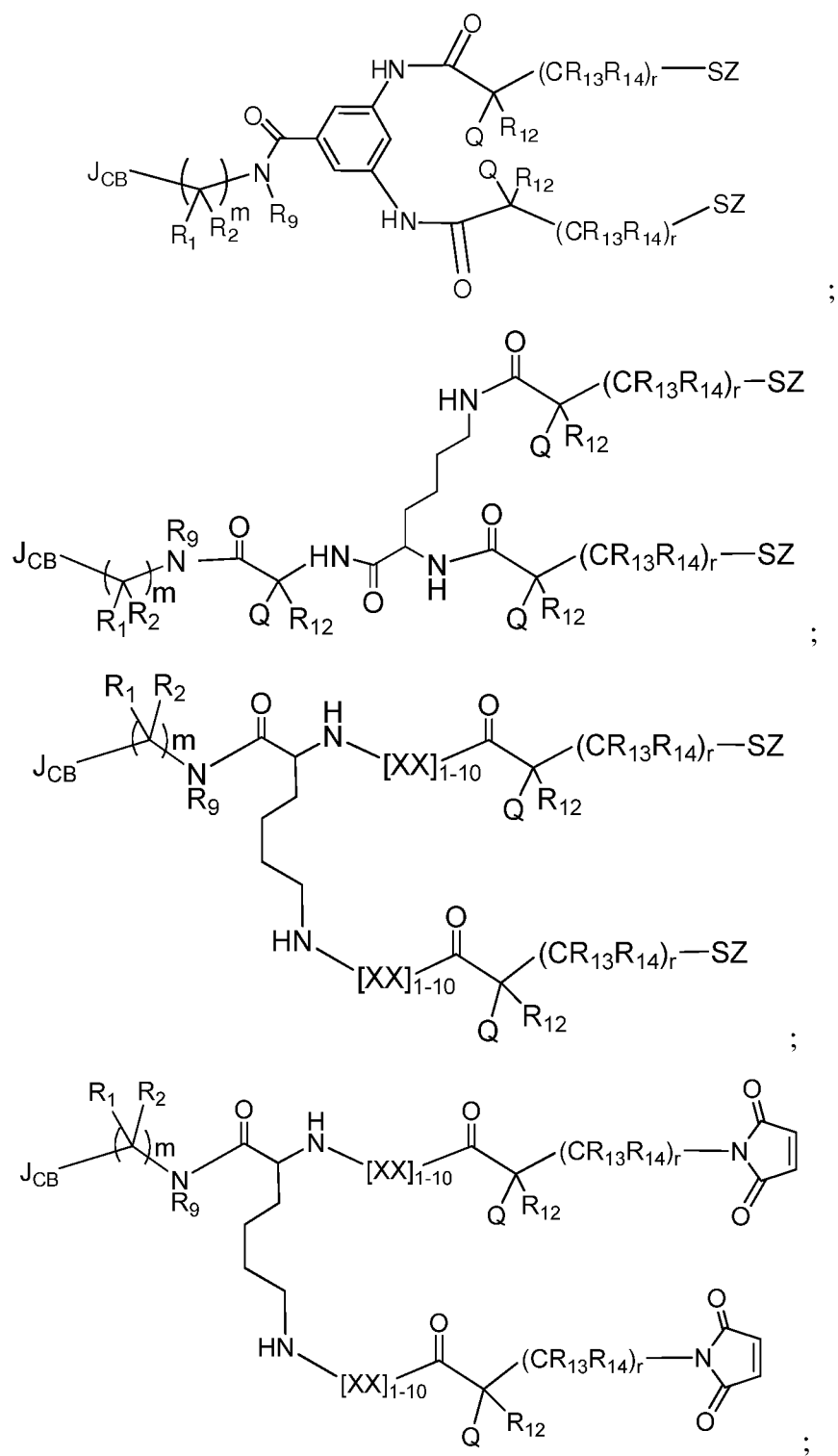
The remainder of the variables in Formula (III) or each of the formulas designated in this specific embodiment are as defined in the third embodiment or its seventh specific embodiment.

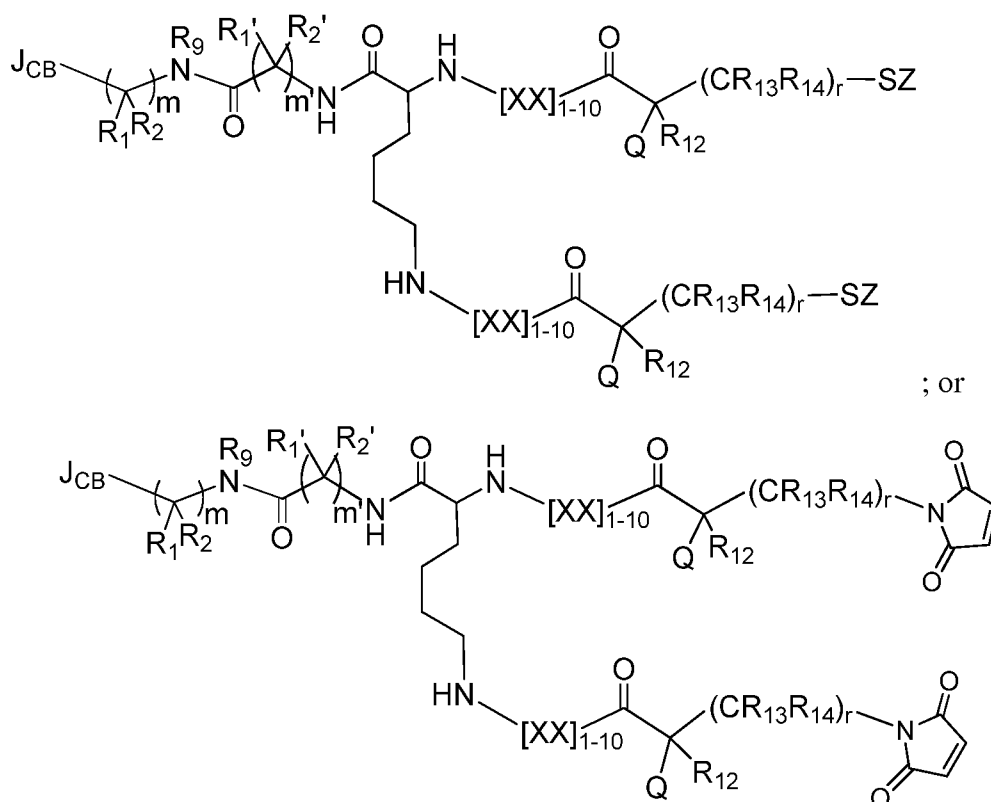
[173] In a ninth specific embodiment, X'' together with the adjacent -C(=O)- group is a reactive ester group; and R^d is a pyridyl or nitropyridyl. The remainder of the variables in Formula (III) or each of the formulas designated in the eighth specific embodiment are as defined in the third embodiment or its seventh or eighth specific embodiment.

[174] In a tenth specific embodiment, the compound is represented by the following formula, or a salt thereof:









The variables in each of the formulas designated in this specific embodiment are as defined in the third embodiment or its seventh, eighth, or ninth specific embodiment.

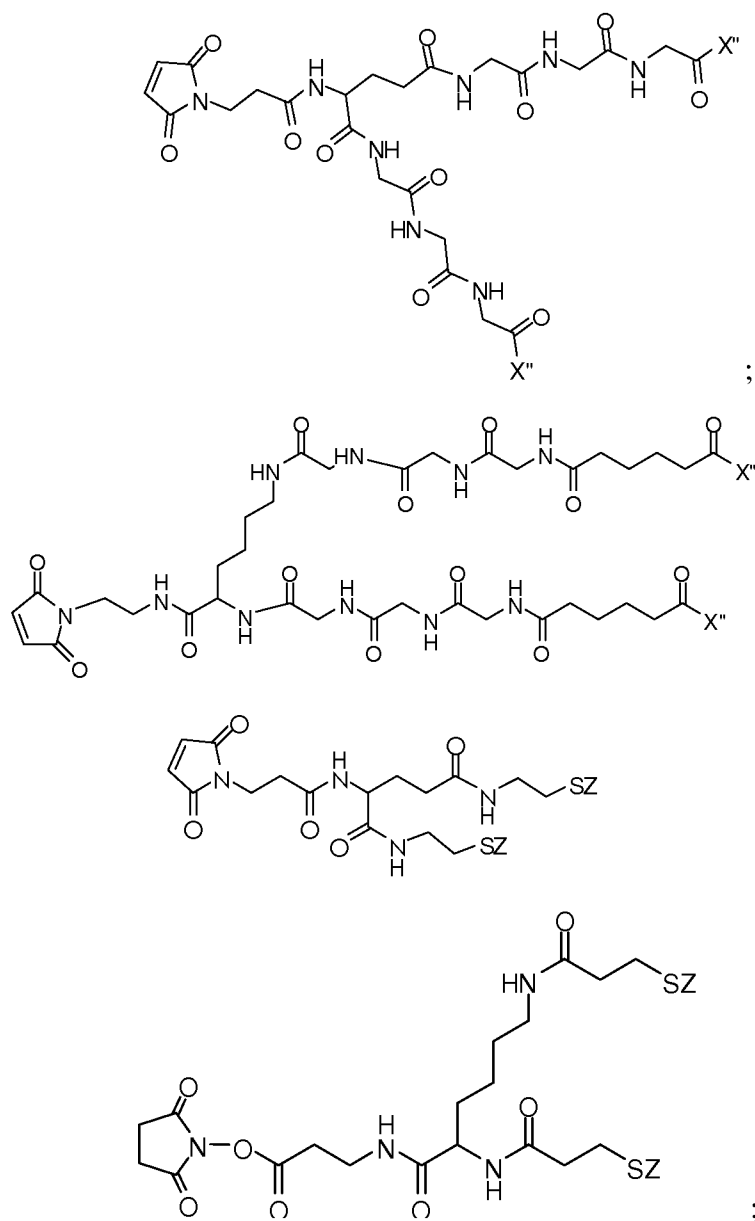
[175] In a eleventh specific embodiment, Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion. The remainder of the variables in Formula (III) or each of the formulas designated in the eighth or tenth embodiment above are as defined in the third embodiment or its eighth, ninth, or tenth specific embodiment.

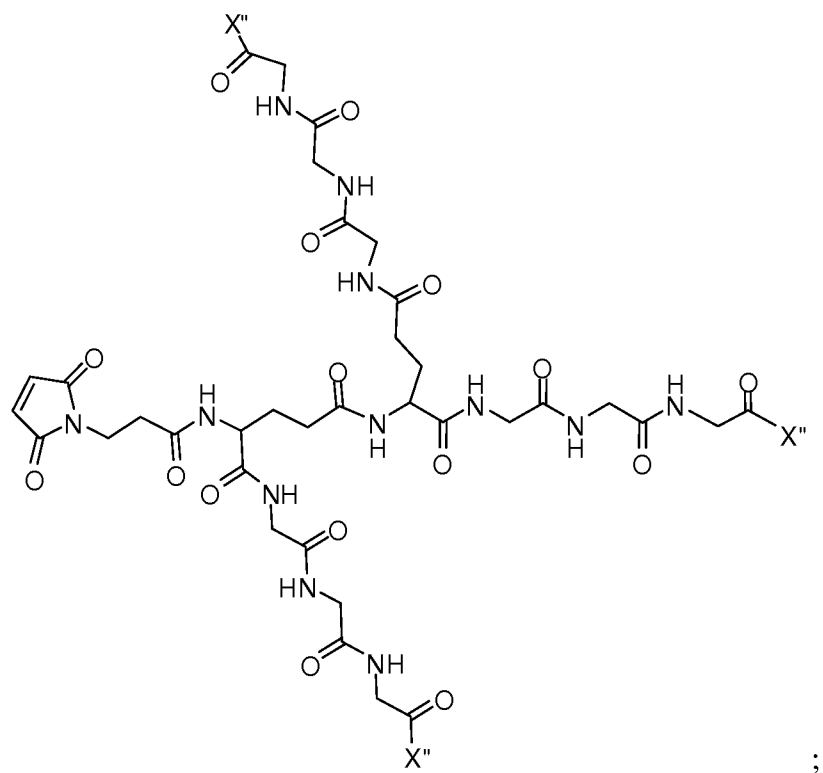
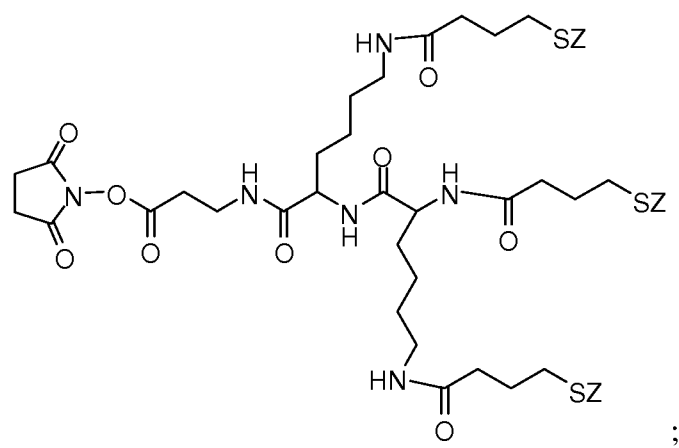
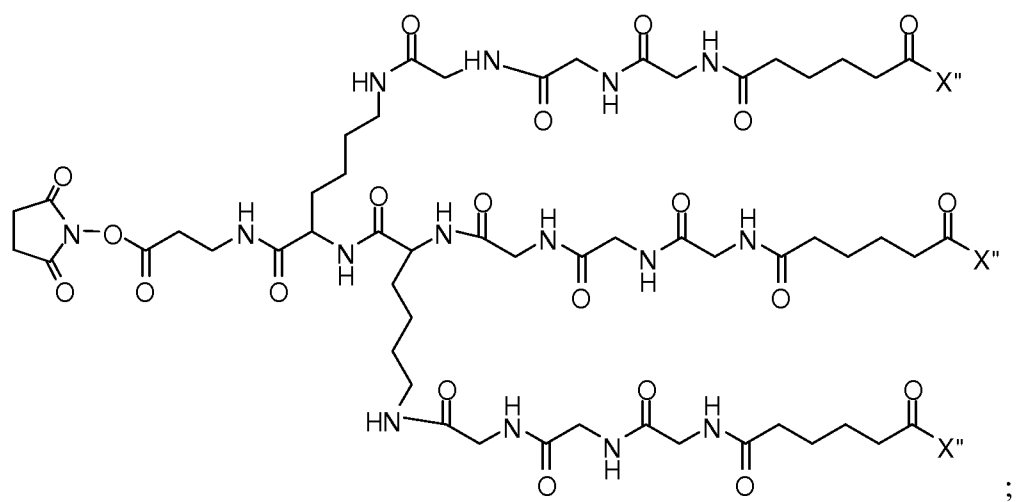
[176] In a twelfth specific embodiment, R_{12} is H; and Q is H, SO_3H or a salt (e.g., pharmaceutically acceptable salt) thereof. The remainder of the variables in Formula (III) or each of the formulas designated in the eighth or tenth embodiment above are as defined in the third embodiment or its eighth, ninth, tenth, eleventh specific embodiment.

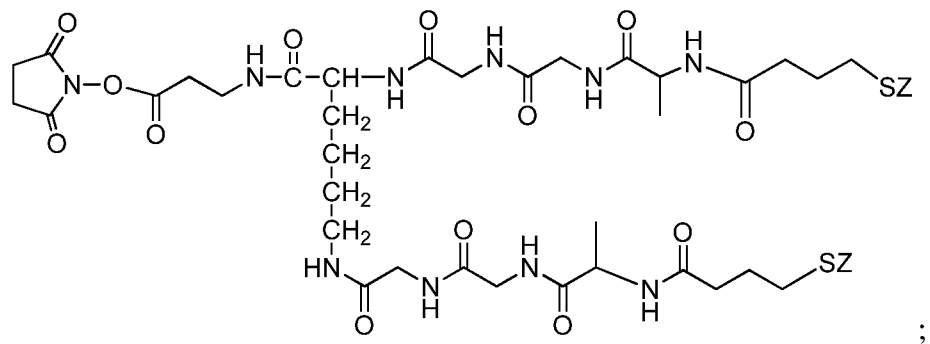
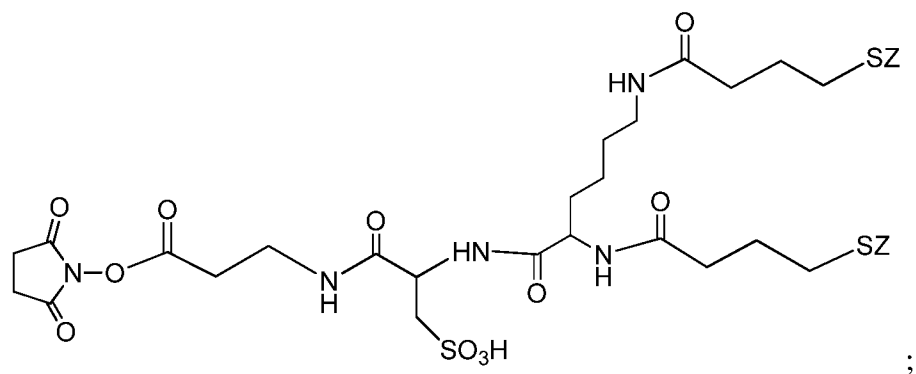
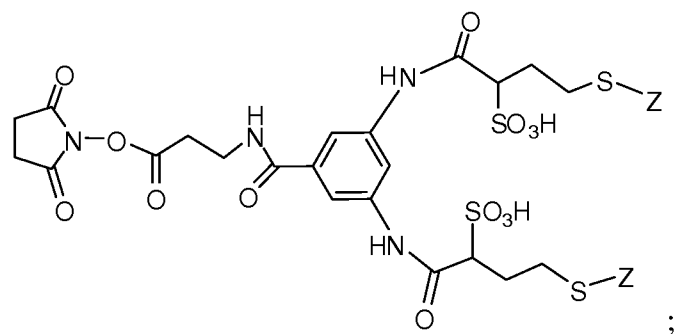
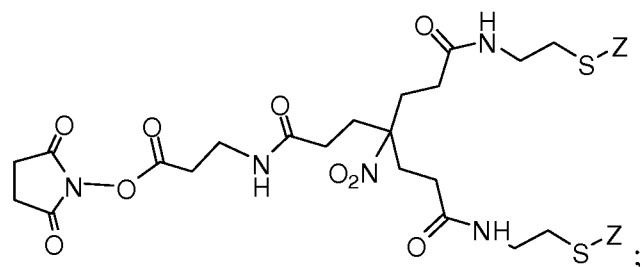
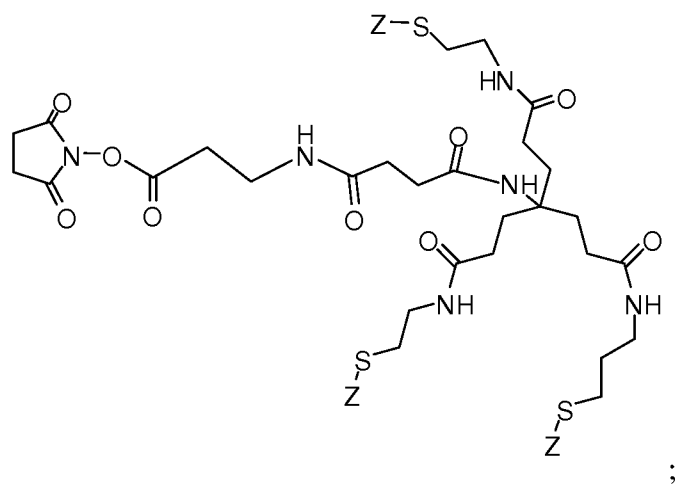
[177] In a thirteenth specific embodiment, R^{b} and R^{c} are both H; and p_2' is an integer from 1 to 5. The remainder of the variables in Formula (III) or each of the formulas designated in the seventh, eighth, or tenth specific embodiment are as defined in the third embodiment or its seventh, eighth, ninth, tenth, eleventh or twelfth specific embodiment.

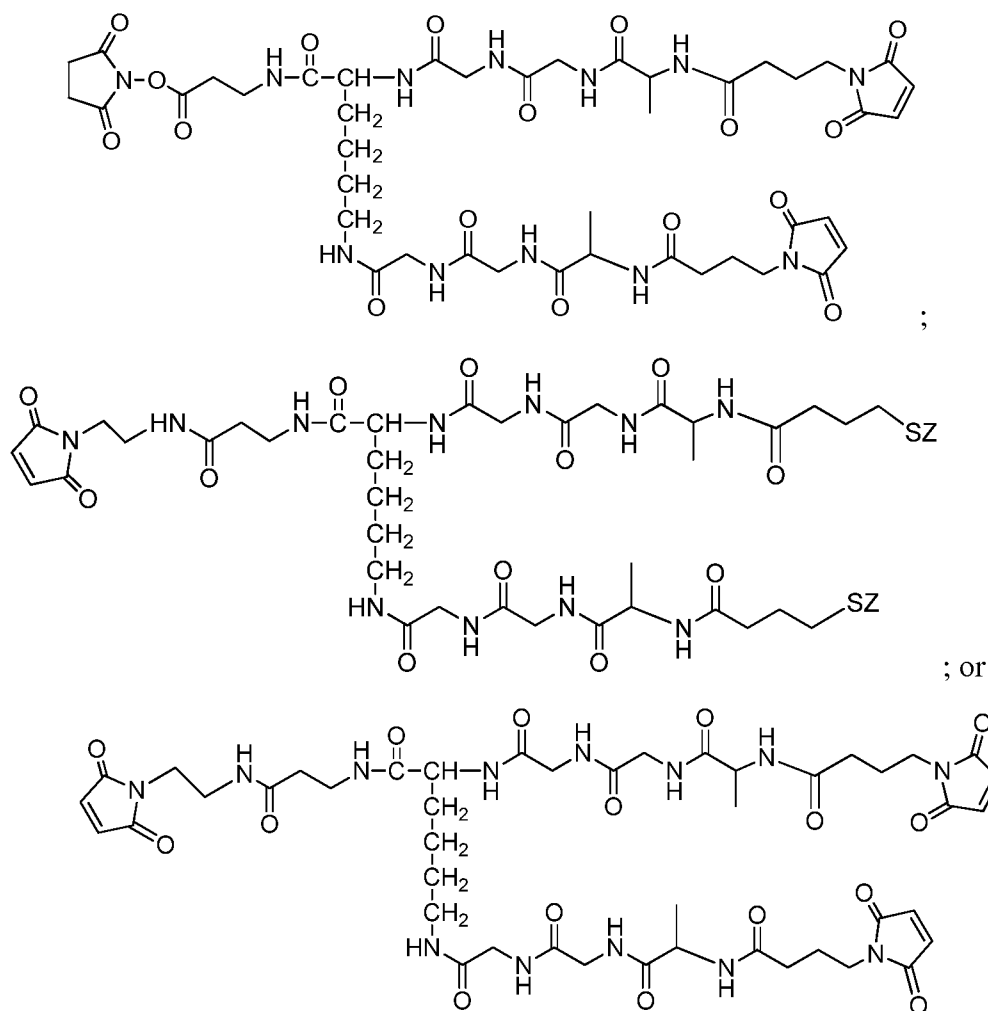
[178] In a fourteenth specific embodiment, R_{13} and R_{14} are both H; and r is 1 to 5. The remainder of the variables in Formula (III) or each of the formulas designated in the eighth, or ninth embodiment are as defined in the third embodiment or its eighth, ninth, tenth, eleventh, twelfth, or thirteenth specific embodiment.

[179] In a fifteenth specific embodiment, wherein the compound is represented by the following formula, or a salt thereof:







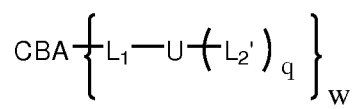


The variables in each of the formulas designated in this specific embodiment are as defined in the seventh specific embodiment of this section.

[180] In a sixteenth specific embodiment, in each of the formulas designated in the fifteenth specific embodiment of this section, X'' together with the adjacent $-(C=O)-$ group forms a reactive ester group; Z is $-SR^d$, and R^d is a pyridyl or nitropyridyl.

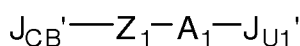
MODIFIED CELL BINDING AGENT

[181] In a fourth embodiment, the present invention further provides a modified cell binding agent represented by Formula (IV) below:

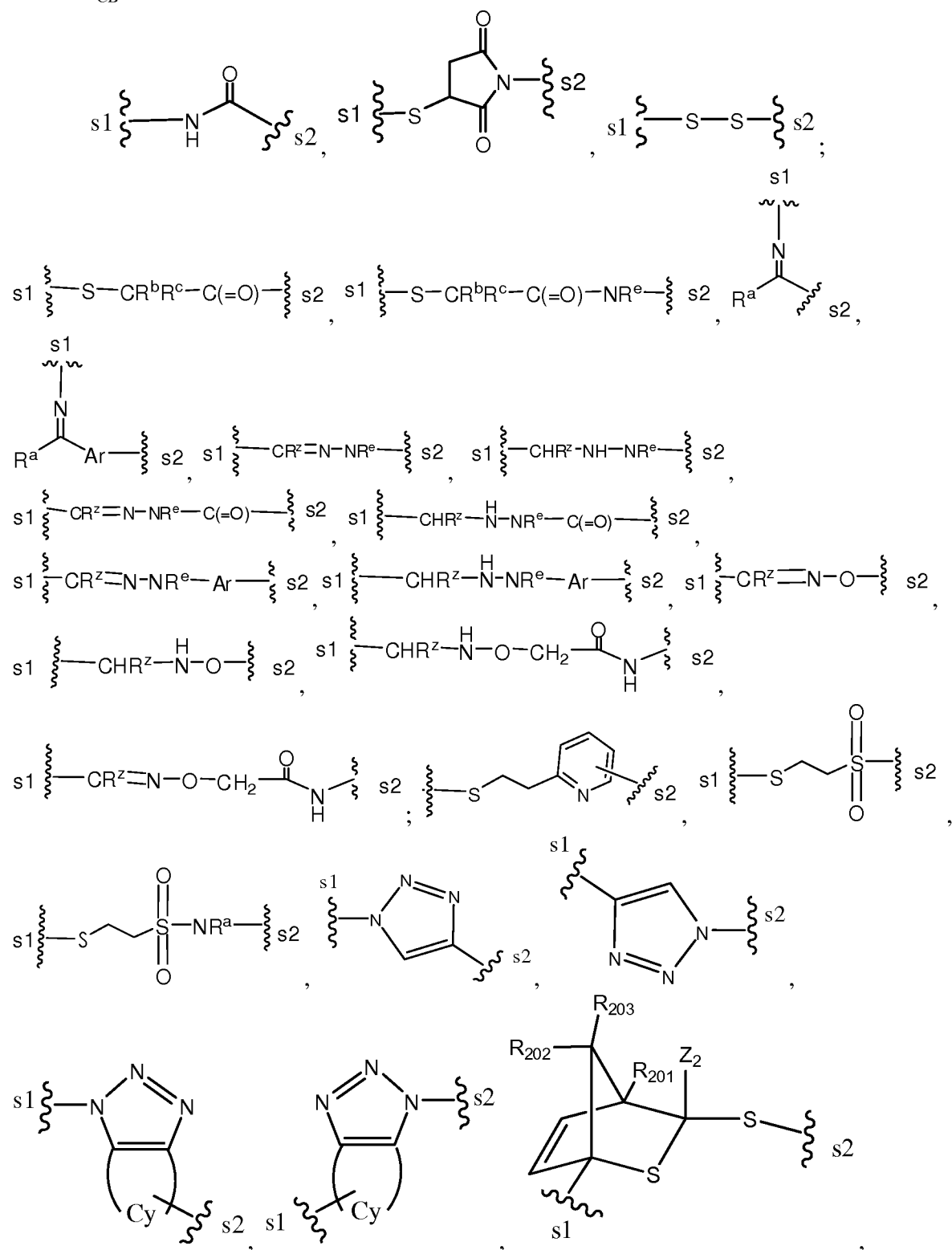


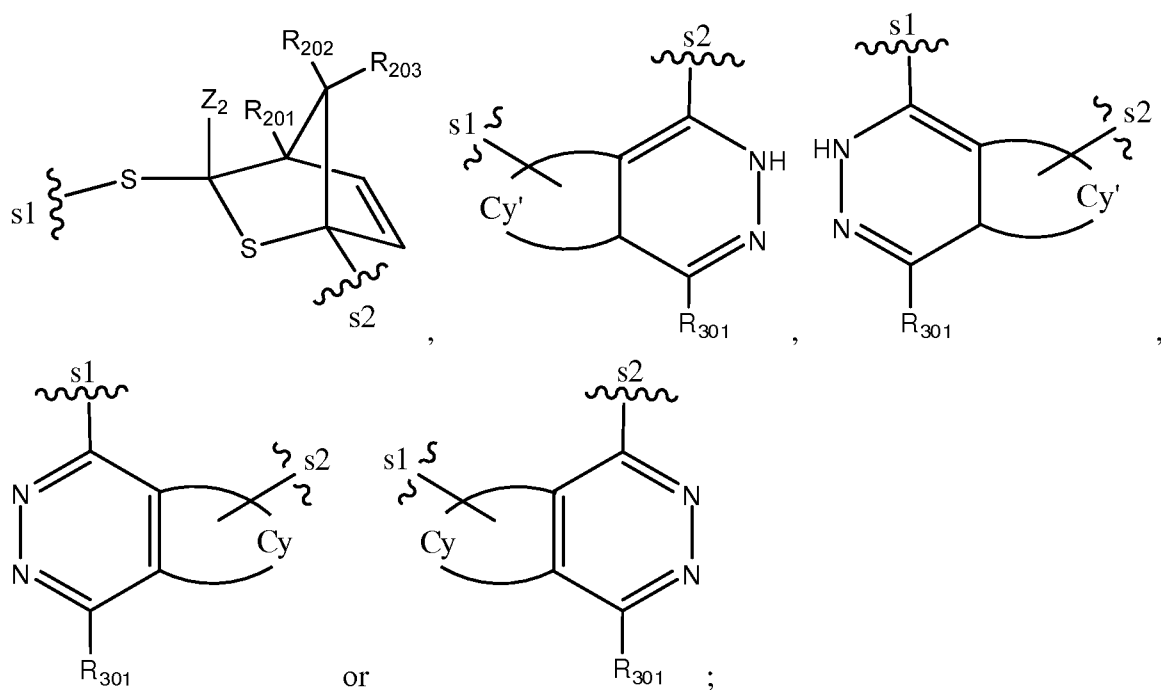
[182] In addition, sixteen specific embodiments for the modified cell binding agent are further described below in this modified cell binding agent section.

[183] In a first specific embodiment, L_1 is represented by the following formula:



in which:

 J_{CB}' is



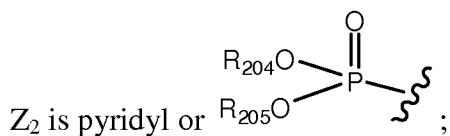
s_1 is the site covalently linked to the CBA;

s_2 is the site covalently linked to the group U;

Ar is an optionally substituted arylene or an optionally substituted heteroarylene;

Cy is an optionally substituted carbocyclic or heterocyclic ring;

R_{201} , R_{202} and R_{203} each are independently H or an optionally substituted alkyl;



R_{204} and R_{205} are each independently optionally substituted alkyl;

R_{301} is H or optionally substituted alkyl;

Cy' is an optionally substituted carbocyclic or heterocyclic ring;

Z_1 is $-NR_9-C(=O)-$ or $-C(=O)-NR_9-$ or absent,

A_1 is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, $-(CH_2-CH_2-O)_{p1}-(CR^bR^c)_{p1'}$ or $-(CR^bR^c)_{p1'}-(O-CH_2-CH_2)_{p1}$,

$J_{U1'}$ is absent, $-NR_9-$ or $-C(=O)-$

p_1 is an integer from 1 to 1000 (specifically, 1 to 24, more specifically 1 to 8 (e.g., 2, 4, 6 or 8))

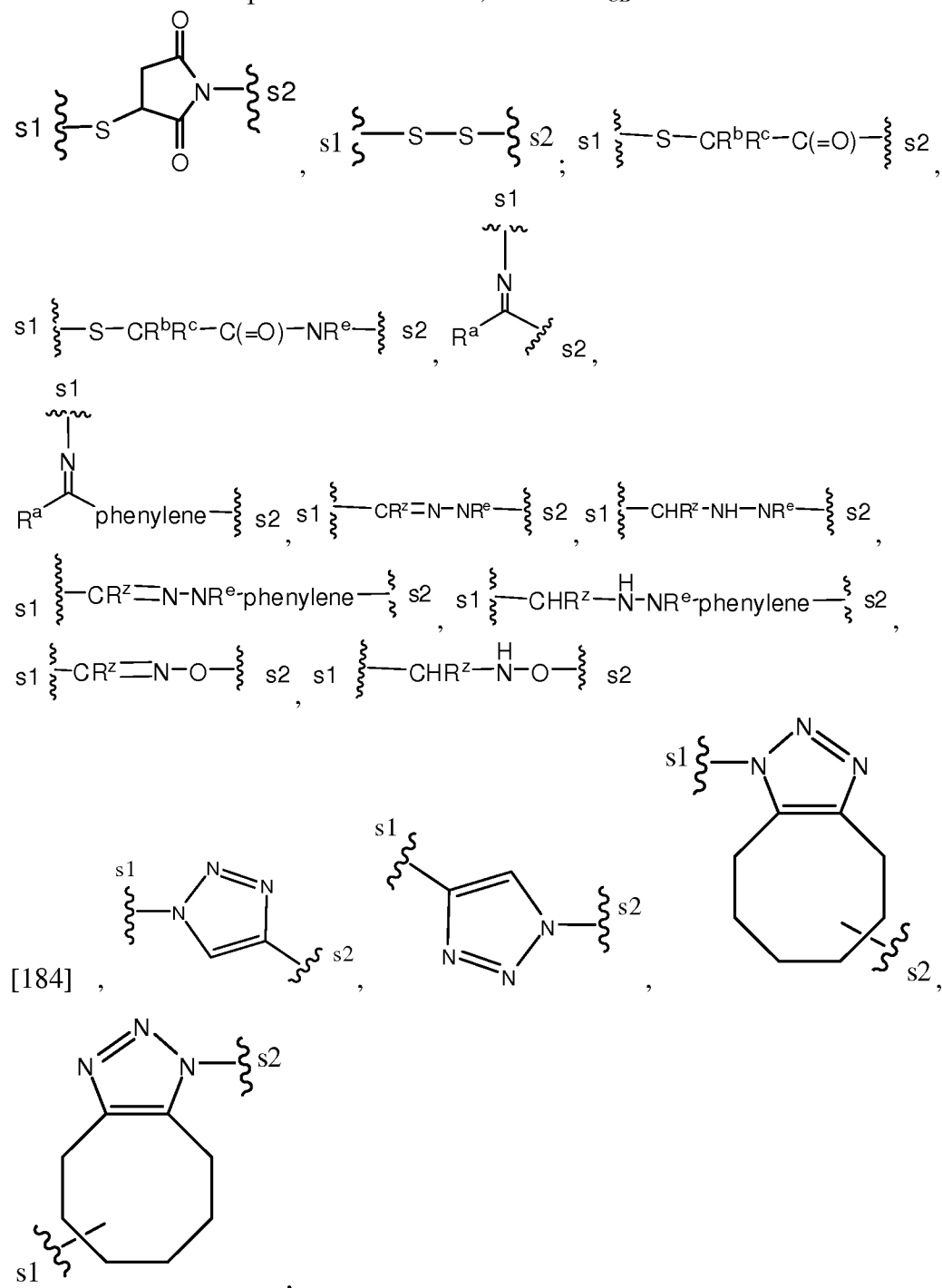
p_1' is an integer from 0 to 10;

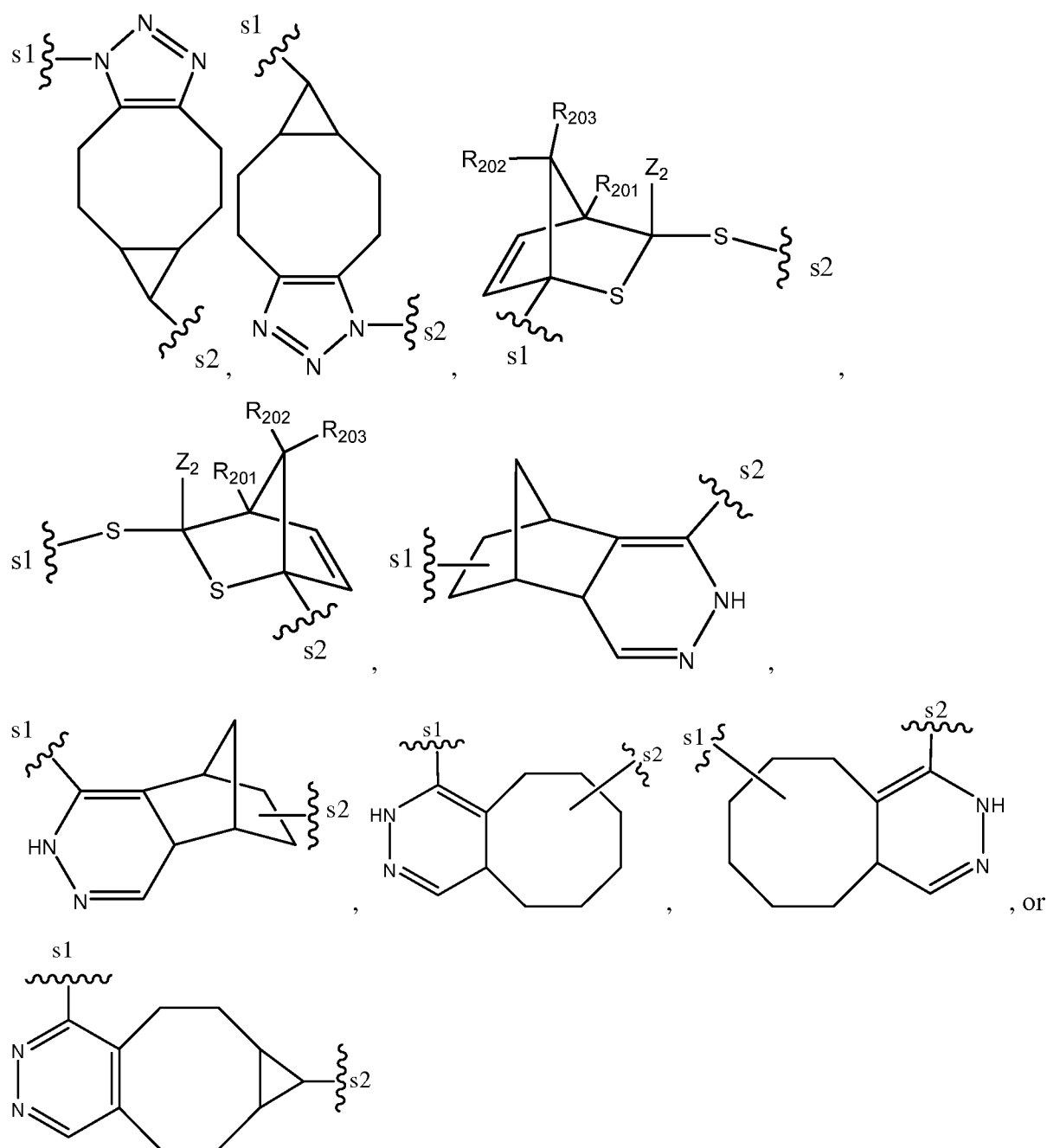
R_9 is H or an optionally substituted alkyl; and

R^a, R^b, R^c, R^e and R^z, for each occurrence, are independently H or an optionally substituted alkyl.

Alternatively, p1 is 1 to 24. In another alternative, p1 is 1 to 8. In yet another alternative, p1 is 2, 4, 6, or 8. The remainder of the variables in Formula (IV) are as described in the fourth embodiment.

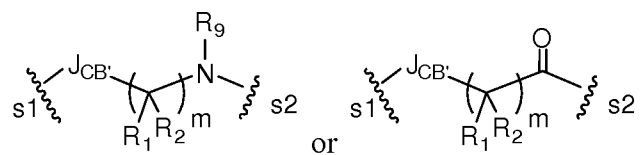
In a second specific embodiment, variable J_{CB} ' is





The remainder of the variables in Formula (IV) or the formula designated in the first specific embodiment are as defined in the fourth embodiment, each of its alternative embodiments, or its first specific embodiment.

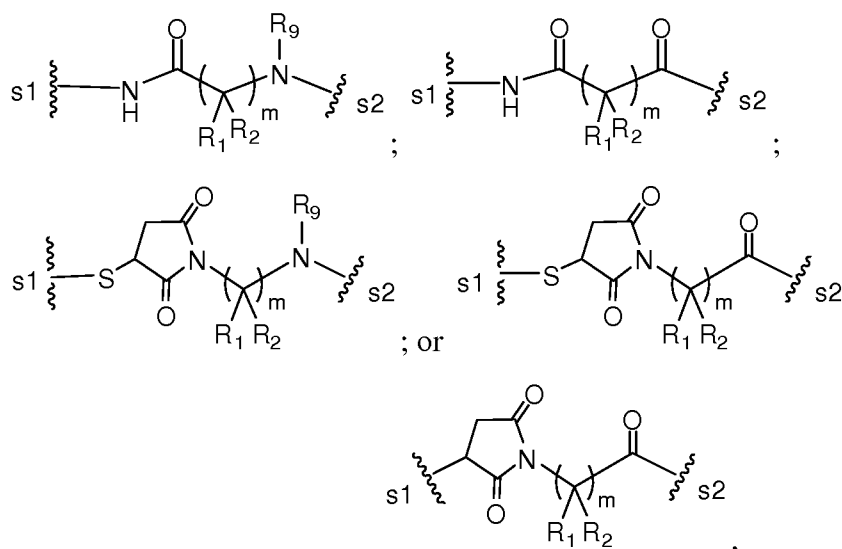
[185] In a third specific embodiment, L_1 is represented by the following formula:



in which R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10. The remainder of the

variables in Formula (IV) or each of the above formulas representing L_1 are as defined in the fourth embodiment or its first or second specific embodiment.

[186] In a fourth specific embodiment, L_1 is represented by the following formula:



in which:

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

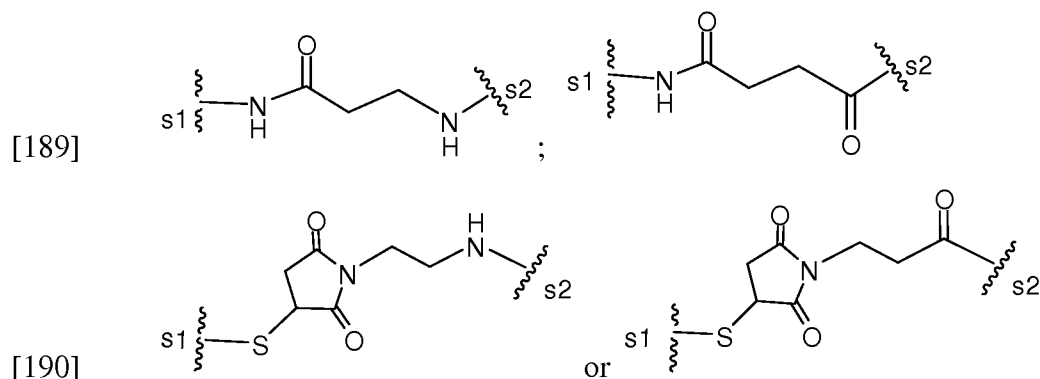
m is an integer from 1 to 5; and

R^9 is H or an alkyl.

The remainder of the variables in Formula (IV) or each of the formulas in this specific embodiment are as defined in the fourth embodiment or its third specific embodiment.

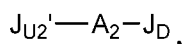
[187] In a fifth specific embodiment, R_1 and R_2 are both H; and R^9 is H. The remainder of the variables in Formula (IV) or each of the formulas designated in the third or fourth specific embodiment are as defined in the fourth embodiment or its third or fourth specific embodiment.

[188] In a sixth specific embodiment, L_1 is represented by the following formula:



The remainder of the variables in Formula (IV) or each of the formulas designated in this specific embodiment are as defined in the fourth embodiment or its fifth specific embodiment.

[191] In a seventh specific embodiment, L_2' is represented by the following formula:

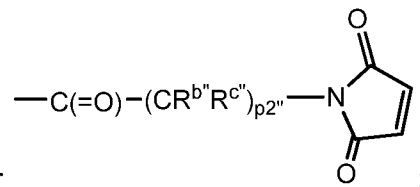


wherein:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_D is $-C(=O)-X''$, $-C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-X''$, $-C(=O)-(CH_2-CH_2-$



$O)_{p2''}-(CR^{b'}R^{c'})_{p2'}-SZ$, $-SZ$, absent, $-C(=O)-(CR^{b''}R^{c''})_{p2''}-SZ$ or

X'' is $-OH$ or a carboxylic acid activating group;

Z is H or $-SR^d$;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl (specifically H);

$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H , an optionally substituted alkyl or a charged substituent or an ionizable group;

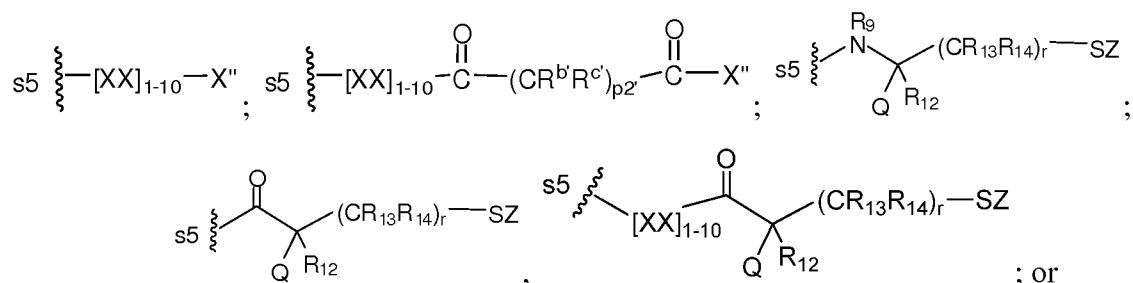
p_2 is an integer from 1 to 1000 (specifically 1 to 14, 1 to 8);

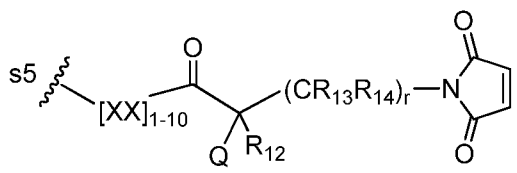
$p_{2'}$ is an integer from 1 to 10 (specifically 1 to 5); and

$p_{2''}$ is an integer from 1 to 10 (specifically 1 to 5).

Alternatively, $R^{b'}$ and $R^{c'}$ are H ; p_2 is 1-14; and $p_{2'}$ is 1 to 5; and in another alternative, $R^{b'}$ and $R^{c'}$ are H ; p_2 is 1-8; and $p_{2'}$ is 1 to 5. The remainder of the variables in Formula (IV) are as defined in the fourth embodiment or its first, second, third, fourth, fifth, or sixth specific embodiment.

[192] In an eighth specific embodiment, L_2' is represented by the following formula:





wherein:

R_{12} , R_{13} , and R_{14} , for each occurrence, are each independently H, an optionally substituted alkyl;

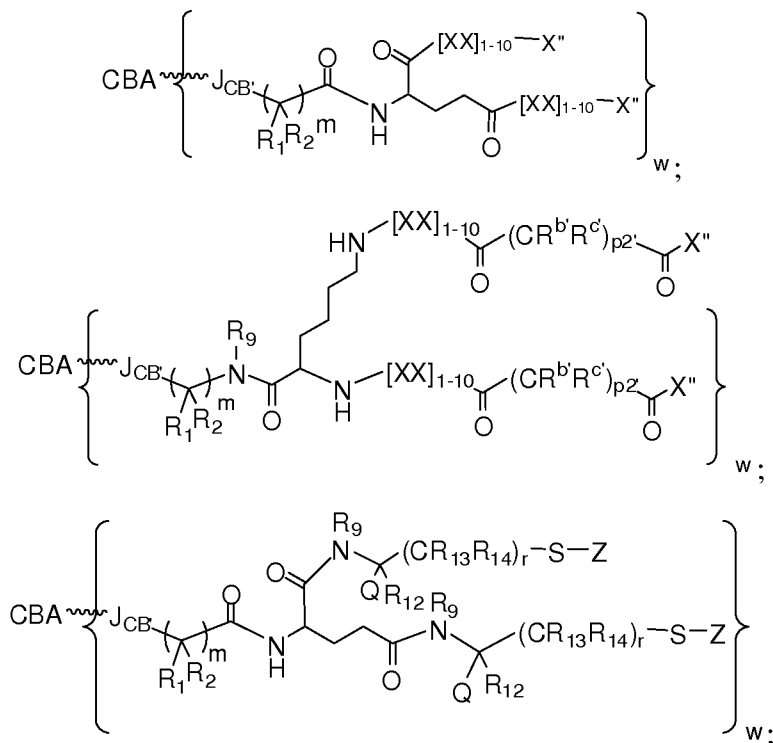
Q is H, a charged substituent or an ionizable group; and

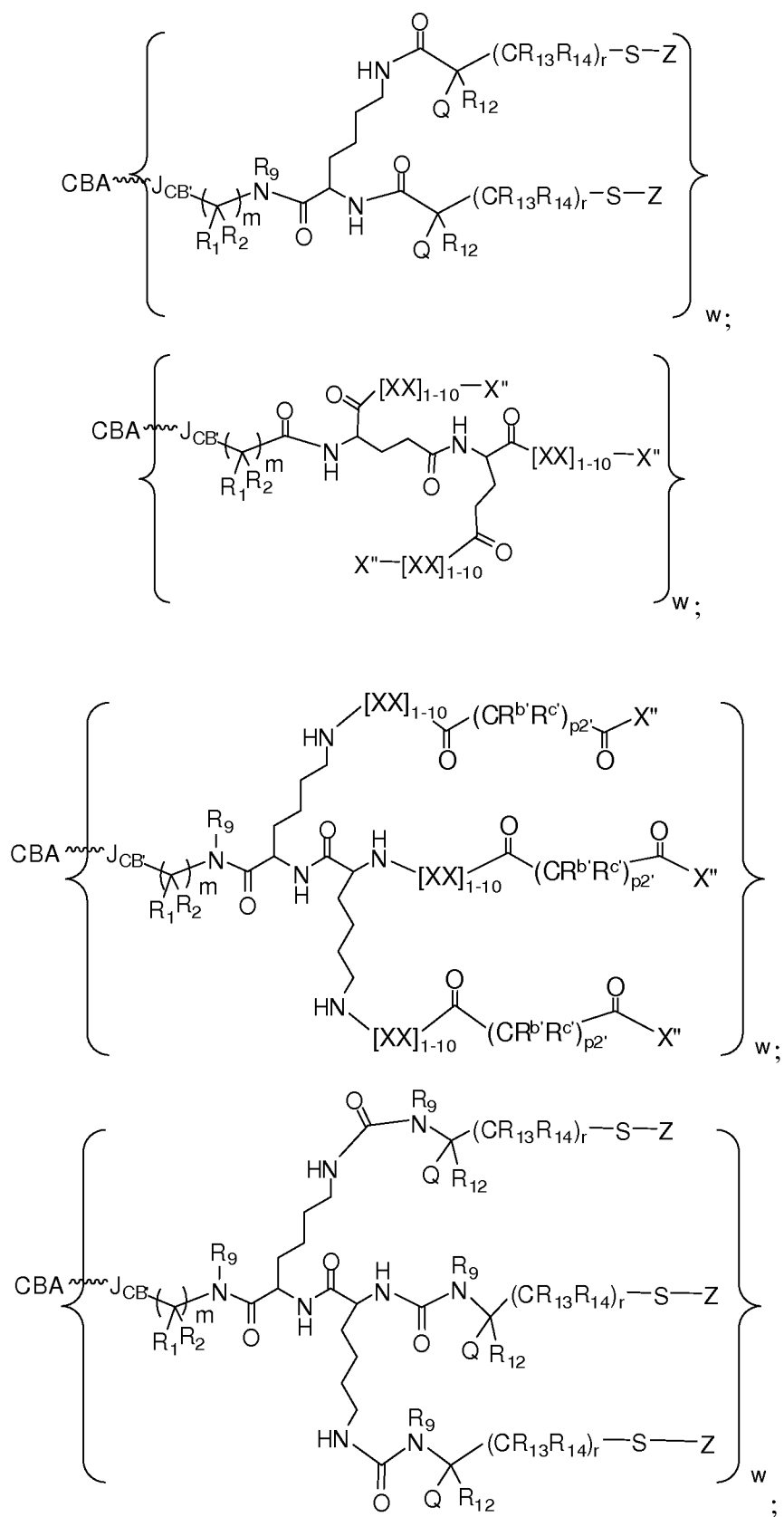
r is an integer from 0 to 10.

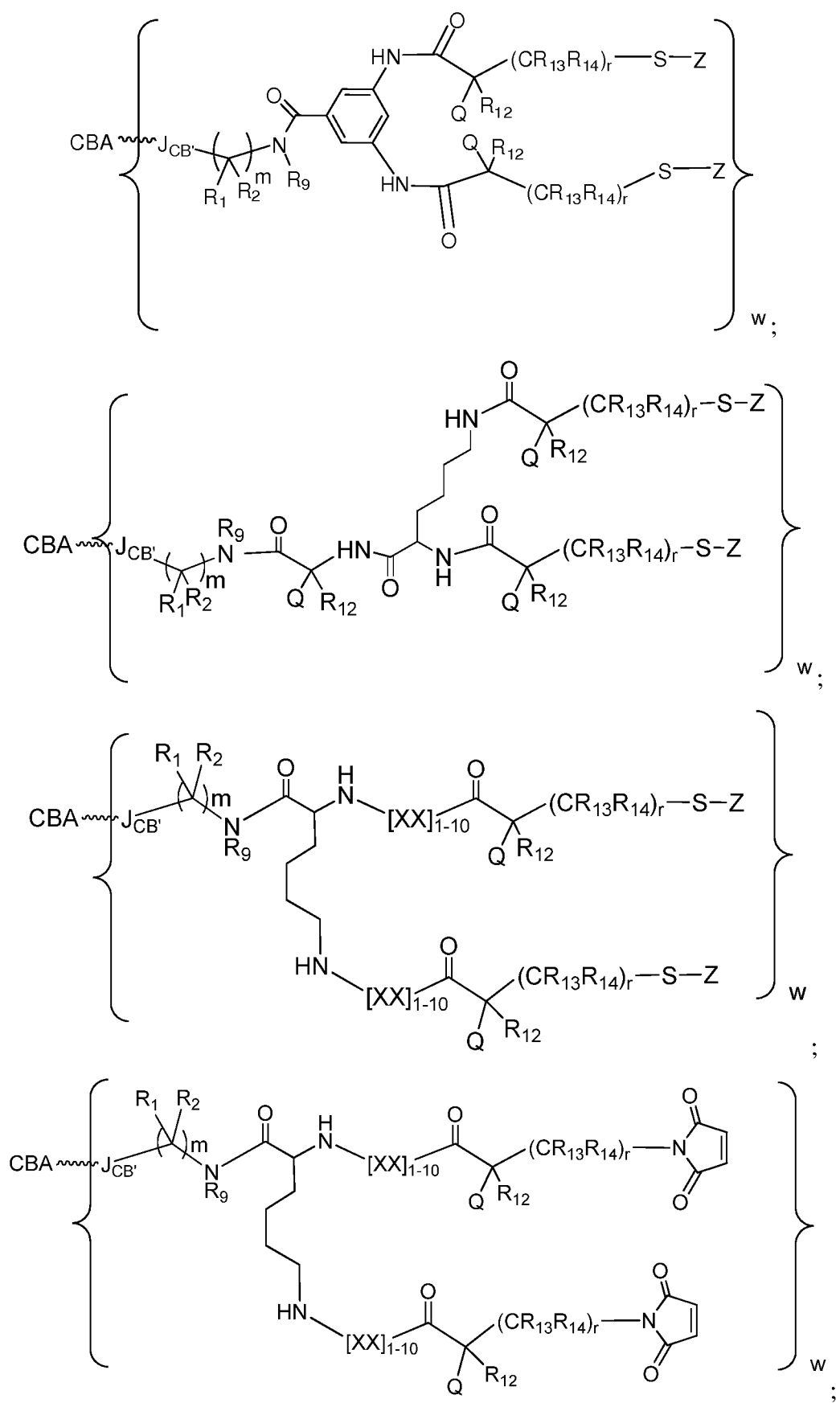
The remainder of the variables in Formula (IV) or each of the formulas designated in this specific embodiment are as defined in the fourth embodiment or its seventh specific embodiment.

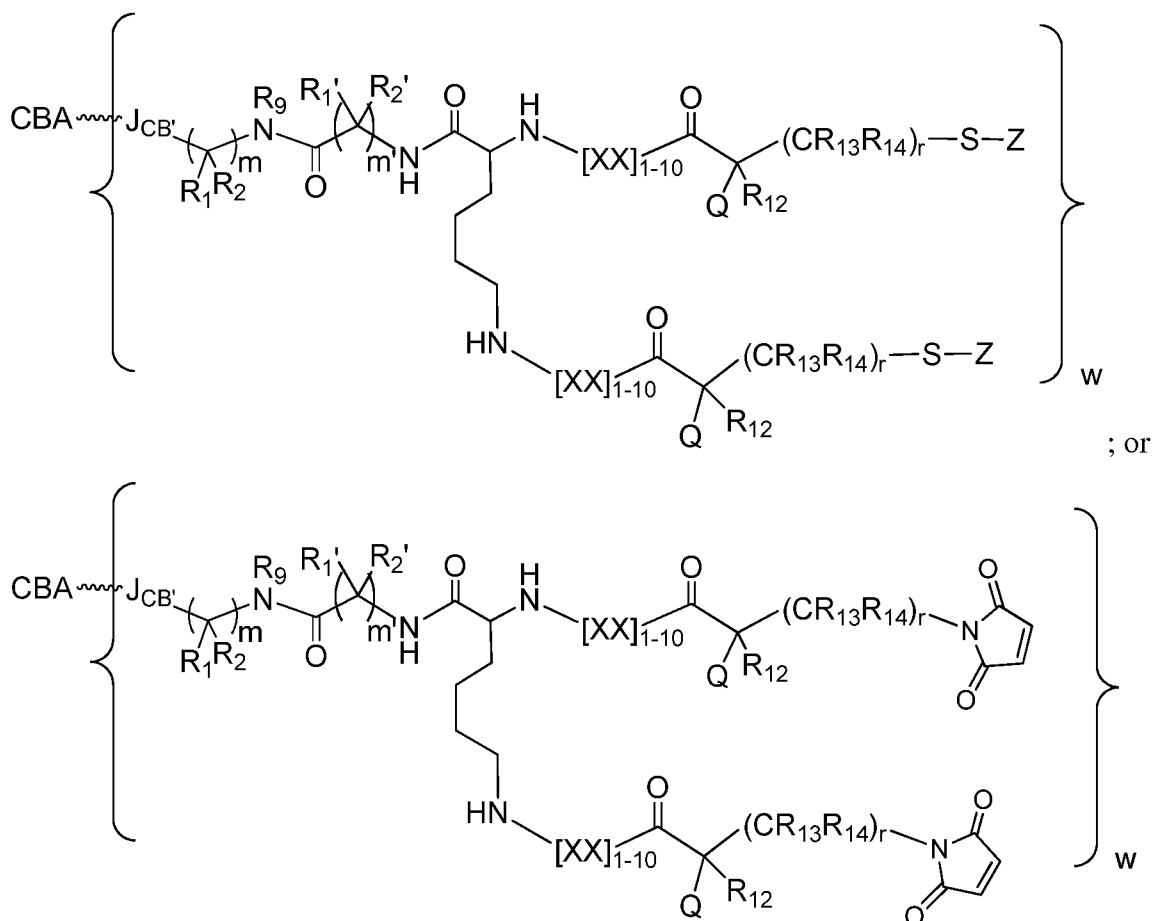
[193] In a ninth specific embodiment, X'' together with the adjacent $-C(=O)-$ group is a reactive ester group; and R^d is a pyridyl or nitropyridyl. The remainder of the variables in Formula (IV) or each of the formulas designated in the eighth specific embodiment are as defined in the fourth embodiment or its seventh or eighth specific embodiment.

[194] In a tenth specific embodiment, the modified cell binding agent is represented by the following formula, or a salt thereof:









The variables in each of the formulas designated in this specific embodiment are as defined in the fourth embodiment or its seventh, eighth, or ninth specific embodiment.

[195] In a eleventh specific embodiment, Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3\text{H}$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion. The remainder of the variables in Formula (IV) or each of the formulas designated in the eighth or tenth embodiment above are as defined in the fourth embodiment or its eighth, ninth, or tenth specific embodiment.

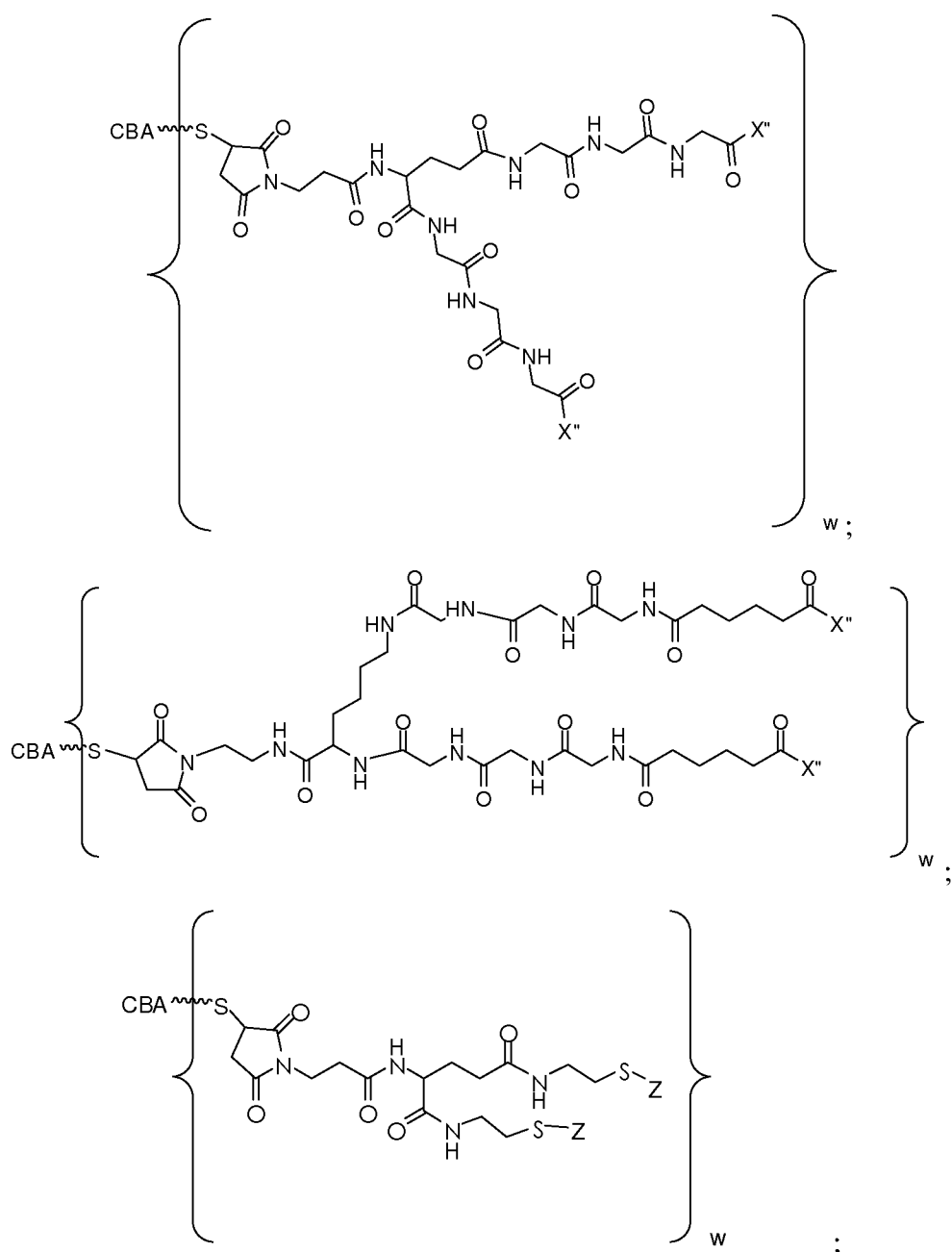
[196] In a twelfth specific embodiment, R_{12} is H; and Q is H, SO_3H or a salt (e.g., pharmaceutically acceptable salt) thereof. The remainder of the variables in Formula (IV) or each of the formulas designated in the eighth or tenth embodiment above are as defined in the fourth embodiment or its eighth, ninth, tenth, eleventh specific embodiment.

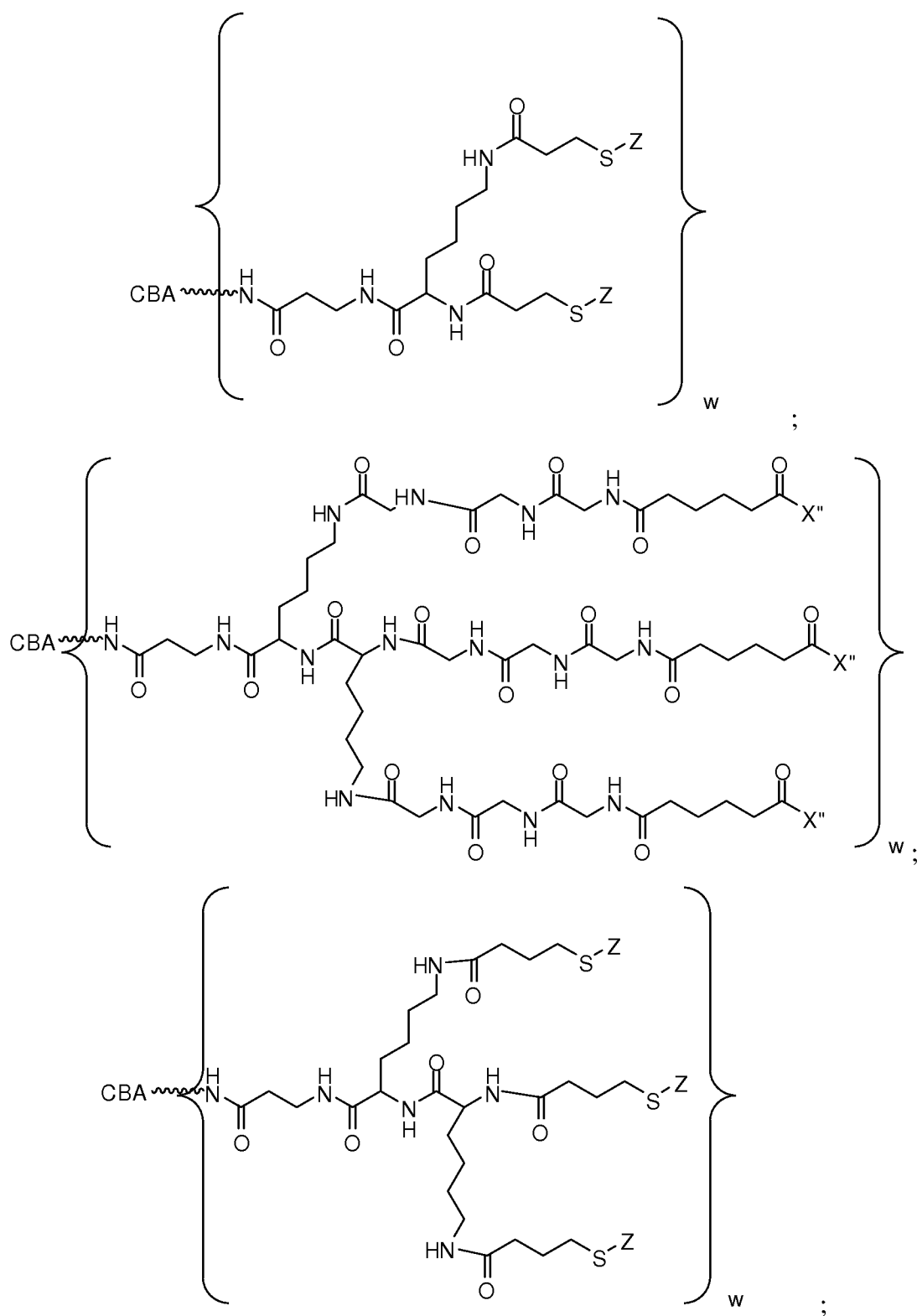
[197] In a thirteenth specific embodiment, $\text{R}^{b'}$ and $\text{R}^{c'}$ are both H; and $p_{2'}$ is an integer from 1 to 5. The remainder of the variables in Formula (IV) or each of the formulas designated in

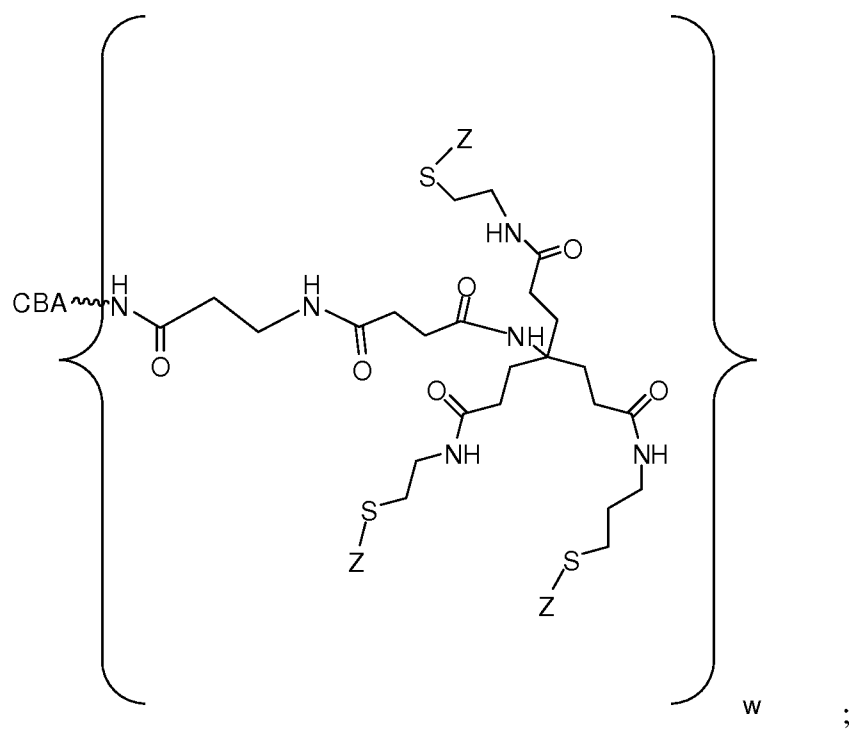
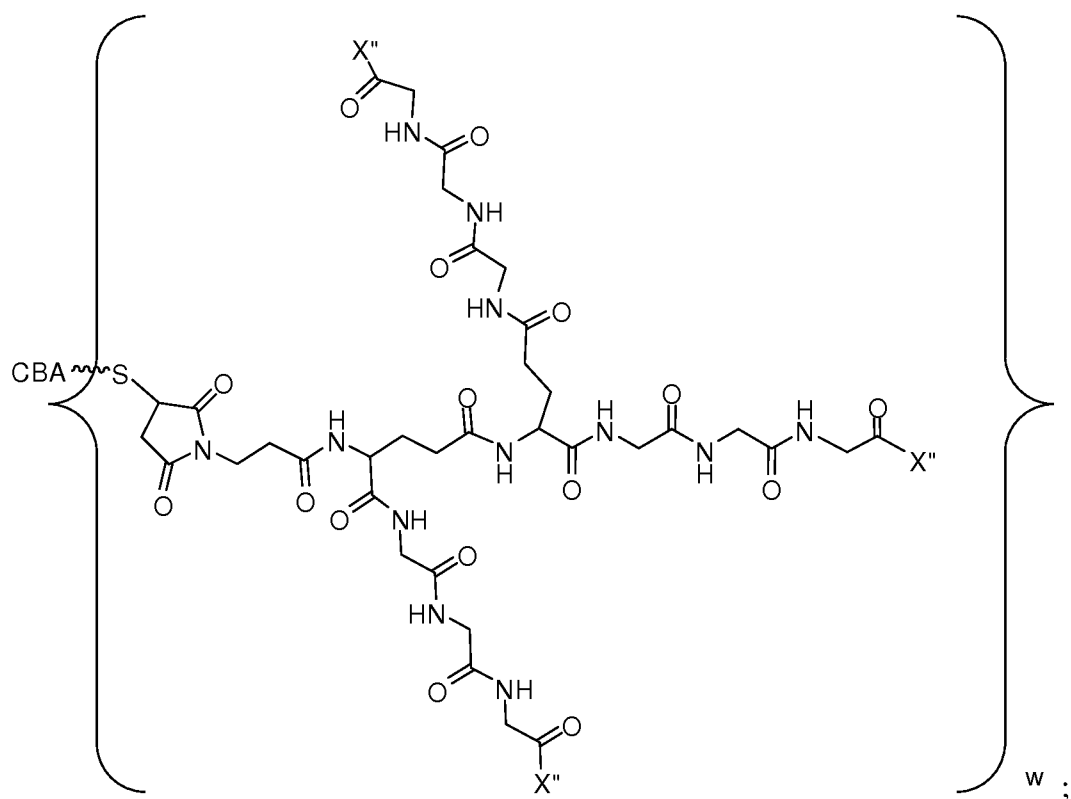
the seventh, eighth, or tenth specific embodiment are as defined in the fourth embodiment or its seventh, eighth, ninth, tenth, eleventh or twelfth specific embodiment.

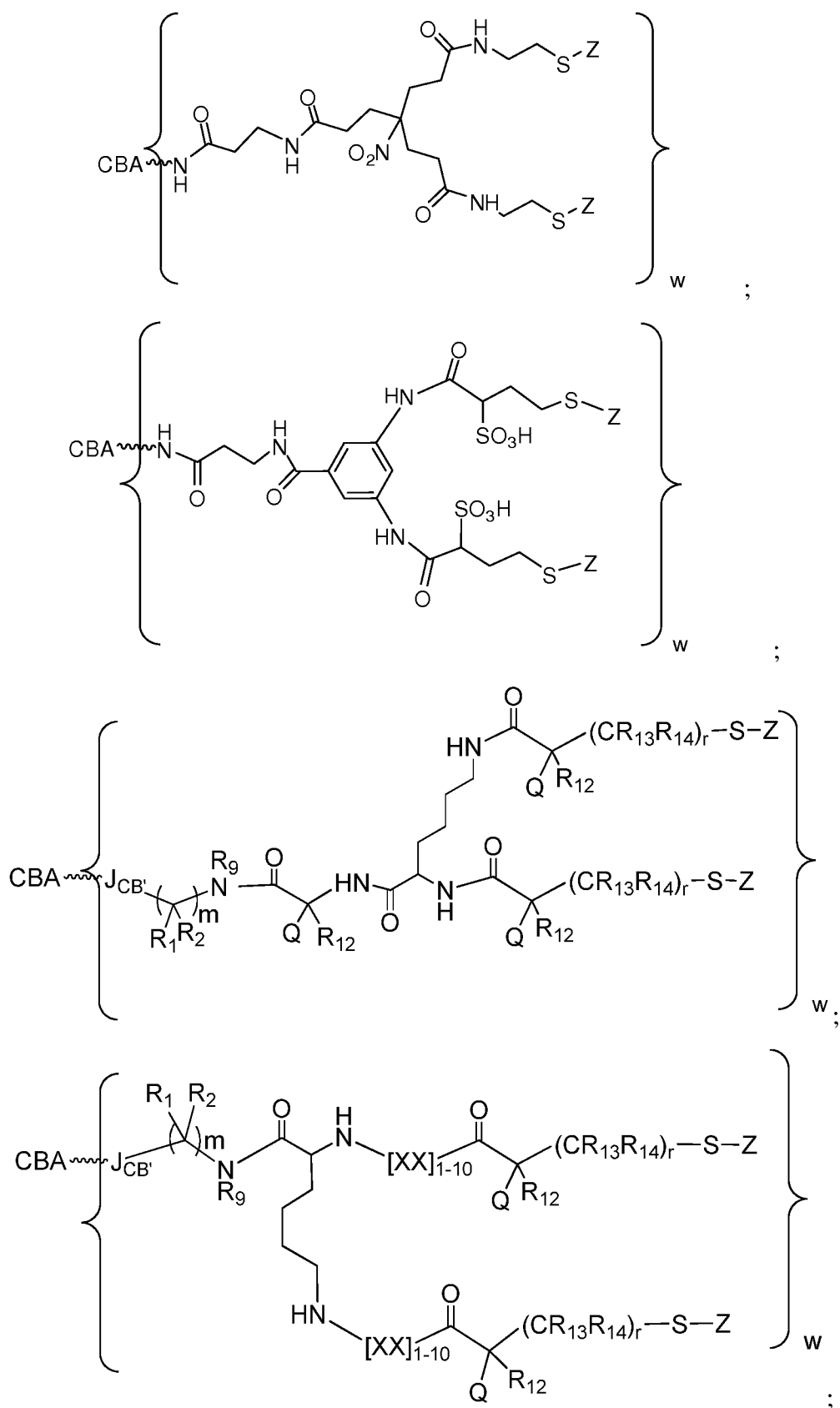
[198] In a fourteenth specific embodiment, R₁₃ and R₁₄ are both H; and r is 1 to 5. The remainder of the variables in Formula (IV) or each of the formulas designated in the eighth, or ninth embodiment are as defined in the fourth embodiment or its eighth, ninth, tenth, eleventh, twelfth, or thirteenth specific embodiment.

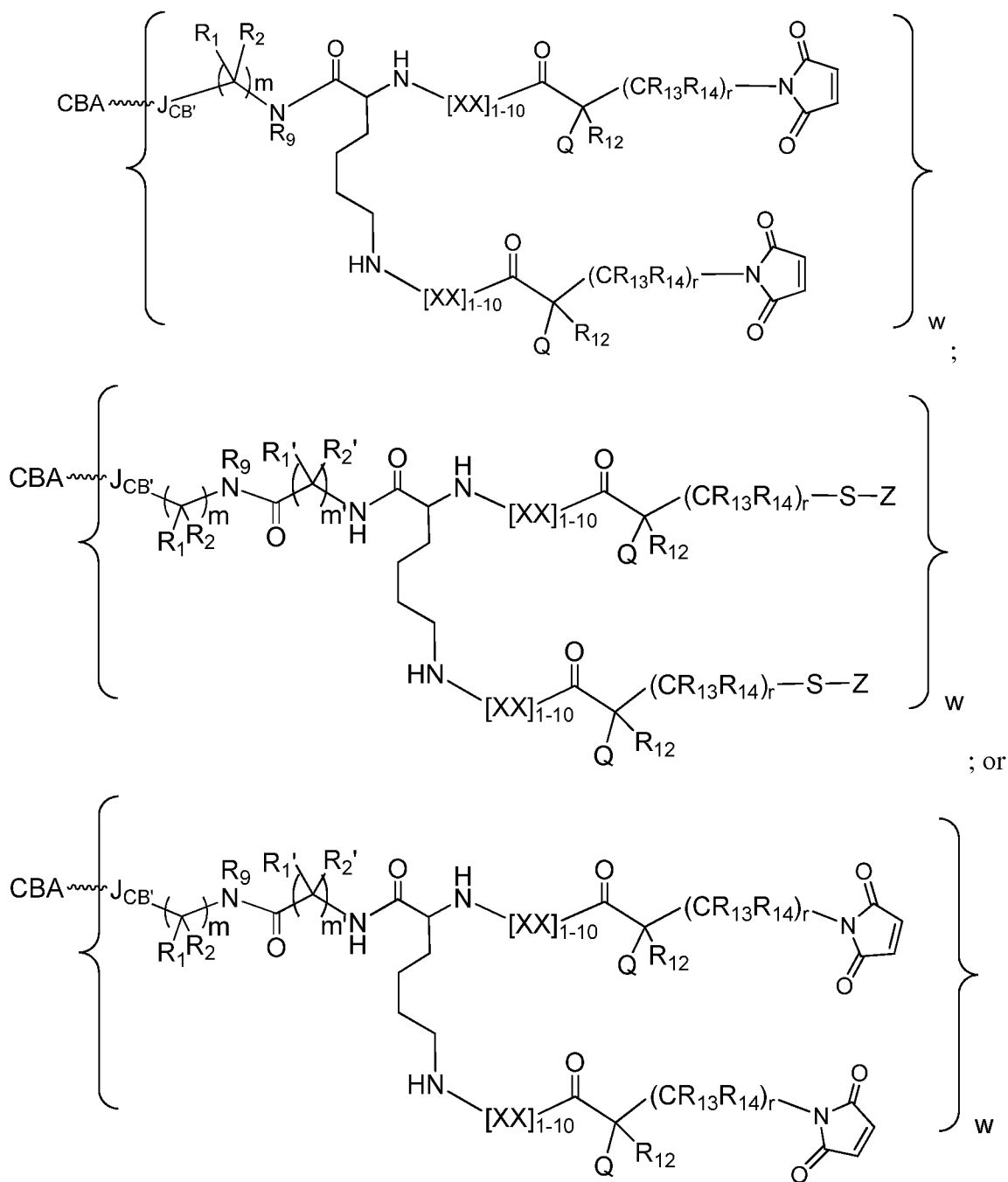
[199] In a fifteenth specific embodiment, wherein the modified cell binding agent is represented by one of the following formulas, or a salt thereof:





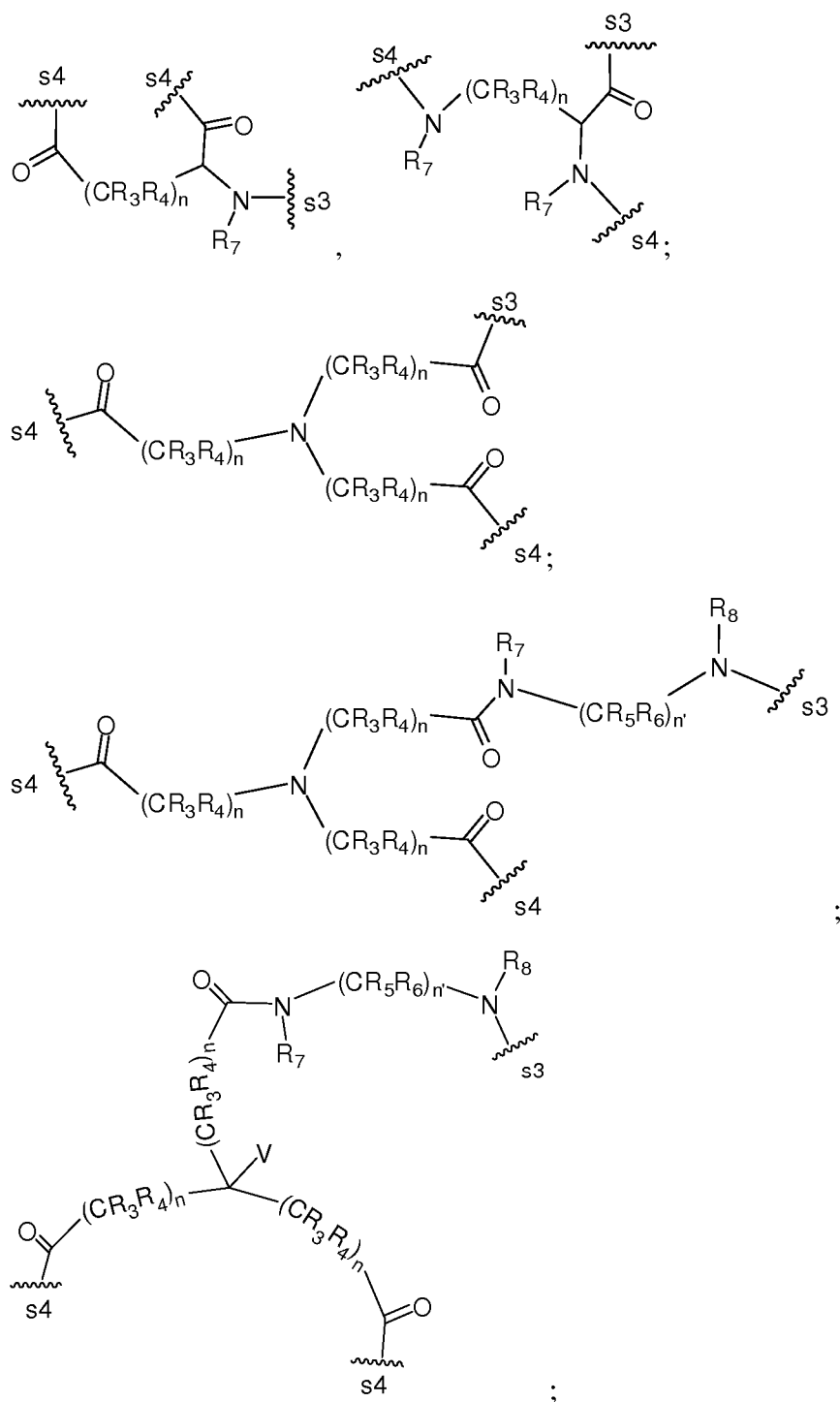


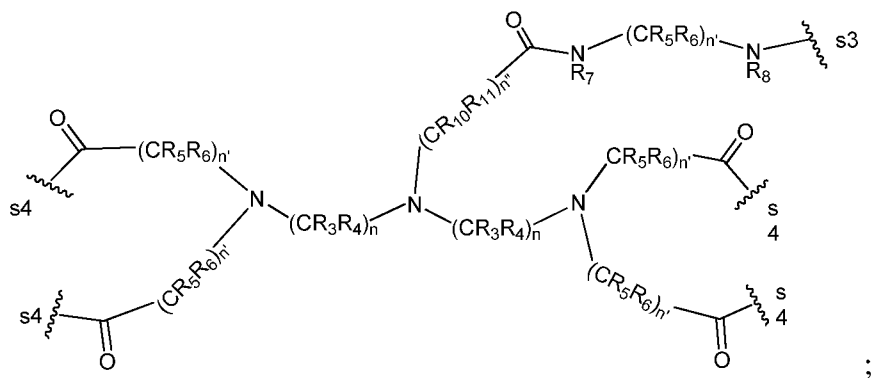
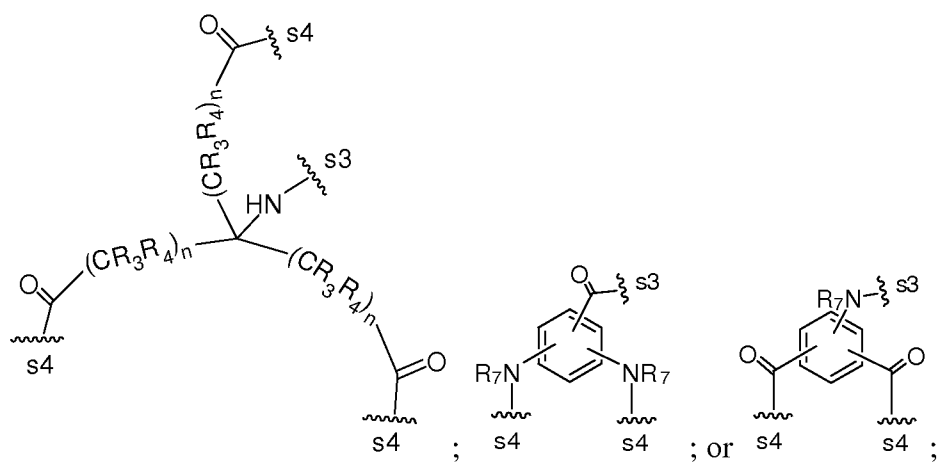
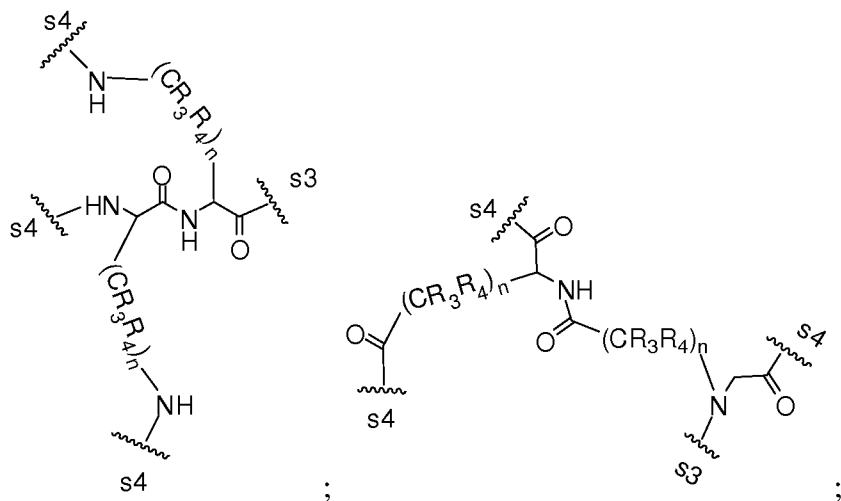
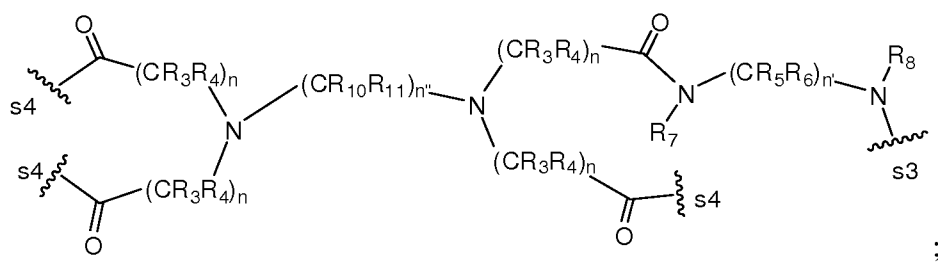


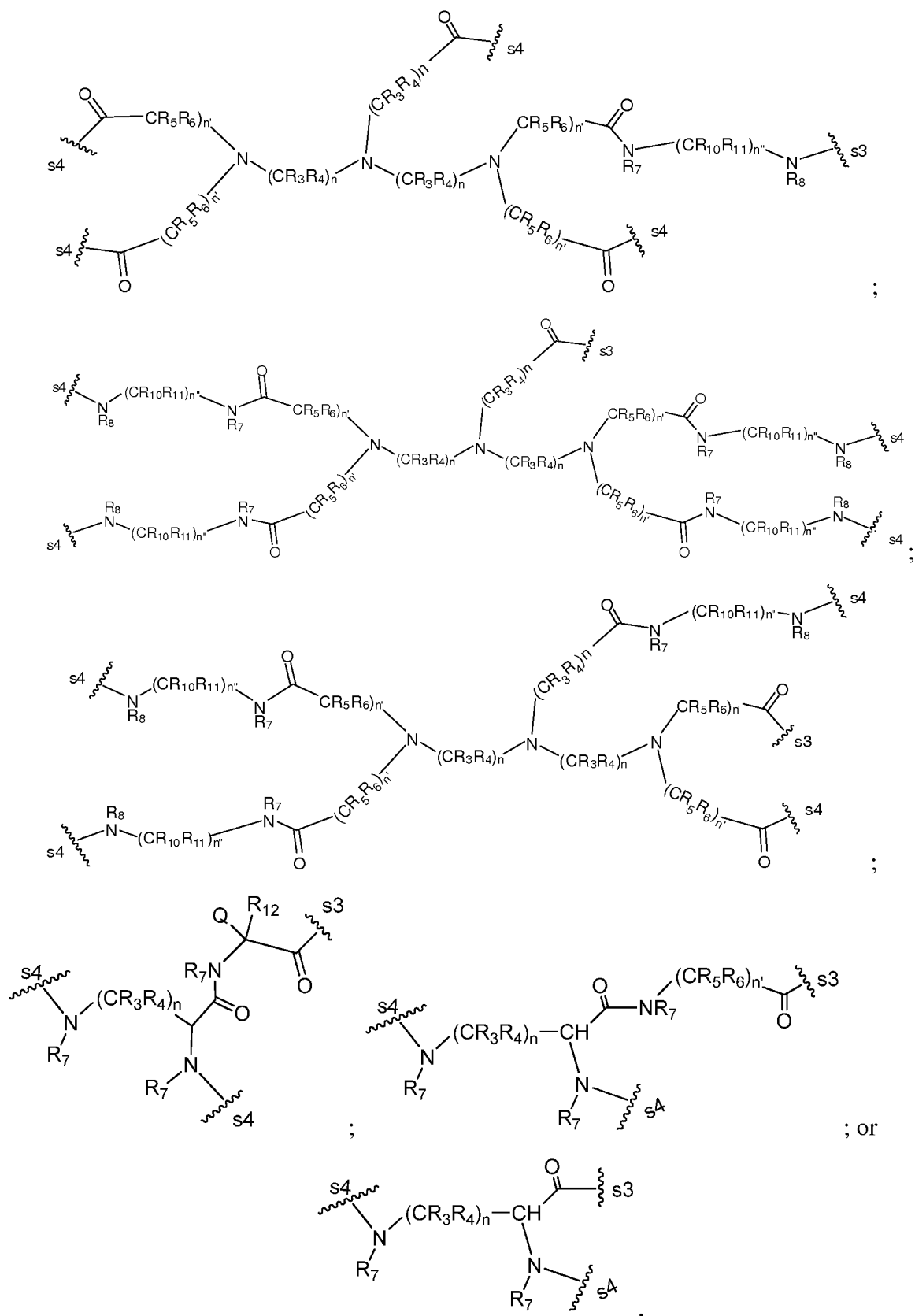


[201] In the fourth embodiment above or each of its sixteen specific embodiments described in this section above, w is specifically an integer between 1 and 6 or alternatively, between 1 and 4.

[202] Furthermore, in the first, second, third, or fourth embodiment described above in the summary section or each of the specific embodiments described in the conjugate section, the cytotoxic compound section, the linker compound section, or the modified cell binding agent section, U in one specific embodiment is represented by the following formula:







in which:

R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} , for each occurrence, are independently H or an optionally substituted alkyl;

n , n' , and n'' are independently an integer from 1 to 10;

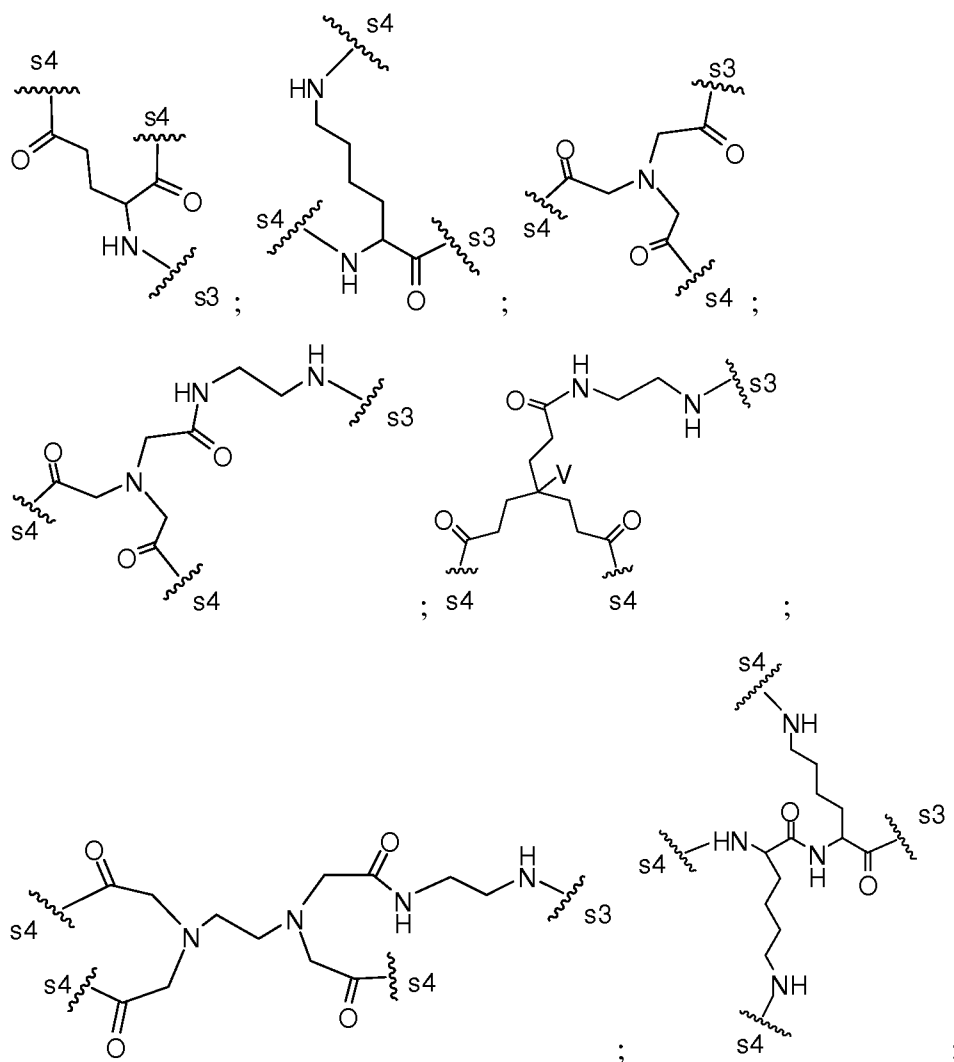
V is H, an optionally substituted alkyl, NO₂, or -NH-C(=O)-R⁵⁰¹;

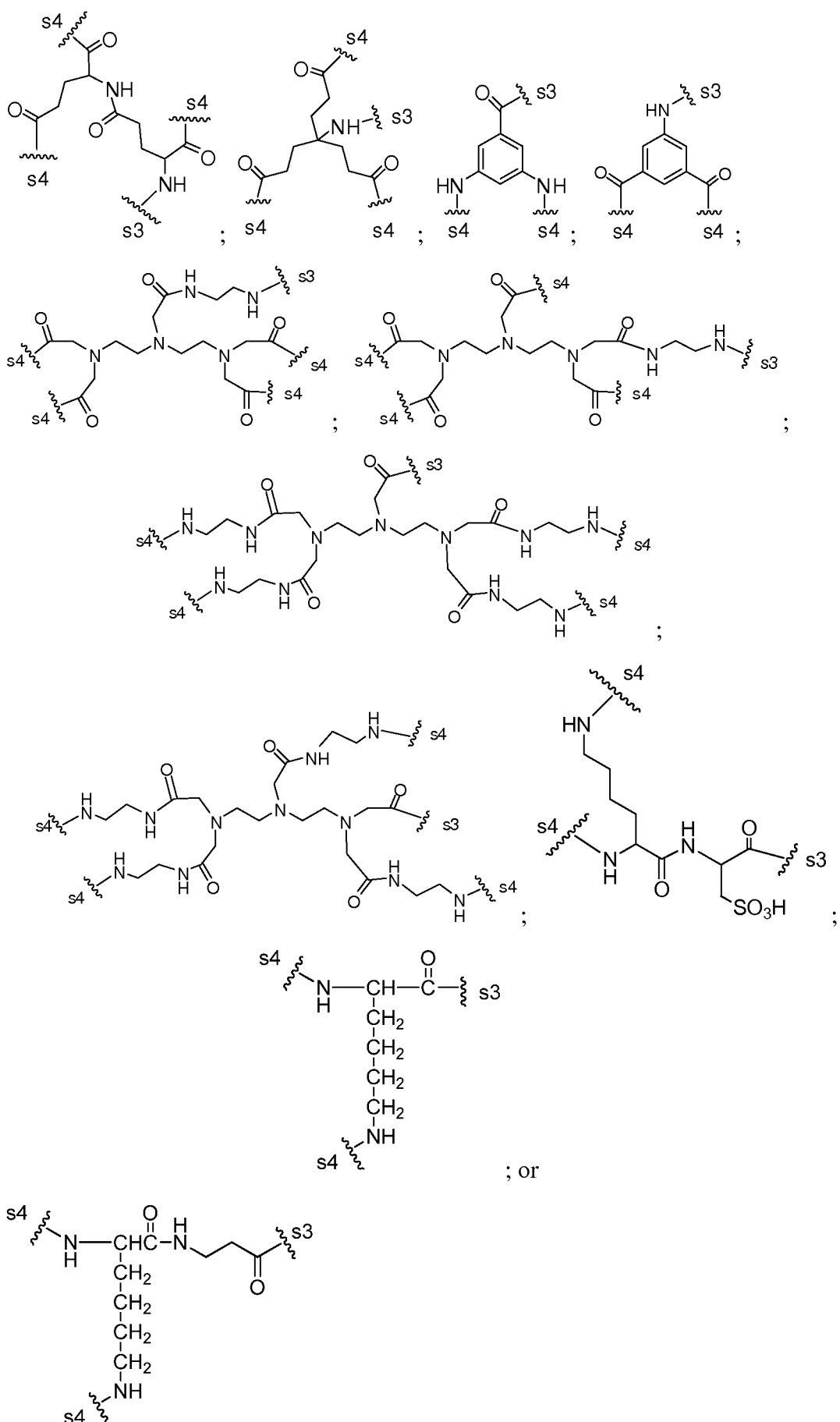
R⁵⁰¹ is H or an optionally substituted alkyl;

s3 is the site covalently linked the group L₁; and

s4 is the site covalently linked to the group L₂'.

Alternatively, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀ and R₁₁ are all H. In another alternative, n, n', and n'' are independently an integer from 1 to 5. In yet another alternative, U is represented by the following formula:

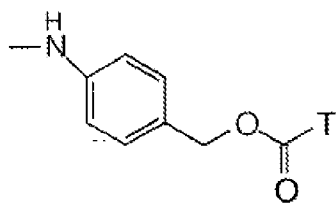




In still another alternative embodiment, V in each of the formulas designated in the above alternative embodiments is H or NO₂.

[203] In one embodiment, L1, L1', D, L2 and L2' do not comprise a self-immolative spacer moiety. In another embodiment, L1, L1', D, L2 and L2' do not comprise a self-immolative spacer moiety connected to a peptide moiety. In yet another embodiment, L1 and L1' do not comprise a self-immolative spacer moiety which spaces and covalently links together the drug moiety and a protein peptide moiety (in the case of L1) or the reactive functional group in L1' and a protein peptide moiety (in the case of L1'). A self-immolative spacer may be defined as a bifunctional chemical moiety which is capable of covalently linking together two spaced chemical moieties into a normally stable tripartate molecule, releasing one of said spaced chemical moieties from the tripartate molecule by means of enzymatic cleavage; and following said enzymatic cleavage, spontaneously cleaving from the remainder of the molecule to release the other of said spaced chemical moieties. In accordance with these embodiments of the present invention, there is no self-immolative spacer in L1, L1', D, L2 and L2', including no self immolative space group covalently linked at one of its ends to a protein peptide moiety to form a tripartide molecule which is stable and pharmacologically inactive in the absence of the target enzyme. Alternatively, there is no self-immolative spacer in L1 covalently linked at one of its ends to a protein peptide moiety and covalently linked at its other end to the chemical reactive site of the drug moiety whose derivatization inhibits pharmacological activity, so as to space and covalently link together the protein peptide moiety and the drug moiety into a tripartate molecule which is stable and pharmacologically inactive in the absence of the target enzyme (or in the case of L1', spaces and links the protein peptide moiety and the reactive functional group in L1'). The tripartide molecule is enzymatically cleavable by such target enzyme at the bond covalently linking the spacer moiety and the protein peptide moiety. Such enzymatic cleavage, in turn, will activate the self-immolating character of the spacer moiety and initiate spontaneous cleavage of the bond covalently linking the spacer moiety to rest of the molecule, e.g., the drug moiety, to thereby effect release of the drug in pharmacologically active form.

[204] Exemplary self-immolative groups which are excluded in these embodiments are represented by Formulas (A)-(I):



Formula (A)

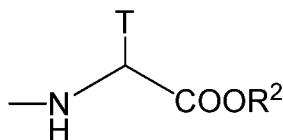
in which T is O, N or S,



Formula (B)

in which T is O, N or S, and

R1 is C1-C5 alkyl;

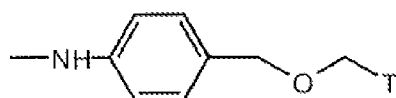


Formula (C)

(J. Med. Chem., 27: 1447 (1984))

in which T is O, N or S, and

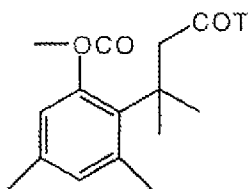
R2 is H or C1-C5 alkyl;



Formula (D)

in which T is O, N or S,

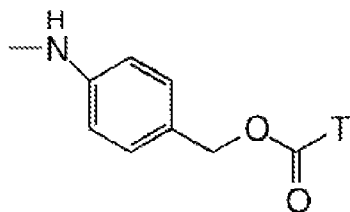
[205]



Formula (E)

in which T is O, S or N.

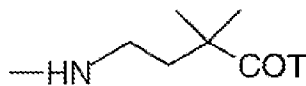
[206] As used herein “C1-C5 alkyl” is meant to include a branched or unbranched hydrocarbon chain having, unless otherwise noted, one to five carbon atoms, including but not limited to methyl, ethyl, isopropyl, n-propyl, sec-butyl, isobutyl, n-butyl and the like. Additional Examples are shown below



Formula (F),



Formula (G),



Formula (H), and



Formula (J).

[207] Alternatively to the exclusion of the self-immolative group from L1 , L1' , D, L2 and L2, or in addition to the exclusion of the self-immolative group from L1 , L1' , D, L2 and L2, these groups do not comprise the peptides gly-phe-leu-gly and ala-leu-ala-leu.

PRODUCTION OF CELL-BINDING AGENT-DRUG CONJUGATES

[208] The conjugates of the present invention can be prepared according to any known method in the art. See, for example, WO 2009/134977, U.S. Patent Nos. 7,811,572, 6,441,163, 7,368,565, 8,163,888, U.S. Publication Nos. 2006/0182750, 2011/0003969, 2012/0253021 and Widdison, W. C. *et al.*, "Semisynthetic maytansine analogues for the targeted treatment of cancer," *J. Med. Chem.* (2006) 49(14):4392-4408. In one embodiment, the conjugates of the present invention can be prepared by reacting a cell-binding agent with a cytotoxic compound of the invention (*e.g.*, cytotoxic compounds of Formula (II)) having a reactive moiety capable of forming a covalent bond with the cell-binding agent to form a cell-binding agent-cytotoxic agent conjugate. The conjugate can optionally be purified. The cytotoxic compound of the invention can be generated *in situ* and used to react with the antibody without purification. Alternatively, the cytotoxic compound of the invention can be generated and purified before conjugating to the cell-binding agent.

[209] In another embodiment, the conjugates of the present invention can be prepared by: a) reacting a cell-binding agent with a reactive functional group attached to one of the spacers linked to a branched scaffold to form a modified cell-binding agent (*e.g.*, that of formula (IV)); b) optionally purifying the modified cell-binding agent; c) conjugating a cytotoxic drug / chemotherapeutic agent to the modified cell-binding agent to form the cell-binding agent-cytotoxic compound conjugate of the present invention; and d) purifying the cell-binding agent-cytotoxic compound conjugate.

[210] In another embodiment, the conjugate of the present invention can be prepared by mixing together a cell-binding agent, a cytotoxic drug / chemotherapeutic agent, and a linker compound (*e.g.*, that of formula (III)). For example, the cell-binding agent may be contacted with a cytotoxic drug / chemotherapeutic agent first to form a mixture comprising the cell-

binding agent and the cytotoxic drug / chemotherapeutic agent, followed by contacting the mixture with a linker compound (*e.g.*, linker compounds of Formula (III)) to form the cell-binding agent-cytotoxic compound conjugate. The conjugate can then be purified.

[211] Any purification methods known in the art can be used to purify the conjugates of the present invention (see, for example, *Bioconjugate Techniques*, 2nd Edition by Greg T. Hermanson, published by Academic Press, Inc., 2008). In one embodiment, the conjugates of the present invention can be purified using tangential flow filtration (TFF), non-adsorptive chromatography, adsorptive chromatography, adsorptive filtration, selective precipitation, high performance liquid chromatography (HPLC), dialysis or any other suitable purification process, as well as combinations thereof.

[212] Any suitable TFF systems may be utilized for purification, including a Pellicon type system (Millipore, Billerica, MA), a Sartoclon Cassette system (Sartorius AG, Edgewood, NY), and a Centrasette type system (Pall Corp., East Hills, NY).

[213] Any suitable adsorptive chromatography resin may be utilized for purification. Preferred adsorptive chromatography resins include hydroxyapatite chromatography, hydrophobic charge induction chromatography (HCIC), hydrophobic interaction chromatography (HIC), ion exchange chromatography, mixed mode ion exchange chromatography, immobilized metal affinity chromatography (IMAC), dye ligand chromatography, affinity chromatography, reversed phase chromatography, and combinations thereof. Examples of suitable hydroxyapatite resins include ceramic hydroxyapatite (CHT Type I and Type II, Bio-Rad Laboratories, Hercules, CA), HA Ultrogel hydroxyapatite (Pall Corp., East Hills, NY), and ceramic fluoroapatite (CFT Type I and Type II, Bio-Rad Laboratories, Hercules, CA). An example of a suitable HCIC resin is MEP Hypercel resin (Pall Corp., East Hills, NY). Examples of suitable HIC resins include Butyl-Sepharose, Hexyl-Sepharose, Phenyl-Sepharose, and Octyl Sepharose resins (all from GE Healthcare, Piscataway, NJ), as well as Macro-prep Methyl and Macro-Prep t-Butyl resins (Biorad Laboratories, Hercules, CA). Examples of suitable ion exchange resins include SP-Sepharose, CM-Sepharose, and Q-Sepharose resins (all from GE Healthcare, Piscataway, NJ), and Unosphere S resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable mixed mode ion exchangers include Bakerbond ABx resin (JT Baker, Phillipsburg NJ). Examples of suitable IMAC resins include Chelating Sepharose resin (GE Healthcare, Piscataway, NJ) and Profinity IMAC resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable dye ligand resins include Blue Sepharose resin (GE Healthcare, Piscataway, NJ) and Affi-gel Blue resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable affinity

resins include Protein A Sepharose resin (*e.g.*, MabSelect, GE Healthcare, Piscataway, NJ), His-Tag metal affinity resins, anti-FLAG affinity resins, and lectin affinity resins, *e.g.* Lentil Lectin Sepharose resin (GE Healthcare, Piscataway, NJ), where the antibody bears appropriate lectin binding sites. Examples of suitable reversed phase resins include C4, C8, and C18 resins (Grace Vydac, Hesperia, CA).

[214] Any suitable non-adsorptive chromatography resin may be utilized for purification. For example, size-exclusion chromatography can be used for purifying the conjugates of the invention. Examples of suitable non-adsorptive chromatography resins include, but are not limited to, SEPHADEX™ G-25, G-50, G-100, SEPHACRYL™ resins (*e.g.*, S-200 and S-300), SUPERDEX™ resins (*e.g.*, SUPERDEX™ 75 and SUPERDEX™ 200), BIO-GEL® resins (*e.g.*, P-6, P-10, P-30, P-60, and P-100), and others known to those of ordinary skill in the art.

[215] In one embodiment, when the cell-binding agent is an epitope-tagged Avibody, the conjugate can be purified using hydroxyl apatite chromatography, size-exclusion chromatography, tangential flow filtration, gel electrophoresis, dialysis, and affinity chromatography, specifically affinity chromatography, more specifically His-tag metal affinity chromatography and anti-FLAG M2 affinity chromatography (see, for example, US 2008/0152586 and US 2012/0171115).

[216] In another embodiment, when the cell-binding agent is a Centyrin, the conjugate can be purified using protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, size-exclusion chromatography, tangential flow filtration, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Alternatively, the conjugate can be purified using HPLC. Specifically, the conjugate can be purified by using affinity chromatography, more specifically His-tag metal affinity chromatography. See, for example, US 2010/0255056, US 2010/0216708 and US 2011/0274623.

[217] In another embodiment, when the cell-binding agent is a DARPin, the conjugate can be purified by affinity chromatography, size exclusion chromatography, hydroxylapatite chromatography, tangential flow filtration, specifically affinity chromatography, more specifically His-Tag affinity chromatography. See, for example, U.S. Patent Publication Nos. 2004/0132028, 2009/0082274, 2011/0118146, and 2011/0224100, WO 02/20565 and WO 06/083275.

[218] The number of cytotoxic compound molecule bound per cell-binding agent (*e.g.*, antibody) molecule can be determined spectroscopically by measuring the ratio of the absorbance at 280 nm and 252 nm. An average of about 0.5 – about 20 cytotoxic compounds/antibody molecule(s) can be linked by the methods described herein. In one embodiment, the average number of linked cytotoxic compound per cell-binding agent in the conjugate (*i.e.*, average w value) is about 0.5 to about 10, about 0.5 to 2 (*e.g.*, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, or 2.1), about 2 to about 8 (*e.g.*, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, or 8.1), about 2.5 to about 7, about 3 to about 5, about 2.5 to about 5.0 (*e.g.*, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0), about 2.5 to about 4.0, about 3.0 to about 4.0, about 3.2 to about 4.2, or about 4.5 to 5.5 (*e.g.*, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, or about 5.5).

IN VITRO EVALUATION OF CYTOTOXICITY

[219] The cytotoxic compounds and cell-binding agent-drug conjugates of the invention can be evaluated for their ability to suppress proliferation of various cancer cell lines *in vitro*. The *in vitro* cytotoxicity assays can be conducted using methods known in the art (*e.g.*, Widdison, W. C. et al., “Semisynthetic maytansine analogues for the targeted treatment of cancer,” *J. Med. Chem.* (2006) 49(14):4392-408). For example, cells to be evaluated can be exposed to the compounds or conjugates for 1-5 days and the surviving fractions of cells measured in direct assays by known methods. IC₅₀ values can then be calculated from the results of the assays.

COMPOSITIONS AND METHODS OF USE

[220] The present invention includes a composition (*e.g.*, a pharmaceutical composition) comprising conjugates (*e.g.*, conjugates of Formula (I), or cytotoxic compounds (*e.g.*, cytotoxic compounds of Formula (II)) described herein, and a carrier (a pharmaceutically acceptable carrier). The present invention also includes a composition (*e.g.*, a pharmaceutical composition) comprising the conjugate of Formula (I), or the cytotoxic compound of Formula

(II), and a carrier (a pharmaceutically acceptable carrier), and further comprising a second therapeutic agent. The present compositions are useful for inhibiting abnormal cell growth or treating a proliferative disorder in a mammal (*e.g.*, human). The present compositions are also useful for treating an autoimmune disorder, a destructive bone disorder, a graft versus host disease, a transplant rejection, an immune deficiency, an inflammatory disease, an infectious disease, a viral disease, a fibrotic disease, a neurodegenerative disorder, a pancreatitis or kidney disease in a mammal (*e.g.*, human).

[221] The present invention includes a method of inhibiting abnormal cell growth or treating a proliferative disorder in a mammal (*e.g.*, human) comprising administering to said mammal a therapeutically effective amount of conjugates (*e.g.*, conjugates of formula (I)) or cytotoxic compounds (*e.g.*, compounds of formula (II)) described herein, or a composition thereof, alone or in combination with a second therapeutic agent. In one embodiment, the proliferative disorder is cancer in general; alternatively, the proliferative disorder is cancer selected from the group consisting of breast cancer, colon cancer, brain cancer, prostate cancer, kidney cancer, pancreatic cancer, ovarian cancer, head and neck cancer, melanoma, colorectal cancer, gastric cancer, squamous cancer, small-cell lung cancer, nonsmall-cell lung cancer, testicular cancer, Merkel cell carcinoma, glioblastoma, neuroblastoma, a cancer of a lymphatic organ, and a hematological malignancy.

[222] Similarly, the present invention provides a method for inducing cell death in selected cell populations comprising contacting target cells or tissue containing target cells with an effective amount of the conjugates of the present invention. The target cells are cells to which the cell-binding agent of the conjugates can bind.

[223] If desired, other active agents, such as other anti-tumor agents, may be administered along with the conjugate.

[224] Cancer therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the Physician's Desk Reference (PDR). The PDR discloses dosages of the agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill in the art and can be determined by the physician. The contents of the PDR are expressly incorporated herein in its entirety by reference. One of skill in the art can review the PDR, using one or more of the following parameters, to determine dosing regimen and dosages of the chemotherapeutic agents and conjugates that can be used in accordance with the teachings

of this invention. These parameters include: Comprehensive index; Manufacturer; Products (by company's or trademarked drug name); Category index; Generic/chemical index (non-trademark common drug names); Color images of medications; Product information, consistent with FDA labeling; Chemical information; Function/action; Indications & Contraindications; Trial research, side effects, warnings.

[225] The present invention also provides methods of treating a non-cancerous condition comprising administering to a subject in need of treatment an effective amount of any of the conjugates described above. Examples of the conditions include, but not limited to, an autoimmune disorder (*e.g.*, systemic lupus, rheumatoid arthritis, and multiple sclerosis), a graft versus host disease, a transplant rejection (*e.g.*, a renal transplant rejection, a liver transplant rejection, a lung transplant rejection, a cardiac transplant rejection, and a bone marrow transplant rejection), an immune deficiency, an inflammatory diseases (*i.e.*, myositis and pancreatitis), a destructive bone disorder, an infectious disease (*e.g.*, viral infections and parasite infections), a viral disease, a fibrotic disease, a neurodegenerative disorder, or a kidney disease. In one embodiment, the condition selected from the group consisting of cancer, rheumatoid arthritis, multiple sclerosis, graft versus host disease, transplant rejection, lupus, myositis, infectious disease, and immune deficiency.

[226] Examples of *in vitro* uses include treatments of autologous bone marrow prior to their transplant into the same patient in order to kill diseased or malignant cells: treatments of bone marrow prior to their transplantation in order to kill competent T cells and prevent graft-versus-host-disease (GVHD); 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.

[227] The conditions of non-clinical *in vitro* use are readily determined by one of ordinary skill in the art.

[228] Examples of clinical *ex vivo* use are to remove tumor 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 autologous or allogenic bone marrow or tissue prior to transplant in order to prevent GVHD. Treatment 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 cytotoxic agent of the invention, concentrations range from about 10 μ M to 1 pM, for about 30 minutes to about 48 hours at about 37°C. The exact conditions of concentration and time of incubation, *i.e.*, the dose, are readily determined by one of ordinary skill in the art. After incubation the bone

marrow cells are washed with medium containing serum and returned to the patient intravenously according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or total-body irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

[229] For clinical *in vivo* use, the cytotoxic compounds or conjugates of the invention will be supplied as a solution or a lyophilized powder that are tested for sterility and for endotoxin levels. Examples of suitable protocols of conjugate administration are as follows.

Conjugates are given weekly for 4 weeks as an intravenous bolus each week. Bolus doses are given in 50 to 1000 ml of normal saline to which 5 to 10 ml of human serum albumin can be added. Dosages will be 10 µg to 2000 mg per administration, intravenously (range of 100 ng to 20 mg/kg per day). After four weeks of treatment, the patient can continue to receive treatment on a weekly basis. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, *etc.*, can be determined by one of ordinary skill in the art as the clinical situation warrants.

[230] Suitable pharmaceutically acceptable carriers, diluents, and excipients are well known and can be determined by those of ordinary skill in the art as the clinical situation warrants. Examples of suitable carriers, diluents and/or excipients include: (1) Dulbecco's phosphate buffered saline, pH about 7.4, containing or not containing about 1 mg/ml to 25 mg/ml human serum albumin, (2) 0.9% saline (0.9% w/v NaCl), and (3) 5% (w/v) dextrose; and may also contain an antioxidant such as tryptamine and a stabilizing agent such as Tween 20.

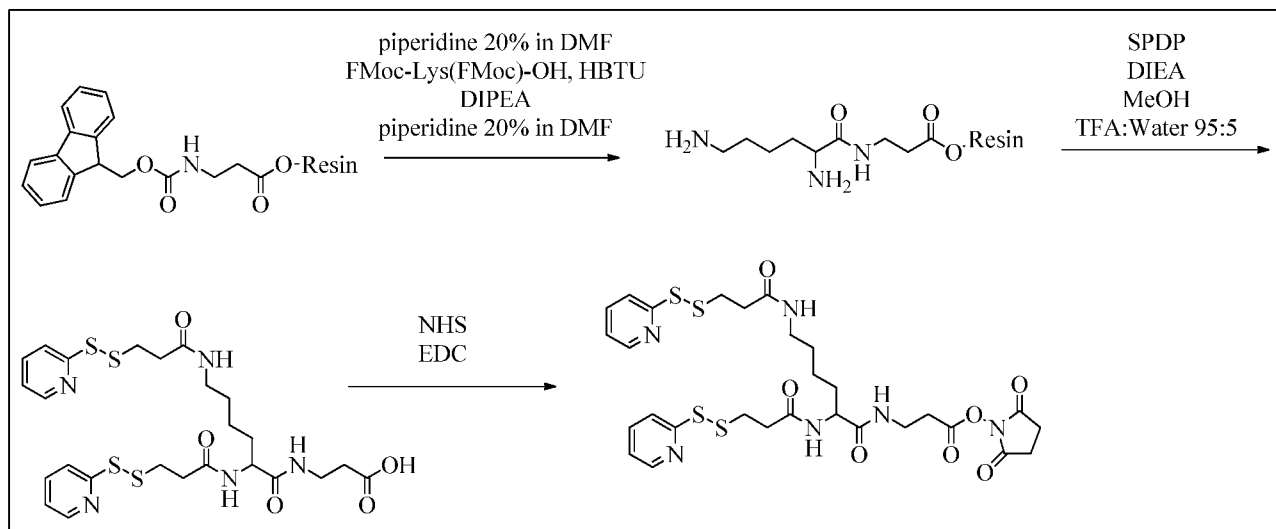
[231] The method for inducing cell death in selected cell populations can be practiced *in vitro*, *in vivo*, or *ex vivo*.

ANALOGUES AND DERIVATIVES

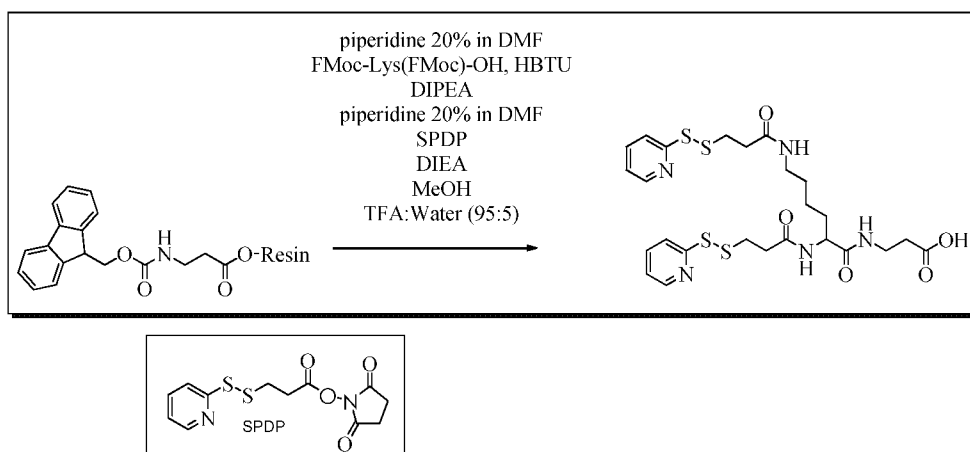
[232] One skilled in the art of cytotoxic agents will readily understand that each of the cytotoxic agents described herein can be modified in such a manner that the resulting compound still retains the specificity and/or activity of the starting compound. The skilled artisan will also understand that many of these compounds can be used in place of the cytotoxic agents described herein. Thus, the cytotoxic agents of the present invention include analogues and derivatives of the compounds described herein.

All references cited herein and in the examples that follow are expressly incorporated by reference in their entireties.

Example 1. Synthesis of di-SPDP-Lys- β -Ala-NHS for Production of Amine-Branched DM_x



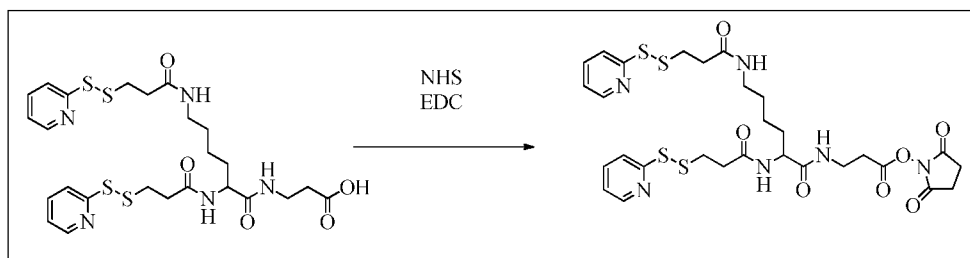
Synthesis of di-SPDP-Lys-beta-Ala-OH:



[233] Wang resin (FMoc-beta-Alanine-resin, 5 g, 3.68 mmol protected amine) was added to a solid-phase peptide reaction vessel. The material was swelled with dimethylformamide (100 mL) overnight then drained under vacuum. The resin was treated with 20% piperidine in DMF (100 mL, 3.68 mmol), agitated with nitrogen bubbling for 40 min, then drained and washed with dimethylformamide (4X 100mL). Resin was agitated during each wash then drain thoroughly. FMoc-Lys(FMoc)-OH (2.171 g, 3.68 mmol) and O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU, 3.48 g, 9.19 mmol) were dissolved in dimethylformamide (80 mL). This solution was transferred to the reaction vessel containing the resin, and treated with diisopropyl ethylamine (DIPEA, 3.20 mL, 18.4 mmol). The suspension was agitated for 2h then resin was washed with dimethyl formamide (4X 100mL) followed by washing with dichloromethane (2X 100 mL). Resin was dried then treated with piperidine 20% in dimethylformamide (100 mL, 3.68 mmol) and allowed to

agitate for 40 min then drained and washed with dimethylformamide (4X 100mL) while agitating resin. The resin was drained then SPDP (1.15 g, 3.68 mmol) and 1-hydroxybenzotriazol (HOBt, 3.5 g, 9.20 mmol) were dissolved in dimethylformamide (50 mL) and added to the reaction vessel followed by diisopropyl ethylamine (3.2 mL, 18.40 mmol) and agitated at room temperature for 3 hrs. in the dark. Resin was washed with dimethylformamide (4X 50mL) followed by washing with dichloromethane (2X 50 mL). The resin was then washed with methanol (100 mL). The reaction vessel was attached to a vacuum flask which was placed under vacuum overnight in the dark. A solution of 95:5 trifluoroacetic acid:deionized water (100 ml) was added to the dry resin with agitation for 4hrs. The reaction was vacuum filtered and filtrate was collected and the filter cask was washed with 95:5 trifluoroacetic acid:deionized water (2x 100 mL) under vacuum. The filtrates were combined and solvent was evaporated under vacuum. Residue was taken up in a minimum volume of dimethylformamide then purified by on an intelliflash system using a 45- g C18 cartridge flow rate 50 mL/min eluting with deionized water with the following gradient starting at 5% acetonitrile for 5 min and a linear ramp to 95% acetonitrile over the next 25 min. desired product fractions eluting between 18-20 min was frozen then lyophilized to give 289 mg, (12% yield) of desired product. MS [M+1] calcd. 611.8 found. 612.4.

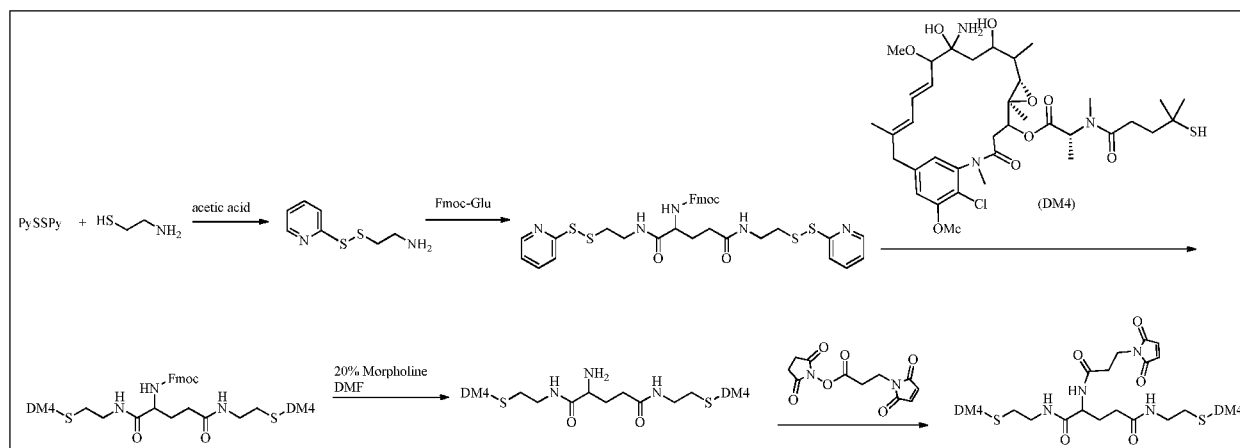
Synthesis of di-SPDP-Lys-b-Ala-NHS (linker 1):



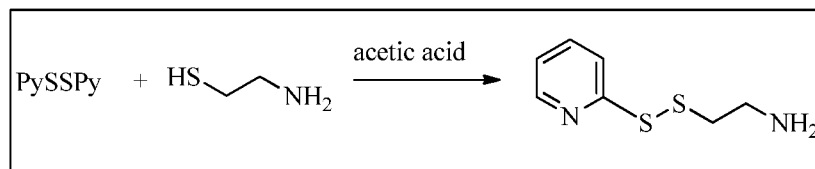
[234] Di-SPDP-Lys-b-Ala (289 mg, 0.472 mmol) was dissolved in a minimal amount of dimethylformamide and was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) HCl salt (EDC, 91 mg, 0.472 mmol) followed by N-hydroxysuccinimide (54.4 mg, 0.472 mmol). The reaction was purified by preparative reverse phase HPLC using a kromasil C18 10 micron 250 x 21.2 mm, flow rate 20 mL/min eluting with deionized water containing 0.1% formic acid with a linear gradient of 5% acetonitrile to 95% acetonitrile over 18 min. Fractions containing pure desired product (R_t 11.8 min) were immediately combined, frozen and lyophilized give 88.4 mg (26.4 % yield) of desired product. MS [M+1] calcd.709.9 found 709.4. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.16 – 1.37 (m, 4H), 1.48 (dt, J = 14.7, 6.0 Hz, 2H),

1.65 (dq, $J = 13.6, 6.7, 6.0$ Hz, 1H), 1.77 – 1.97 (m, 4H), 2.18 (t, $J = 7.2$ Hz, 2H), 2.24 – 2.36 (m, 2H), 2.71 – 2.91 (m, 11H), 2.98 (q, $J = 6.2$ Hz, 3H), 3.17 – 3.48 (m, 3H), 3.92 – 4.10 (m, 1H), 4.22 (q, $J = 6.0$ Hz, 1H), 7.14 – 7.28 (m, 2H), 7.71 – 7.88 (m, 5H), 8.21 (d, $J = 6.5$ Hz, 1H), 8.32 (d, 1H), 8.39 – 8.51 (m, 2H).

Example 2. Production of Disulfide Branched DM4

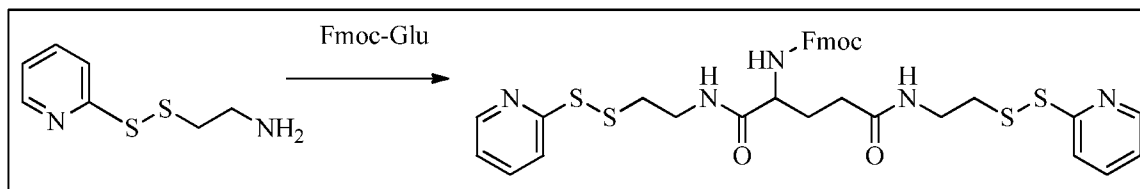


Synthesis of 2-(pyridin-2-yl)disulfany)ethanamine:



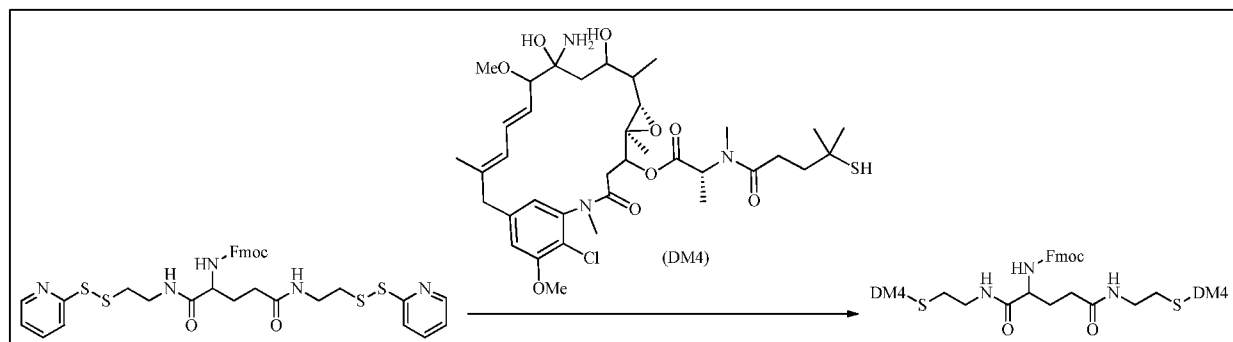
[235] A 100mL round bottom flask was equipped with a magnetic stir bar and charged with 2,2'-dithiodipyridine (2.141 g, 9.72 mmol), ethanol (5.56 ml) and acetic acid (0.371 ml, 6.48 mmol). The brown suspension was allowed to stir for 10 min under argon at room temperature. A solution of 2-aminoethanethiol HCl salt (0.736 g, 6.48 mmol) in ethanol (1.85 ml) was added drop wise over ~2 min. After 3.5 h solvent was evaporated under vacuum to give an oil that was purified by on an intelliflash system using a 40 g rediflash silica cartridge eluting at 40 mL/min with dichloromethane and a linear gradient of 0% -100% ethyl acetate over 20 min, remaining at 100 % ethyl acetate for 5 min then eluting with ethyl acetate with a linear gradient of 0% - 100 % methanol over 5 min. The fraction containing desired compound (R_t 27 min) was concentrated under vacuum to give 985 mg (82 % yield) of a white oily solid. HRMS $[M+H]$ calcd. 187.0358 found 187.0356. 1H NMR (400MHz, $CDCl_3$) 2.32(t, $J = 6.2$ Hz, 2H), 2.47(d, $J=6.4$ Hz, 2H), 4.04(s, 2H), 6.50 (ddd, $J=7.4, 5.0, 1.0$ Hz, 1H), 6.84(dt, $J= 8.1, 0.9$ Hz, 1H), 7.03-6.94(m, 1H), 7.75-7.68(m, 1H)

Synthesis of Fmoc-Glu(amidoethyl-2-(pyridin-2-ylidisulfanyl))-(amidoethyl-2-(pyridin-2-ylidisulfanyl)):



[236] Fmoc-Glu-OH (317 mg, 0.859 mmol) was dissolved in a minimal volume of dimethylformamide and treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) HCl salt (EDC, 823 mg, 4.29 mmol) and 1-Hydroxy-7-azabenzotriazole (HOAt, 580 mg, 4.29 mmol) followed by a solution of 2-(pyridin-2-ylidisulfanyl)ethanamine (400mg, 2.147 mmol) in a minimal volume of dimethylformamide. After 3h the reaction was purified on an Intelliflash system using a 24 g rediflash silica cartridge at a flow rate of 35 mL/min, eluting with dichloromethane for 2 min then with a linear gradient of 0- 100% ethyl acetate over the next 13 min followed by elution with ethyl acetate with a linear gradient of 0% methanol to 100% methanol over the next 8 min. The product containing fraction (R_t 22 min) was concentrated under vacuum to give 269 (89 % yield) of desired product. HRMS $[M+H]$ calcd. 706.1645 found 706.1643.

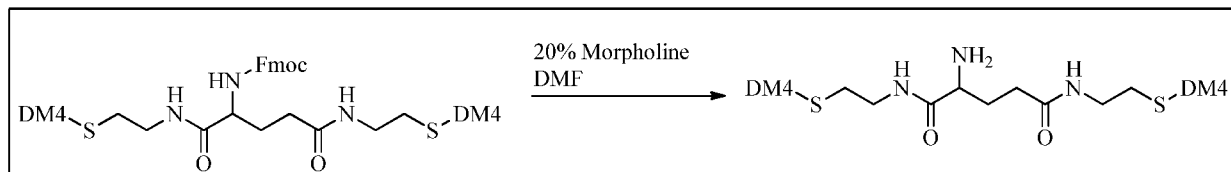
FMoc-Glu(amido-ethyl-2-sulfanyl DM4)-amidoethyl-s-sulfanyl-DM4):



[237] Fmoc-Glu(amidoethyl-2-(pyridin-2-ylidisulfanyl))-(amidoethyl-2-(pyridin-2-ylidisulfanyl)) (48.4 mg, 0.069 mmol) was dissolved in minimal volume of dimethylformamide and treated with a solution DM4 (mg, 0.137) in dimethylacetamide (9.4 ml). After 30 min the mixture was purified by reverse phase chromatography on an Intelliflash system equipped with a 450 g C18 eluted at 50 mL/min with 9:5 deionized water containing 0.1% formic acid: acetonitrile for 5 min then with deionized water containing 0.1% formic acid using a linear gradient of between 5% and 95% acetonitrile over the next 25 min then at 95% acetonitrile for the next 15 min. The fraction containing pure desired

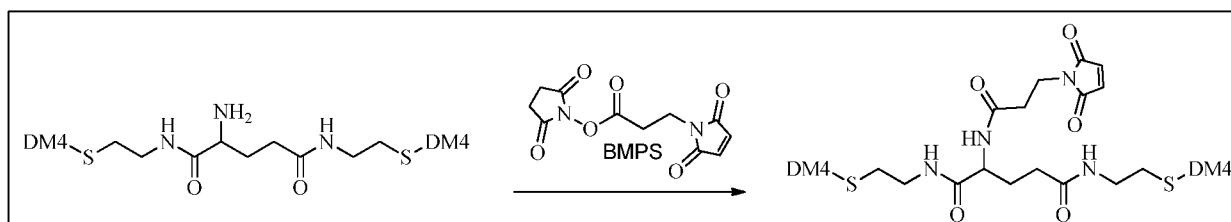
product (R_t 35 min) was frozen and lyophilized to give 96.2mg (68 % yield) of desired product. MS $[M+Na]$ calcd. 2065.7 found 2066.9.

H-Glu(amido-ethyl-2-sulfanyl DM4)-amidoethyl-s-sulfanyl-DM4):



[238] FMoc-Glu(amido-ethyl-2-sulfanyl DM4)-amidoethyl-s-sulfanyl-DM4 (89.6mg, 0.044 mmol) was treated with 20% morpholine in dimethylformamide (2 mL) and allowed to stir under argon at room temperature. After 2 h the material was purified by HPLC using a 250 x 21.2 mm C8, 10 micron, Kromasil column eluting at a flow rate of 20 mL/min with deionized water containing 0.1% formic acid with a gradient of between 0%-95% acetonitrile over 18 min. The fraction containing pure product (R_t 8.8 min) was frozen and lyophilized to give 65 mg (81 % yield). HRMS $[M+1]$ calcd. 1820.7115 found 1820.7101.

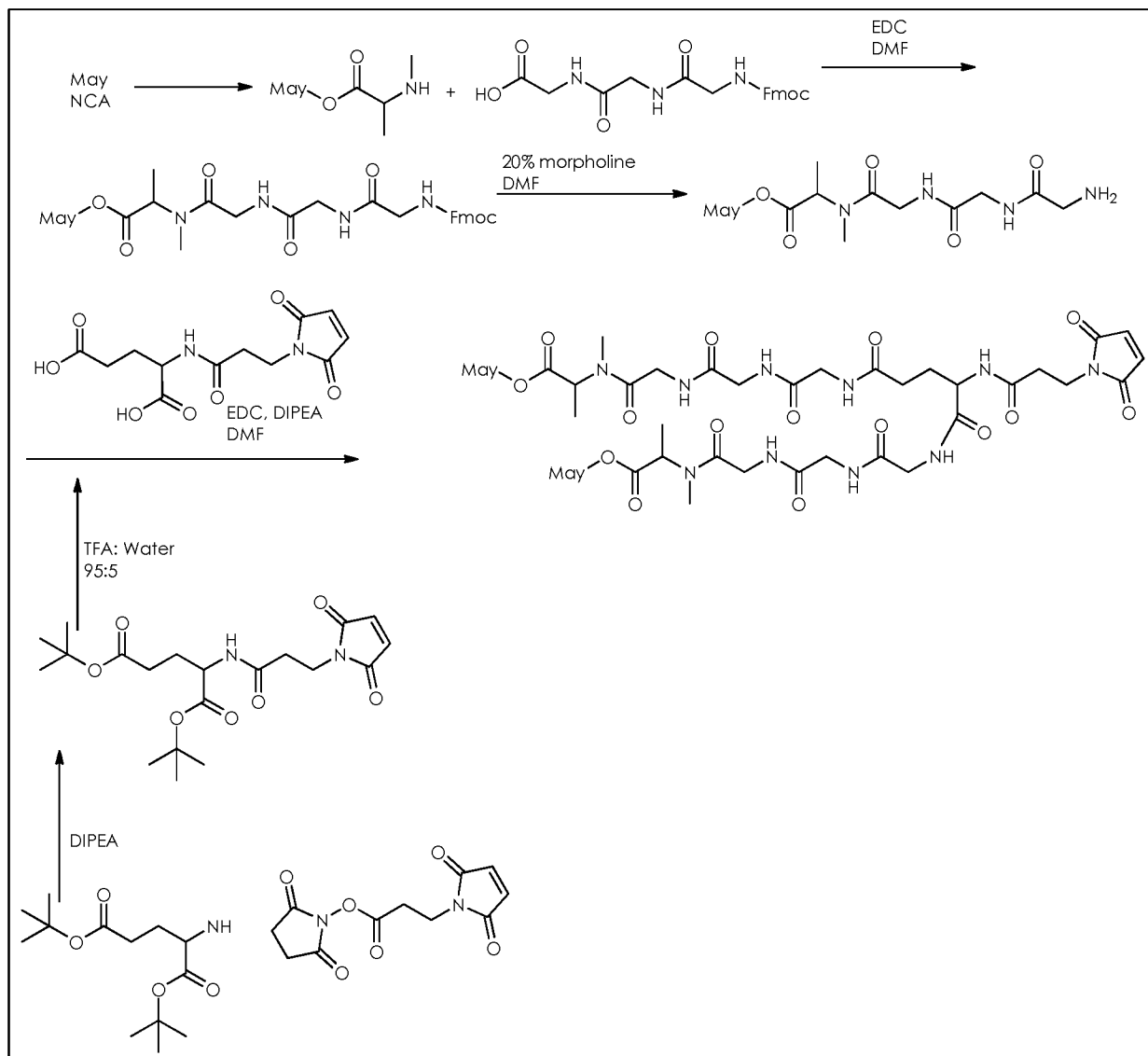
BMP-Glu(amido-ethyl-2-sulfanyl DM4)-amidoethyl-s-sulfanyl-DM4) (linker 2-(DM4)₂):



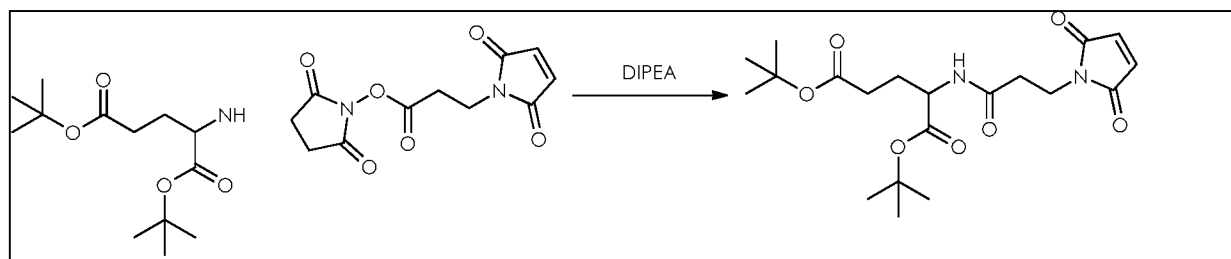
[239] H-Glu(amido-ethyl-2-sulfanyl DM4)-amidoethyl-s-sulfanyl-DM4) (65mg, 0.036 mmol) was dissolved in a minimal volume of dimethylformamide and treated with BMPS (13.39 mg, 0.054 mmol) followed by diisopropyl ethylamine (9.35 μ l, 0.054 mmol). After 2 h the material was purified by HPLC using a 250 x 21.2 10 micron, Kromasil column, eluting at 20 mL/min with deionized water using a gradient of between 5% - 95% acetonitrile over 18 min. The fraction containing desired product (R_t 17.7 min) was immediately frozen and lyophilized to yield disulfide branched maytansinoid 13.15mg (18.7 % yield) as a white solid. HRMS $[M+1]$ calcd 986.3729 found 986.3721. 1H NMR (400 MHz, DMSO- d_6) δ 0.78 (s, 6H), 1.06 – 1.20 (m, 18H), 1.25 (d, J = 13.7 Hz, 3H), 2.72 – 2.75 (m, 1H), 1.37 – 1.52 (m, 4H), 3.93 – 3.95 (m, 1H), 1.59 (s, 4H), 1.62 – 1.78 (m, 4H), 1.78 – 1.96 (m, 4H), 1.98 – 2.11 (m, 4H), 2.13 – 2.29 (m, 3H), 2.30 – 2.35 (m, 1H), 2.40 (t, J = 7.5 Hz, 3H), 2.54 – 2.68 (m, 5H), 2.72 (s, 4H), 2.80 (d, J = 9.6 Hz, 2H), 3.10 (s, 5H), 3.15 – 3.22 (m, 4H), 3.25 (s, 5H),

3.43 (s, 4H), 3.59 (t, $J = 7.4$ Hz, 2H), 3.92 (s, 5H), 4.00 – 4.15 (m, 3H), 4.53 (dd, $J = 12.1$, 2.8 Hz, 2H), 5.32 (q, $J = 6.7$ Hz, 3H), 5.49 – 5.68 (m, 3H), 5.91 (s, 2H), 6.43 – 6.55 (m, 3H), 6.56 – 6.69 (m, 3H), 6.87 (s, 2H), 6.99 (s, 2H), 7.18 (d, $J = 1.8$ Hz, 2H), 7.83 – 7.96 (m, 1H), 7.96 – 8.06 (m, 1H), 8.12 (d, $J = 7.9$ Hz, 1H).

Example 3. Production of Peptide Branched Maytansinoid

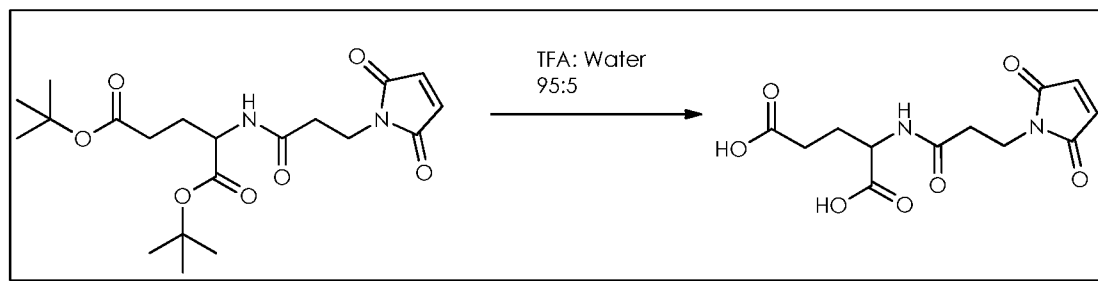


Synthesis of BMPS-ditBu-2-aminopentanedioate:



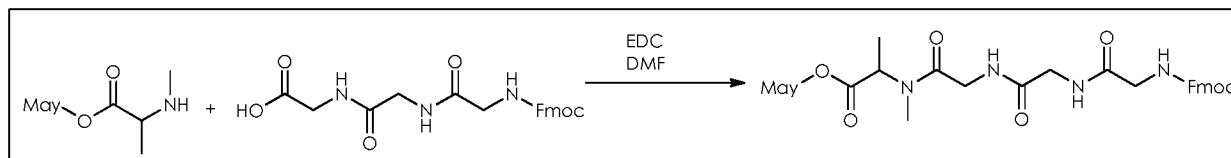
[240] H-Glu(OtBu)-OtBu HCl salt (556 mg, 1.878 mmol) was dissolved in anhydrous dimethylformamide and treated with diisopropyl ethylamine (656 μ l, 3.76 mmol) followed by a solution of BMPS (500 mg, 1.878 mmol) in dimethylformamide (6 mL) and allowed to proceed at room temp under argon. After 3h the material was purified using an Intelliflash system equipped with a 450 g C18 Redisep cartridge and eluting at 50 mL/min with deionized water containing 0.1% formic acid using 5% acetonitrile for 5 min then eluting with a linear gradient of between 5% - 95% acetonitrile over the next 20 min. The fraction containing desired product (R_t 24.3 min) was frozen and lyophilized to give desired product 706 mg (92 % yield) as a white solid. HRMS $[M+1]$ calcd. 411.2126 found 411.2129.

Synthesis of BMP-Glu-OH:



[241] BMPS-Glu(OtBu)-OtBu (706mg, 1.72 mmol) was treated with 95:5 TFA: deionized water (5mL). After stirring for 1h under argon at room temperature solvent was removed under vacuum, residue was taken up in 1:2 toluene:acetonitrile (6 mL) vortexed then evaporated under vacuum to give desired product 500 mg, (100 % yield). HRMS $[M+1]$ calcd. 299.0874 found 299.0876

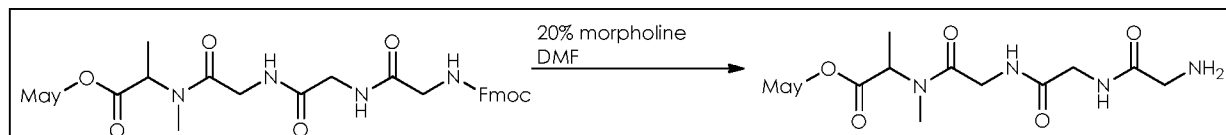
Synthesis of May-NMA-Gly₃-FMoc:



[242] Fmoc-Gly₃-OH (285 mg, 0.692 mmol) was dissolved in a minimal volume of dimethylformamide and treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) HCl salt (EDC, 442 mg, 2.307 mmol) and a solution of MayNMA (300mg, 0.461 mmol) in dimethylformamide. MayNMA was in turn prepared as described in US 7,598,375. The reaction was allowed to proceed at room temperature under argon for 6 h. Then purified by HPLC using a 250 x 21.2 mm, C8, 10 micron Kromasil column, eluting at 20 mL/min using deionized water containing formic acid using a linear gradient of between 5% - 95% acetonitrile over 18 min. The fraction containing desired product (R_t 13.6 min) was frozen

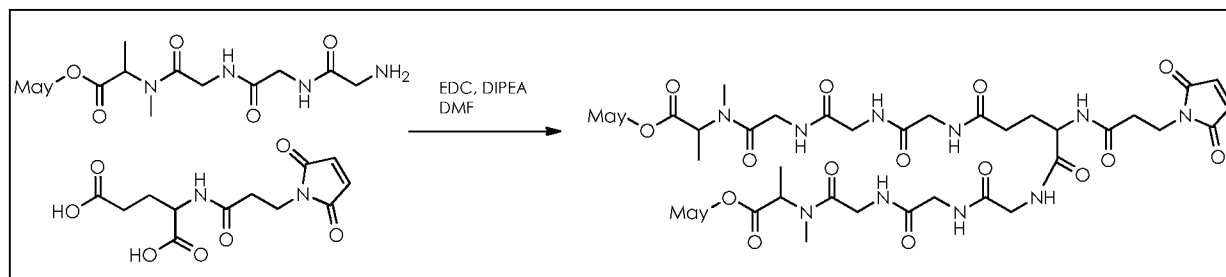
then lyophilized to give 142 mg (29.5 % yield) of desired product as a white solid. HRMS [M+1] calcd. 1065.3983 found 1065.3956.

Synthesis of MayNMA-Gly₃-H:



[243] May-NMA-Gly₃-FMoc (142mg, 0.136 mmol) was treated with 20% morpholine dimethylformamide (1mL) at room temperature under argon. After 2 h the reaction was purified using an Intelliflash system equipped with a 450 g C18 redisepp cartridge, eluting at 50 mL/min with 5% acetonitrile for 3 min then with a linear gradient of between 5% - 95% acetonitrile over the next 17 min. The fraction containing desired product (R_t 18.2 min) was frozen and lyophilized to give 55 mg (49.2 % yield) of desired product as an oil. HRMS [M+1] calcd. 821.3483 found 821.3486.

Synthesis of BMP-Glu(Gly₃-May-NMA)-Gly₃-May-NMA (linker3-(MayNMA)₂):



BMP-Glu-OH (2118-20) (9.08 mg, 0.030 mmol) was dissolved in anhydrous dimethylformamide and treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) HCl salt (EDC, 29.2 mg, 0.152 mmol) and 1-Hydroxy-7-azabenzotriazole (HOAt, 20.57 mg, 0.152 mmol) followed by MayNMA-Gly₃-H (50mg, 0.061 mmol). After stirring over night at room temperature the reaction was purified by HPLC using a 250 x 21.2 mm, C8, 10 micron Kromasil column, eluting at 20 mL/min using deionized water containing formic acid using a linear gradient of between 5% - 95% acetonitrile over 18 min. The fraction containing desired product (R_t 10.5 min) was frozen then lyophilized to give 11.6 mg (20 % yield) of white solid. HRMS [M+1] calcd. 1903.7482 found 1903.7493. HRMS [M+1] calcd. 1903.7482; found 1903.7493. ¹H NMR (400 MHz, DMSO-d₆) δ 0.76 (s, 5H), 1.12 (d, J = 6.3 Hz, 5H), 1.19 (d, J = 6.7 Hz, 5H), 1.25 (d, J = 13.7 Hz, 2H), 1.45 (dt, J = 9.9, 5.0 Hz, 3H), 1.58 (s, 4H), 1.71 (dd, J = 13.6, 7.1 Hz, 2H), 1.86 (s, 2H), 2.00 – 2.07 (m, 2H), 2.12 – 2.19

(m, 2H), 2.30 – 2.35 (m, 1H), 2.40 (t, J = 7.5 Hz, 2H), 2.52 – 2.58 (m, 1H), 2.62 – 2.74 (m, 6H), 2.77 (d, J = 9.6 Hz, 2H), 3.11 (s, 7H), 3.24 (s, 5H), 3.37 – 3.52 (m, 4H), 3.59 (t, J = 7.4 Hz, 2H), 3.66 – 3.83 (m, 7H), 3.92 (s, 5H), 3.95 – 4.01 (m, 3H), 4.01 – 4.11 (m, 3H), 4.16 (q, J = 7.2 Hz, 1H), 4.55 (dd, J = 12.0, 3.0 Hz, 2H), 5.27 (q, J = 6.8 Hz, 2H), 5.53 – 5.65 (m, 2H), 5.93 (s, 2H), 6.52 (s, 5H), 6.87 (s, 2H), 6.99 (s, 2H), 7.11 – 7.20 (m, 2H), 7.96 – 8.28 (m, 7H).

Example 4. **Indirect Binding Assay**

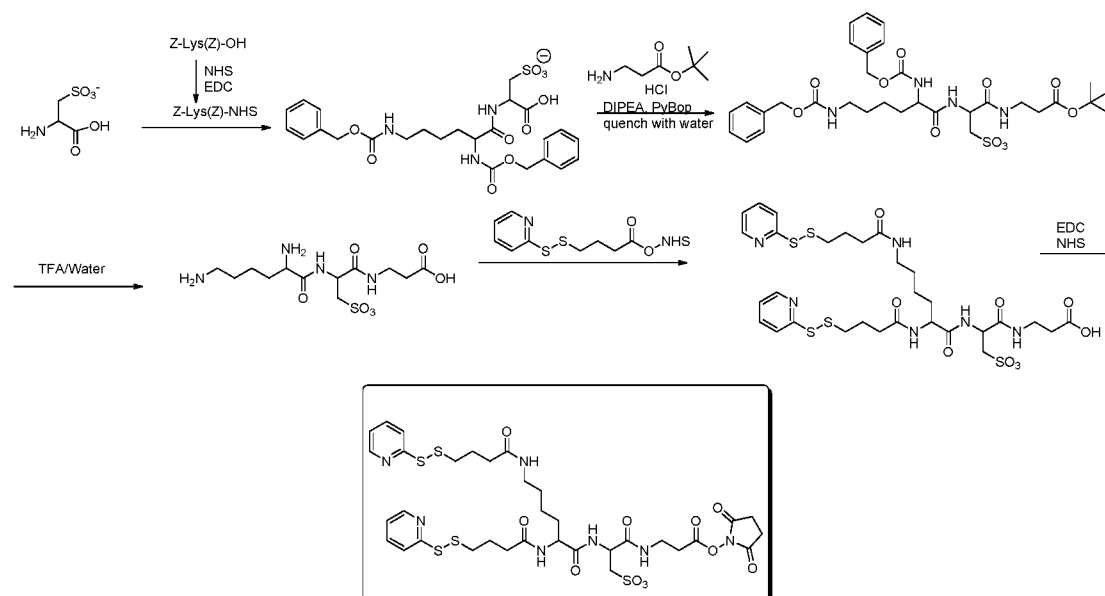
[244] Serial dilutions (triplicates) of Ab and conjugates in FACS Buffer (alpha-MEM + 2% goat serum) were made. Cells were re-suspended in FACS Buffer at the cell density of 5×10^5 cells/ml. 100 μ L of cells was plated in duplicate at 5×10^4 cells/well with a total volume of 200 μ L. The cells were mixed in the well of the plate and incubated on ice for 2-3 hours. The plates were spun down at 1200 rpm in the pre-chilled centrifuge for 5 mins. The supernatant was removed by quickly inverting plate. The cells were resuspended by gentle vortex in a plate with a cover and washed with 200 μ L/well of cold FACS Buffer. The washing solution was removed by repeating the centrifuge step. FITC labeled secondary antibody was diluted to 1:100 in FACS Buffer and 100 μ L of the diluted antibody was added to each well on the assay plate. The plate was incubated for 1 hour on ice without exposing to light and then spun down and washed as described above. The cells were resuspended in 200 μ L of Fixer (1xPBS with 1% Formaldehyde). The reading was taken on the FACS Calibur. As shown in Figures 24 and 25, the conjugates bind equally well as the naked antibody with similar K_d values.

Example 5. **Cytotoxicity Assay**

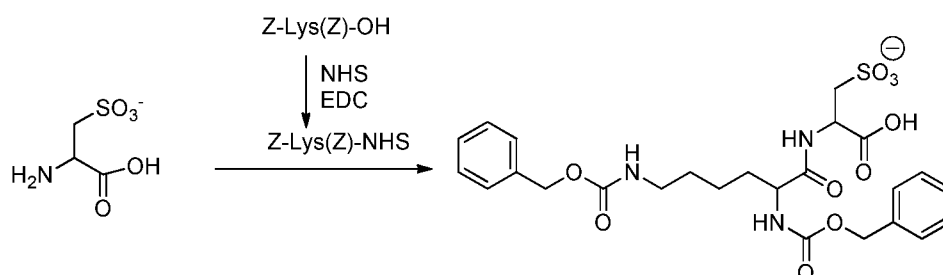
[245] Cells were plated at 2000 to 10,000 cells/well (depending on growth characteristics) in 96 well flat bottom tissue culture plates in 100 μ L volume. Conjugates/Drugs were titrated in a reservoir then added to the cells on the plate, total well volume 200 μ L (100 μ L cells, 100 μ L Conjugate/ drug titration). Each dilution of drug was in triplicate. Appropriate controls of un-dosed cells and medium only were included. Only the inner 60 wells were used; the outer wells were filled with buffer (HBSS or PBS to reduce evaporation). The plates were incubated at 37°C. with 5% CO₂ for 4 to 7 days depending on the growth characteristics of the cells tested. Develop when the untreated control reached confluence or when media started to change color and was becoming depleted. At the end of the incubation period WST-8 (Cat# CK04-20 Dojindo Molecular Technologies) was added at 20

μL /well to each assay well, and the plate was returned to the incubator for 2-7 hours depending on the dye turnover rate of the particular cells used. The plate was read on a plate reader at 450-650 nm. 450-650 reading was subtracted to correct for background and then the signal of the media alone is subtracted from all the wells. The results of the samples was subtracted by those of the untreated sample and plotted. In Figures 26-28, the conjugate containing a branched linking group (Ab-linker2-(DM4)₂ or Ab-linker3-(MayNMA)₂) is more potent than a conjugate with non-branched linking group, Ab-SPDB-DM4. In addition, the potency of the conjugates significantly decreases when the cells were blocked with naked antibodies, indicating target-specific cytotoxicity of the conjugates.

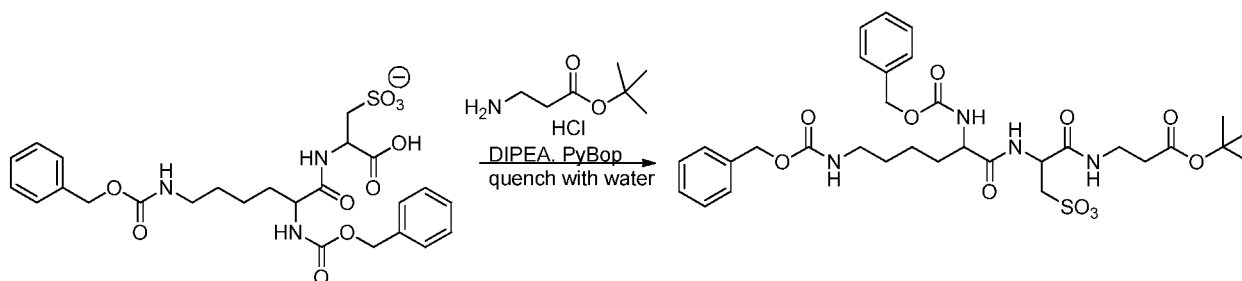
Example 6.



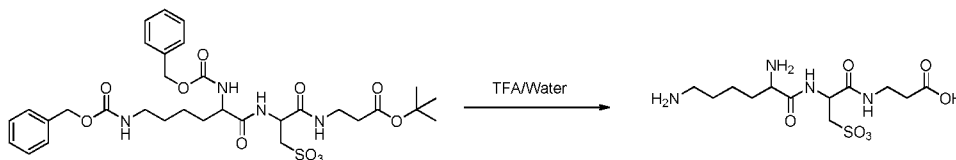
N-hydroxysuccinimide ester of Z-Lys(Z)-OH: Z-Lys(Z)-OH (2g, 4.83mmol) was dissolved in dimethylformamide (10 mL) and treated with 1-hydroxypyrrolidine-2,5-dione (0.833 g, 7.24 mmol) followed by EDC (0.925 g, 4.83 mmol). The reaction was allowed to proceed at room temperature, under argon atmosphere overnight to give crude N-hydroxysuccinimide ester which was used without purification.



Z-Lys(Z)-Cysteic Acid: Cysteic Acid (2.434 g, 14.47 mmol) was weighed into a 250 mL beaker equipped with a stir bar and dissolved in a solution of deionized water (70 ml) and dimethylformamide (30 ml). The mixture was stirred while 1M NaOH was added to bring the pH to 8.4. Crude Z-Lys(Z)-NHS (2.468 g, 4.82 mmol) in dimethylformamide (1 mL) was added dropwise while keeping the pH between 7.5-8.4 with the addition of 1M NaOH. After addition the reaction was allowed to stir for 1h. The crude mixture was concentrated under vacuum and purified using a Combiflash Rf 200i, PF 30 C18 HP 450g column with flow rate of 125 ml/min, eluting with deionized water containing 0.1% formic acid with a 5% to 95% linear acetonitrile gradient over 23 min. Fractions containing desired product were combined, frozen and lyophilized to give 2.09 g (77 % yield) of desired product. MS [M – H] calcd 564.17; found 564.17.

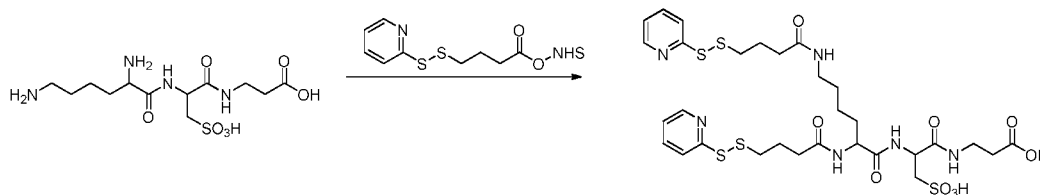


Z-Lys(Z)-Cys-β-Ala-OtBu: H-β-Ala-OtBu (241 mg, 1.328 mmol) was dissolved in dimethylsulfoxide (10 mL), then treated with diisopropyl ethylamine (309 μl, 1.771 mmol) followed by Z-Lys(Z)-Cysteic (497 mg, 0.718 mmol, 81 % yield). After all solids were in solution the reaction was treated with PyBOP (461 mg, 0.886 mmol). The reaction was allowed to proceed at room temperature under an argon atmosphere for 1 h and was quenched with water. The crude material was purified using a Combiflash Rf 200i, PF 30 C18 HP 450g column with flow rate of 125ml/min. Eluting with deionized water containing 0.1% formic acid using a linear gradient of acetonitrile 10% to 95% over 35 min. Fractions containing desired product were combined, frozen and lyophilized to give 497mg (81% yield) of desired product as an oily solid. HRMS [M-H]⁻ calcd. 693.3; found 693.3.

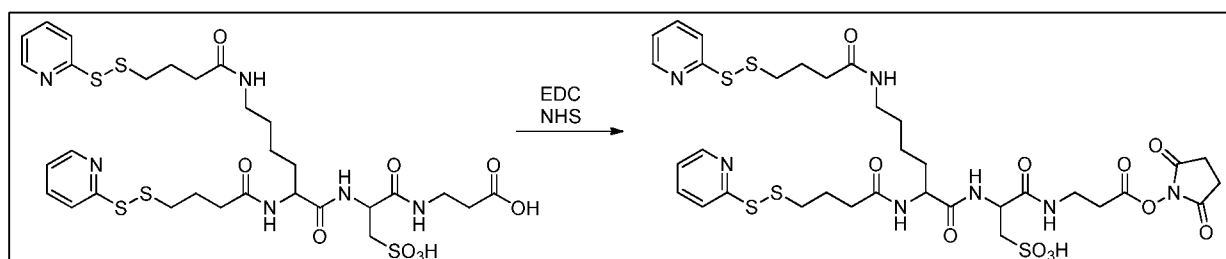


Lys-Cys-β-Ala-OH: Z-Lys(Z)-Cysteic Acid-β-Ala-OtBu (497mg, 0.718 mmol) was treated with 95:5 TFA:Water (10 mL) under an argon atmosphere overnight. The material was co-evaporated with toluene (3x 5 mL). The crude product was purified using a Combiflash Rf 200i, PF 30 C18 AQ 175g column with flow rate of 75ml/min. Eluting with

deionized water containing 0.1% formic acid for 5 min then using a linear gradient of acetonitrile 0% - 20% over 15 min then 20% to 95% over 10 min. Fractions containing desired material were combined, frozen then lyophilized to give 74 mg (28.0 % yield) of desired product as a white solid. HRMS $[M+H]^+$ calcd. 369.14; found 369.14.



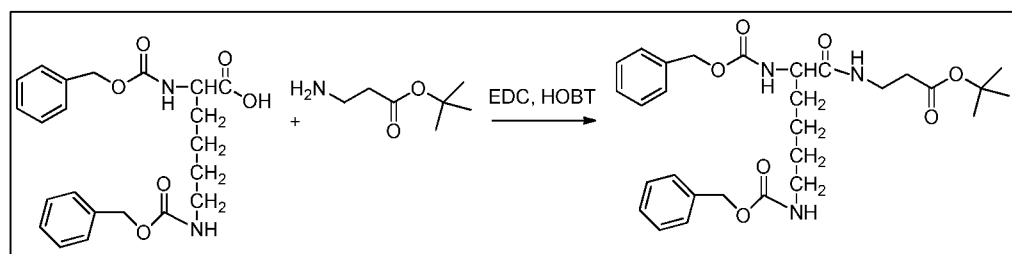
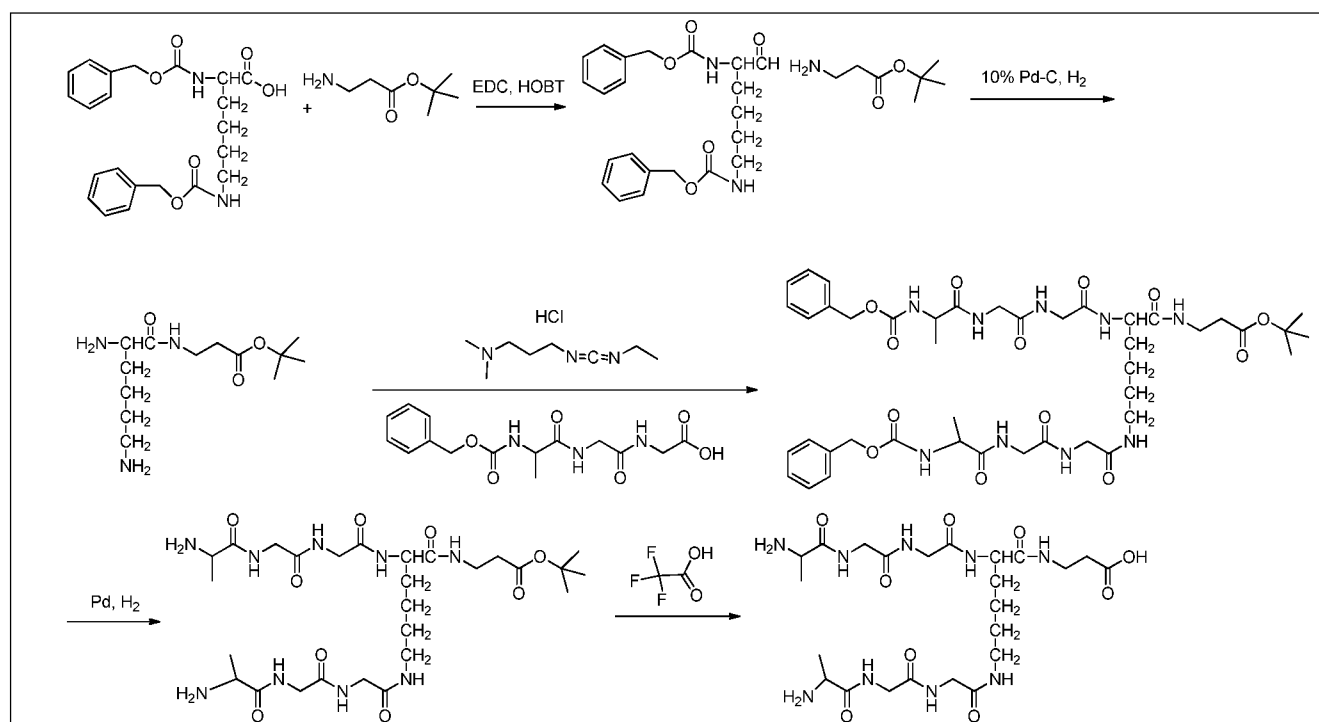
SPDB-Lys(SPDB)-Cys- β -Ala: Lys-Cys- β -Ala-OH (37mg, 0.101 mmol) was dissolved in dimethyl sulfoxide (1 mL) and treated with a solution of SPDB (72.3 mg, 0.222 mmol) dissolved in dimethyl sulfoxide (1 mL) and followed by diisopropyl ethylamine (0.035 ml, 0.201 mmol). The reaction was allowed to proceed at room temperature under an Argon atmosphere for 3h then was purified by semi-preparative C18 HPLC using a XB-C18 21.2 x 150mm, 5 μ m column with a flow rate of 21.2mL/min. Eluting with deionized water containing 0.1% formic acid with an acetonitrile gradient as follows: 5% acetonitrile for 3 min then a linear gradient from 3% - 90% acetonitrile over 17 min. The desired product eluted at 6.4 minutes and was collected, frozen then lyophilized to give 39.7mg (49.9 % yield) of desired product as a white solid. HRMS $[M+H]^+$ calcd. 791.2; found 791.2.



N-hydroxysuccinimide ester of SPDB-Lys(SPDB)-Cys- β -Ala (linker 4): SPDB-Lys(SPDB)-Cys- β -Ala-OH (39.7mg, 0.050 mmol) was dissolved in dimethylformamide (1 mL), to which was added EDC (9.63 mg, 0.050 mmol) and N-hydroxysuccinimide (5.78 mg, 0.050 mmol). The reaction was allowed to proceed at room temperature under argon atmosphere for 2 h then purified by semi-preparative C18 HPLC using a XB-C18 21.2x150mm, 5 μ m column with a flow rate of 21.2mL/min. eluting with deionized water containing 0.1% formic acid using a gradient of acetonitrile 5% for 4 min then a linear gradient of 5% - 95% over 17 min. The desired product eluted at 5.6 minutes and was collected, immediately frozen and lyophilized to give 12.7 mg (28.5 % yield) of product as a white solid. MS $[M+H]^+$ calcd. 888.19; found 888.18. ^1H NMR (400 MHz, DMSO- d_6) δ 1.16

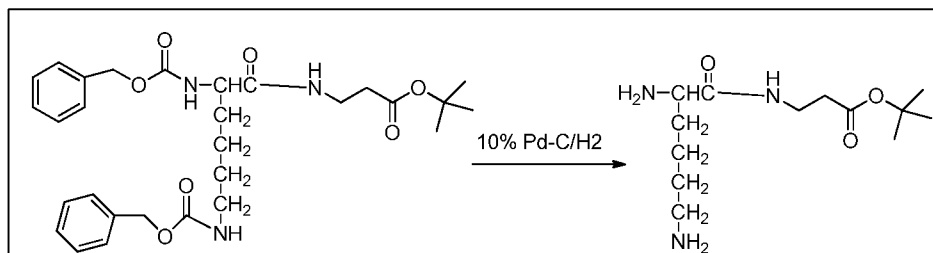
– 1.37 (m, 4H), 1.48 (dt, $J = 14.7, 6.0$ Hz, 2H), 1.65 (dq, $J = 13.6, 6.7, 6.0$ Hz, 1H), 1.77 – 1.97 (m, 4H), 2.18 (t, $J = 7.2$ Hz, 2H), 2.24 – 2.36 (m, 2H), 2.71 – 2.91 (m, 11H), 2.98 (q, $J = 6.2$ Hz, 3H), 3.17 – 3.48 (m, 3H), 3.92 – 4.10 (m, 1H), 4.22 (q, $J = 6.0$ Hz, 1H), 7.14 – 7.28 (m, 2H), 7.71 – 7.88 (m, 5H), 8.21 (d, $J = 6.5$ Hz, 1H), 8.32 (d, 1H), 8.39 – 8.51 (m, 2H).

Example 7.

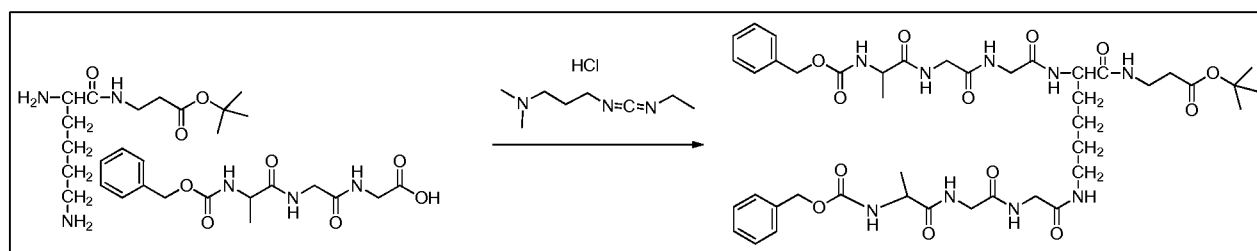


Z-Lys(Z)-b-Ala-OtBu: Z-L-Lys(Z)-OH (10.0 g, 24 mmol) was dissolved in dimethyl formamide (100 mL) to which was added beta-alanine t-butyl ester HCl salt (5.0 g, 27 mmol), EDC (6.0 g, 31 mmol) and HOBT (4.0 g, 25 mmol). After reacting for 2 h the reaction was purified directly on a 1200 g C18 cartridge on an intelliflash system, eluting with deionized water containing 0.2% formic acid with a gradient of 5 – 95% acetonitrile over 50 min at a flow rate of 100 mL/min. Fractions containing desired product were combined, frozen and lyophilized to give 8 g, (58 % yield) of desired product as a white solid. HRMS [MS + H]⁺ Calcd. 542.2861; found 542.2862. ¹H NMR (400 MHz, Chloroform-*d*) δ 1.35 (t, *J* = 7.6 Hz, 2H), 1.44 (s, 9H), 1.50 (dt, *J* = 13.7, 6.7 Hz, 2H), 1.57 – 1.69 (m, 1H), 1.70 – 1.88 (m, 2H),

2.42 (t, $J = 6.2$ Hz, 2H), 3.12 – 3.20 (m, 2H), 3.46 (q, $J = 6.1$ Hz, 2H), 4.09 (q, $J = 7.2$ Hz, 1H), 4.88 (s, 1H), 5.08 (d, $J = 6.7$ Hz, 4H), 5.51 (d, $J = 7.8$ Hz, 1H), 6.58 (d, $J = 7.2$ Hz, 1H), 7.24 – 7.39 (m, 10H).

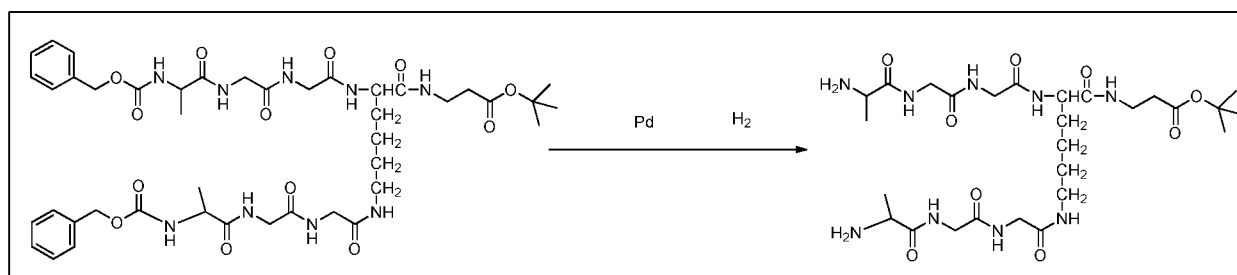


H-Lys-b-Ala-OtBu: Z-Lys(Z)-b-Ala-OtBu (7.7 g, 14.2 mmol) was dissolved in 1:2 methanol:dimethoxyethane (150 mL) containing deionized water (8 mL) in a 250 mL capacity PAR shaker flask, to which was added 10% Palladium on carbon (500 mg). The flask was shaken under 40 PSI hydrogen pressure for 3 h then the mixture was vacuum filtered through celite filter aid. Filtrate solvent was removed under vacuum to give 3.8 g (96% yield) of desired product as a thick oil. HRMS $[MS + H]^+$ 274.2125; found 274.2122. 1H NMR (400 MHz, Acetonitrile- d_3) δ 1.21 (d, $J = 6.9$ Hz, 7H), 1.38 (s, 9H), 1.49 – 1.73 (m, 2H), 2.38 (t, $J = 6.8$ Hz, 2H), 2.76 (d, $J = 0.8$ Hz, 2H), 2.90 (s, 2H), 3.10 (t, $J = 6.8$ Hz, 2H), 3.30 (dt, $J = 9.4, 6.8$ Hz, 2H), 3.48 (qd, $J = 7.0, 1.3$ Hz, 2H), 3.76 (s, 1H), 3.82 – 3.86 (m, 5H), 4.15 (dd, $J = 9.3, 4.9$ Hz, 1H).

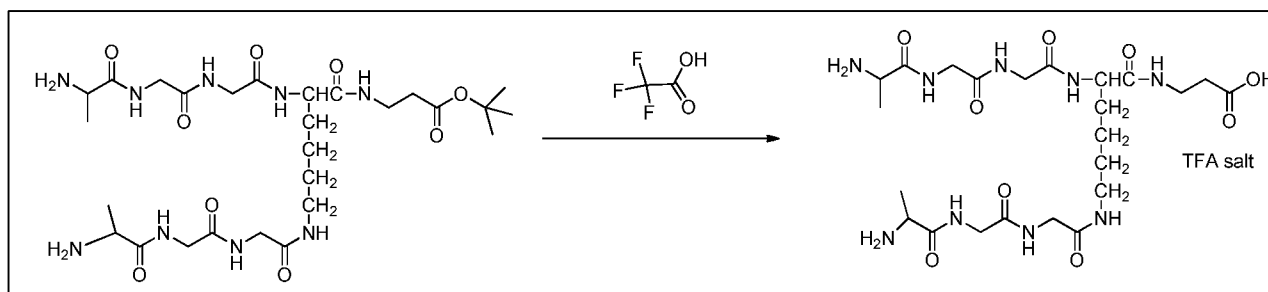


(Z-Ala-Gly-Gly)₂-Lys-b-Ala-OtBu: H-Lys-b-Ala-OtBu (4.65 g, 17 mmol) and Z-Ala-Gly-Gly-OH (14.34 g, 42 mmol) were dissolved in anhydrous dimethyl formamide (60 mL) to which was added EDC (9.78 g, 51 mmol) and the mixture was magnetically stirred for 3 h. approximately 5 mL of deionized water was added to solubilize material then the sample was vacuum filtered and loaded onto a 450 g C18 cartridge using the Intelliflash system. The cartridge was pre-equilibrated at 90% deionized water containing 0.2% formic acid and 10% acetonitrile. The cartridge was eluted with deionized water containing 0.2% formic acid and a linear gradient of 10 – 95% acetonitrile over 40 min. Fractions containing desired product were combined, frozen and lyophilized to give 8 g (51% yield) of a white solid. HRMS

$[M+Na]^+$ calcd. 934.4281; found 934.4260. 1H NMR (400 MHz, Acetonitrile- d_3) δ 1.39 (s, 3H), 1.41 (s, 3H), 1.49 (s, 9H), 1.73 (dddd, $J = 44.5, 17.6, 8.5, 4.8$ Hz, 2H), 2.47 (t, $J = 6.9$ Hz, 2H), 3.20 (q, $J = 6.7$ Hz, 2H), 3.29 – 3.48 (m, 2H), 3.59 (s, 6H), 3.79 – 3.97 (m, 8H), 4.14 – 4.32 (m, 3H), 5.02 – 5.24 (m, 4H), 6.87 (s, 1H), 7.34 – 7.51 (m, 10H), 7.52 – 7.69 (m, 1H).



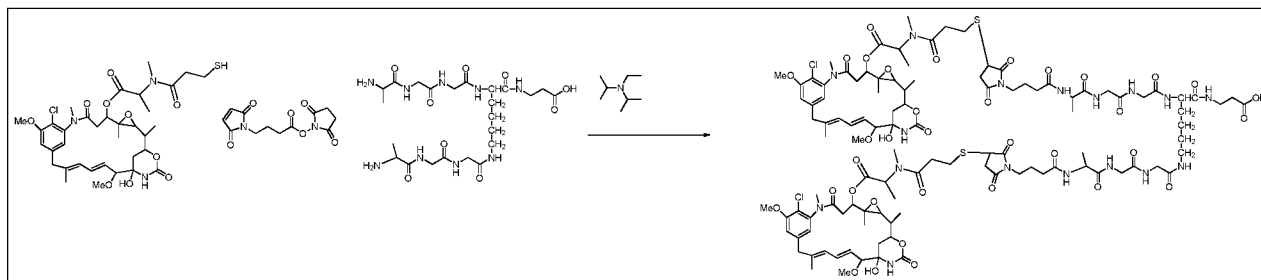
(H-Ala-Gly-Gly)₂-Lys-b-Ala-OtBu: (Z-Ala-Gly-Gly)₂-Lys-b-Ala-OtBu (11 g, 12 mmol) was vigorously stirred with 9:1 methanol:water (250 mL) and dimethylformamide (50 mL). Some material did not go into solution. 10%Pd-C (430 mg) was then added and the sample was hydrogenated in a 500 mL Parr hydrogenator flask at 40 PSI of hydrogen for 3 h. The mixture was vacuum filtered through celite filter aid. Deionized water 60 ml was added to the filtrate and the combined solution was frozen then lyophilized. About 2 h into the lyophilization the material thawed but was left on the lyophilizer for 3 days giving 5 g (64% yield) of white solid. HRMS $[M + H]^+$ calcd. 644.3740; found 644.3726.



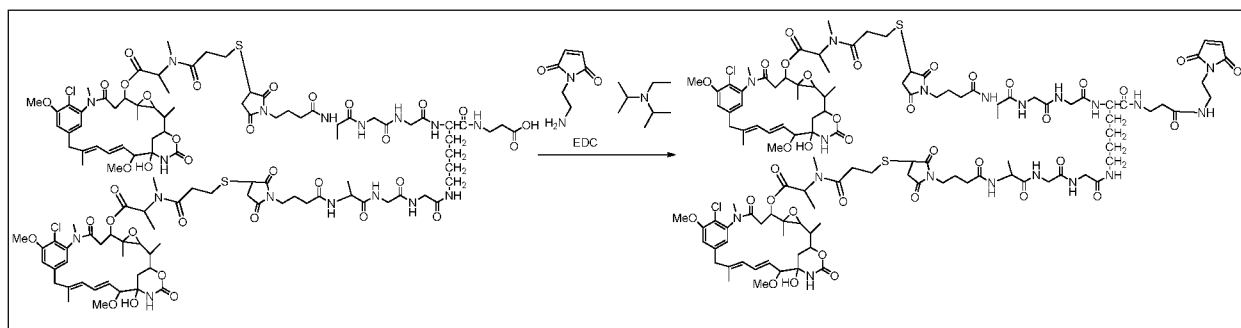
(H-Ala-Gly-Gly)-Lys-OH: (H-Ala-Gly-Gly)₂-Lys-OtBu (2.8 g, 4.3 mmol) was taken up in 9:1 TFA:deionized water (20 mL) and magnetically stirred for 2 h. Solvent was evaporated under vacuum and the residue was taken up in deionized 2:1 acetonitrile:deionized water (50 mL), frozen and lyophilized to give 2.6 grams (95% yield) of an oily solid as the TFA salt. HRMS $[M + H]^+$ calcd. 588.3101; found 588.3100. 1H NMR (400 MHz, Deuterium Oxide) δ 1.29 (ddt, $J = 16.1, 9.3, 6.0$ Hz, 1H), 1.47 (q, $J = 7.3$ Hz, 1H), 1.52 (d, $J = 7.1$ Hz, 3H), 1.70 (dddd, $J = 26.3, 17.6, 9.1, 5.3$ Hz, 1H), 2.57 (t, $J = 6.5$ Hz, 1H), 2.82 (d, $J = 0.8$ Hz, 0H), 2.98

(s, 0H), 3.16 (t, $J = 6.9$ Hz, 1H), 3.43 (dtd, $J = 18.4, 13.6, 6.5$ Hz, 1H), 3.90 (d, $J = 27.5$ Hz, 2H), 3.97 – 4.07 (m, 2H), 4.09 – 4.23 (m, 2H).

Example 8.

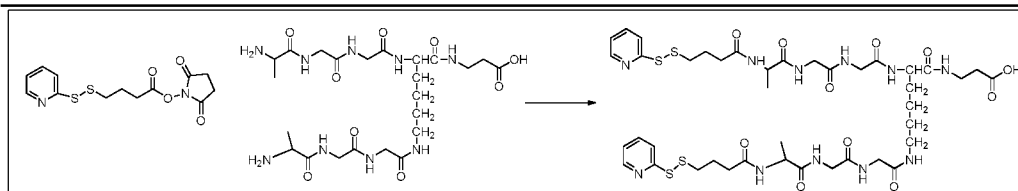


(DM1-GMB-Ala-Gly2)-Lys-b-Ala-OH: DM1 (101 mg, 140 mmol) and H-Ala-Gly-Gly)₂-Lys-b-Ala-OH (40 mg, 0.068 mmol) were dissolved in anhydrous dimethyl formamide (1.0 mL) to which was added deionized water (10 μ L). The mixture was magnetically stirred vigorously GMBS (39 mg, 0.14 mmol) in anhydrous dimethyl formamide was added followed by diisopropyl ethyl amine (50 μ L, 0.28 mmol) and the reaction was stirred for 1 hour then purified on a 27 mm x 220 mm C18 load and lock HPLC column flow rate 40 mL/min eluting with deionized water containing 0.2% formic acid and a linear gradient of 5 – 95% acetonitrile over 30 min. Fractions containing pure desired product were combined and frozen then lyophilized to give 40 mg (24% yield) of desired product as white solid. MS [$M + Na$]⁺ calcd. 2414.9; found 2415.2. ¹H NMR (400 MHz, DMSO-*d*₆) δ 0.78 (d, $J = 2.1$ Hz, 6H), 1.12 (d, $J = 6.3$ Hz, 6H), 1.18 (t, $J = 6.5$ Hz, 12H), 1.25 (d, $J = 12.8$ Hz, 3H), 1.36 (t, $J = 7.6$ Hz, 2H), 1.45 (tt, $J = 10.0, 5.5$ Hz, 5H), 1.59 (s, 6H), 1.64 (dd, $J = 12.1, 5.9$ Hz, 4H), 2.00 – 2.14 (m, 6H), 2.29 – 2.38 (m, 3H), 2.71 (s, 5H), 2.79 (dd, $J = 9.7, 1.8$ Hz, 3H), 2.85 (d, $J = 6.2$ Hz, 2H), 2.86 – 2.96 (m, 4H), 2.96 – 3.06 (m, 4H), 3.10 (s, 6H), 3.14 – 3.22 (m, 4H), 3.25 (s, 8H), 3.49 (d, $J = 9.0$ Hz, 4H), 3.65 (d, $J = 5.8$ Hz, 2H), 3.72 (dd, $J = 12.1, 5.7$ Hz, 6H), 3.85 (dd, $J = 9.0, 3.9$ Hz, 1H), 3.88 – 3.97 (m, 7H), 4.01 – 4.19 (m, 3H), 4.19 – 4.28 (m, 2H), 4.52 (dd, $J = 12.1, 2.9$ Hz, 2H), 5.26 – 5.36 (m, 2H), 5.56 (td, $J = 10.0, 8.9, 3.3$ Hz, 2H), 5.92 (s, 2H), 6.49 – 6.61 (m, 6H), 6.88 (s, 2H), 7.17 (dd, $J = 8.5, 1.8$ Hz, 2H), 7.70 (t, $J = 5.6$ Hz, 1H), 7.88 (d, $J = 8.0$ Hz, 1H), 7.95 (t, $J = 5.5$ Hz, 1H), 7.99 – 8.14 (m, 4H), 8.21 (dt, $J = 17.2, 6.0$ Hz, 2H).



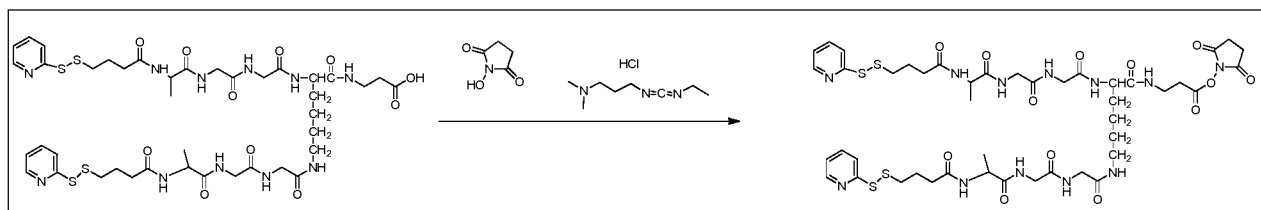
(DM1-GMB-Ala-Gly-Gly)-Lys-b-Ala-Mal: (DM1-GMB-Ala-Gly-Gly)₂-Lys-b-Ala-OH (5.3 mg, 0.0022 mmol) was dissolved in anhydrous dimethylformamide (0.25 mL) to which was added EDC (2.0 mg, 0.014 mmol). After 2 min 2-amino-ethyl-maleimide HCl salt (1 mg, 0.0057 mmol) was added and the solution was stirred at room temperature for 15 min. The reaction mixture was purified by HPLC on a XB-C18 21.2x150 mm, 5 μ m column with a flow rate of 21.2 mL/min. eluting with deionized water containing 0.1% formic acid using a gradient of acetonitrile 5% for 4 min then a linear gradient of 5% - 95% over 17 min. Fractions containing desired product were combined, frozen and lyophilized to give 2 mg (35 % yield) of white solid. MS [M + Na]⁺ calcd. 2537.0; found 2537.3.

Example 9.



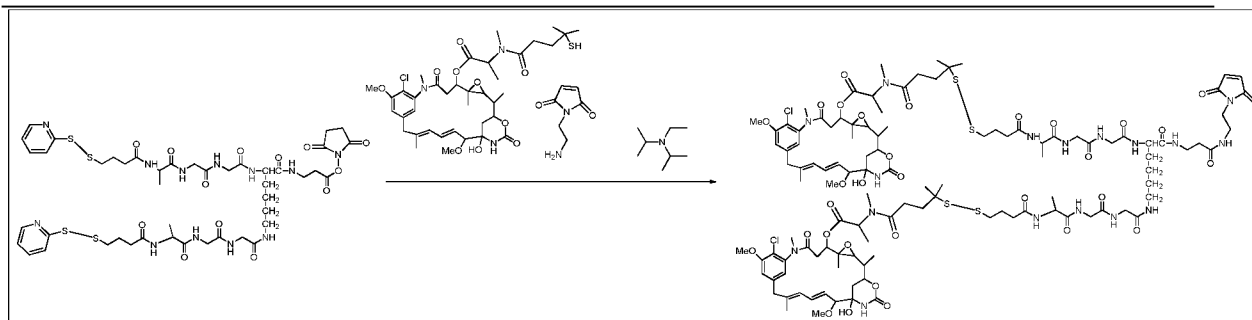
(SPDB-A-GG)₂-Lys-b-Ala-OH: (H-Ala-Gly-Gly)₂-Lys-b-Ala-OH (450 mg, 0.76 mmol) was dissolved in anhydrous dimethyl formamide (11 mL) to which was added SPDB (683 mg, 2.1 mmol) and diisopropyl amine (450 μ L, 3 mmol). After 2h the reaction was injected directly onto a 19 mm x 150 mm C18 HPLC column eluting with deionized water containing 0.2% formic acid with a 5 – 95% acetonitrile linear gradient with a flow rate of 20 mL/min. Fractions containing desired product were combined, frozen and lyophilized to give 230 mg, (30% yield) of desired product as a white solid. HRMS [M + Na]⁺ calcd. 1032.3171; found 1032.3194. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.19 (d, *J* = 7.1 Hz, 3H), 1.23 (d, *J* = 8.1 Hz, 1H), 1.36 (h, *J* = 6.7, 5.7 Hz, 1H), 1.41 – 1.55 (m, 1H), 1.60 (dt, *J* = 13.4, 7.5 Hz, 1H), 1.84 (p, *J* = 7.4 Hz, 2H), 2.25 (td, *J* = 7.3, 2.6 Hz, 2H), 2.35 (t, *J* = 7.0 Hz, 1H), 2.83 (t, *J* = 7.3 Hz, 2H), 3.01 (q, *J* = 6.6 Hz, 1H), 3.22 (dh, *J* = 19.9, 6.6 Hz, 2H), 3.61 – 3.76 (m, 4H), 4.19 (dtd,

$J = 32.4, 8.5, 7.8, 5.2$ Hz, 2H), 7.23 (dd, $J = 7.3, 4.8$ Hz, 1H), 7.73 – 7.98 (m, 3H), 8.02 (t, $J = 5.8$ Hz, 1H), 8.09 – 8.25 (m, 2H), 8.42 – 8.48 (m, 1H).



(SPDB-Ala-Gly-Gly)₂-Lys-b-Ala-ONHS: (SPDB-Ala-Gly-Gly)₂-Lys-b-Ala-OH (200 mg, 0.19 mmol) N-hydroxysuccinimide (68 mg, 0.59 mmol) and EDC (114 mg, 0.59 mmol) were dissolved in anhydrous dimethyl formamide (2 mL) and magnetically stirred at ambient temperature for 2 hours, and the reaction mixture was purified by HPLC on a 27 cm x 220 cm C18 column flow rate 40 mL/min eluting with deionized water containing 0.1% formic acid using a 5 – 95% acetonitrile linear gradient over 28 min. Fractions containing pure desired product were combined, frozen and lyophilized to give 82 mg (39% yield) of desired product as a white solid. HRMS $[M + Na]^+$ calcd. 1129.3334; found 1129.3325. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.19 (d, $J = 7.1$ Hz, 6H), 1.26 (d, $J = 9.3$ Hz, 1H), 1.36 (p, $J = 7.0, 6.5$ Hz, 2H), 1.49 (td, $J = 9.0, 4.7$ Hz, 1H), 1.63 (dt, $J = 13.2, 6.6$ Hz, 1H), 1.85 (p, $J = 7.4$ Hz, 6H), 2.26 (td, $J = 7.3, 2.1$ Hz, 4H), 2.32 (t, $J = 7.3$ Hz, 2H), 2.72 (t, $J = 7.2$ Hz, 2H), 2.77 – 2.89 (m, 9H), 3.01 (q, $J = 6.7$ Hz, 2H), 3.34 (ddq, $J = 26.2, 13.0, 6.5$ Hz, 3H), 3.65 (d, $J = 5.8$ Hz, 2H), 3.70 (d, $J = 5.7$ Hz, 3H), 3.74 (d, $J = 5.7$ Hz, 2H), 4.20 (dtd, $J = 28.4, 8.5, 7.9, 5.1$ Hz, 3H), 7.19 – 7.27 (m, 2H), 7.68 (t, $J = 5.6$ Hz, 1H), 7.76 (d, $J = 8.0$ Hz, 2H), 7.79 – 7.86 (m, 2H), 7.91 (d, $J = 8.0$ Hz, 1H), 8.01 (q, $J = 6.2$ Hz, 2H), 8.11 (dt, $J = 12.4, 6.3$ Hz, 3H), 8.18 (t, $J = 5.8$ Hz, 2H), 8.45 (dd, $J = 4.9, 1.7$ Hz, 2H). ¹³C NMR (101 MHz, DMSO) δ 17.73, 17.84, 22.70, 24.02, 24.48, 25.43, 28.72, 30.34, 31.57, 32.17, 33.43, 34.25, 36.92, 37.49, 37.51, 38.41, 41.98, 42.09, 42.19, 48.32, 48.40, 52.48, 119.14, 121.07, 137.73, 149.51, 159.27, 167.24, 168.30, 168.51, 169.00, 169.17, 170.07, 171.39, 171.48, 171.76, 172.83, 172.95, 173.93.

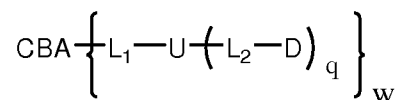
Example 10.



(SPDB-Ala-Gly-Gly)₂-Lys-β-Ala-Mal: (SPDB-Ala-Gly-Gly)₂-Lys-β-Ala-ONHS (22 mg, 0.020 mmol) was dissolved in anhydrous dimethyl formamide (400 μL) to which was added DM4 (30 mg, 0.038) with magnetic stirring. After 2 min deionized water (20 μL) was added then after 15 min diisopropylethyl amine (10 μL, mmol) and H₂N-(CH₂)₂-Mal HCl salt (3.7 mg, 0.022 mmol) were added. After an additional 30 min, sample was injected directly onto a 19 mm x 150 mm C18 HPLC column eluting with deionized water containing 0.2% formic acid with a 5-95% linear acetonitrile gradient. Flow rate was 20 mL/min. Fractions containing pure desired product were combined and frozen then lyophilized to give 19 mg (38% yield) of product as a white solid. MS [M + Na]⁺ calcd.2491.0; found 2491.3.

We claim:

1. A conjugate represented by the following formula, or a pharmaceutically acceptable salt thereof:



wherein:

CBA is a cell binding agent;

L₁ is a spacer;

U is a branched scaffold;

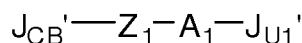
L₂, for each occurrence, is independently a spacer

D, for each occurrence, is independently a cytotoxic drug moiety;

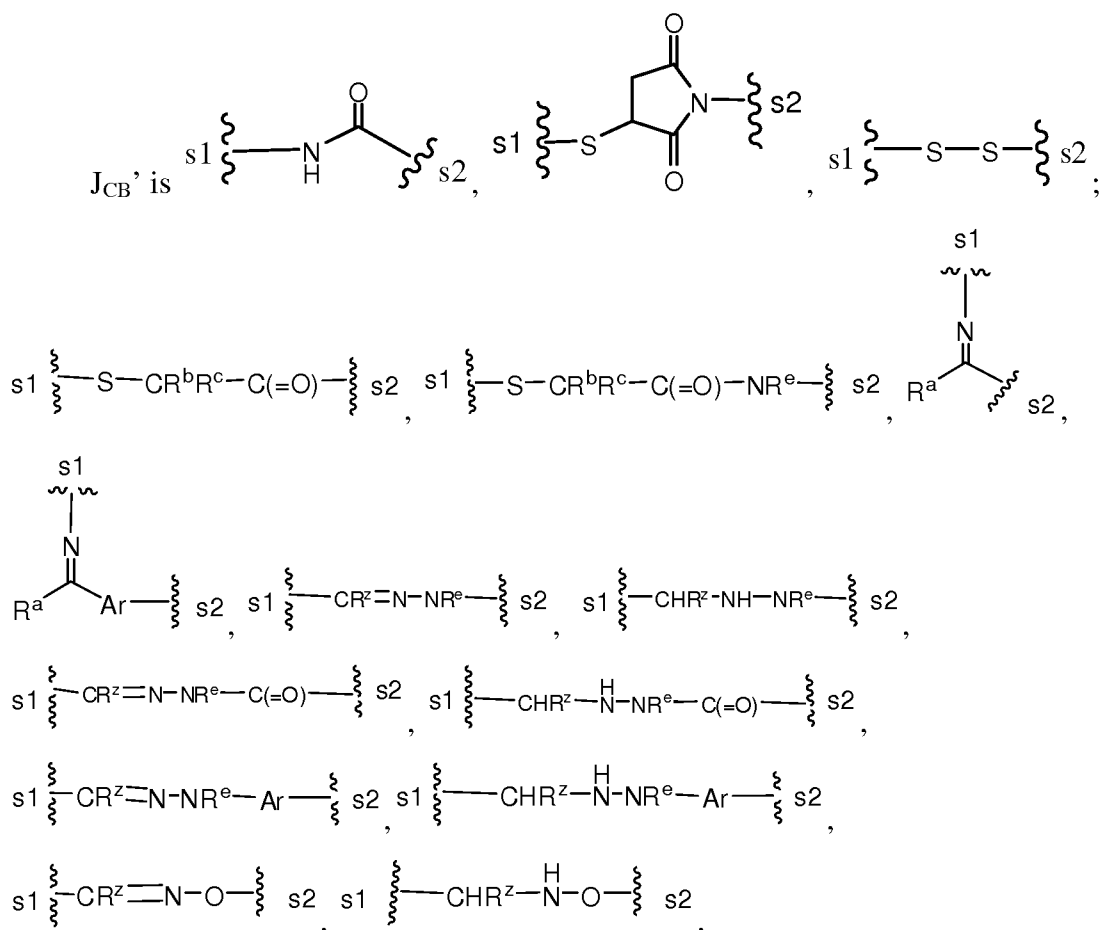
q is an integer from 2 to 5; and

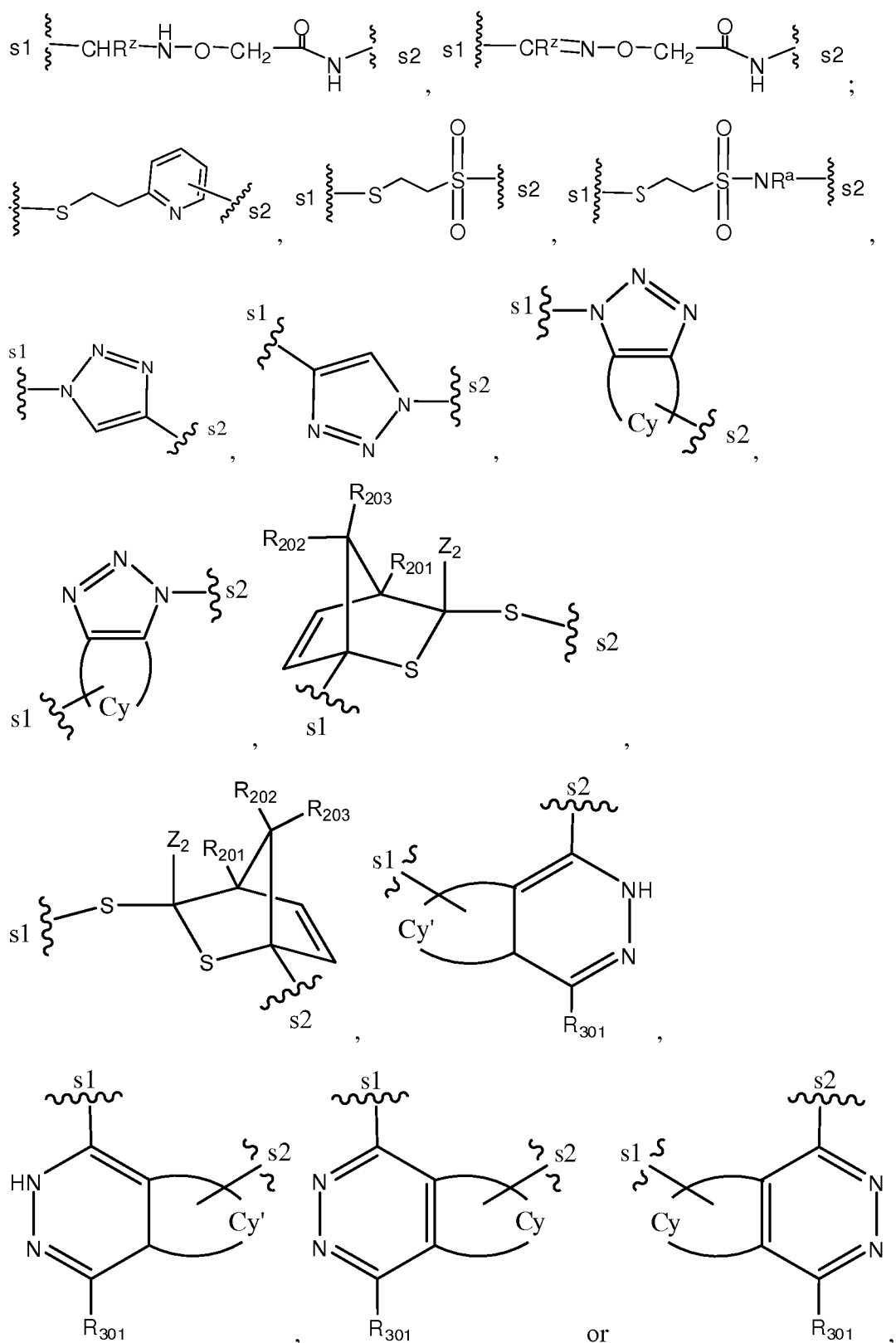
w is an integer between 1 and 10.

2. The conjugate of claim 1, wherein L₁ is represented by the following formula:

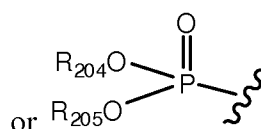


wherein:





in which s1 is the site covalently linked to the CBA, s2 is the site covalently linked to the group U, Ar is an optionally substituted arylene or an optionally substituted heteroarylene, Cy is an optionally substituted carbocyclic or heterocyclic ring, R₂₀₁, R₂₀₂ and R₂₀₃ each are independently H or an optionally substituted alkyl; Z₂ is pyridyl


 , wherein R₂₀₄ and R₂₀₅ are each independently optionally substituted alkyl, R₃₀₁ is H or optionally substituted alkyl; and Cy' is an optionally substituted carbocyclic or heterocyclic ring;

Z₁ is -NR₉-C(=O)- or -C(=O)-NR₉- or absent,

A₁ is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, -(CH₂-CH₂-O)_{p1}-(CR^bR^c)_{p1'}- or -(CR^bR^c)_{p1'}-(O-CH₂-CH₂)_{p1}-,

J_{U1}' is absent, -NR₉- or -C(=O)-

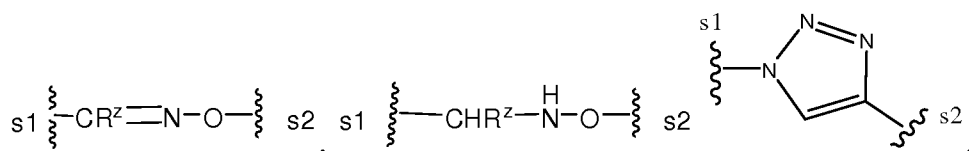
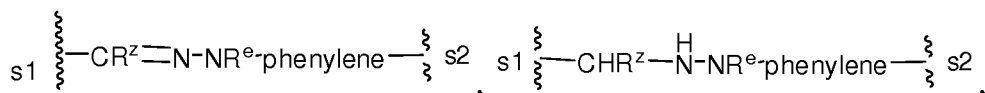
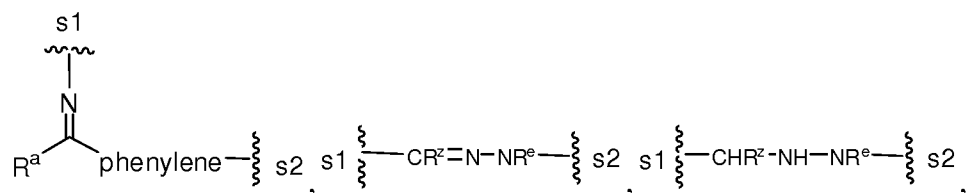
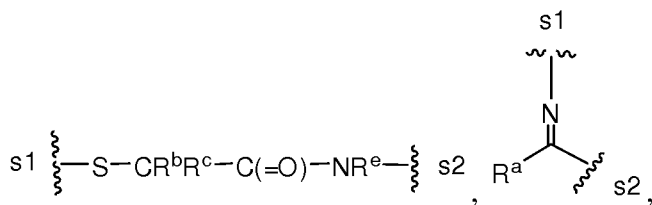
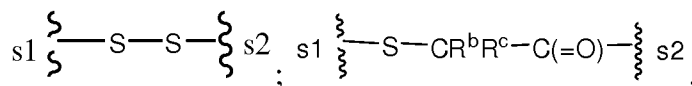
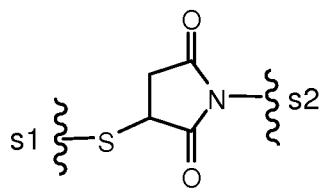
p₁ is an integer from 1 to 1000

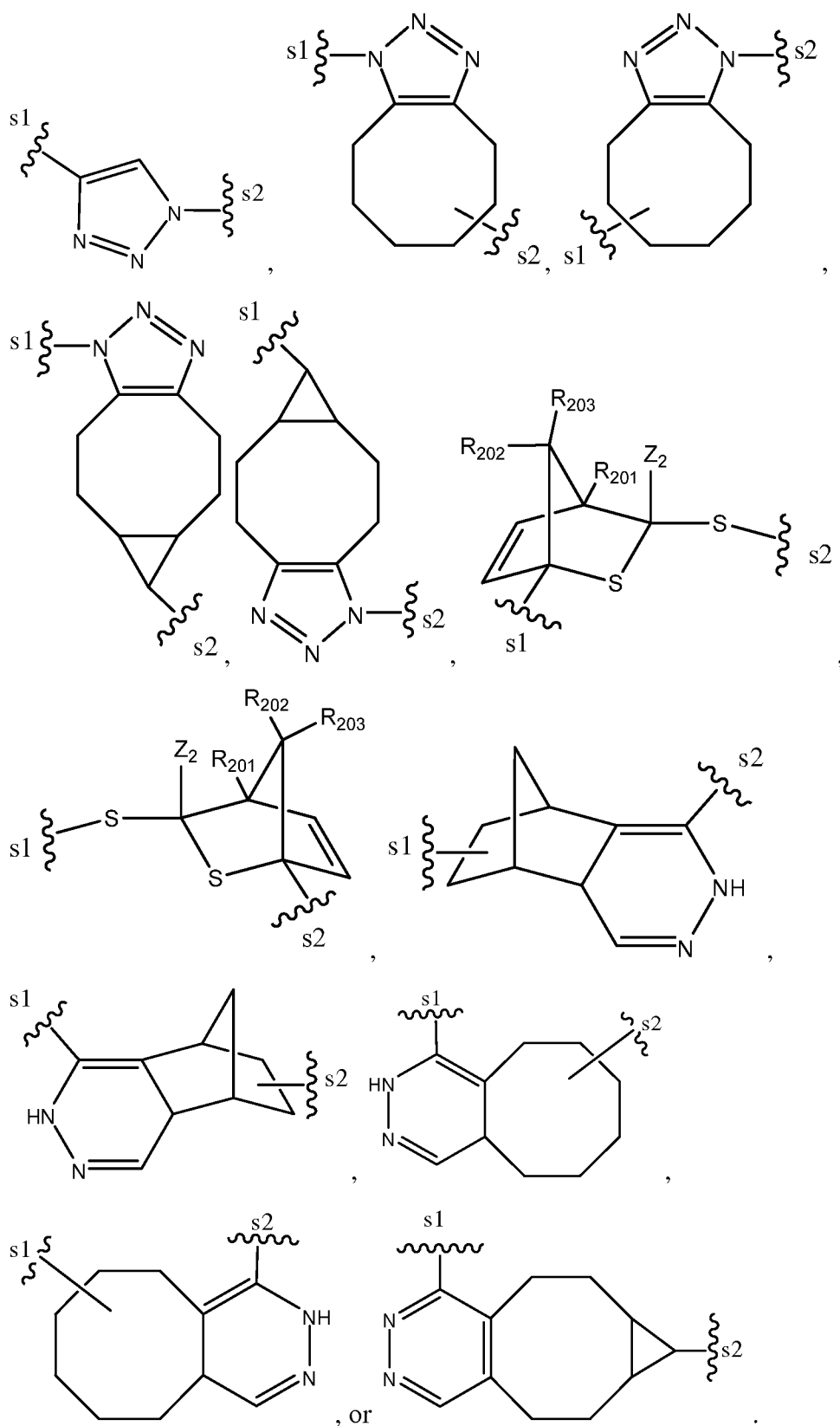
p₁' is an integer from 0 to 10;

R₉ is H or an optionally substituted alkyl; and

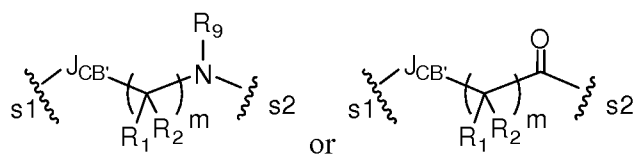
R^a, R^b, R^c, R^e, and R^z, for each occurrence, are independently H or an optionally substituted alkyl.

3. The conjugate of claim 2, wherein J_{CB}' is



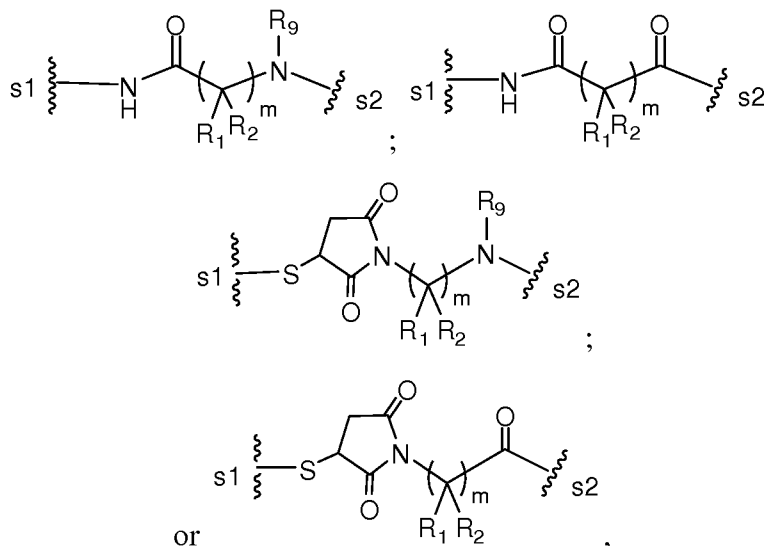


4. The conjugate of any one of claims 1-3, wherein L_1 is represented by the following formula:



wherein R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10.

5. The conjugate of any one of claims 1-4, wherein L_1 is represented by the following formula:



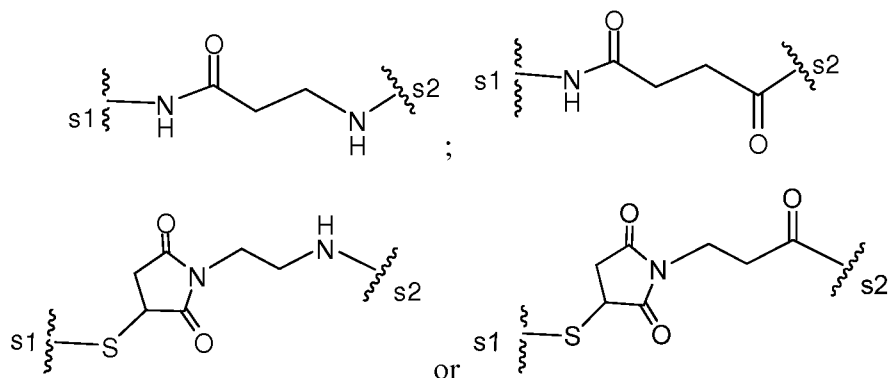
wherein:

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

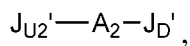
m is an integer from 1 to 5; and

R^9 is H or an alkyl.

6. The conjugate of claim 4 or 5, wherein R_1 and R_2 are both H; and R^9 is H.
7. The conjugate of claim 6, wherein L_1 is represented by the following formula:



8. The conjugate of any one of claims 1-7, wherein L_2 is represented by the following formula:

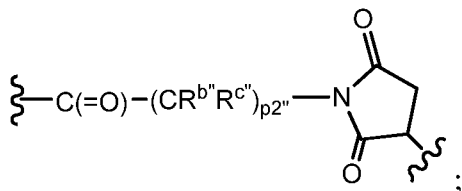


wherein:

J_{U2}'is -C(=O)-, -NR₉- or absent;

A₂ is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or [XX]₁₋₁₀;

J_D' is -C(=O)-, -C(=O)-(CR^bR^c)_{p2}·-C(=O)-, -C(=O)-(CH₂-CH₂-O)_{p2}-(CR^bR^c)_{p2}·- S-, -S-, absent, -C(=O)-(CR^{b''}R^{c''})_{p2}·-S- or



[XX], for each occurrence, is independently an amino acid residue;

R^{b'} and R^{c'}, for each occurrence, are independently H or an optionally substituted alkyl ;

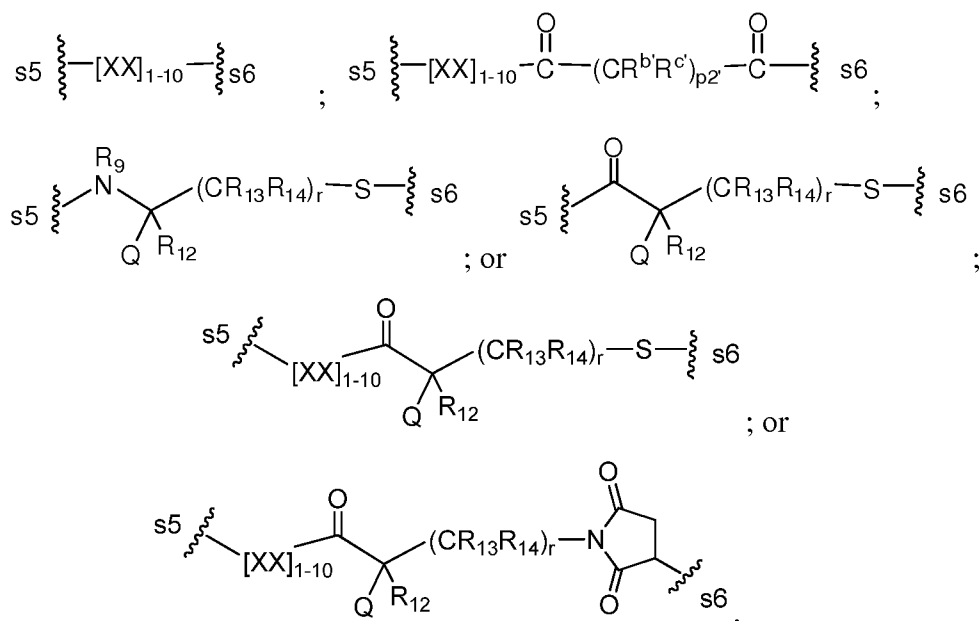
R^{b''} and R^{c''}, for each occurrence, are independently H, an optionally substituted alkyl or a charged substituent or an ionizable group;

p2 is an integer from 1 to 1000;

p2' is an integer from 1 to 10; and

p2" is an integer from 1 to 10 (specifically 1 to 5).

9. The conjugate of claim 8, wherein L_2 is represented by the following formula:



wherein:

R_{12} , R_{13} , and R_{14} , for each occurrence, are each independently H, an optionally substituted alkyl;

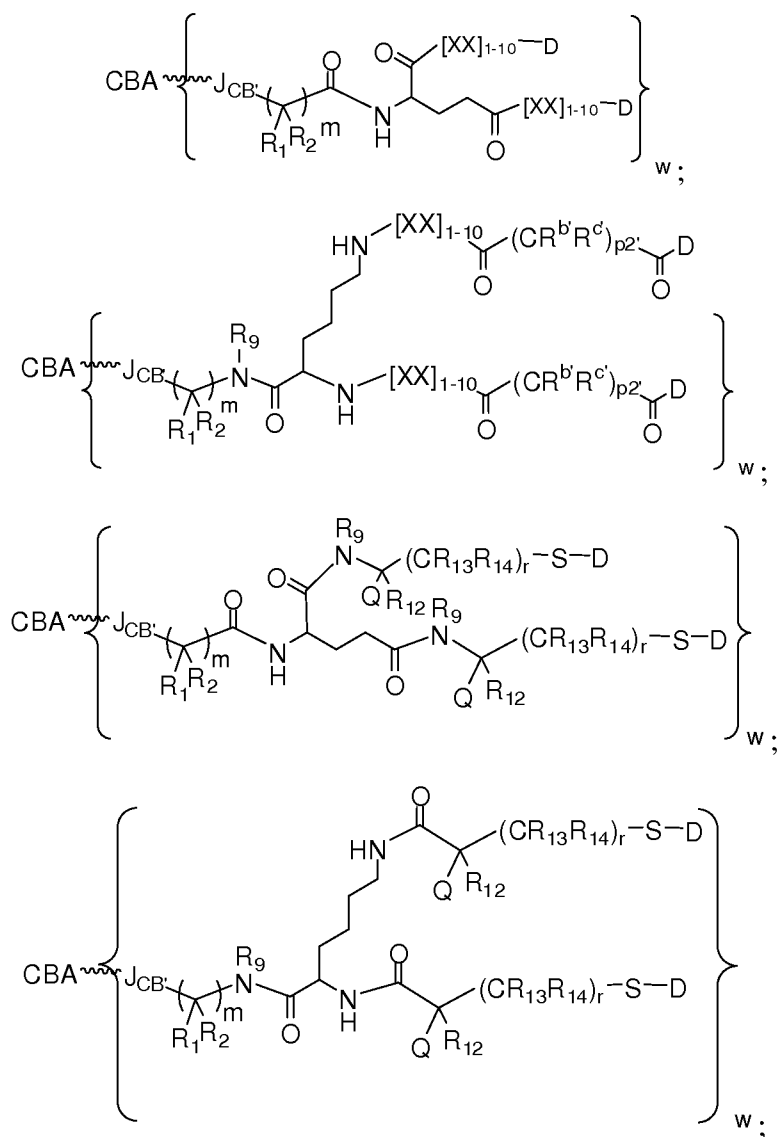
Q is H, a charged substituent or an ionizable group;

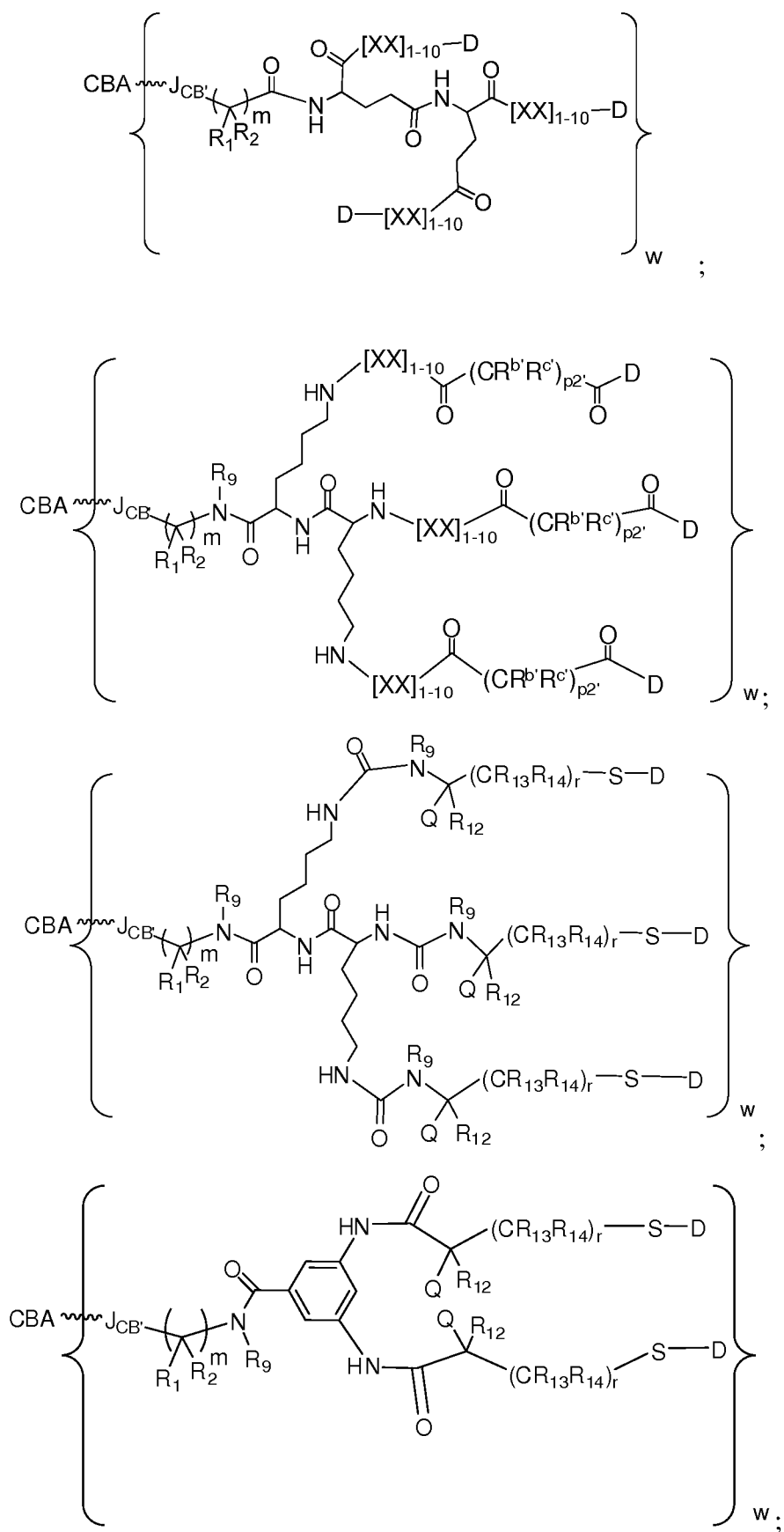
r is an integer from 0 to 10;

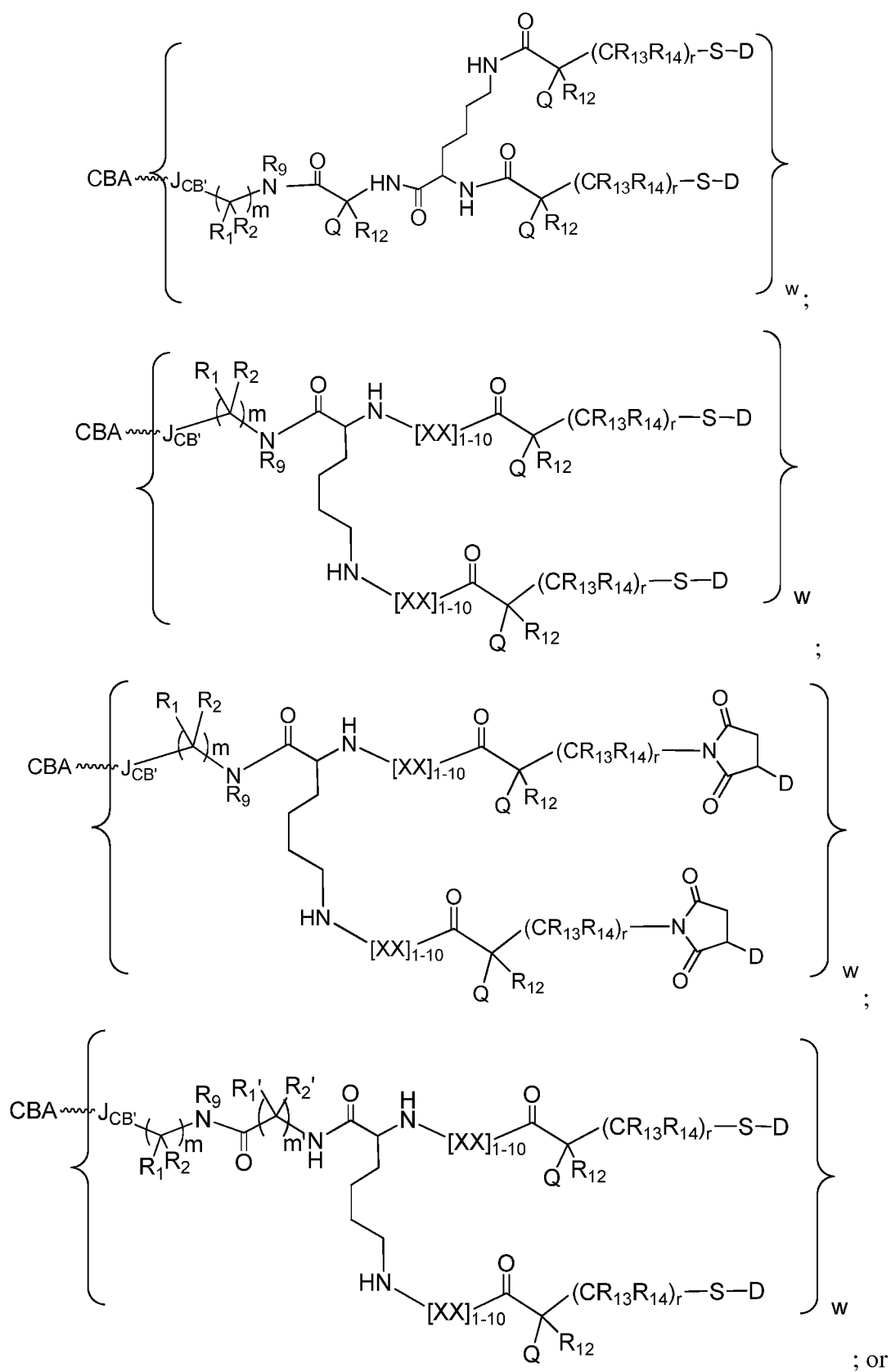
s5 is the site covalently linked to the group U; and

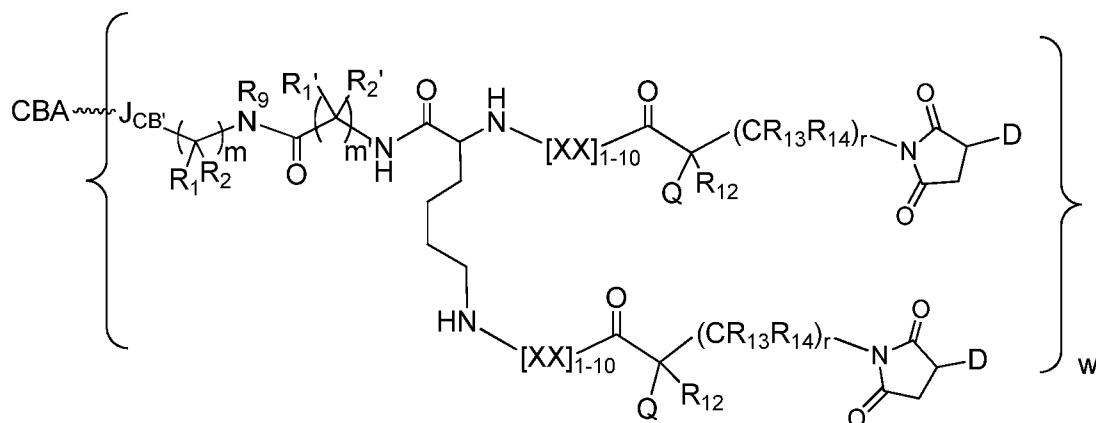
s6 is the site covalently linked to the group D.

10. The conjugate of claim 8 or 9, wherein the conjugate is represented by the following formula, or a pharmaceutically acceptable salt thereof:



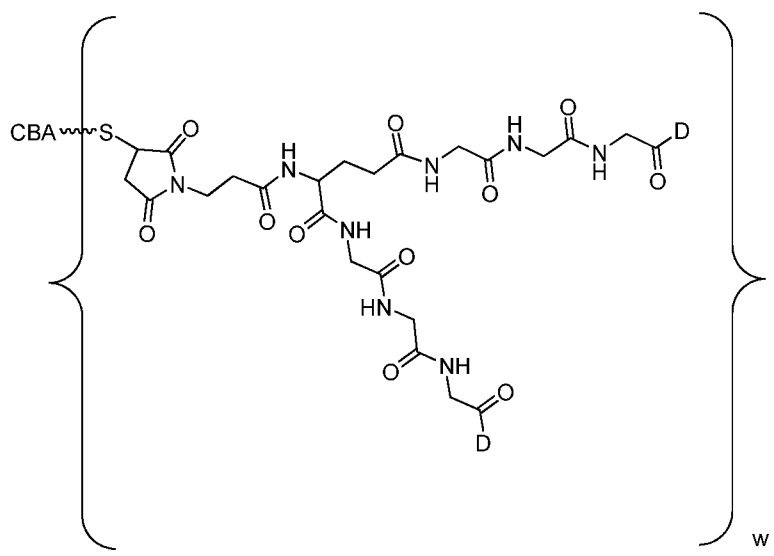


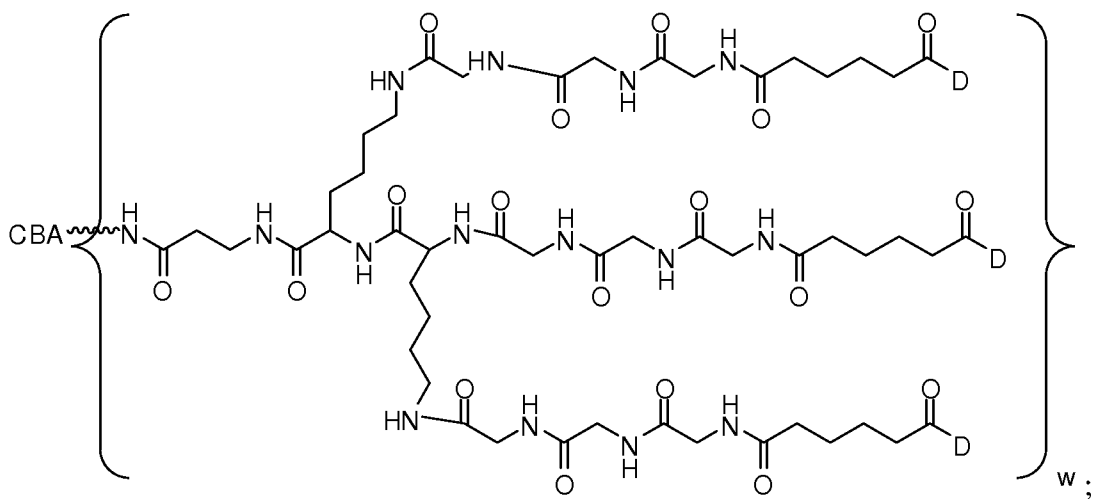
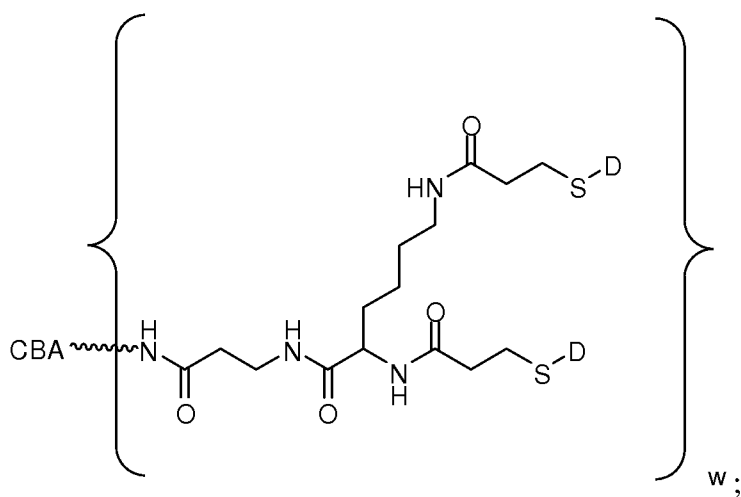
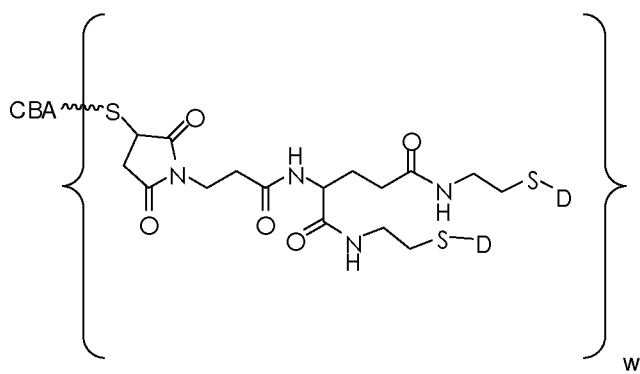
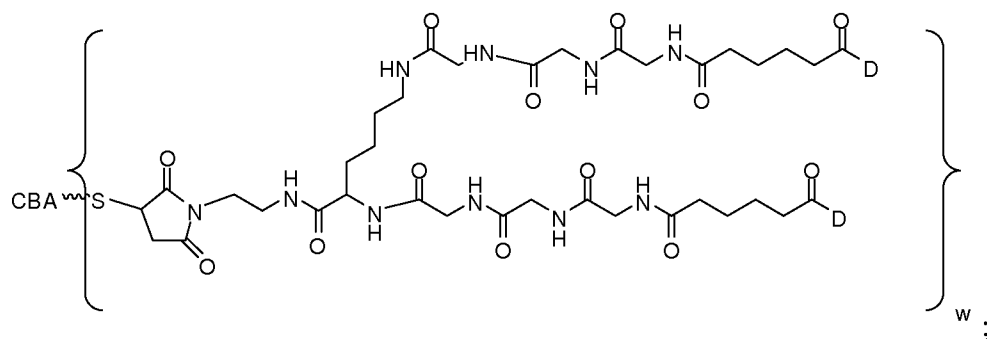


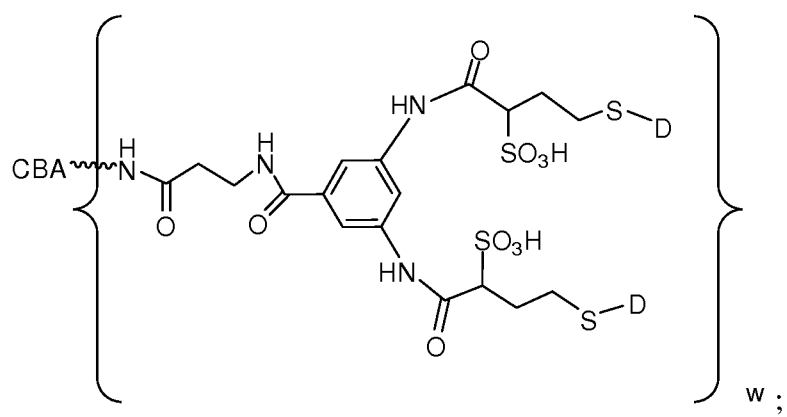
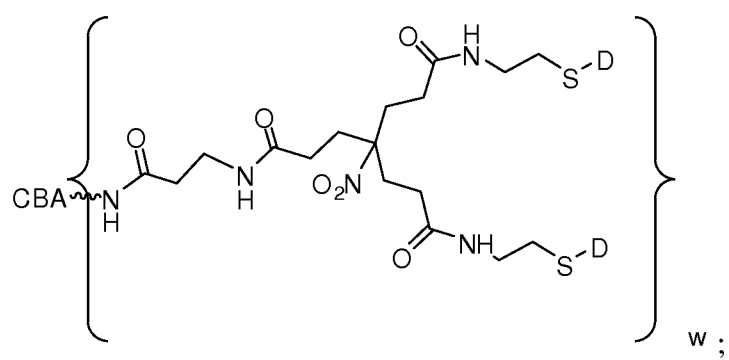
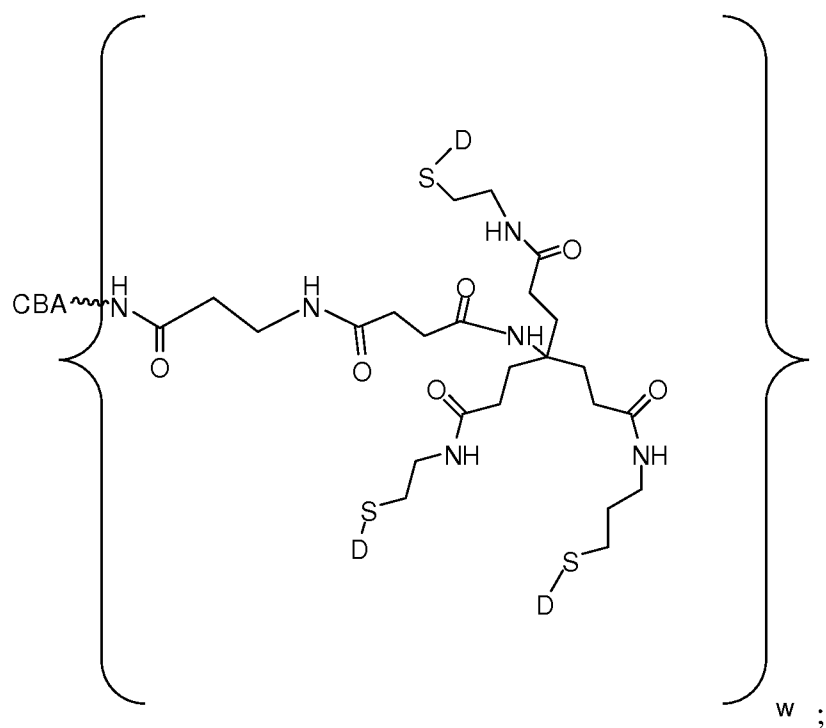


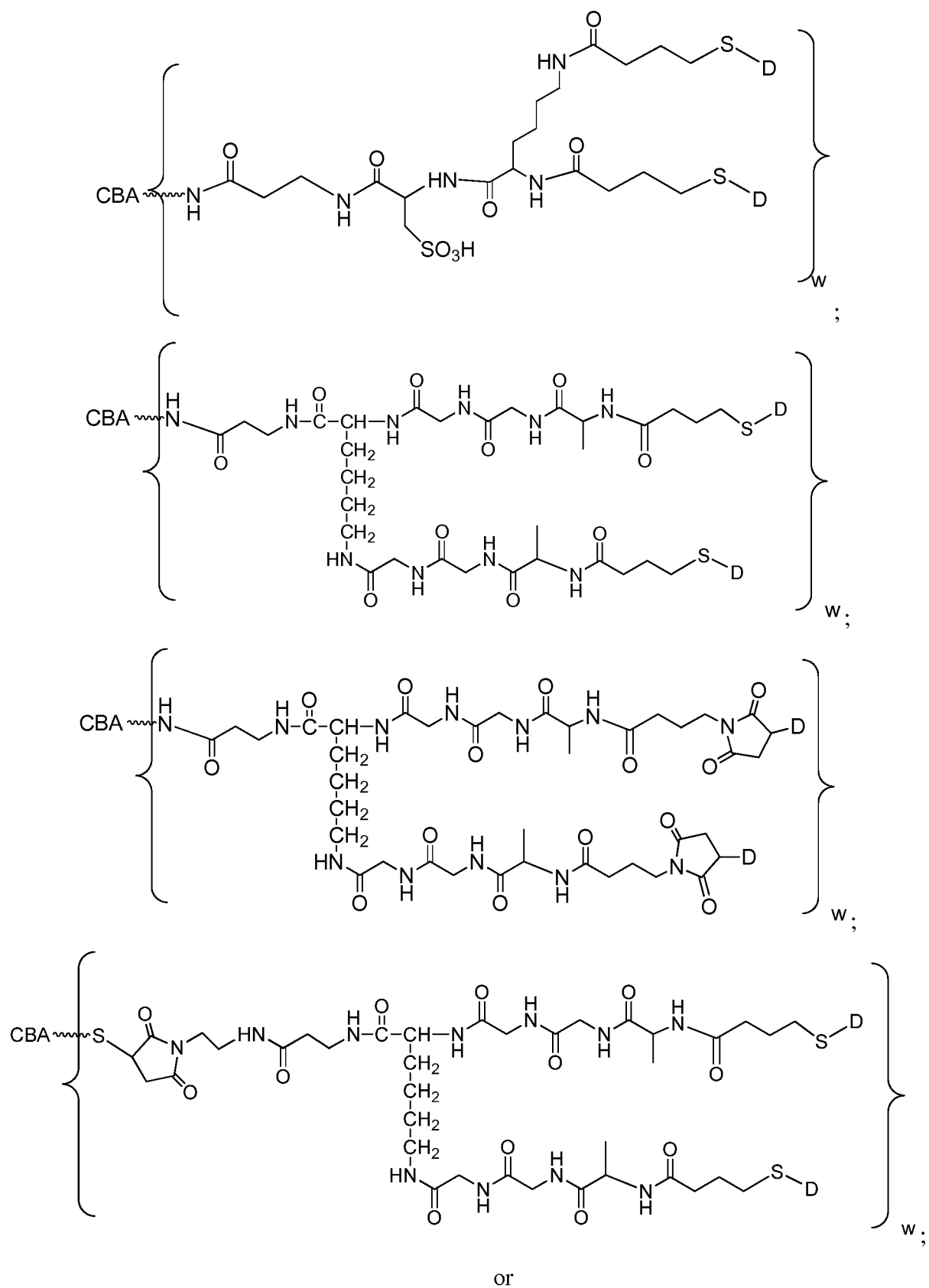
11. The conjugate of claim 9 or 10, wherein Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion.
12. The conjugate of any one of claims 9-11, wherein R_{12} is H; and Q is H, SO_3H or a pharmaceutically acceptable salt thereof.
13. The conjugate of any one of claims 8 -12, wherein $\text{R}^{\text{b}'}$ and $\text{R}^{\text{c}'}$ are both H; and $p2'$ is an integer from 1 to 5.
14. The conjugate of any one of claim 9-13, wherein R_{13} and R_{14} are both H; and r is 1 to 5.
15. The conjugate of any one of claims 8-14, wherein $[\text{XX}]_{1-10}$ is $[\text{XX}]_{2-5}$.
16. The conjugate of any one of claims 8-15, wherein $[\text{XX}]$, for each occurrence, is the residue of an amino acid independently selected from the group consisting of: histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-proline, N-methyl-serine, N-methyl-tyrosine, hydroxyproline, γ -carboxyglutamate, selenocysteine, O-phosphoserine, homoserine, norleucine, methionine sulfoxide,

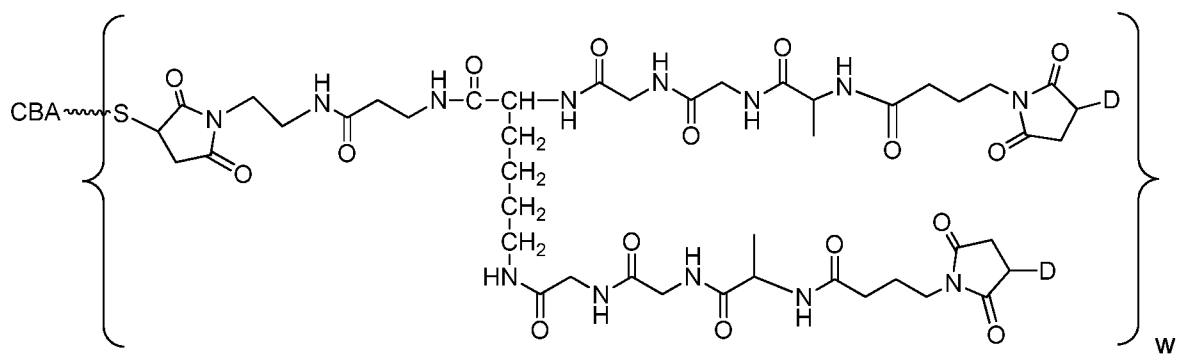
- methionine methyl sulfonium, citrulline, ornithine, cysteine sulfonic acid, cysteine sulfinic acid, 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, and β -alanine, each independently as an L or D isomer.
17. The conjugate of any one of claims 8-16, wherein [XX]₁₋₁₀ is selected from the group consisting of: Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Lle-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu, β -Ala-Leu-Ala-Leu, Gly-Phe-Leu-Gly, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, and Ala-Val-Ala.
 18. The conjugate of any one of claims 8-16, wherein [XX]₁₋₁₀ is Gly-Gly-Gly, Gly-Gly-Ala, Val-Ala, Glu-Ala, or Glu(OMe)-Ala.
 19. The conjugate of claims 1-18, wherein the CBA is bound to L₁ by a residue of a thiol group from a cysteine on the CBA, a residue of a amino group from a lysine on the CBA, or a residue of a carboxylic group from glutamic acid or Aspartic acid on the CBA.
 20. The conjugate of claim 19, wherein the CBA is bounded to L₁ by a residue of a thiol group from a cysteine on the CBA.
 21. The conjugate of claim 1, wherein the conjugate is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:



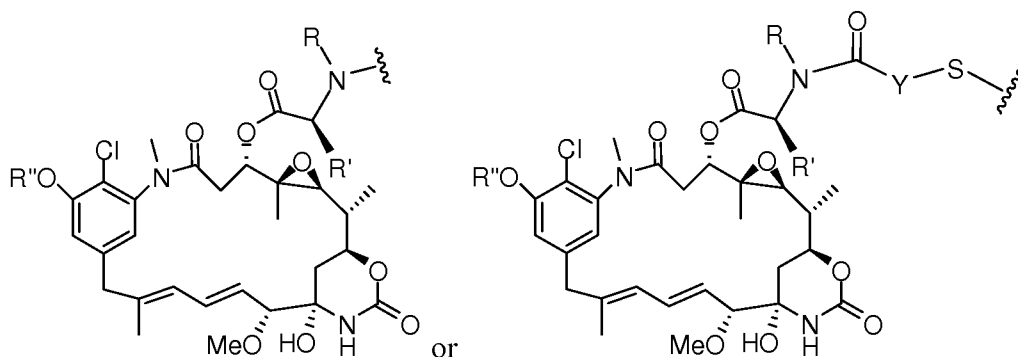








22. The conjugate of any one of claims 1-21, wherein D is a maytansinoid.
23. The conjugate of any one of claims 1-21, wherein D is represented by:

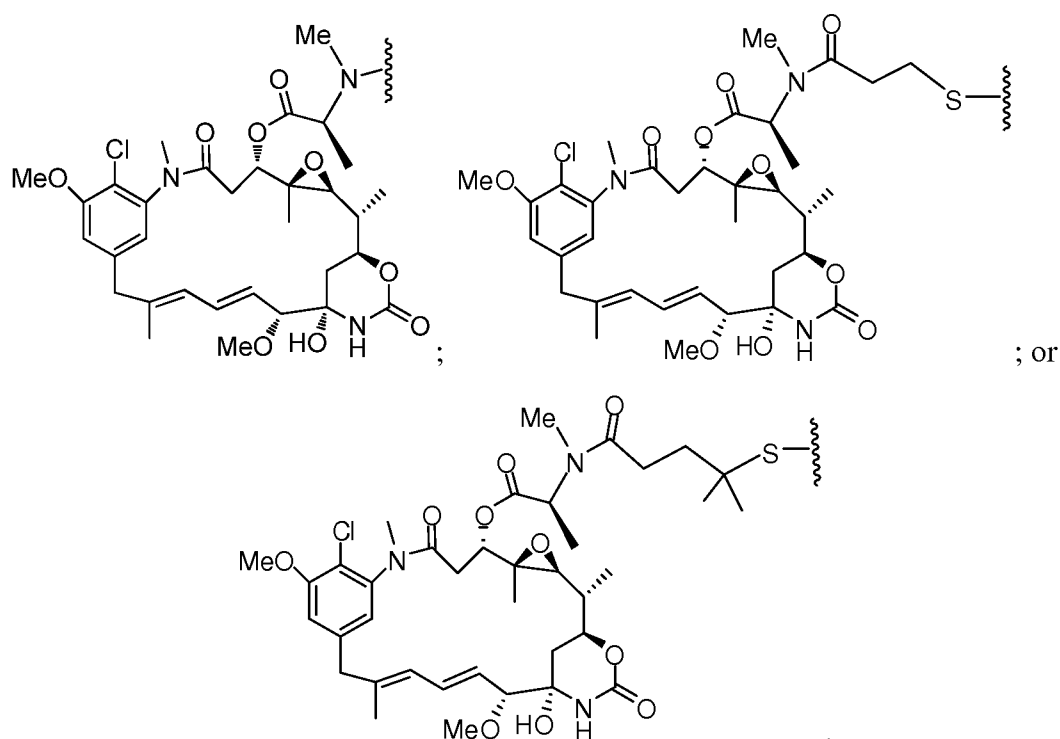


wherein:

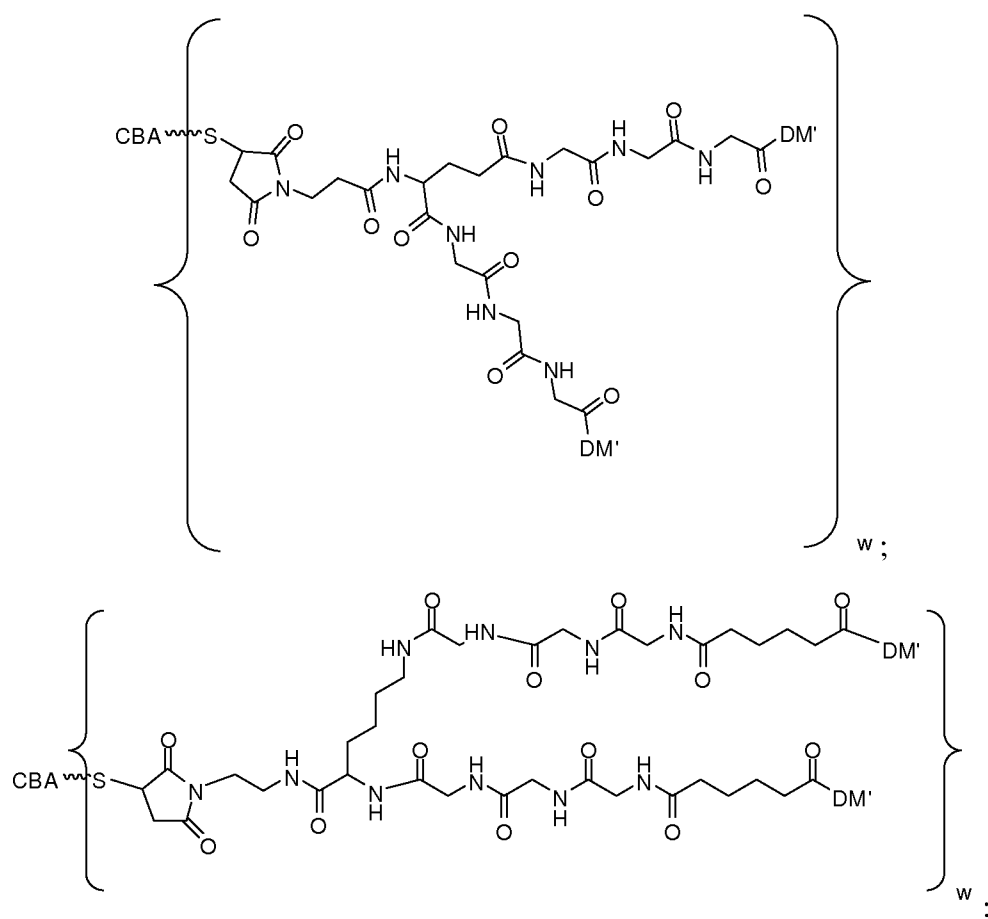
R, R' and R'', for each occurrence, are independently H or an optionally substituted alkyl; and

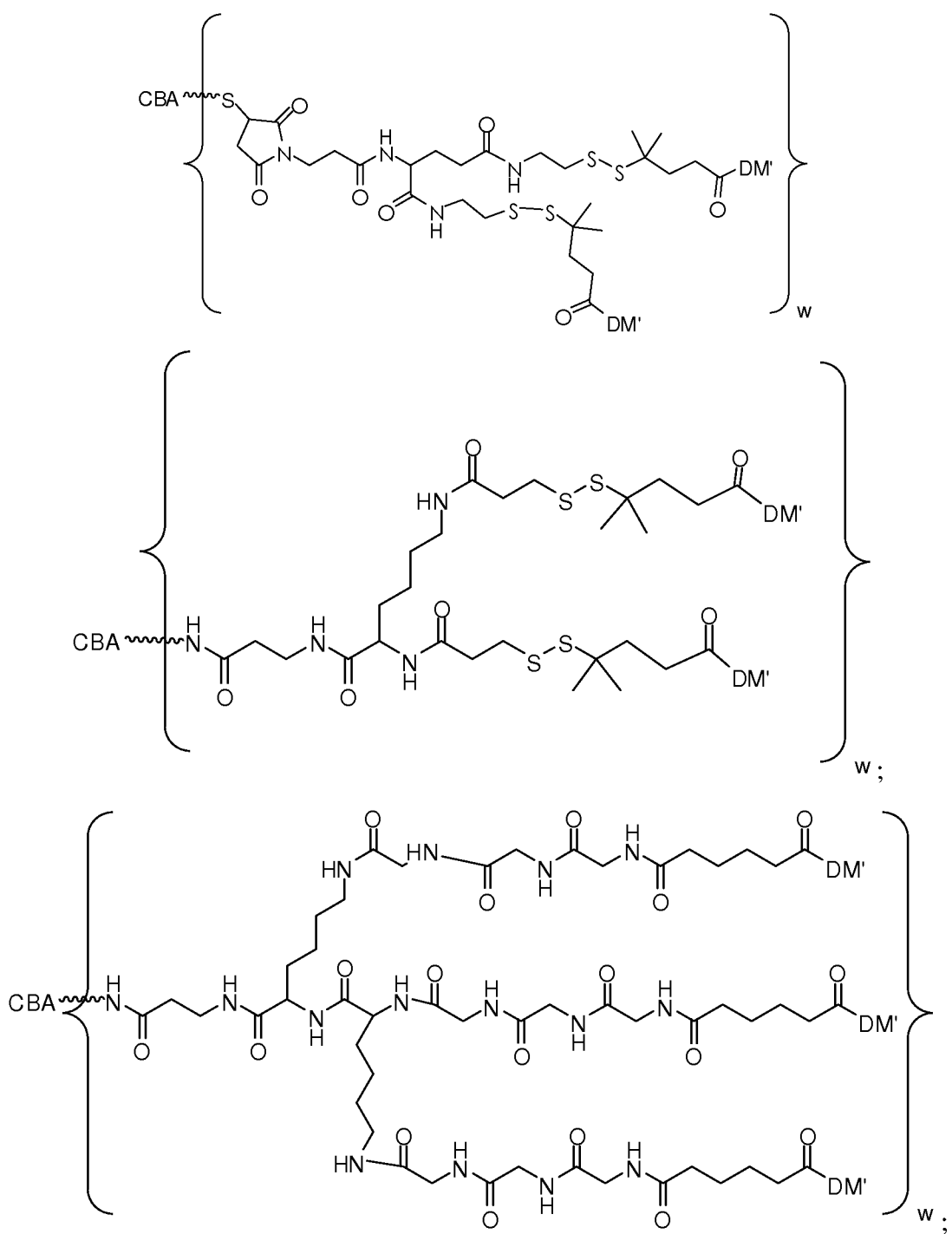
Y is an optionally substituted alkylene, an optionally substituted alkenylene, or an optionally substituted alkynylene.

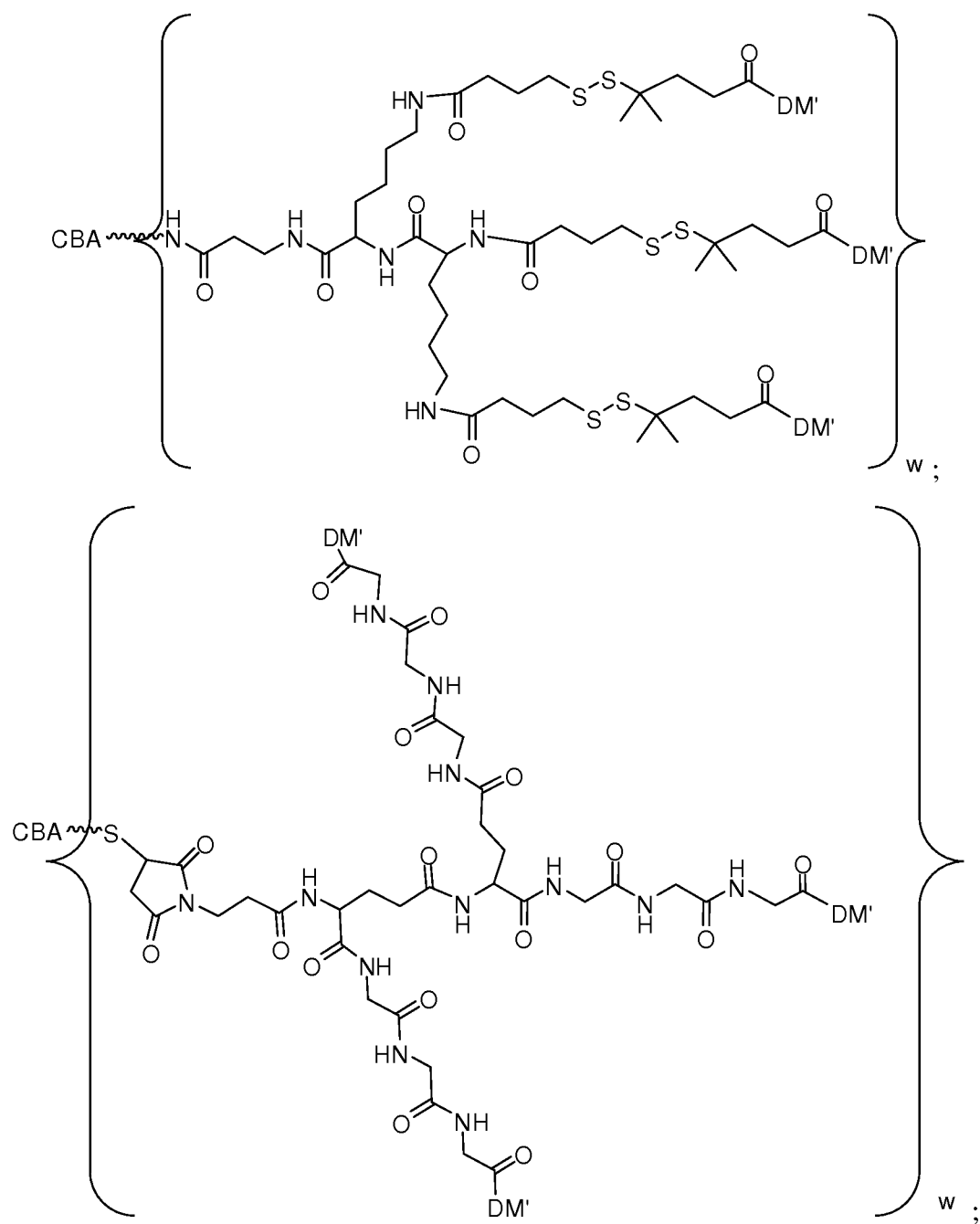
24. The conjugate of claim 23, wherein Y is represented by $-(CR_{15}R_{16})_rCR_{17}R_{18}-$, wherein R_{15} to R_{18} , for each occurrence, are independently H or an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted cycloalkyl, an optionally substituted heterocyclyl, an optionally substituted aryl or an optionally substituted heteroaryl; and r is an integer between 0 to 15.
25. The conjugate of claim 24, wherein R_{15} to R_{18} , for each occurrence, are independently H or an optionally substituted alkyl.
26. The conjugate of claim 25, wherein R_{15} to R_{18} are all H and r is 1.
27. The conjugate of claim 25, wherein R_{15} and R_{16} are H, r is 2, and R_{17} and R_{18} are both methyl.
28. The conjugate of any one of claims 1-27, wherein D is represented by the following formula:

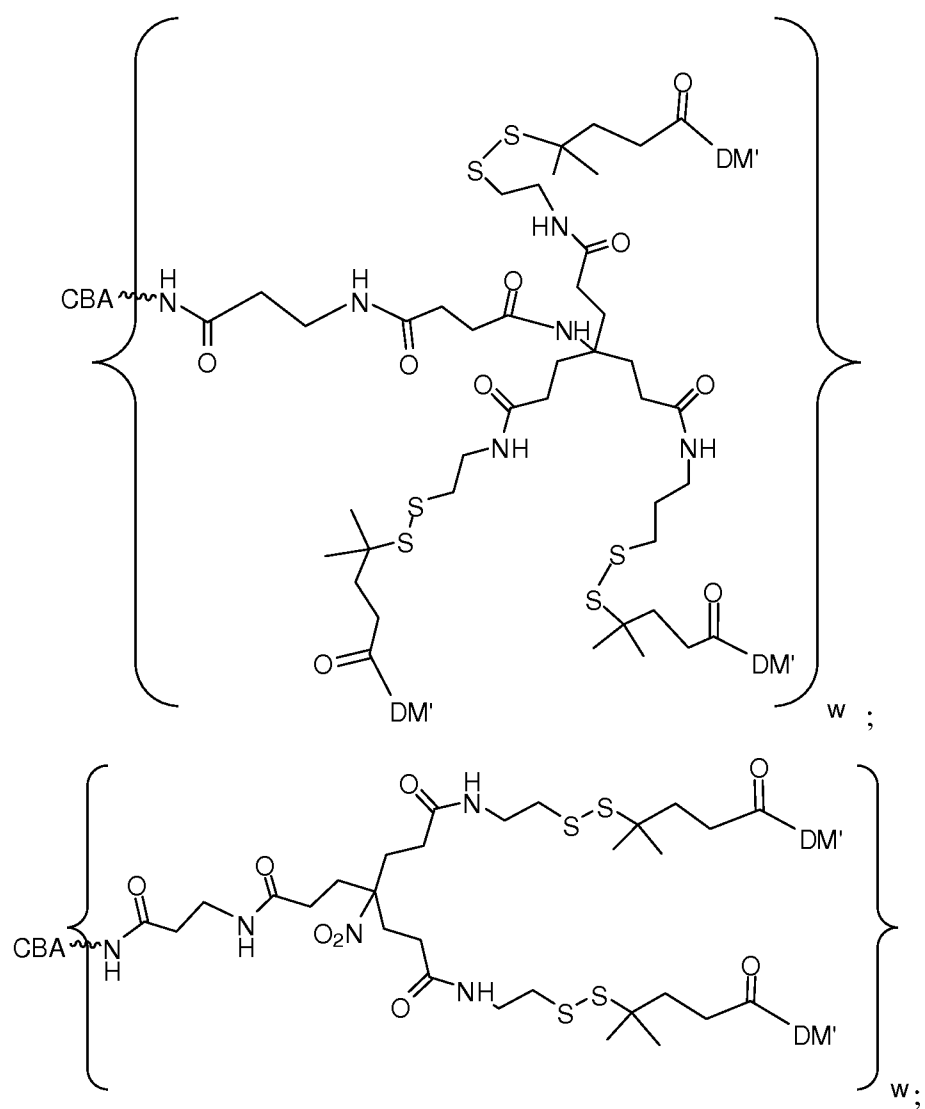


29. The conjugate of claim 1, wherein the conjugate is represented by the following formula, or a pharmaceutically acceptable salt thereof:

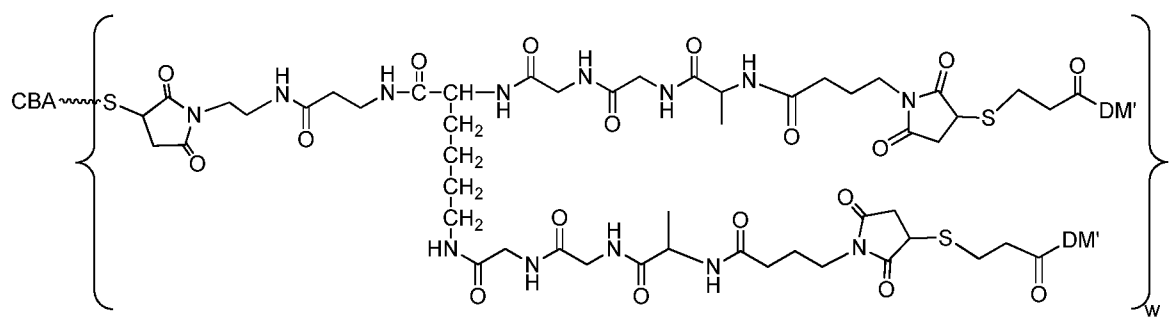
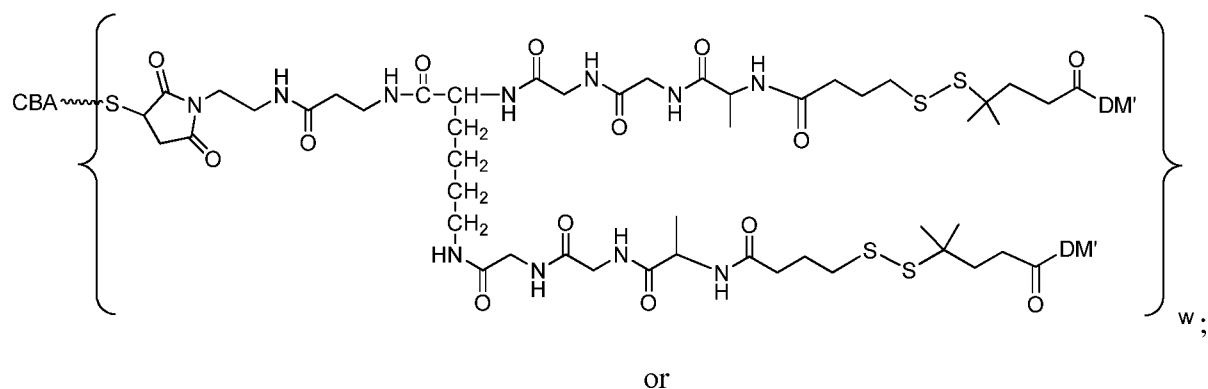




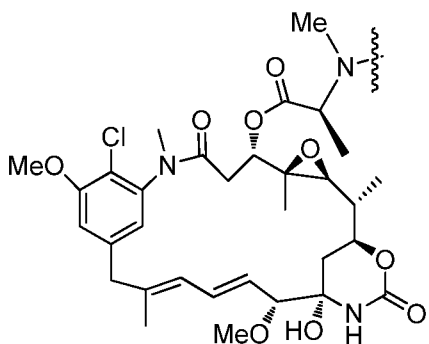






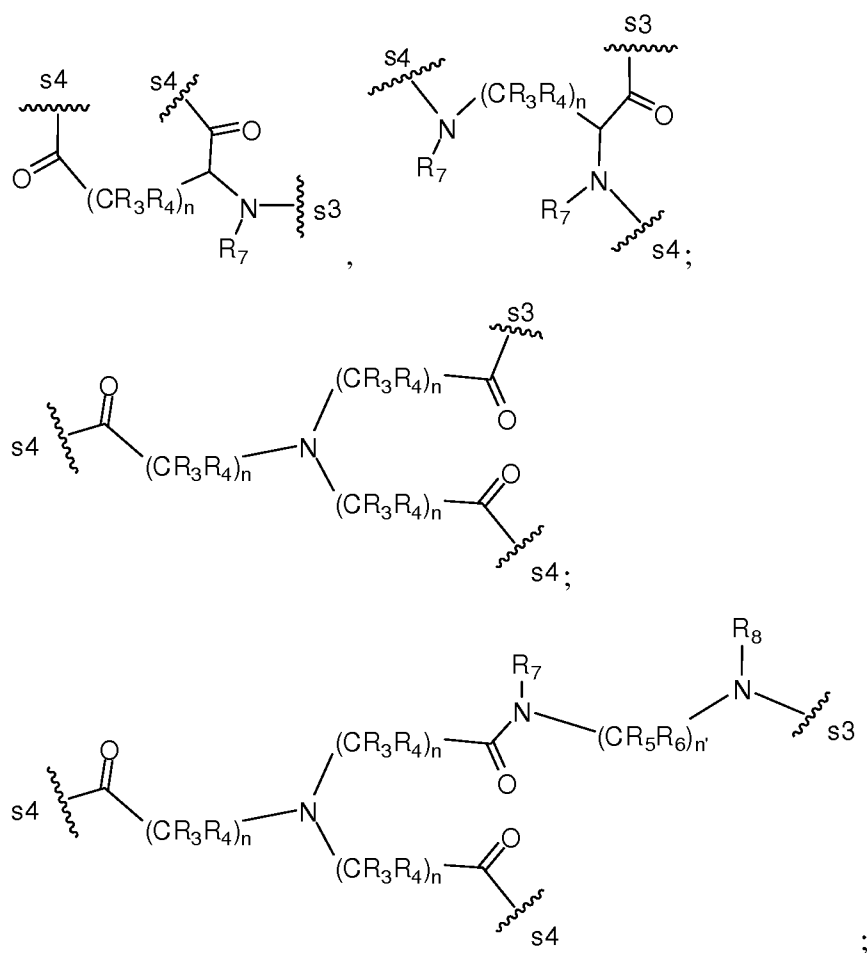


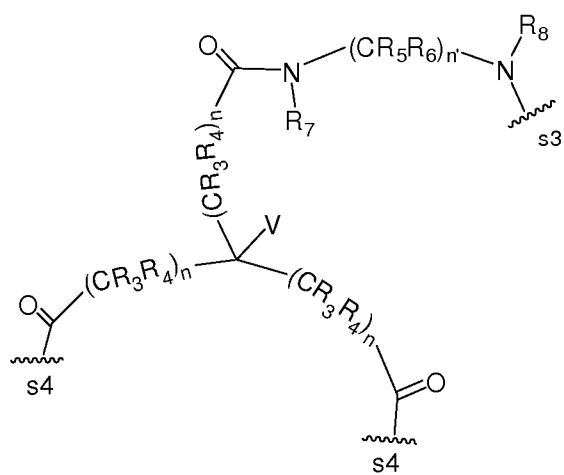
wherein DM' is represented by the following formula:



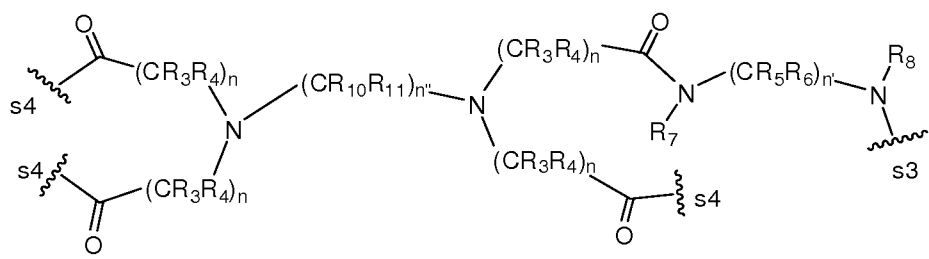
30. The conjugate of any one of claims 1-29, wherein the cell-binding agent is an antibody, a single chain antibody, an antibody fragment that specifically binds to the target cell, a monoclonal antibody, a single chain monoclonal antibody, a monoclonal antibody fragment that specifically binds to a target cell, a chimeric antibody, a chimeric antibody fragment that specifically binds to the target cell, a domain antibody, a domain antibody fragment that specifically binds to the target cell, a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, or a nutrient-transport molecule.
31. The conjugate of claim 30, wherein the cell-binding agent is a monoclonal antibody, a single chain monoclonal antibody, or a monoclonal antibody fragment that specifically binds to a target cell.

32. The conjugate of 30 or 31, wherein the antibody is a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment.
33. The conjugate of any one of claims 30, wherein the antibody is a humanized antibody, a humanized single chain antibody, or a humanized antibody fragment.
34. The conjugate of any one of claims 1-29, wherein the cell-binding agent is a minibody, a diabody, a tribody, a tetrabody, a nanobody, a probody, a domain body, a unibody, a bispecific antibody, an ankyrin repeat protein (*e.g.*, a DARPin), a Centyrin, or an Avibody.
35. The conjugate of any one of claims 1-9, 11-20, 22-28, and 30-34, wherein U is represented by the following formula:

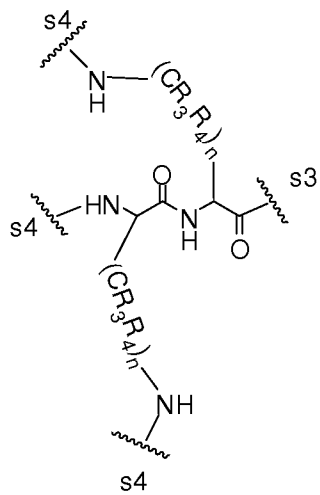




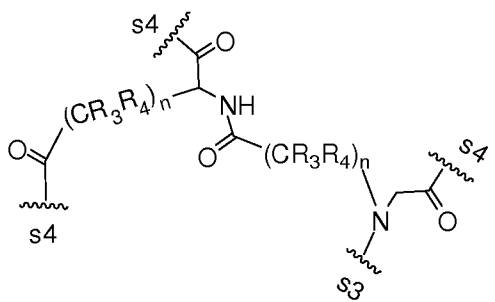
;



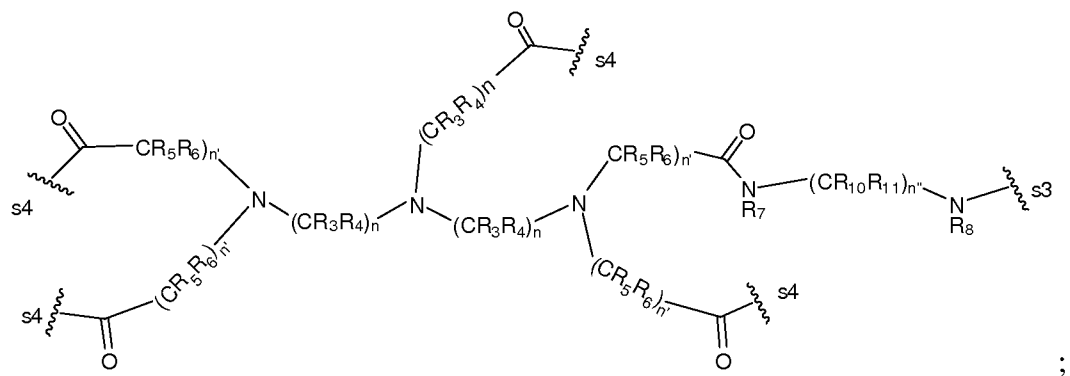
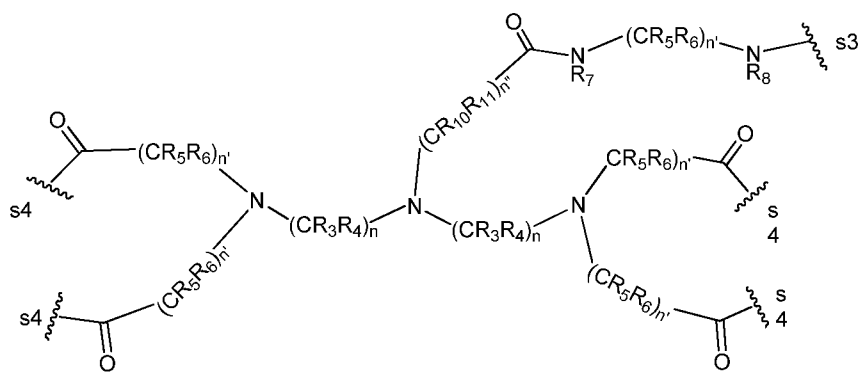
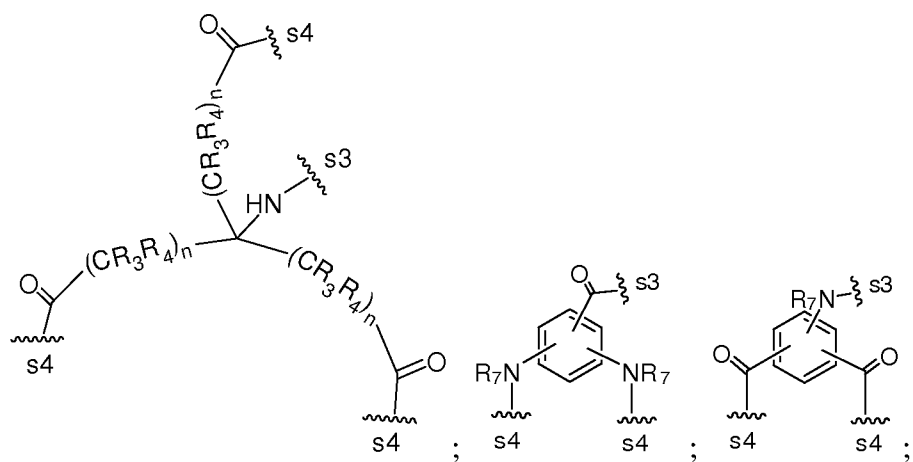
;

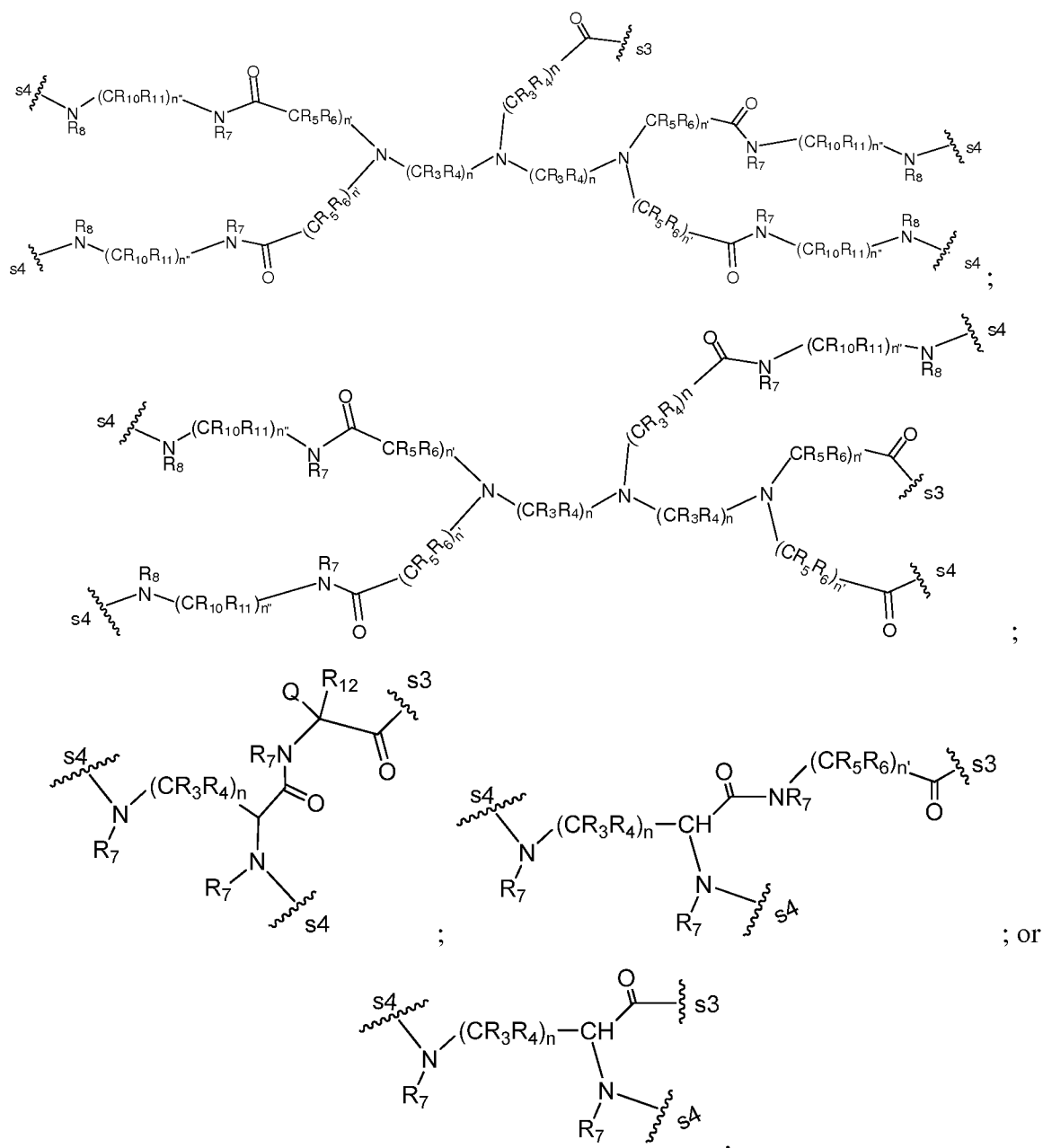


;



;





wherein:

R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} , for each occurrence, are independently H or an optionally substituted alkyl;

n , n' , and n'' are independently an integer from 1 to 10;

V is H, an optionally substituted alkyl, NO_2 , or $-\text{NH}-\text{C}(=\text{O})-\text{R}^{501}$;

R^{501} is H or an optionally substituted alkyl;

R_{12} is H, an optionally substituted alkyl;

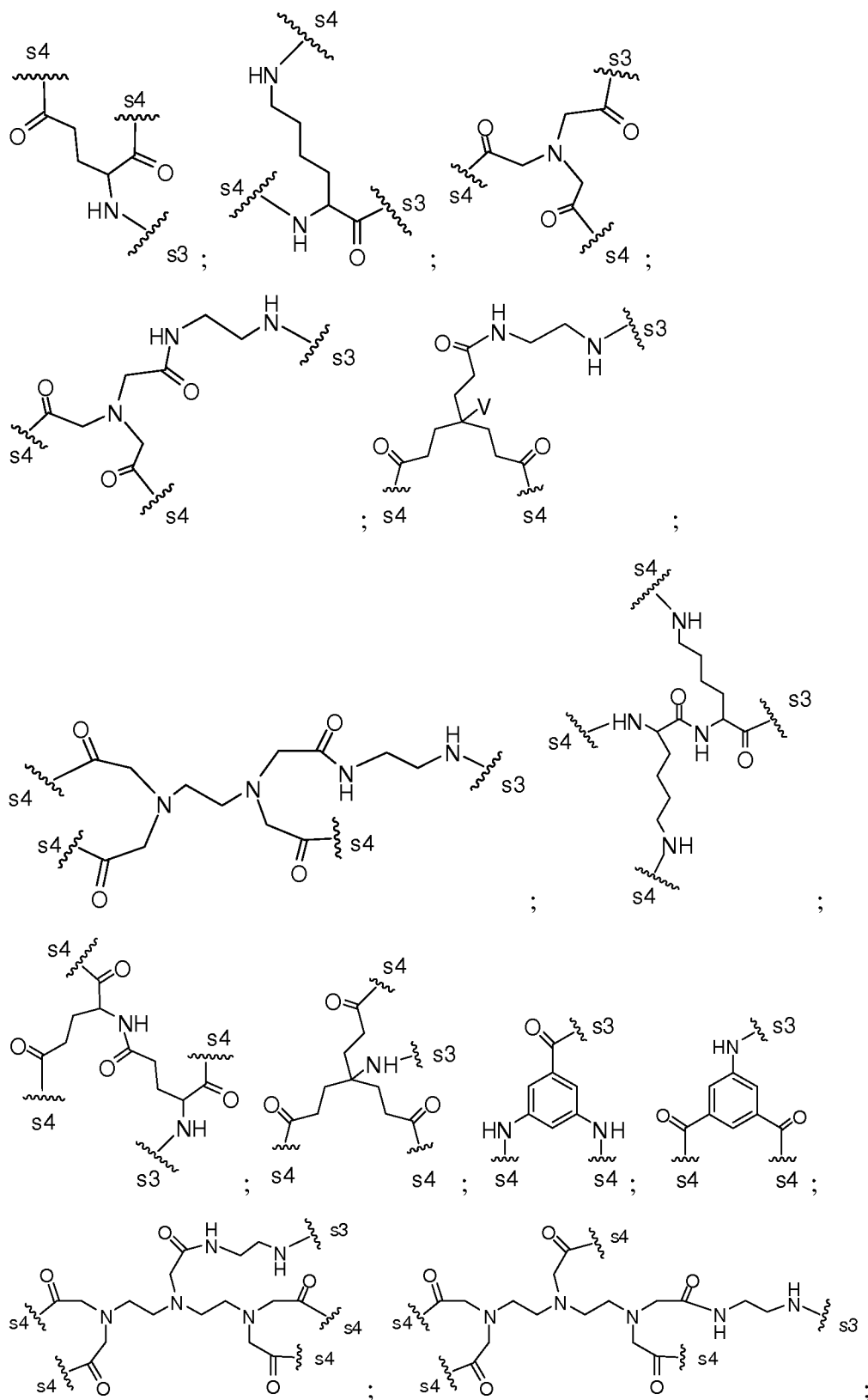
Q is H, a charged substituent or an ionizable group;

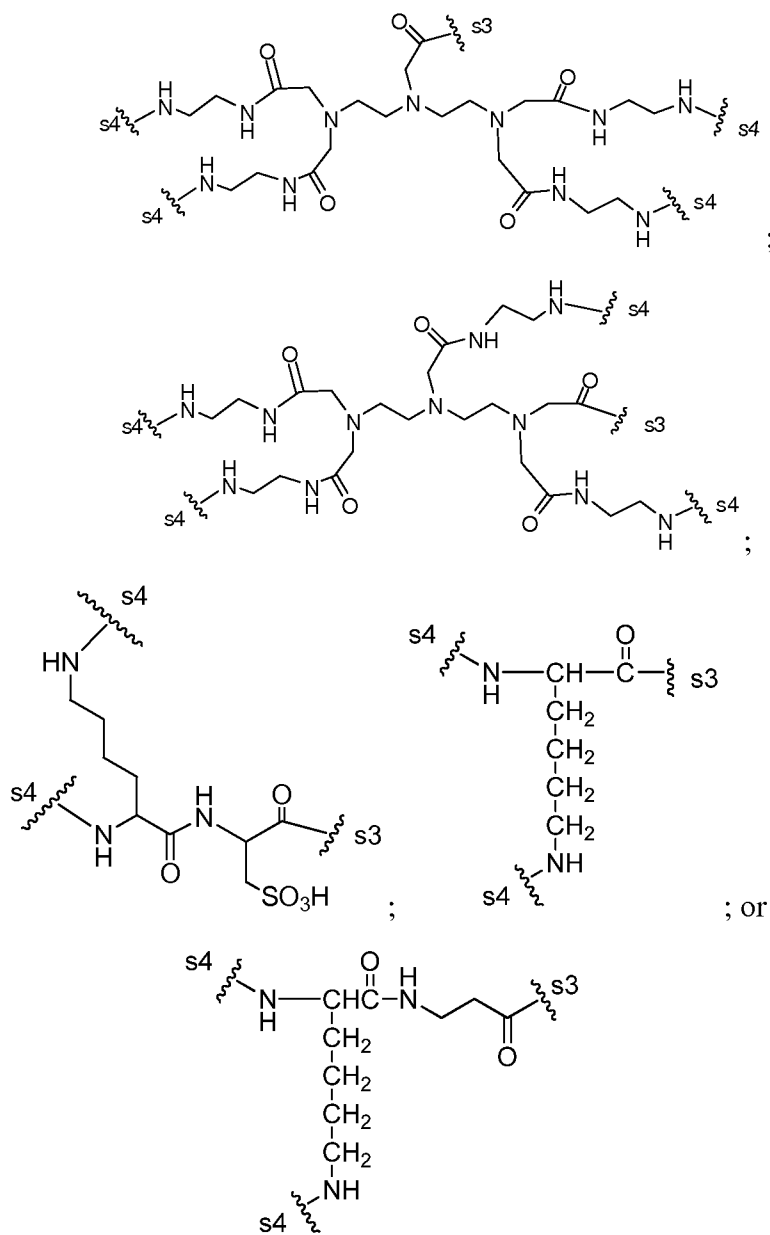
S_3 is the site covalently linked the group L_1 ; and

S_4 is the site covalently linked to the group L_2 .

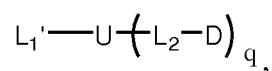
36. The conjugate of claim 35, wherein R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} are all H.

37. The conjugate of claim 35 or 36, wherein n, n', and n'' are independently an integer from 1 to 5.
38. The conjugate of claim 35, wherein U is represented by the following formula:





39. The conjugate of any one of claims 35-38, wherein V is H or NO₂.
40. A cytotoxic compound represented by the following formula, or a salt thereof:



wherein:

L₁' is a spacer attached to a reactive functional group that can form a covalent bond with a cell-binding agent;

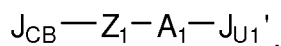
U is a branched scaffold;

L₂, for each occurrence, is independently a spacer

D, for each occurrence, is independently a cytotoxic drug moiety;

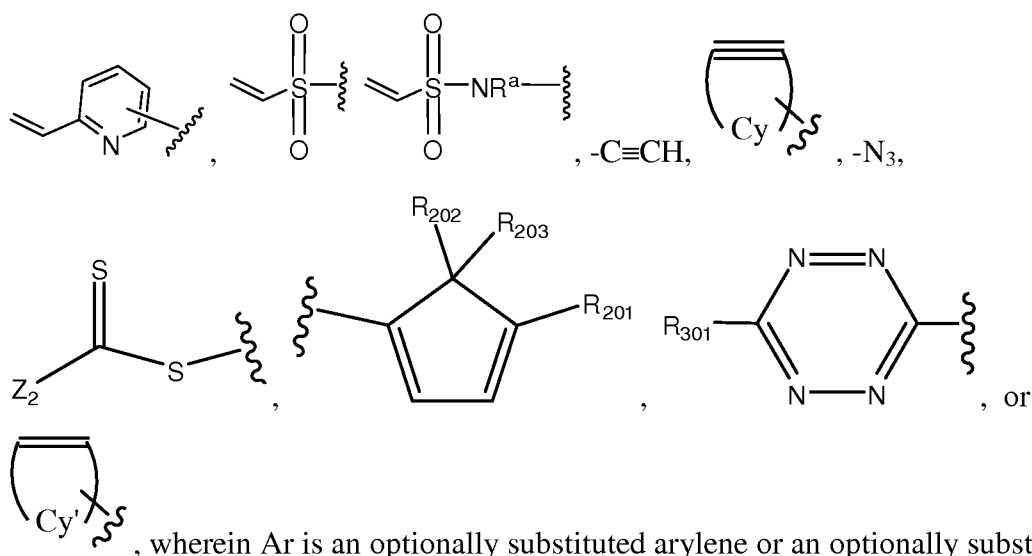
q is an integer from 2 to 5.

41. The cytotoxic compound of claim 40, wherein L_1' is represented by the following formula:



wherein:

J_{CB} is $-COX''$, maleimide, $-SZ$, $X'-CR^bR^c-C(=O)-$, $X'-CR^bR^c-C(=O)-NR^e-$, $R^a-C(=O)-$, $R^a-C(=O)-Ar-$, NH_2-NR^e- , $NH_2-NR^e-C(=O)-$, NH_2-NR^e-Ar- , NH_2-O- ,



heteroarylene, Cy is an optionally substituted cycloalkyne or an optionally substituted heterocycloalkyne, R_{201} , R_{202} and R_{203} each are independently H or an

optionally substituted alkyl; Z_2 is pyridyl or $R_{204}O-P(=O)(R_{205}O)-$, wherein R_{204} and R_{205}

are each independently optionally substituted alkyl, and Cy' is an optionally substituted strained cycloalkene or an optionally substituted strained heterocycloalkene;

X' is a halogen;

X'' is $-OH$ or a carboxylic acid activating group;

Z is H or $-SR^d$;

R^a , R^b , R^c and R^e , for each occurrence, are independently H or an optionally substituted alkyl;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

Z_1 is $-\text{NR}_9-\text{C}(=\text{O})-$ or $-\text{C}(=\text{O})-\text{NR}_9-$ or absent;

A_1 is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, $-(\text{CH}_2-\text{CH}_2-\text{O})_{p1}-(\text{CR}^b\text{R}^c)_{p1'}$ or $-(\text{CR}^b\text{R}^c)_{p1'}-(\text{O}-\text{CH}_2-\text{CH}_2)_{p1}-$;

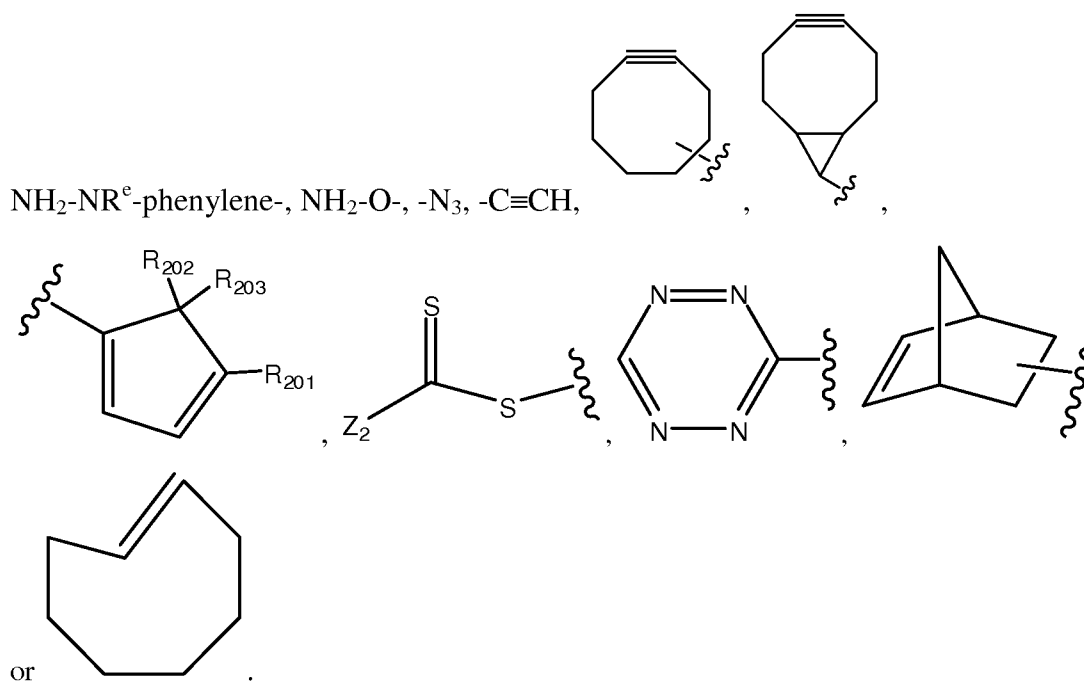
$J_{U1'}$ is absent, $-\text{NR}_9-$ or $-\text{C}(=\text{O})-$;

$p1$ is an integer from 1 to 1000;

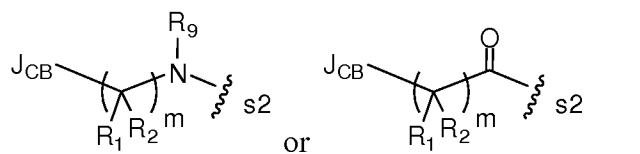
$p1'$ is an integer from 0 to 10; and

R_9 is H or an optionally substituted alkyl.

42. The cytotoxic compound of claim 41, wherein J_{CB} is $-\text{COX}''$, maleimide, $-\text{SZ}$, $X'-\text{CR}^b\text{R}^c-\text{C}(=\text{O})-$, $X'-\text{CR}^b\text{R}^c-\text{C}(=\text{O})-\text{NR}^e-$, $R^a-\text{C}(=\text{O})-$, $R^a-\text{C}(=\text{O})$ -phenylene-, NH_2-NR^e- ,



43. The cytotoxic compound of any one of claims 40-42, wherein L_1' is represented by the following formula:



wherein R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10.

44. The cytotoxic compound of claim 43, wherein:

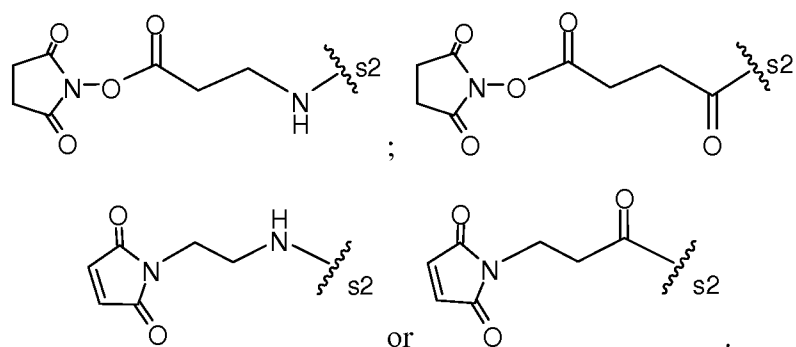
J_{CB} is $-\text{COX}''$ or a maleimide;

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

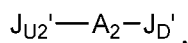
m is an integer from 1 to 5; and

R⁹ is H or an alkyl.

45. The cytotoxic compound of claim 43 or 44, wherein R₁ and R₂ are both H; and R⁹ is H.
46. The cytotoxic compound of claim 45, wherein L₁' is represented by the following formula:



47. The cytotoxic compound of any one of claims 40-46, wherein L₂ is represented by the following formula:

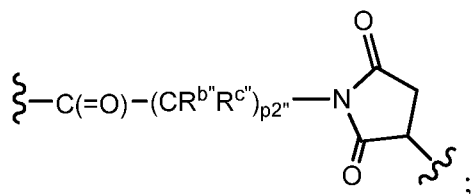


wherein:

J_{U2}' is -C(=O)-, -NR₉- or absent;

A₂ is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or [XX]₁₋₁₀;

J_D' is -C(=O)-, -C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-, -C(=O)-(CH₂-CH₂-O)_{p2}-(CR^{b'}R^{c'})_{p2'}-S-, -S-, absent, -C(=O)-(CR^{b''}R^{c''})_{p2''}-S- or



[XX], for each occurrence, is independently an amino acid residue;

R^{b'} and R^{c'}, for each occurrence, are independently H or an optionally substituted alkyl;

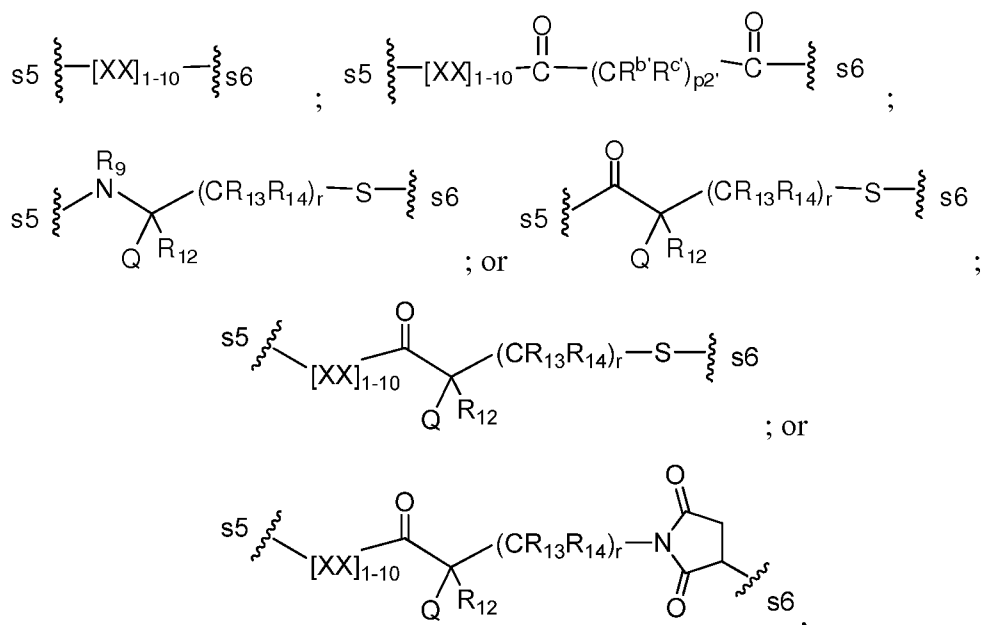
R^{b''} and R^{c''}, for each occurrence, are independently H, an optionally substituted alkyl or a charged substituent or an ionizable group;

p₂ is an integer from 1 to 1000;

p₂' is an integer from 1 to 10;

p₂'' is an integer from 1 to 10 (specifically 1 to 5).

48. The conjugate of claim 47, wherein L₂ is represented by the following formula:



wherein:

R_{12} , R_{13} , and R_{14} , for each occurrence, are each independently H, an optionally substituted alkyl;

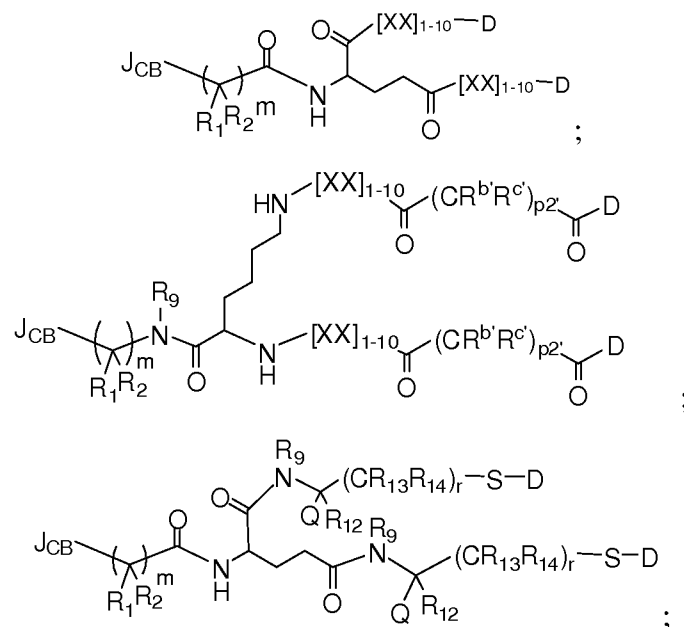
Q is H, a charged substituent or an ionizable group;

r is an integer from 0 to 10;

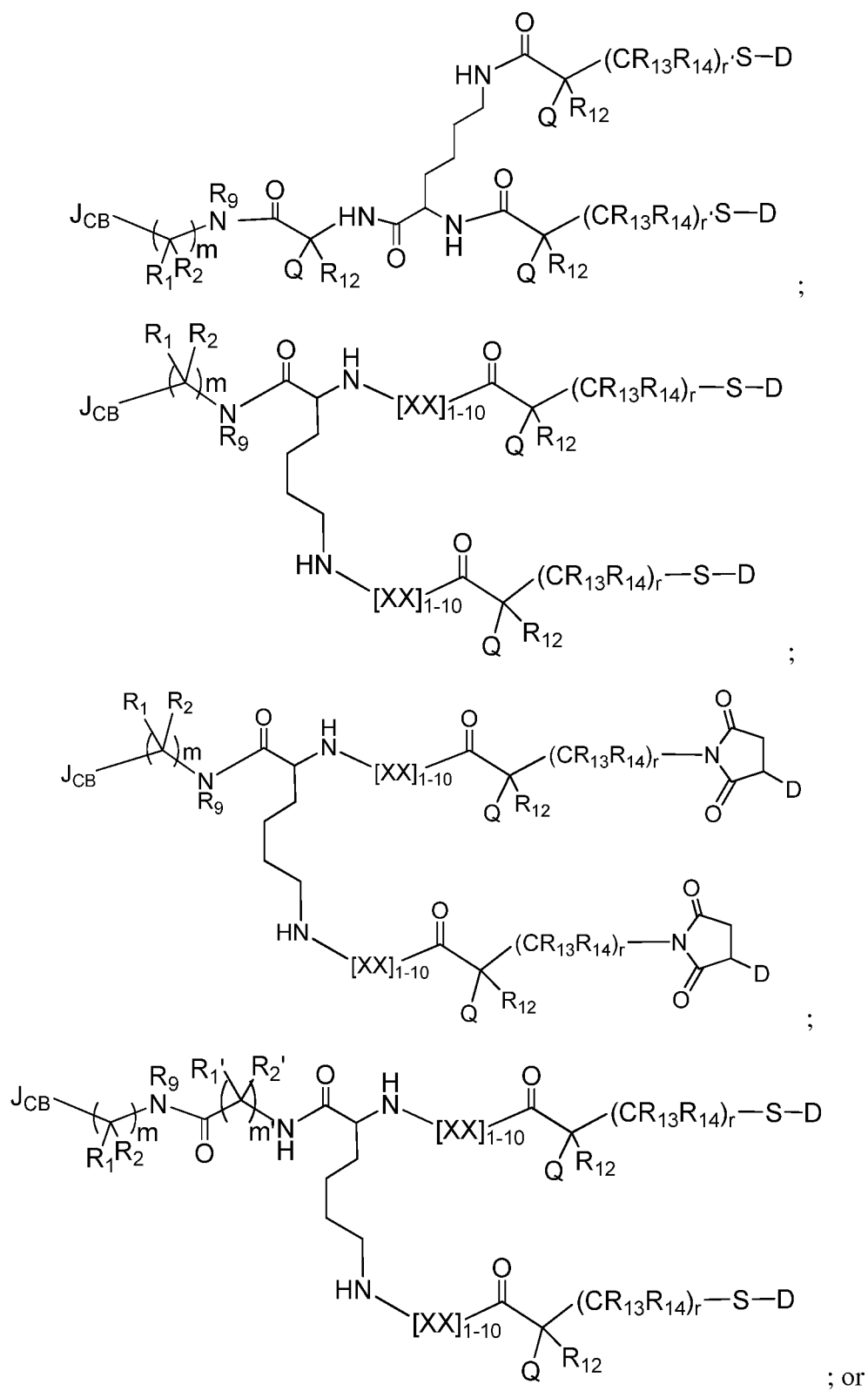
s5 is the site covalently linked to the group U; and

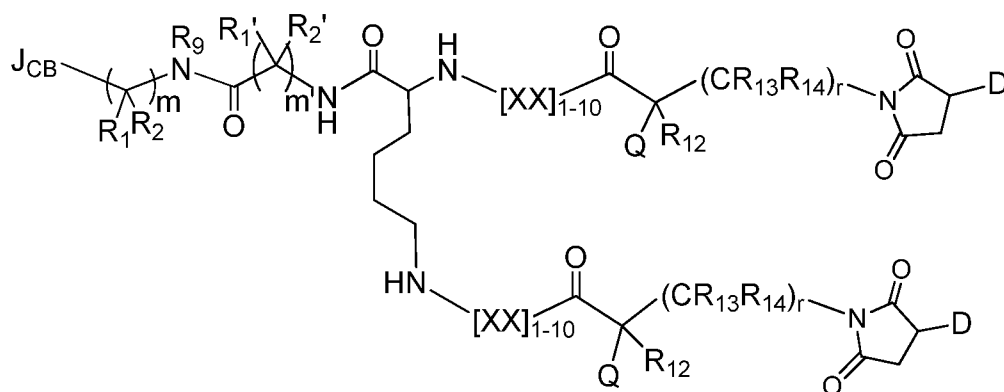
s6 is the site covalently linked to the group D.

49. The cytotoxic compound of claim 47 or 48, wherein the cytotoxic compound is represented by the following formula, or a salt thereof:



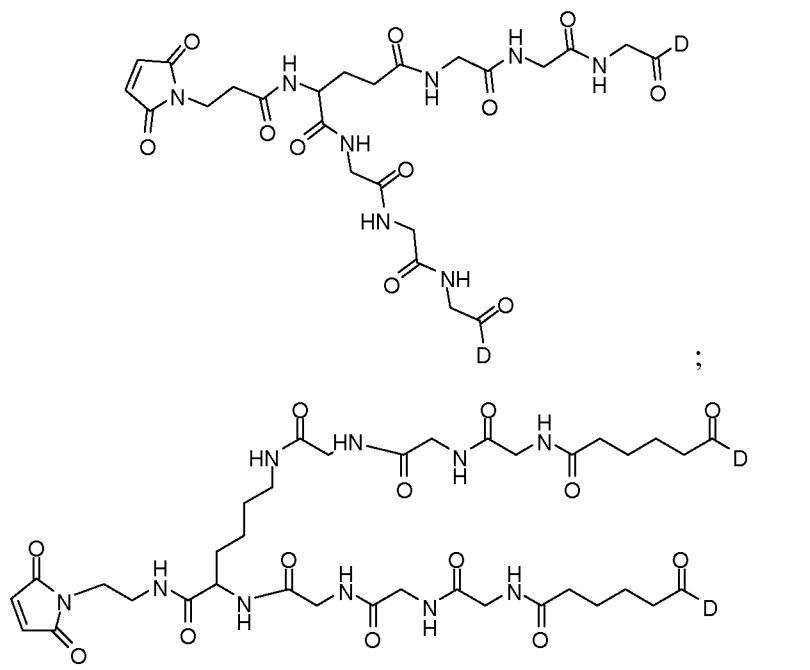


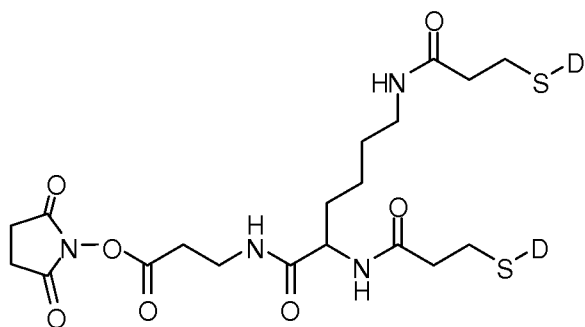
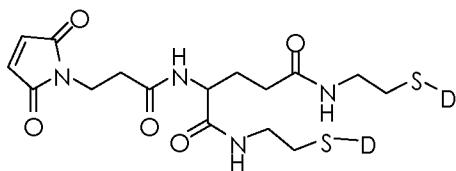




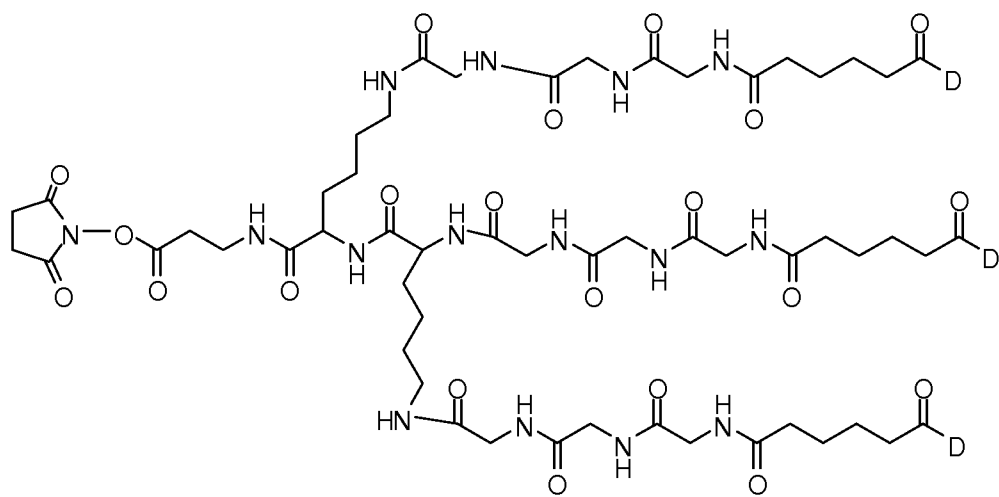
50. The cytotoxic compound of claim 48 or 49, wherein Q is i) H; ii) $-\text{SO}_3\text{H}$, $-\text{Z}'-\text{SO}_3^-$, $-\text{OPO}_3\text{H}_2$, $-\text{Z}'-\text{OPO}_3\text{H}_2$, $-\text{PO}_3\text{H}_2$, $-\text{Z}'-\text{PO}_3\text{H}_2$, $-\text{CO}_2\text{H}$, $-\text{Z}'-\text{CO}_2\text{H}$, $-\text{NR}_{101}\text{R}_{102}$, $-\text{Z}'-\text{NR}_{101}\text{R}_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$ or $-\text{Z}'-\text{N}^+\text{R}_{103}\text{R}_{104}\text{R}_{105}\text{X}^-$; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{101} and R_{102} , are each independently H or an optionally substituted alkyl; R_{103} to R_{105} are each independently an optionally substituted alkyl; and X^- is a pharmaceutically acceptable anion.
51. The cytotoxic compound of any one of claims 48-50, wherein R_{12} is H; and Q is H, SO_3H or a pharmaceutically acceptable salt thereof.
52. The cytotoxic compound of any one of claims 47-51, wherein $\text{R}^{b'}$ and $\text{R}^{c'}$ are both H; and $p2'$ is an integer from 1 to 5.
53. The cytotoxic compound of any one of claim 47-52, wherein R_{13} and R_{14} are both H; and r is 1 to 5.
54. The cytotoxic compound of any one of claims 47-53, wherein $[\text{XX}]_{1-10}$ is $[\text{XX}]_{2-5}$.
55. The cytotoxic compound of any one of claims 47-54, wherein $[\text{XX}]$, for each occurrence, is the residue of an amino acid independently selected from the group consisting of: histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-proline, N-methyl-serine, N-methyl-tyrosine, hydroxyproline, γ -carboxyglutamate, selenocysteine, O-phosphoserine, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, citrulline, ornithine, cysteine

- sulfonic acid, cysteine sulfinic acid, 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, and β -alanine, each independently as an L or D isomer.
56. The cytotoxic compound of any one of claims 47-54, wherein [XX]₁₋₁₀ is selected from the group consisting of: Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu, β -Ala-Leu-Ala-Leu, Gly-Phe-Leu-Gly, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, and Ala-Val-Ala.
57. The cytotoxic compound of claim 47-54, wherein [XX]₁₋₁₀ is Gly-Gly-Gly, Gly-Gly-Ala, Val-Ala, Glu-Ala, or Glu(OMe)-Ala.
58. The cytotoxic compound of claim 40, wherein the cytotoxic compound is represented by one of the following formulas, or a salt thereof:

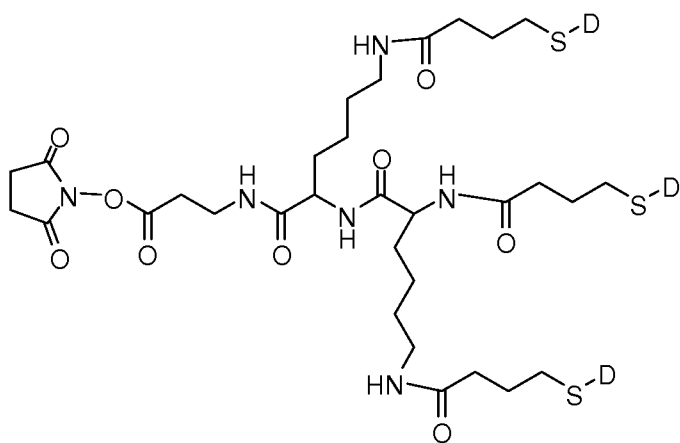




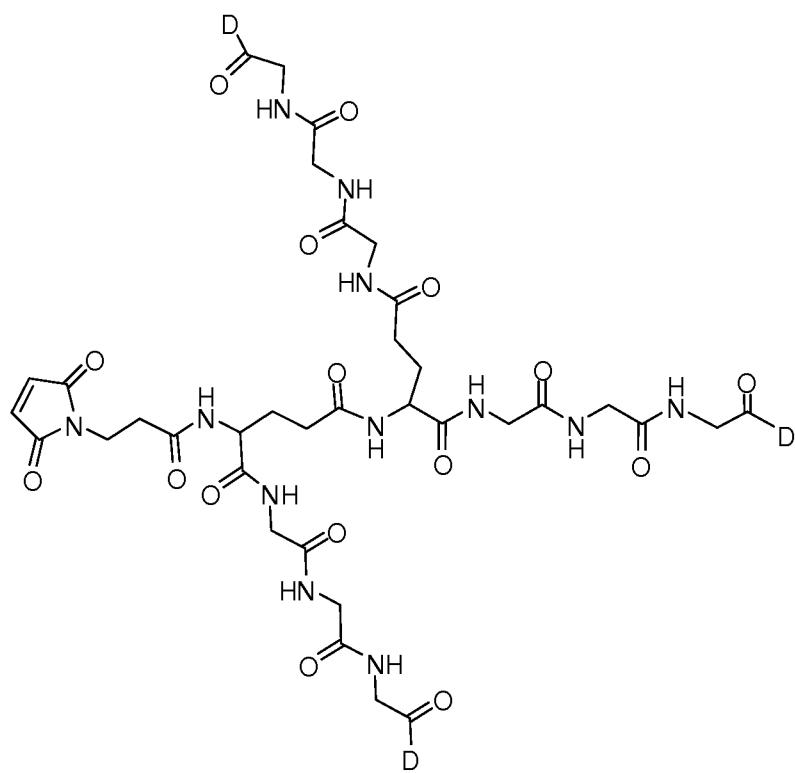
;



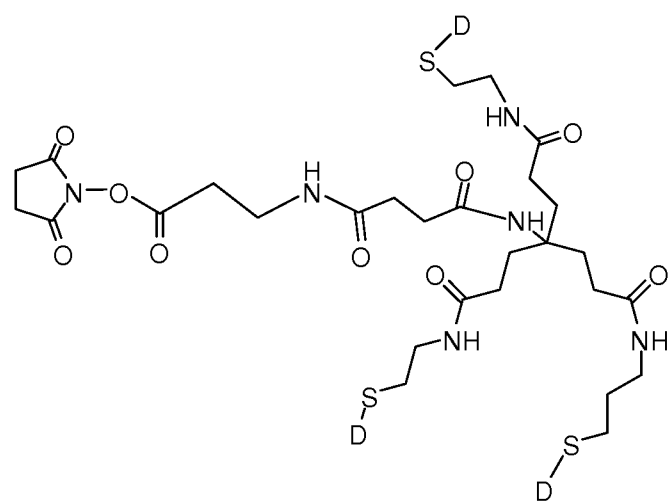
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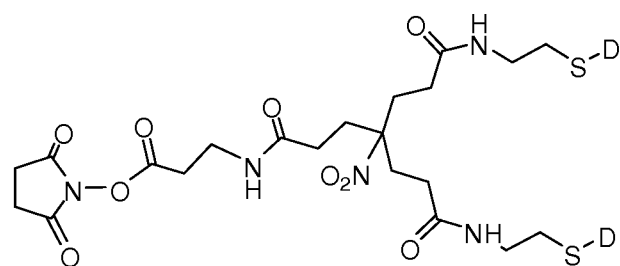
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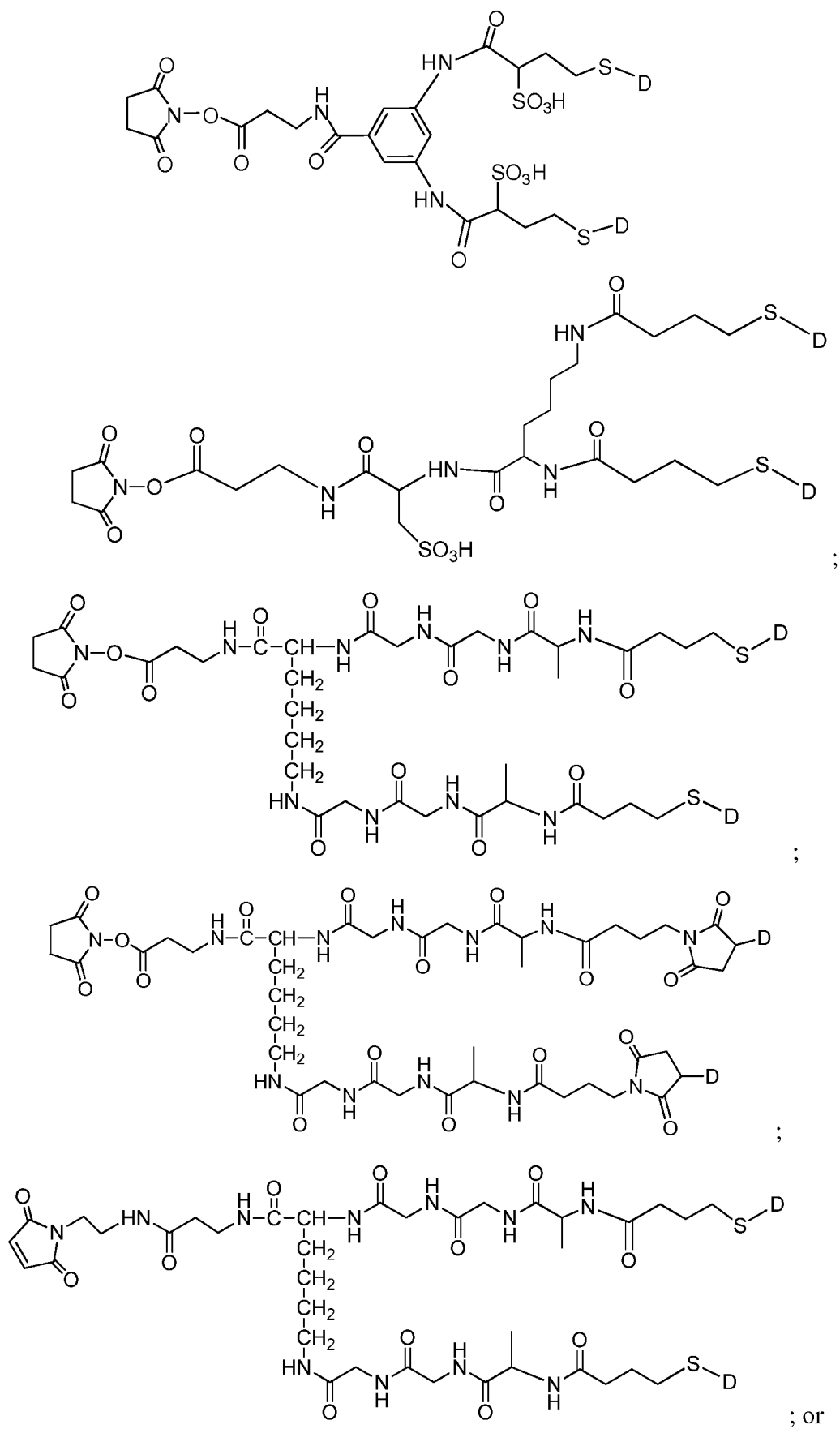
;

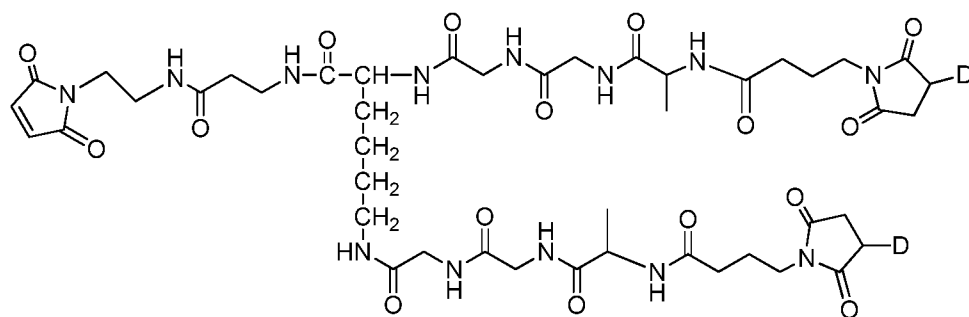


;

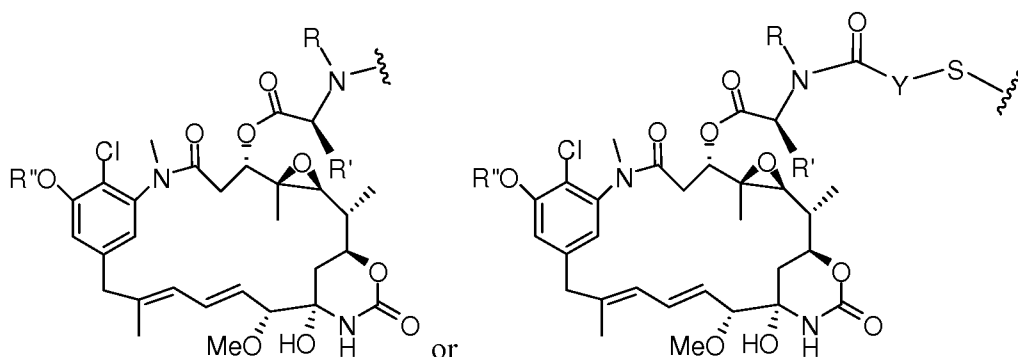


;





59. The cytotoxic compound of any one of claims 40-58, wherein D is a maytansinoid.
 60. The cytotoxic compound of any one of claims 40-59, wherein D is represented by:

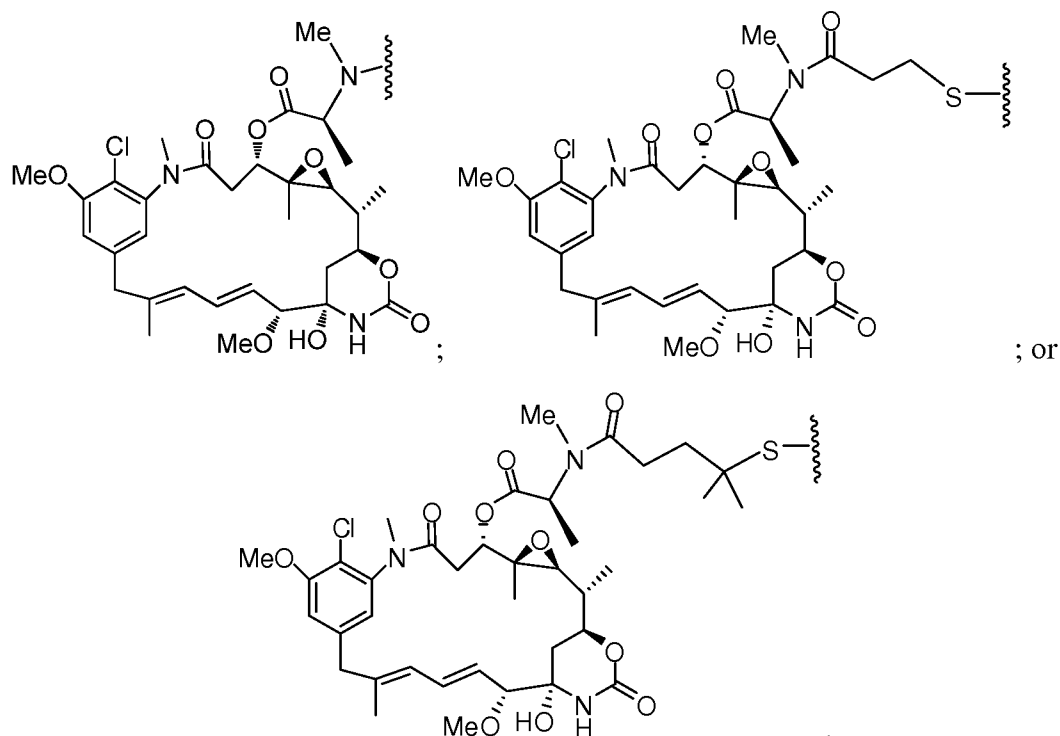


wherein:

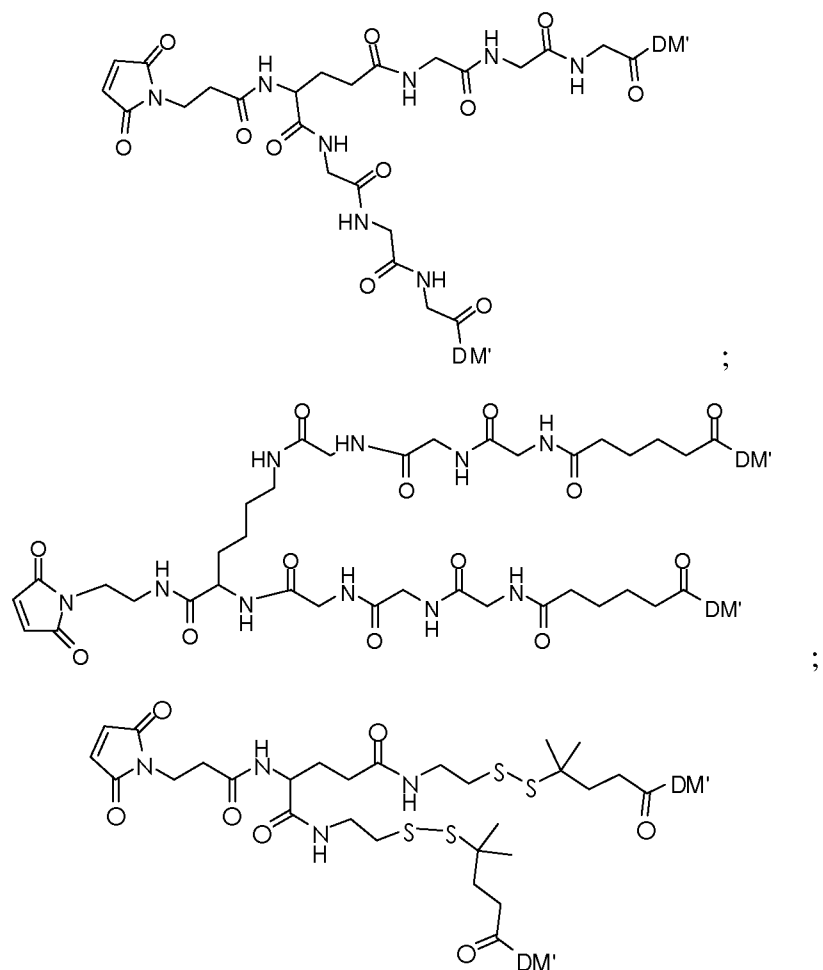
R, R' and R'', for each occurrence, are independently H or an optionally substituted alkyl; and

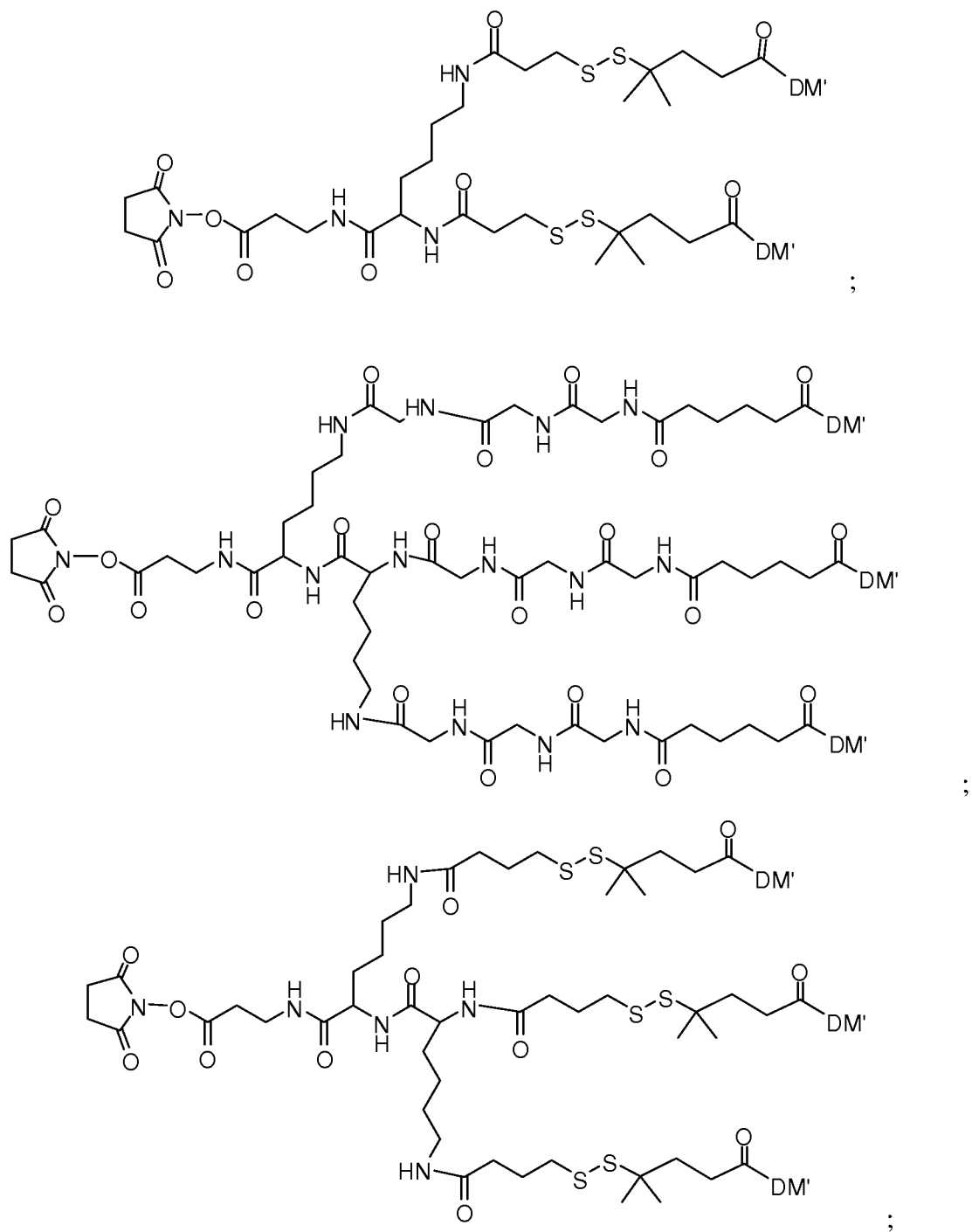
Y is an optionally substituted alkylene, an optionally substituted alkenylene, or an optionally substituted alkynylene.

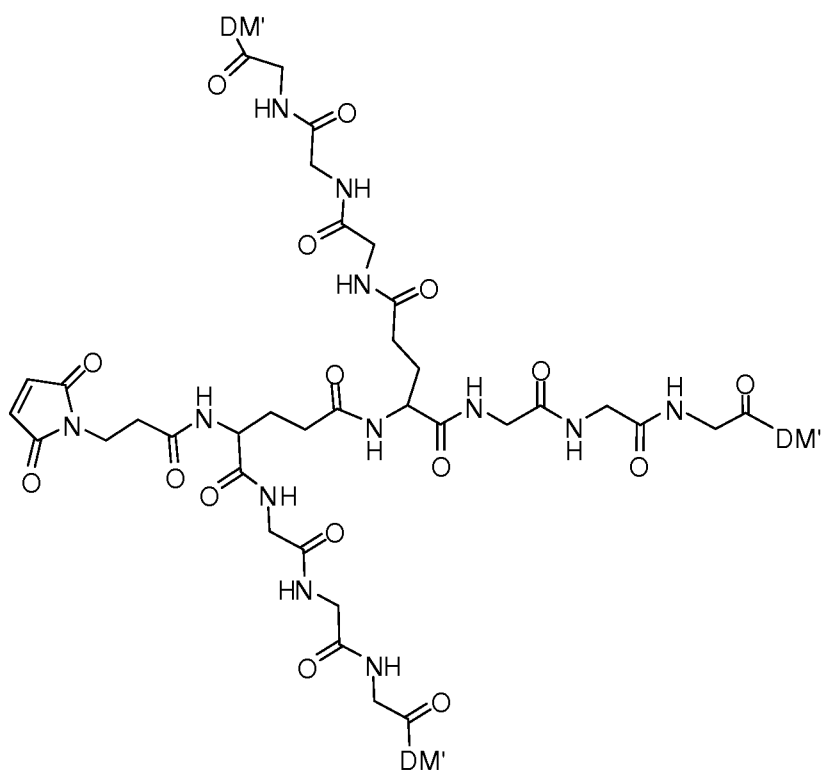
61. The cytotoxic compound of claim 60, wherein Y is represented by –
 $(CR_{15}R_{16})_rCR_{17}R_{18}-$, wherein R_{15} to R_{18} , for each occurrence, are independently H or an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted cycloalkyl, an optionally substituted heterocyclyl, an optionally substituted aryl or an optionally substituted heteroaryl; and r is an integer between 0 to 15.
 62. The cytotoxic compound of claim 61, wherein R_{15} to R_{18} , for each occurrence, are independently H or an optionally substituted alkyl.
 63. The cytotoxic compound of claim 62, wherein R_{15} to R_{18} are all H and r is 1.
 64. The cytotoxic compound of claim 62, wherein R_{15} and R_{16} are , r is 2, and R_{17} and R_{18} are both methyl.
 65. The cytotoxic compound of any one of claims 40-60, wherein D is represented by the following formula:



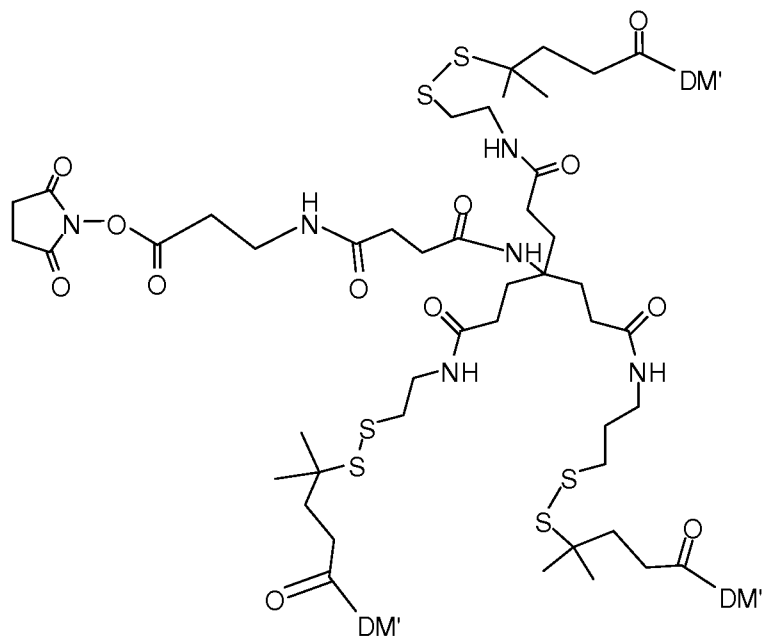
66. The cytotoxic compound of claim 40, wherein the cytotoxic compound is represented by the following formula, or a salt thereof:



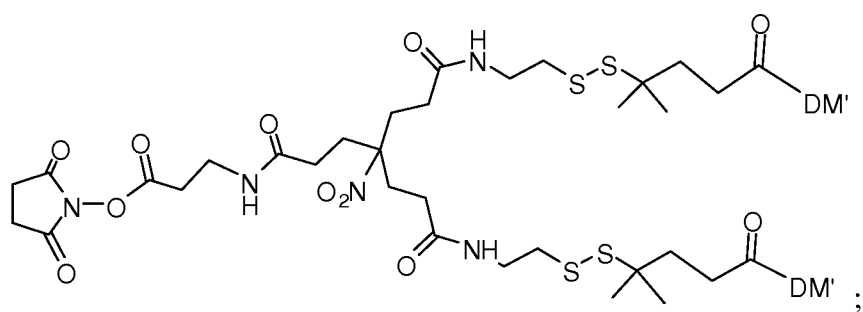




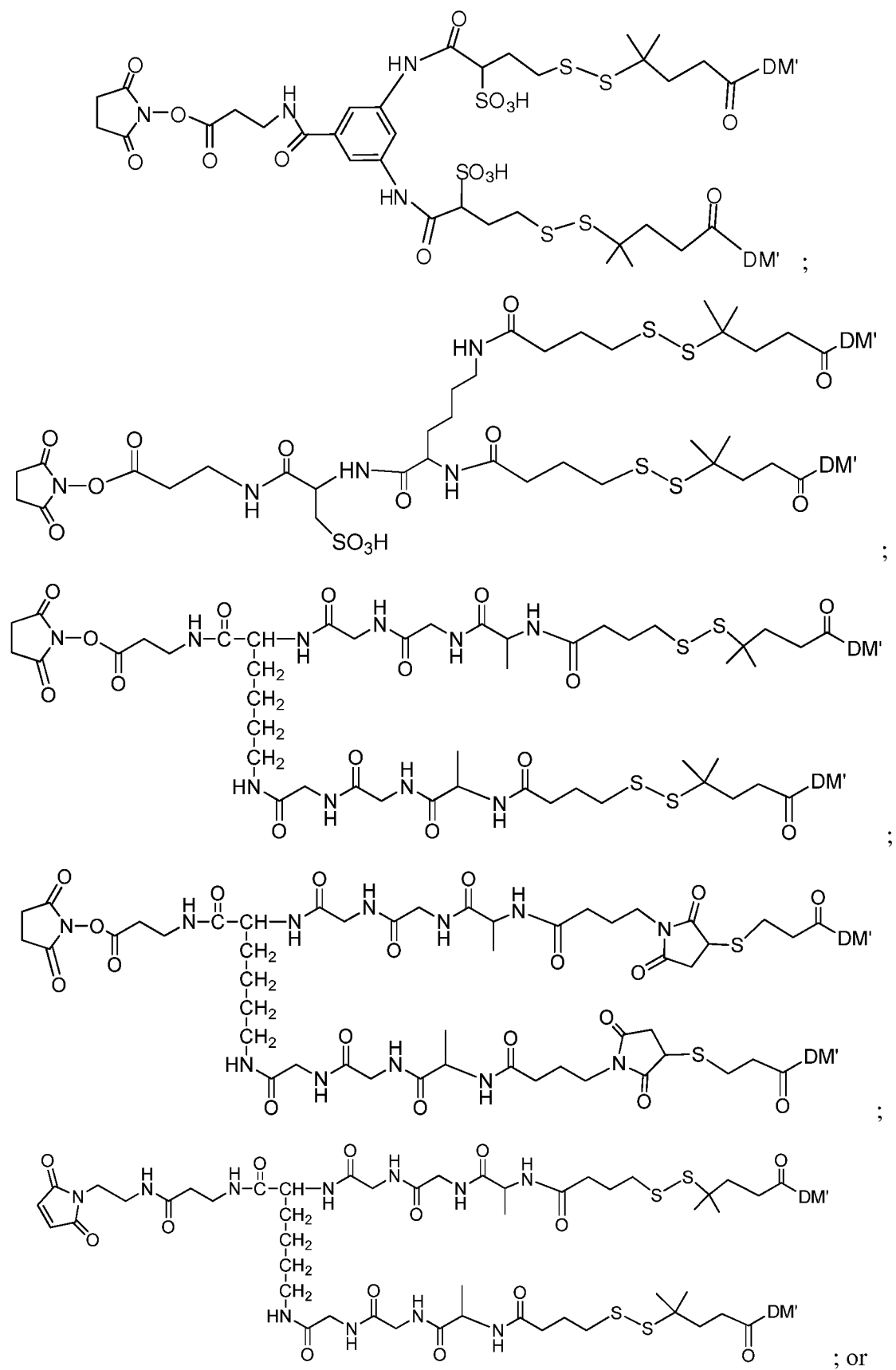
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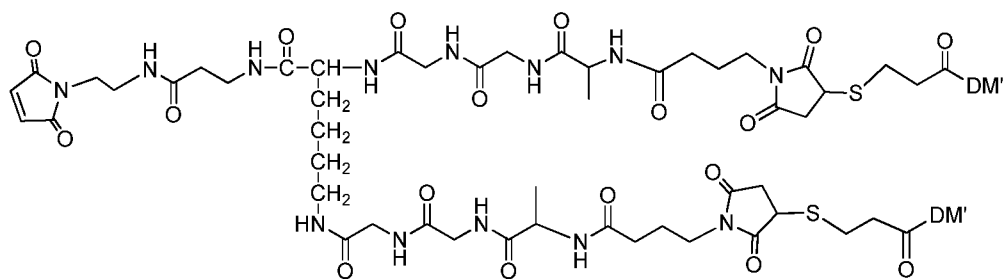


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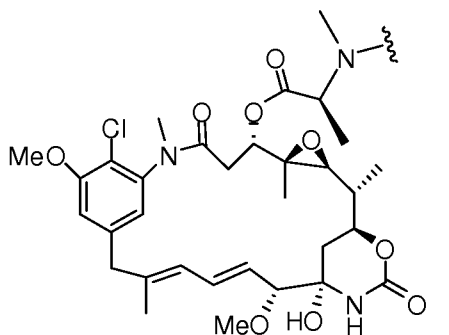


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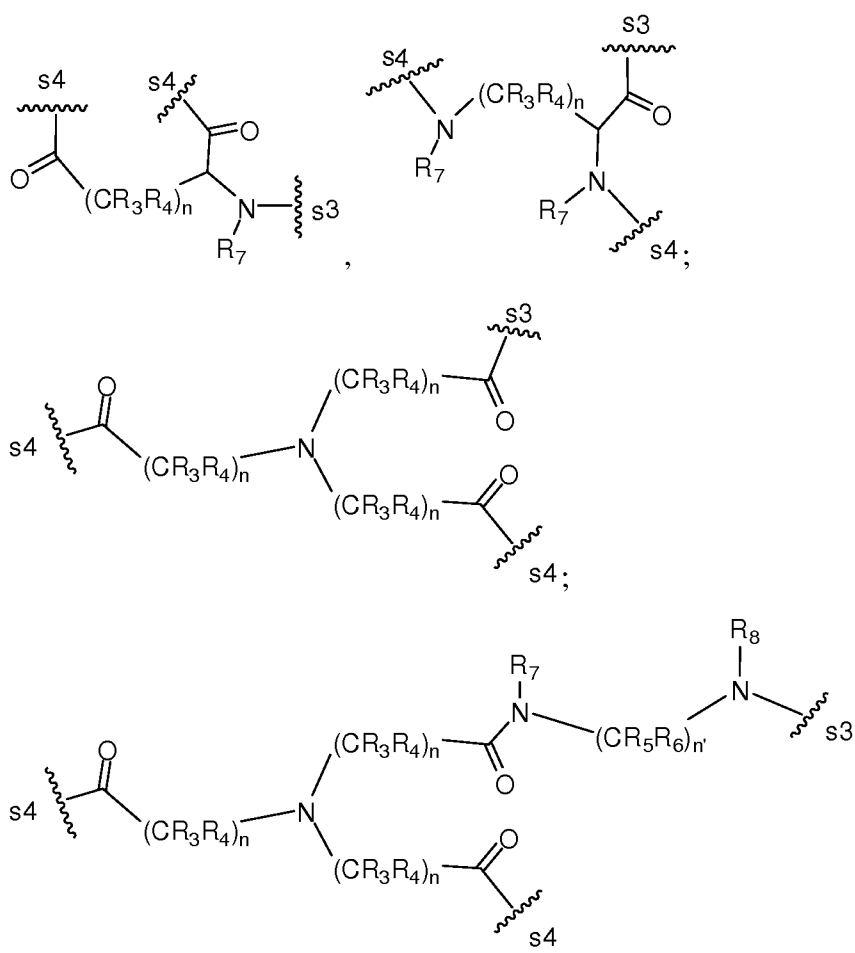


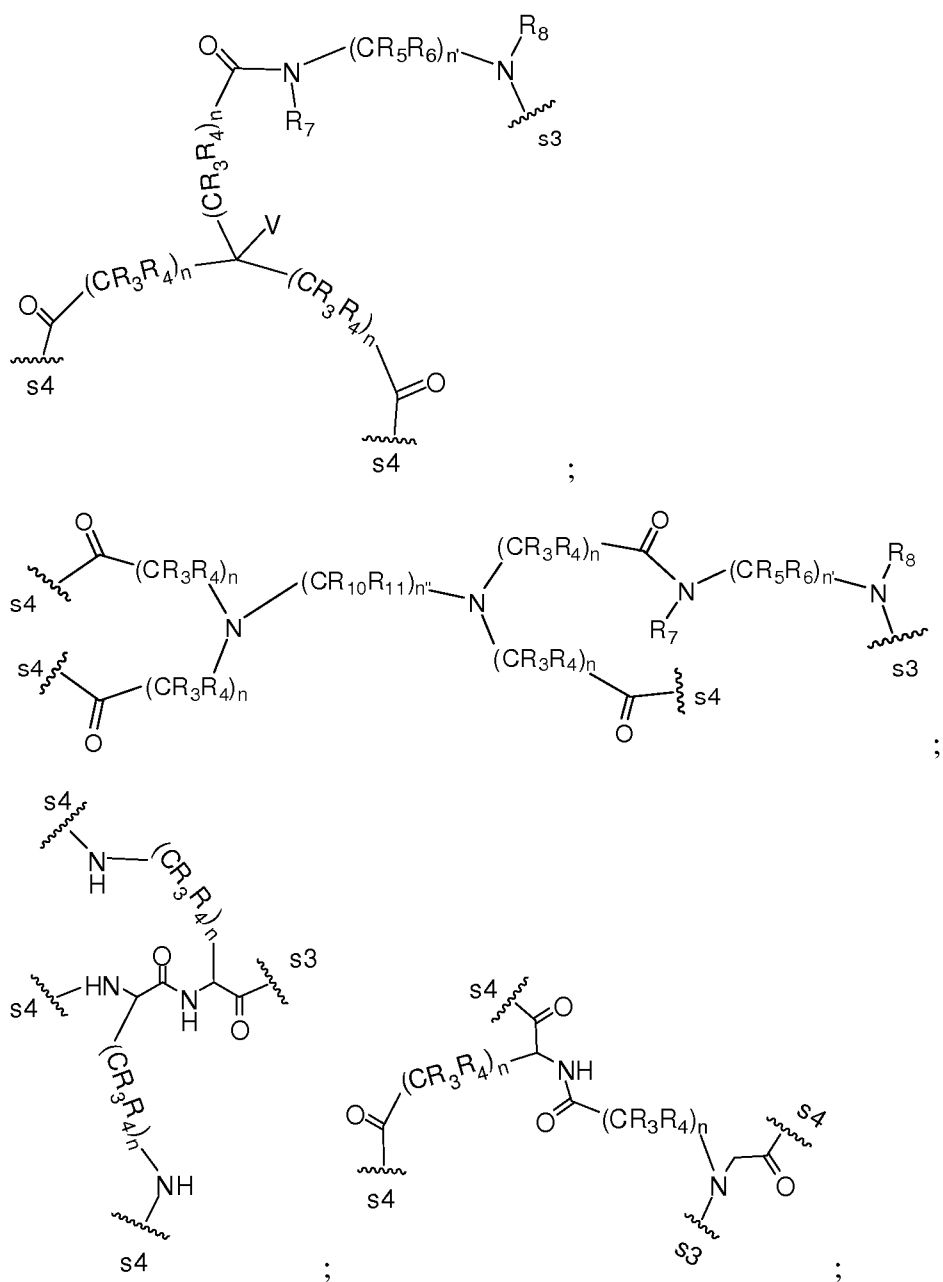


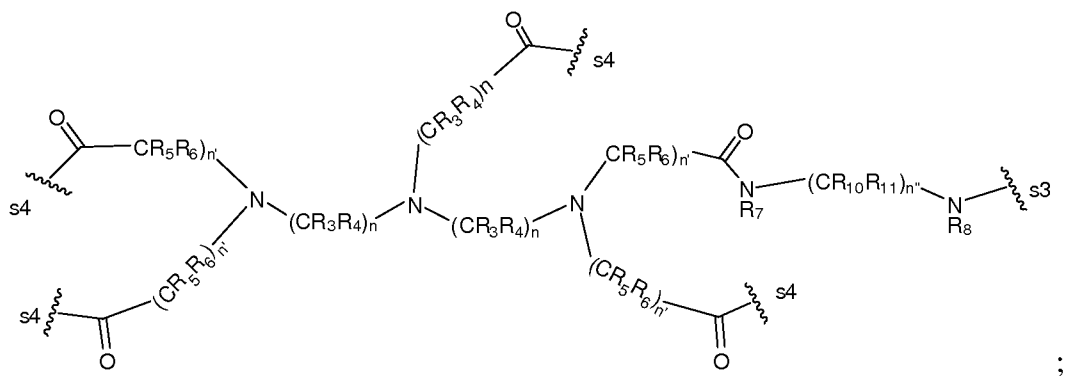
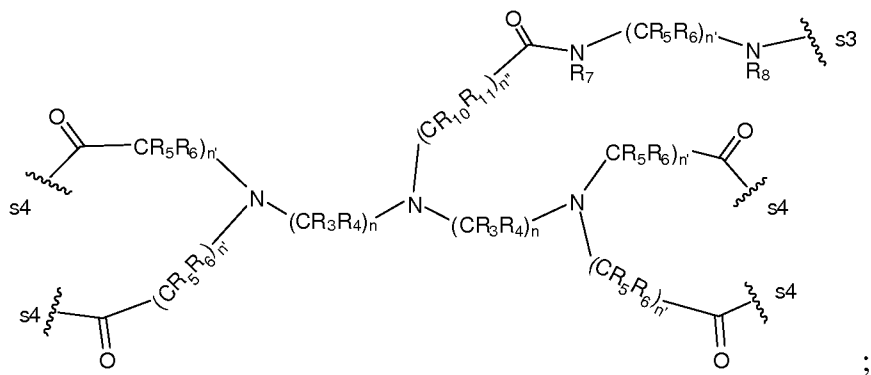
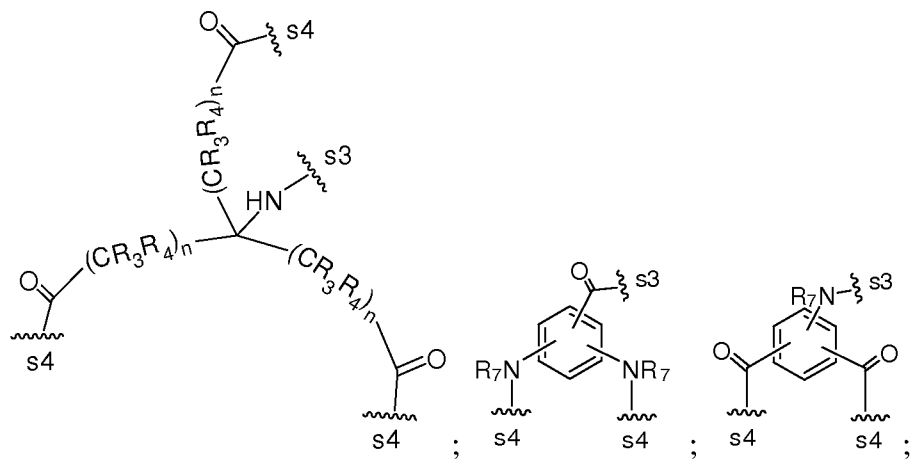
wherein: DM' is represented by the following formula:

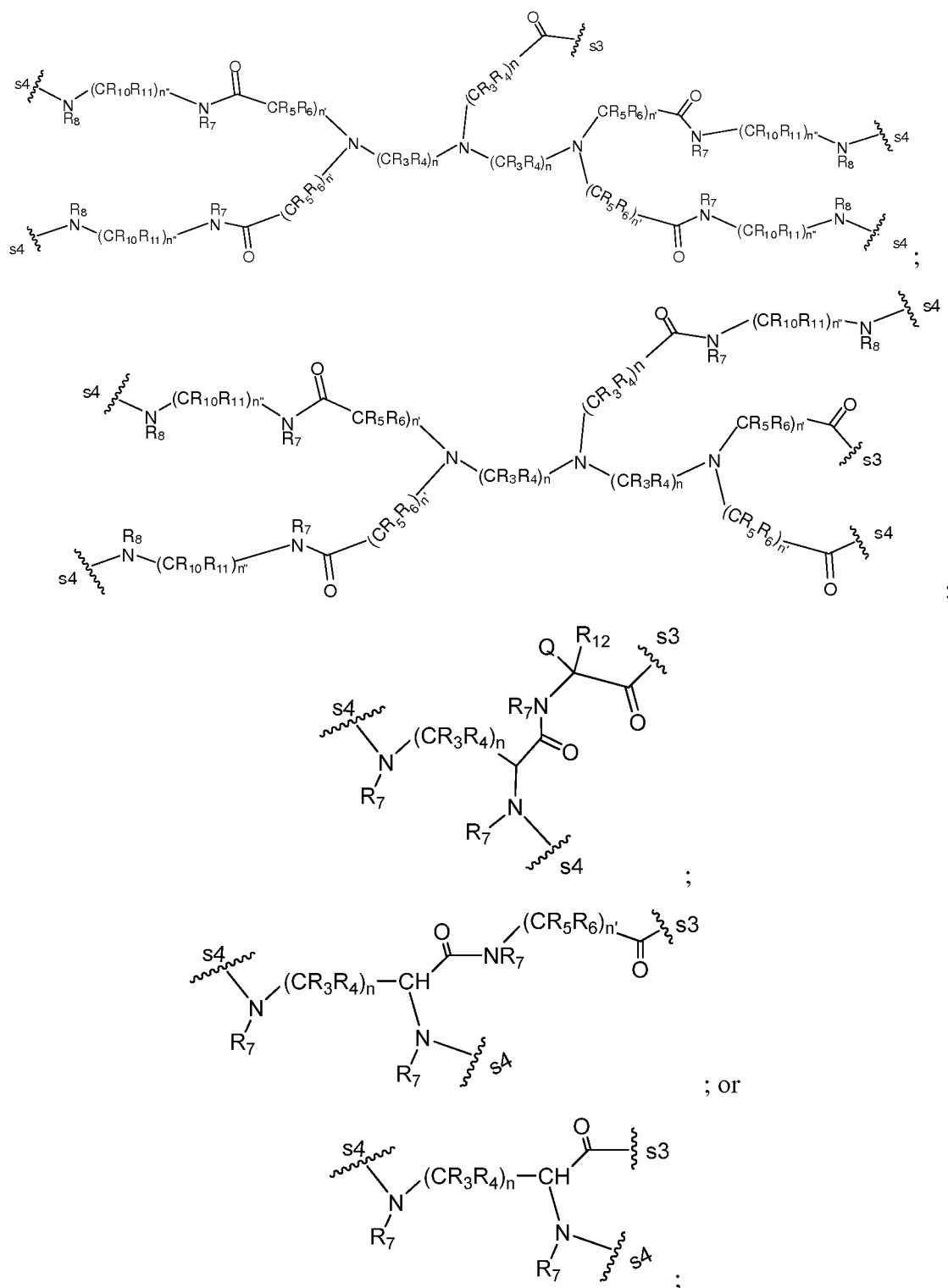


67. The cytotoxic compound of any one of claims 40-48, 50-57, and 59-65, wherein U is represented by the following formula:









wherein:

$R_3, R_4, R_5, R_6, R_7, R_8, R_{10}$ and R_{11} , for each occurrence, are independently H or an optionally substituted alkyl;

$n, n',$ and n'' are independently an integer from 1 to 10;

V is H, an optionally substituted alkyl, NO_2 , or $-\text{NH}-\text{C}(=\text{O})-\text{R}^{501}$;

R^{501} is H or an optionally substituted alkyl;

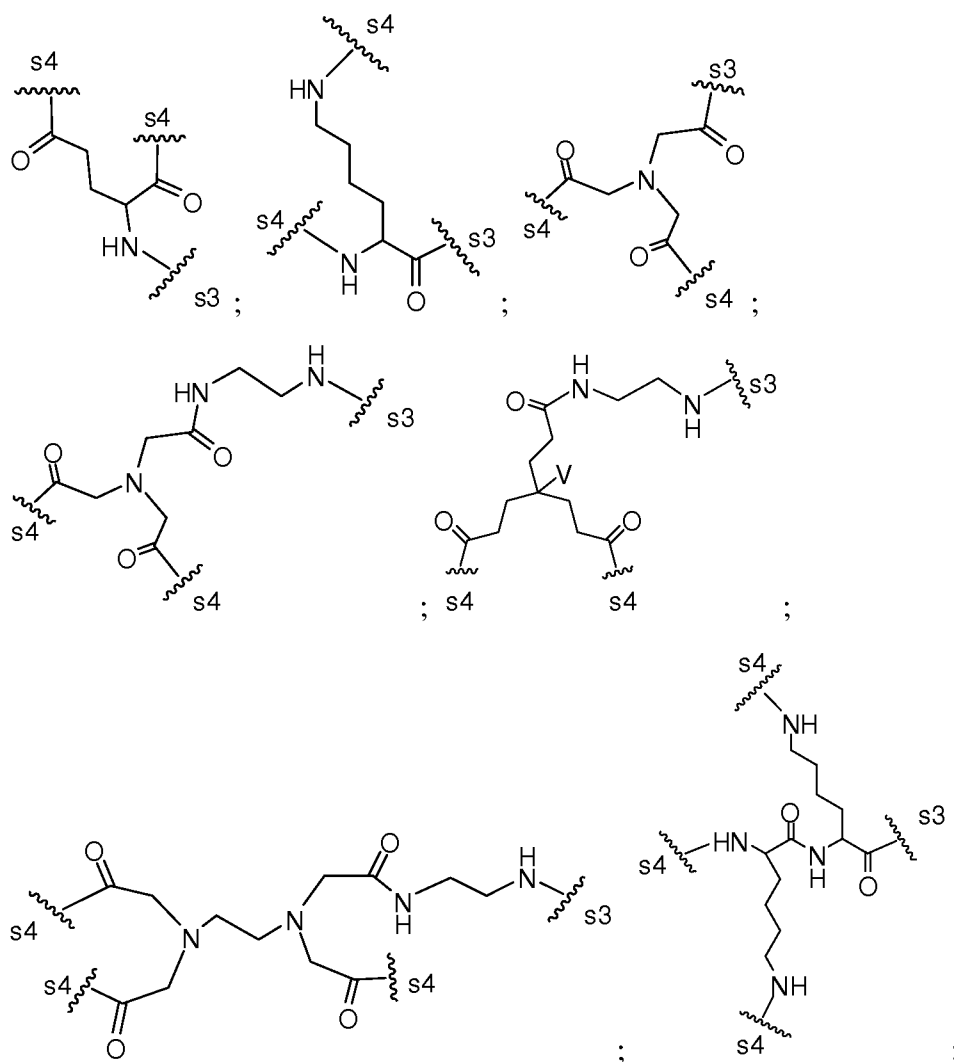
R_{12} is H, an optionally substituted alkyl;

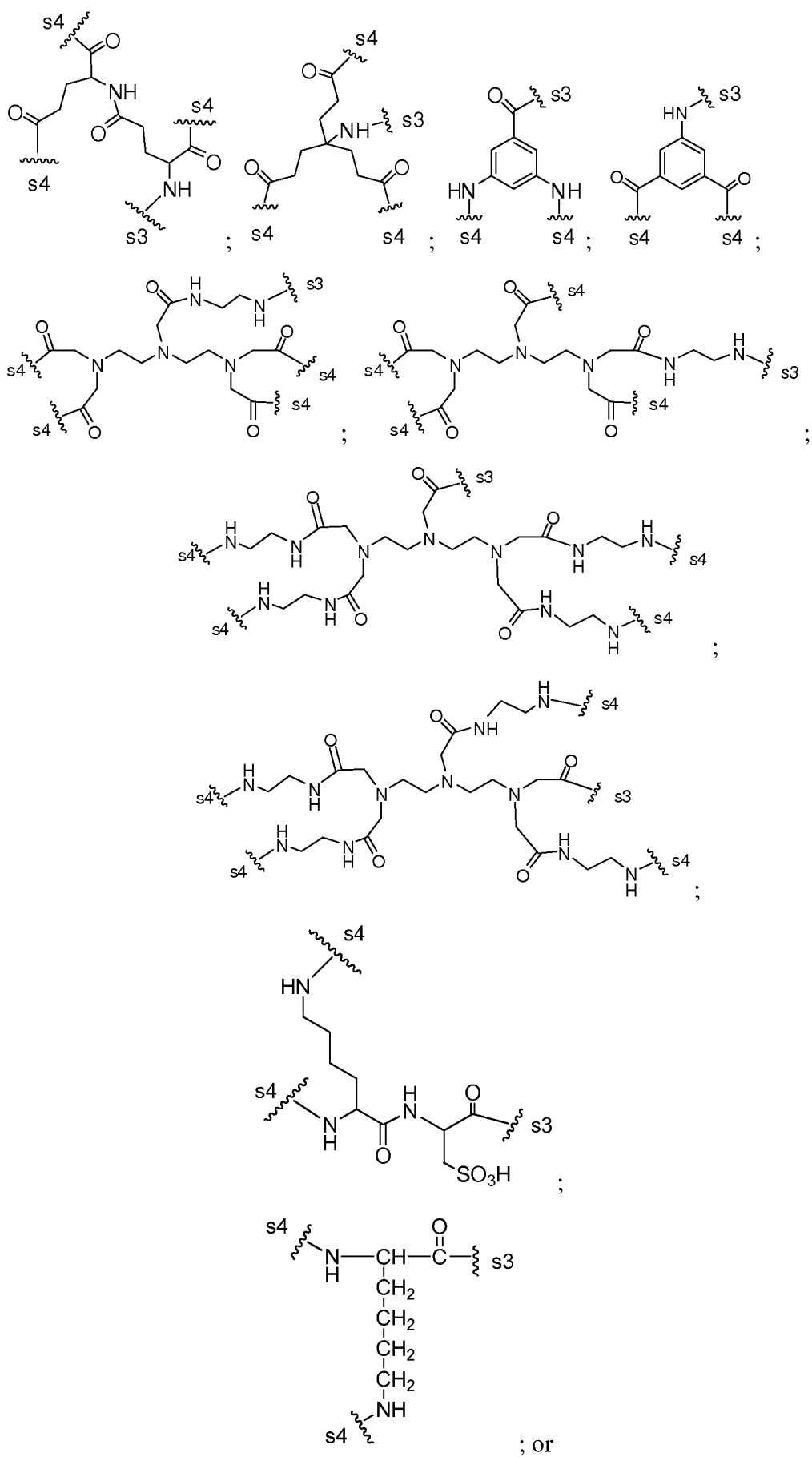
Q is H, a charged substituent or an ionizable group;

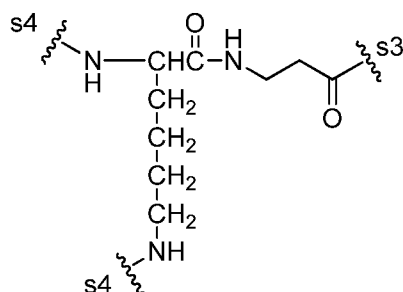
s3 is the site covalently linked the group L_1 ; and

s4 is the site covalently linked to the group L_2 .

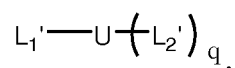
68. The cytotoxic compound of claim 67, wherein R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} are all H.
69. The cytotoxic compound of claim 67 or 68, wherein n, n' and n'' are independently an integer from 1 to 5.
70. The cytotoxic compound of claim 67, wherein U is represented by the following formula:







71. The cytotoxic compound of any one of claims 67-70, wherein V is H or NO₂.
72. A linker compound represented by the following formula, or a salt thereof:



wherein:

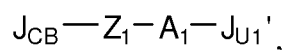
L_1' is a spacer attached to a reactive functional group that can form a covalent bond with a cell binding agent;

U is a branched scaffold;

L_2' , for each occurrence, is independently a spacer attached to a reactive functional group that can form a covalent bond with a cytotoxic agent; and

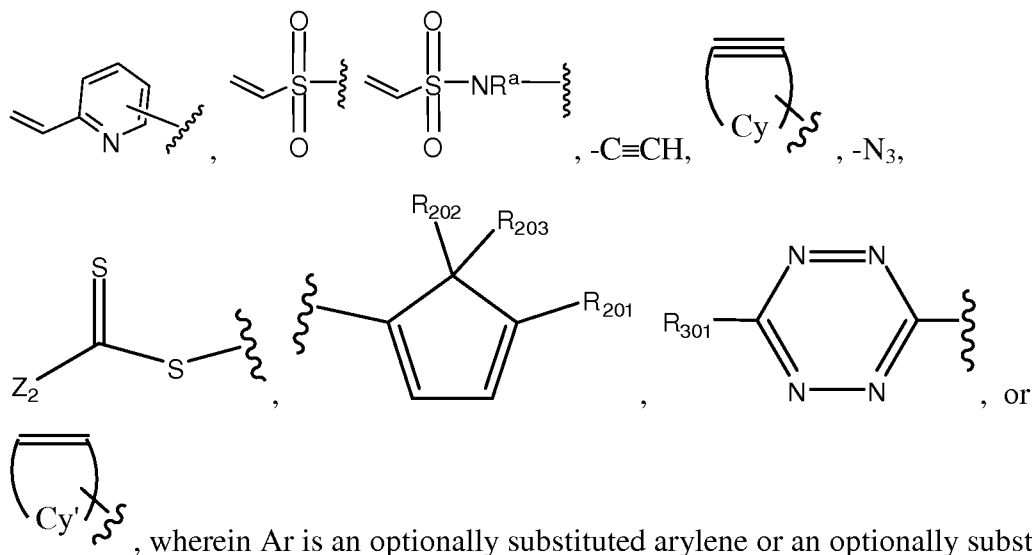
q is an integer from 2 to 5.


73. The linker compound of claim 72, wherein L_1' is represented by the following formula:

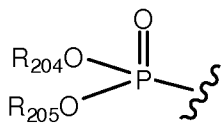


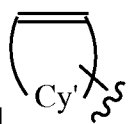
wherein:

J_{CB} is -COX'', maleimide, -SZ, X'-CR^bR^c-C(=O)-, X'-CR^bR^c-C(=O)-NR^e-, R^a-C(=O)-, R^a-C(=O)-Ar-, NH₂-NR^e-, NH₂-NR^e-C(=O)-, NH₂-NR^e-Ar-, NH₂-O-,



heteroarylene,  is an optionally substituted cycloalkyne or an optionally substituted heterocycloalkyne, R_{201} , R_{202} and R_{203} each are independently H or an

optionally substituted alkyl; Z_2 is pyridyl or , wherein R_{204} and R_{205}

are each independently optionally substituted alkyl, and  is an optionally substituted strained cycloalkene or an optionally substituted strained heterocycloalkene;

X' is a halogen;

X'' is $-OH$ or a carboxylic acid activating group;

Z is H or $-SR^d$;

R^a , R^b , R^c and R^e , for each occurrence, are independently H or an optionally substituted alkyl;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

Z_1 is $-NR_9-C(=O)-$ or $-C(=O)-NR_9-$ or absent;

A_1 is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, $-(CH_2-CH_2-O)_{p1}-(CR^bR^c)_{p1'}$ or $-(CR^bR^c)_{p1'-(O-CH_2-CH_2)_{p1}}$;

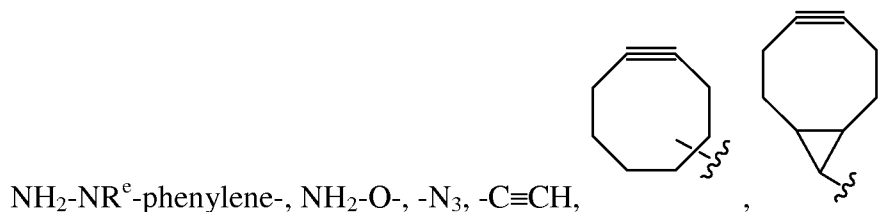
$J_{U1'}$ is absent, $-NR_9-$ or $-C(=O)-$;

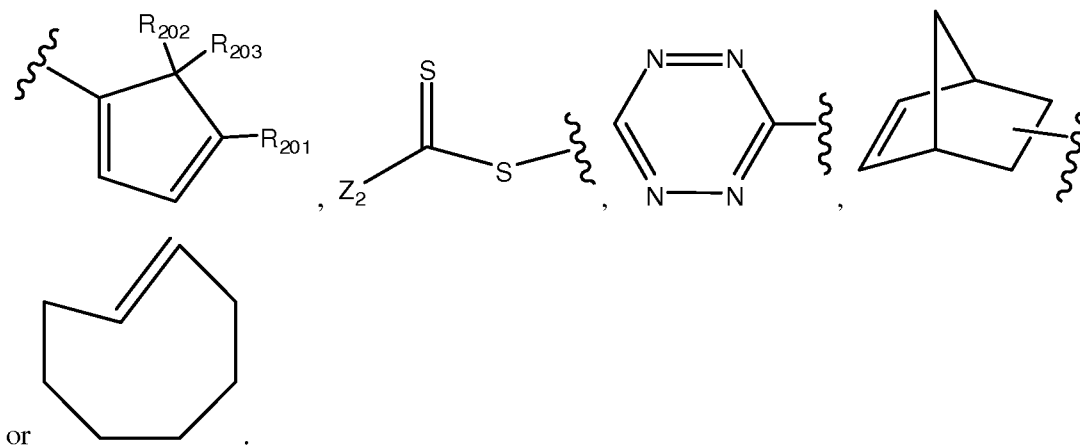
$p1$ is an integer from 1 to 1000 ;

$p1'$ is an integer from 0 to 10; and

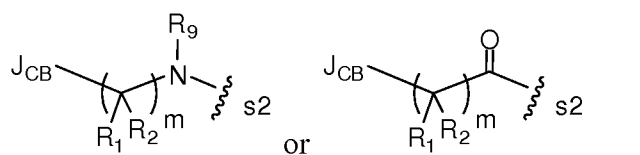
R_9 is H or an optionally substituted alkyl.

74. The linker compound of claim 73, wherein J_{CB} is $-COX''$, maleimide, $-SZ$, $X'-CR^bR^c-C(=O)-$, $X'-CR^bR^c-C(=O)-NR^e-$, $R^a-C(=O)-$, $R^a-C(=O)-phenylene-$, NH_2-NR^e- ,





75. The linker compound of any one of claims 72-74, wherein L_1' is represented by the following formula:



wherein R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10.

76. The linker compound of claim 75, wherein:

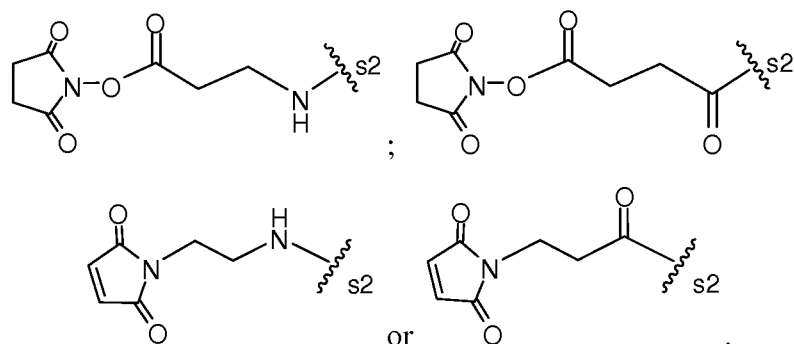
J_{CB} is $-\text{COX}''$ or a maleimide;

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

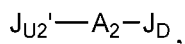
R_9 is H or an alkyl; and

m is an integer from 1 to 5.

77. The linker compound of claim 75 or 76, wherein R_1 and R_2 are both H; and R_9 is H.
78. The linker compound of claim 77, wherein L_1' is represented by the following formula:



79. The linker compound of any one of claims 72-78, wherein L_2' is represented by the following formula:

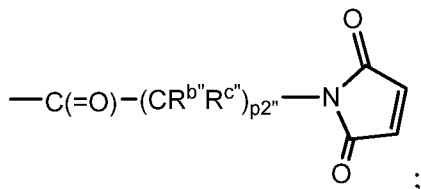


wherein:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_D is $-C(=O)-X''$, $-C(=O)-(CR^{b'}R^{c'})_{p2'}-C(=O)-X''$, $-C(=O)-(CH_2-CH_2-O)_{p2''}-(CR^{b'}R^{c'})_{p2'''}-SZ$, $-SZ$, absent, $-C(=O)-(CR^{b''}R^{c''})_{p2'''}-SZ$ or



X'' is $-OH$ or a carboxylic acid activating group;

Z is H or $-SR^d$;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl;

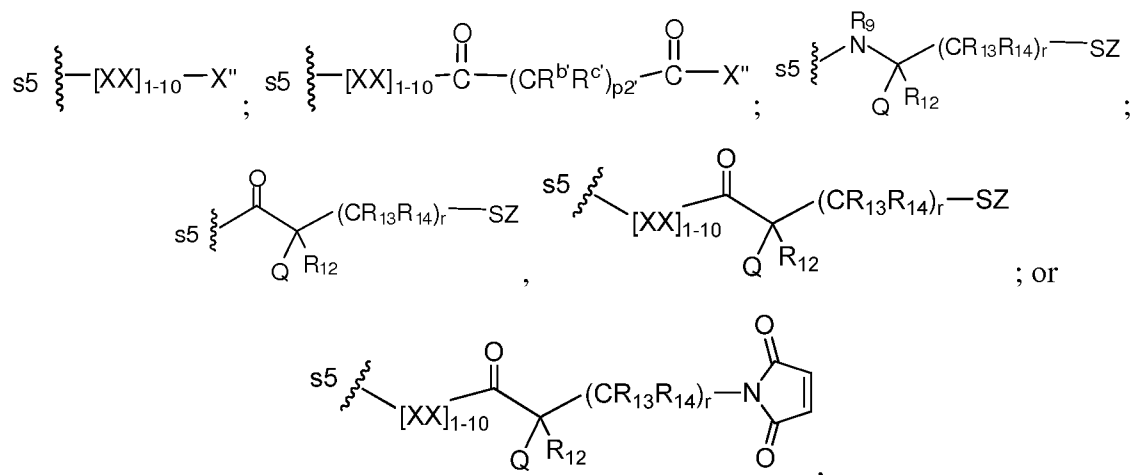
$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H , an optionally substituted alkyl or a charged substituent or an ionizable group;

p_2 is an integer from 1 to 1000;

p_2' is an integer from 1 to 10;

p_2'' is an integer from 1 to 10 (specifically 1 to 5).

80. The linker compound of claim 79, wherein L_2' is represented by the following formula:



wherein:

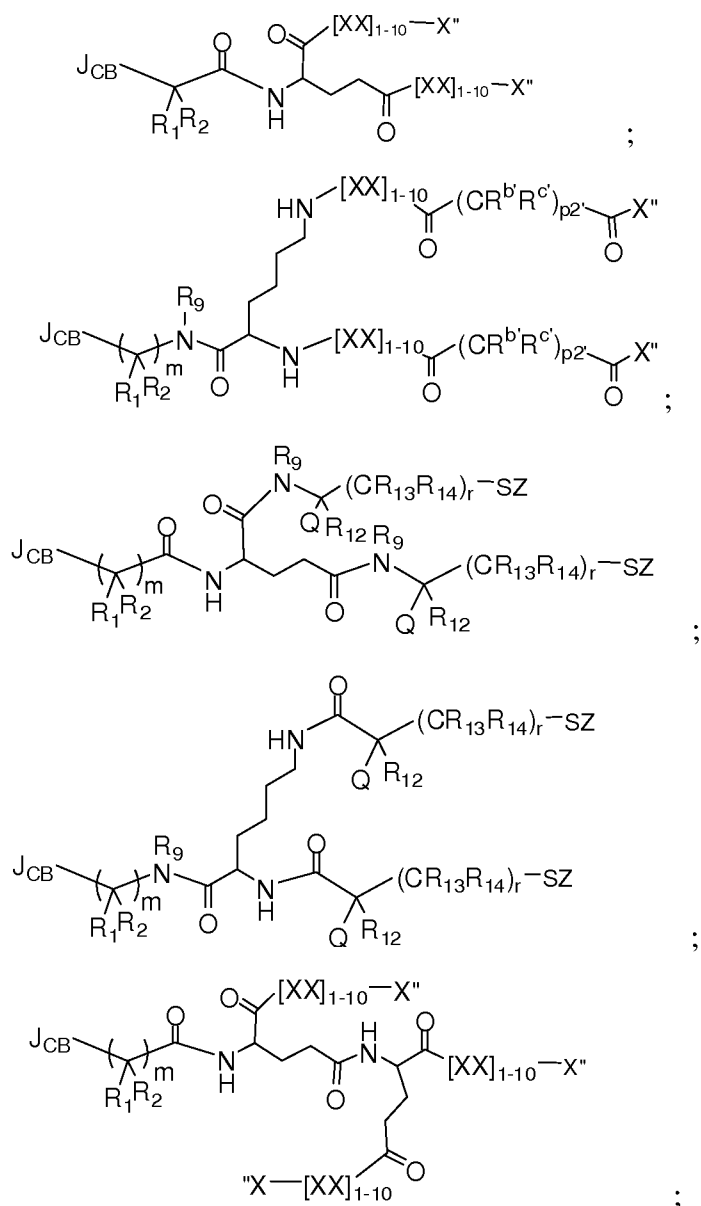
R₁₂, R₁₃, and R₁₄, for each occurrence, are each independently H, an optionally substituted alkyl;

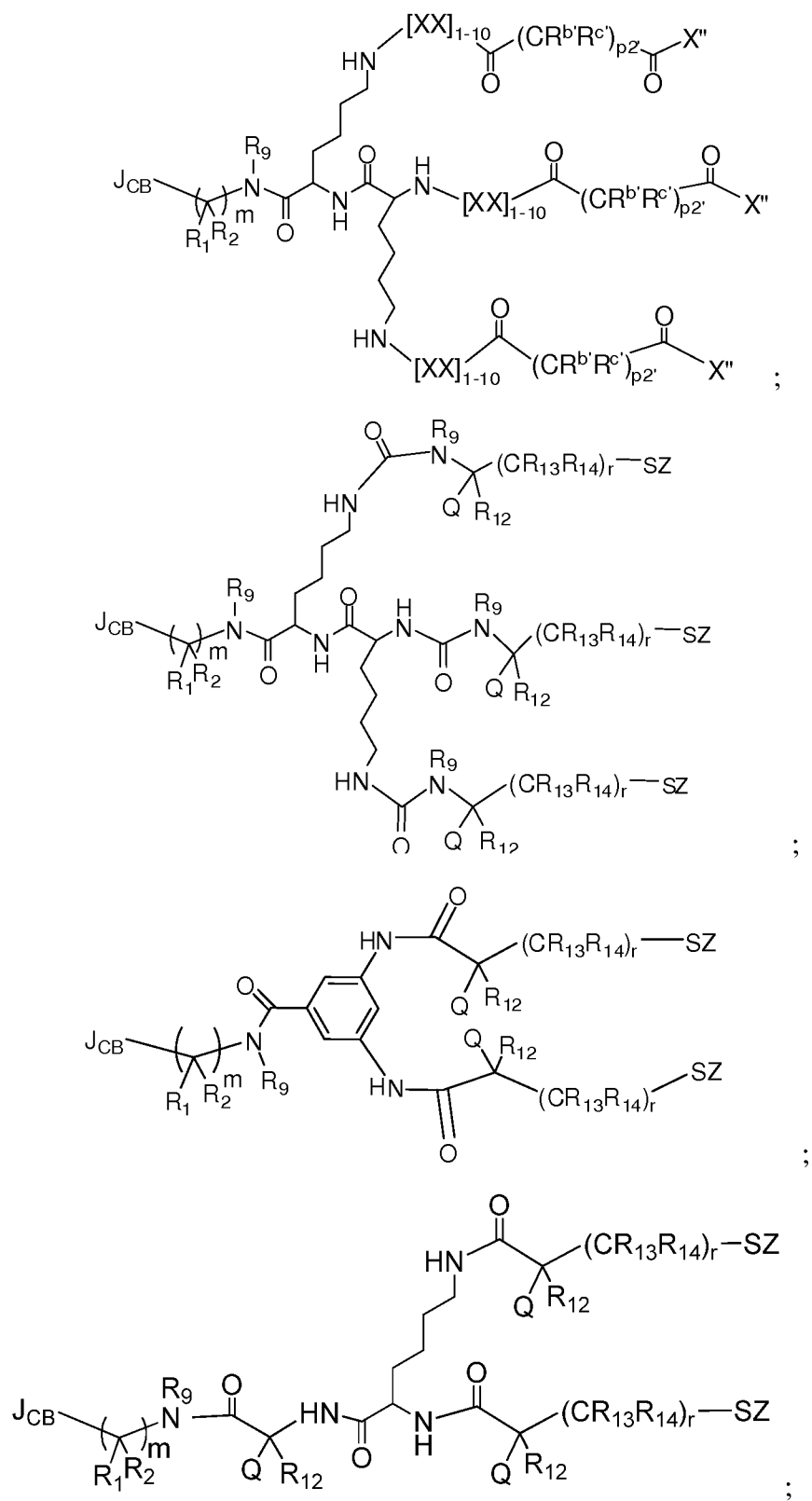
Q is H, a charged substituent or an ionizable group;

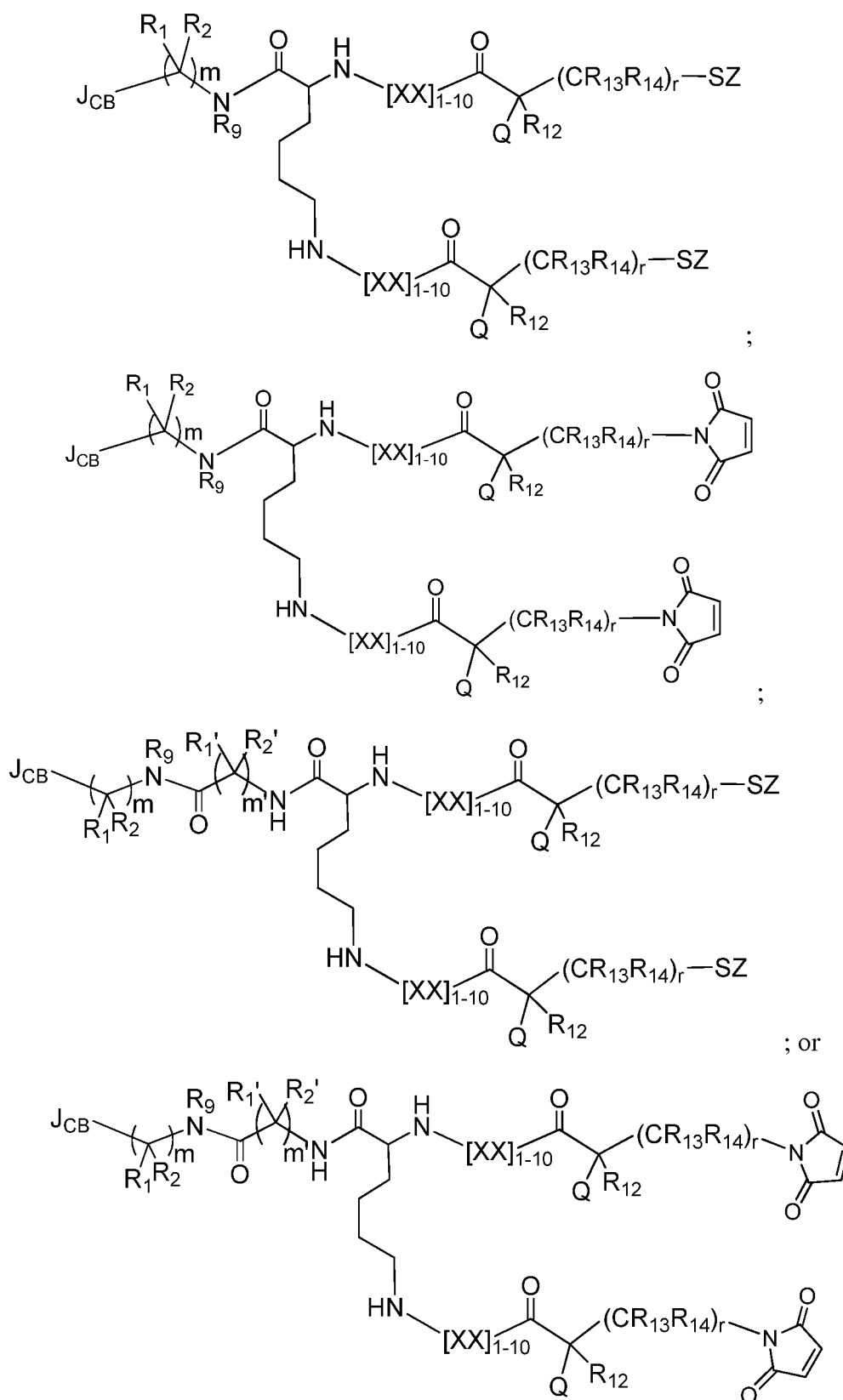
r is an integer from 0 to 10; and

s5 is the site covalently linked to the group U.

81. The linker compound of claim 79 or 80, wherein X'' together with the adjacent –C(=O)- group is a reactive ester group; Z is SR^d; and R^d is a pyridyl or nitropyridyl.
82. The linker compound of any one of claims 79-81, wherein the compound is represented by the following formula, or a salt thereof:



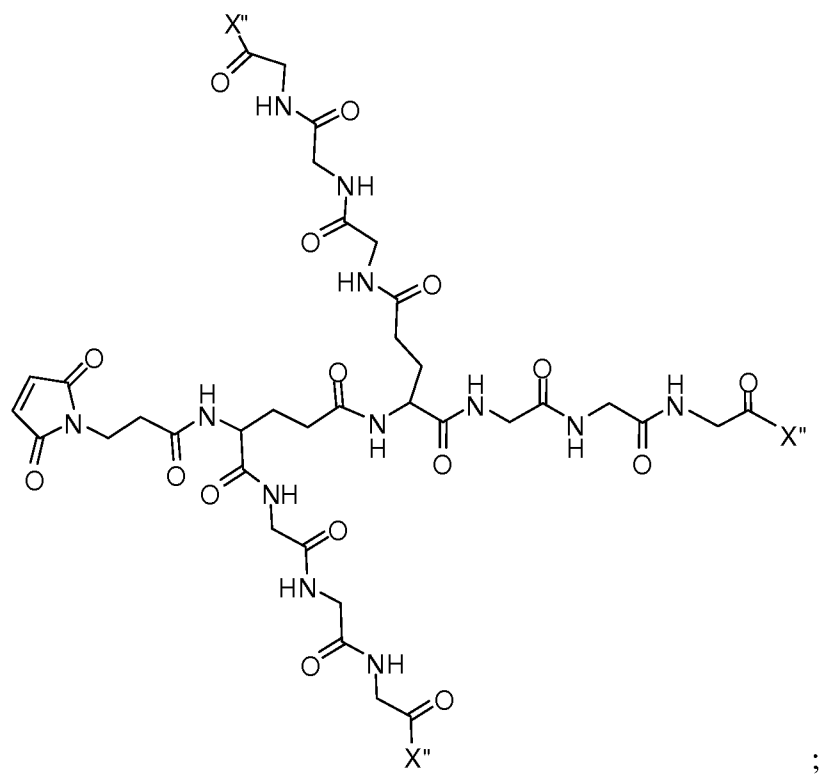
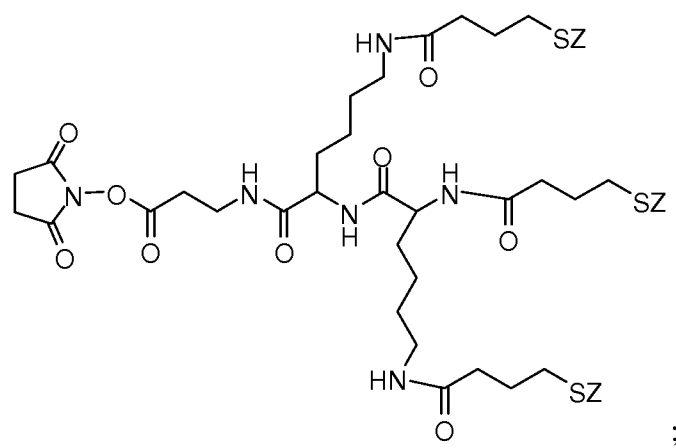
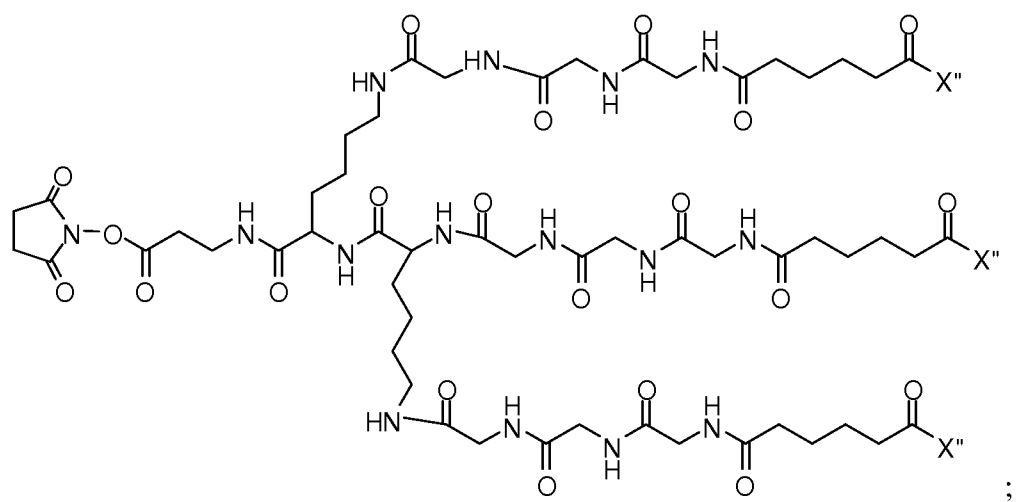


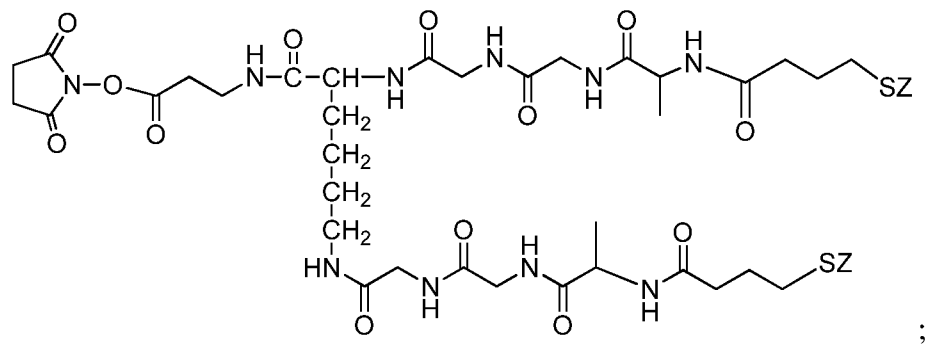
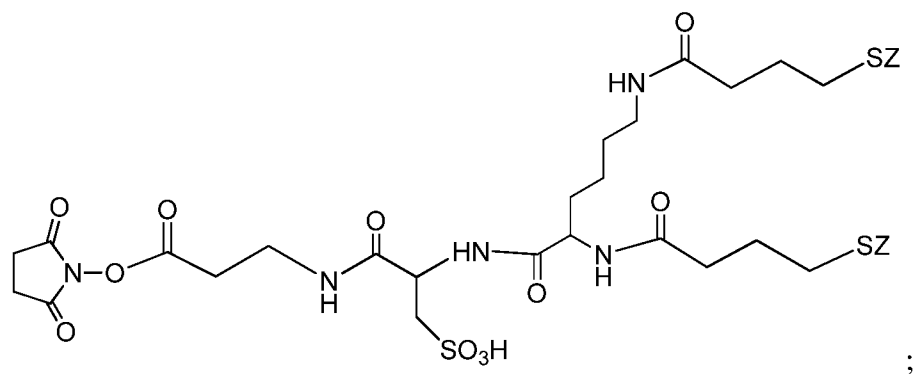
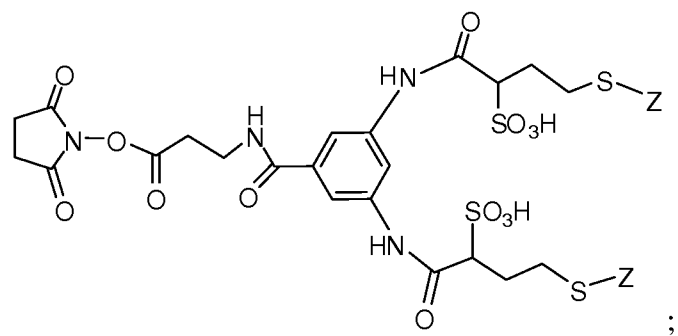
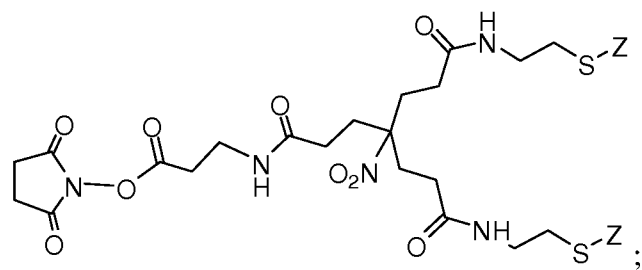
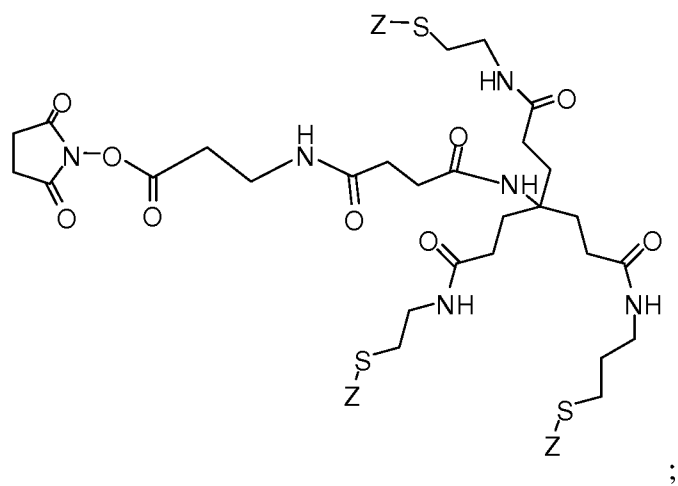


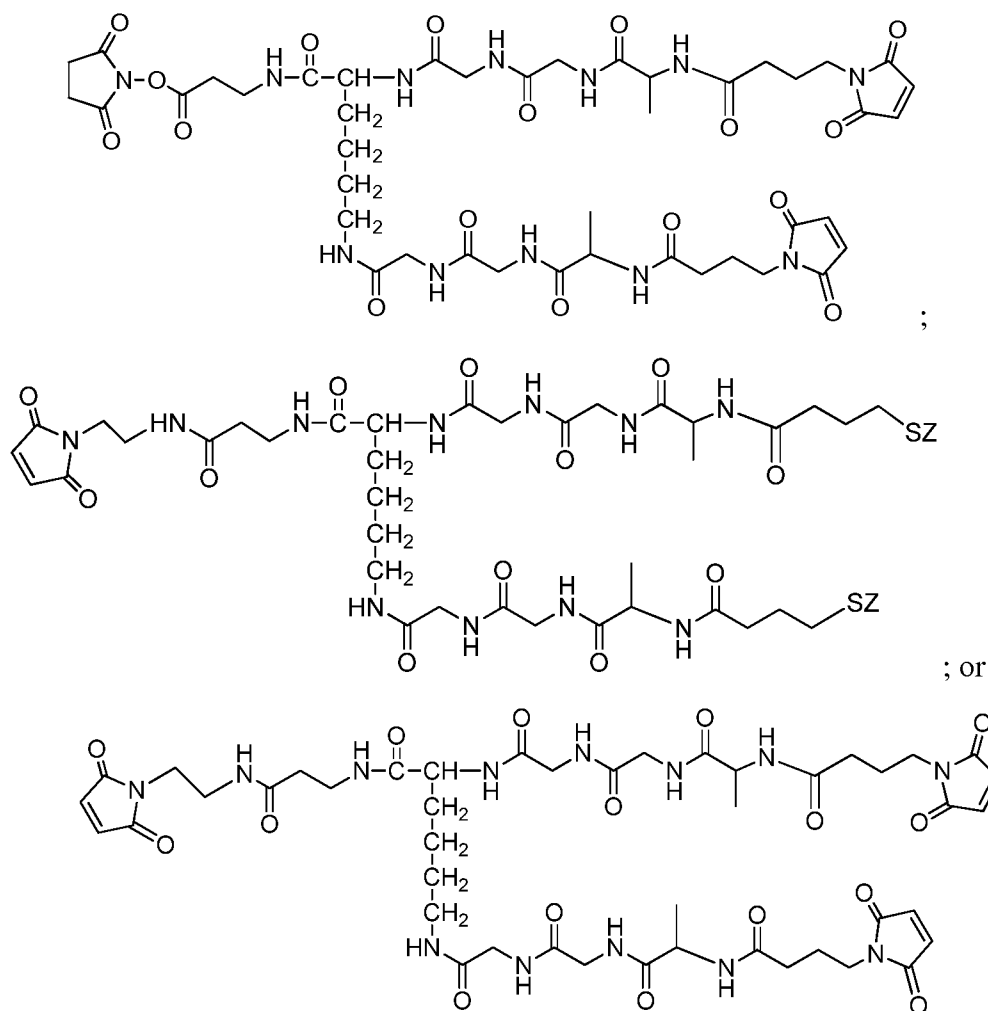
83. The linker compound of any one of claims 80-82, wherein Q is i) H ; ii) $-SO_3H$, $-Z'-SO_3$, $-OPO_3H_2$, $-Z'-OPO_3H_2$, $-PO_3H_2$, $-Z'-PO_3H_2$, $-CO_2H$, $-Z'-CO_2H$, $-NR_{101}R_{102}$, $-Z'-NR_{101}R_{102}$, or a pharmaceutically acceptable salt thereof; or iii) $-N^+R_{103}R_{104}R_{105}X^-$ or $-Z'-N^+R_{103}R_{104}R_{105}X^-$; Z' is an optionally substituted alkylene, an optionally

substituted cycloalkylene or an optionally substituted phenylene; R₁₀₁ and R₁₀₂, are each independently H or an optionally substituted alkyl; R₁₀₃ to R₁₀₅ are each independently an optionally substituted alkyl; and X⁻ is a pharmaceutically acceptable anion.

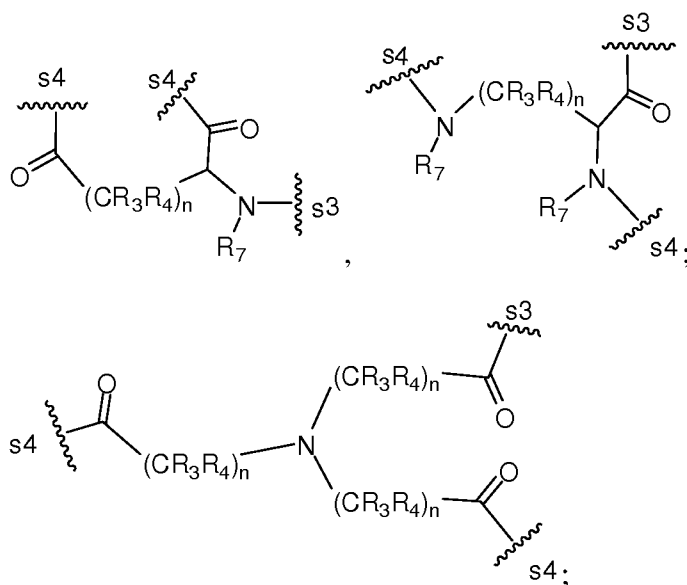
84. The linker compound of any one of claims 80-83, wherein R₁₂ is H; and Q is H, SO₃H or a pharmaceutically acceptable salt thereof.
85. The linker compound of any one of claims 79-84, wherein R^{b'} and R^{c'} are both H; and p2' is an integer from 1 to 5.
86. The linker compound of any one of claims 80-85, wherein R₁₃ and R₁₄ are both H; and r is 1 to 5.
87. The linker compound of any one of claims 79-86, wherein [XX]₁₋₁₀ is [XX]₂₋₅.
88. The linker compound of any one of claims 79-87, wherein [XX], for each occurrence, is the residue of an amino acid independently selected from the group consisting of: histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-proline, N-methyl-serine, N-methyl-tyrosine, hydroxyproline, γ-carboxyglutamate, selenocysteine, O-phosphoserine, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, citrulline, ornithine, cysteine sulfonic acid, cysteine sulfinic acid, 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, and β-alanine, each independently as an L or D isomer.
89. The linker compound of any one of claims 79-87, wherein [XX]₁₋₁₀ is selected from the group consisting of: Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu, β-Ala-Leu-Ala-Leu, Gly-Phe-Leu-Gly, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala,

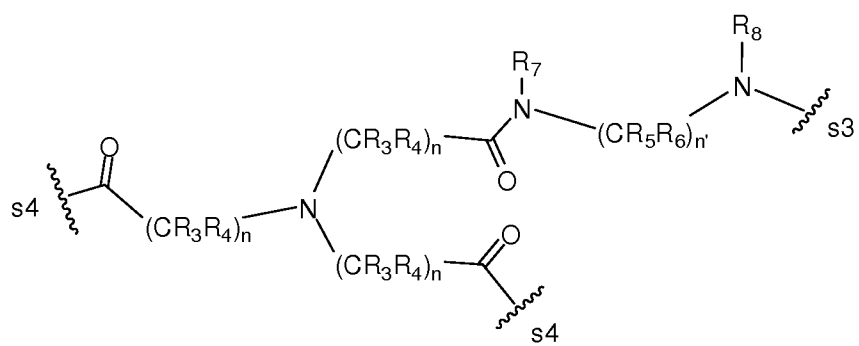




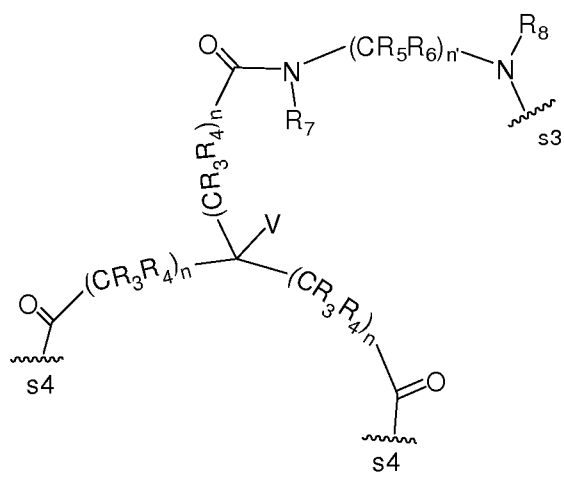


92. The linker compound of claim 91, wherein X'' together with the adjacent $-(C=O)-$ group forms a reactive ester group; Z is $-SR^d$, and R^d is a pyridyl or nitropyridyl.
93. The linker compound of any one of claims 72-81 and 83-90, wherein U is represented by the following formula:

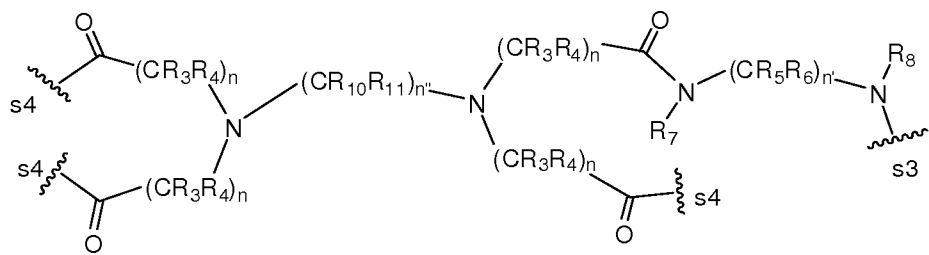




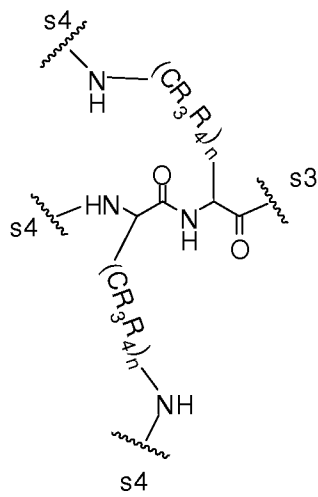
;



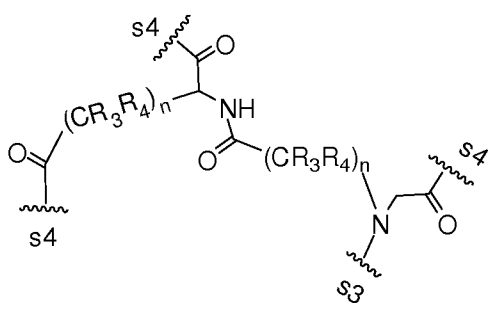
;



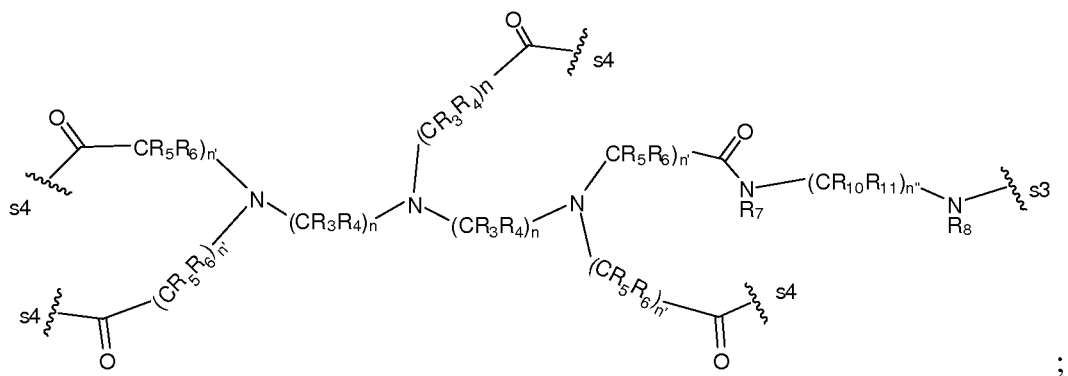
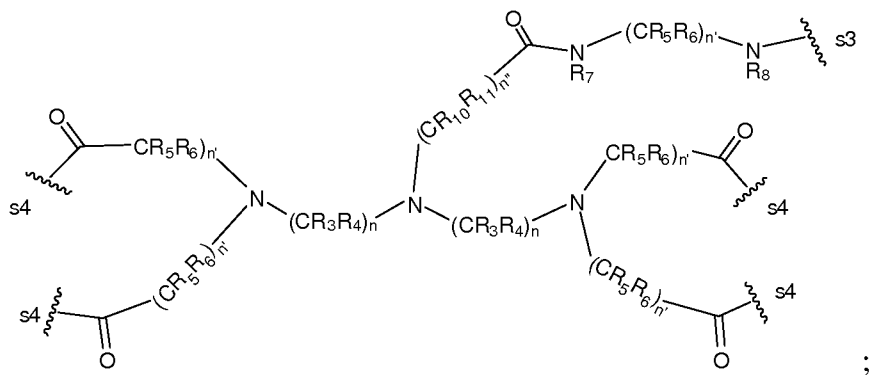
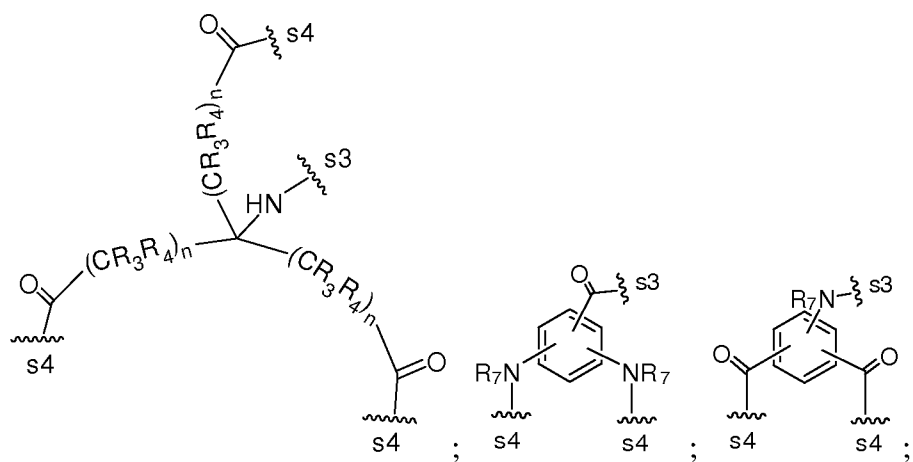
;

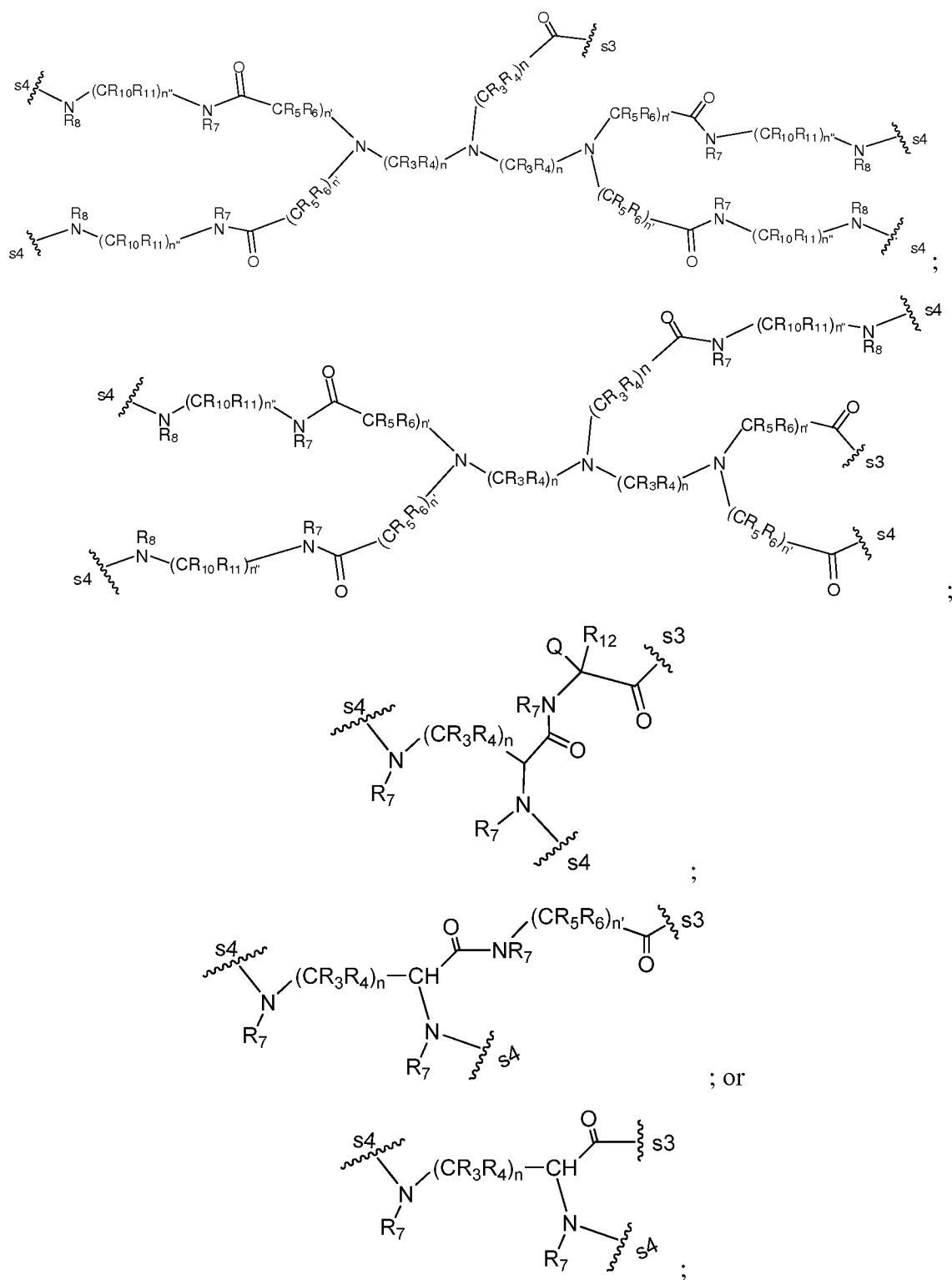


;



;





R₃, R₄, R₅, R₆, R₇, R₈, R₁₀ and R₁₁, for each occurrence, are independently H or an optionally substituted alkyl;

V is H, an optionally substituted alkyl, NO₂, or -NH-C(=O)-R⁵⁰¹;

R^{501} is H or an optionally substituted alkyl;

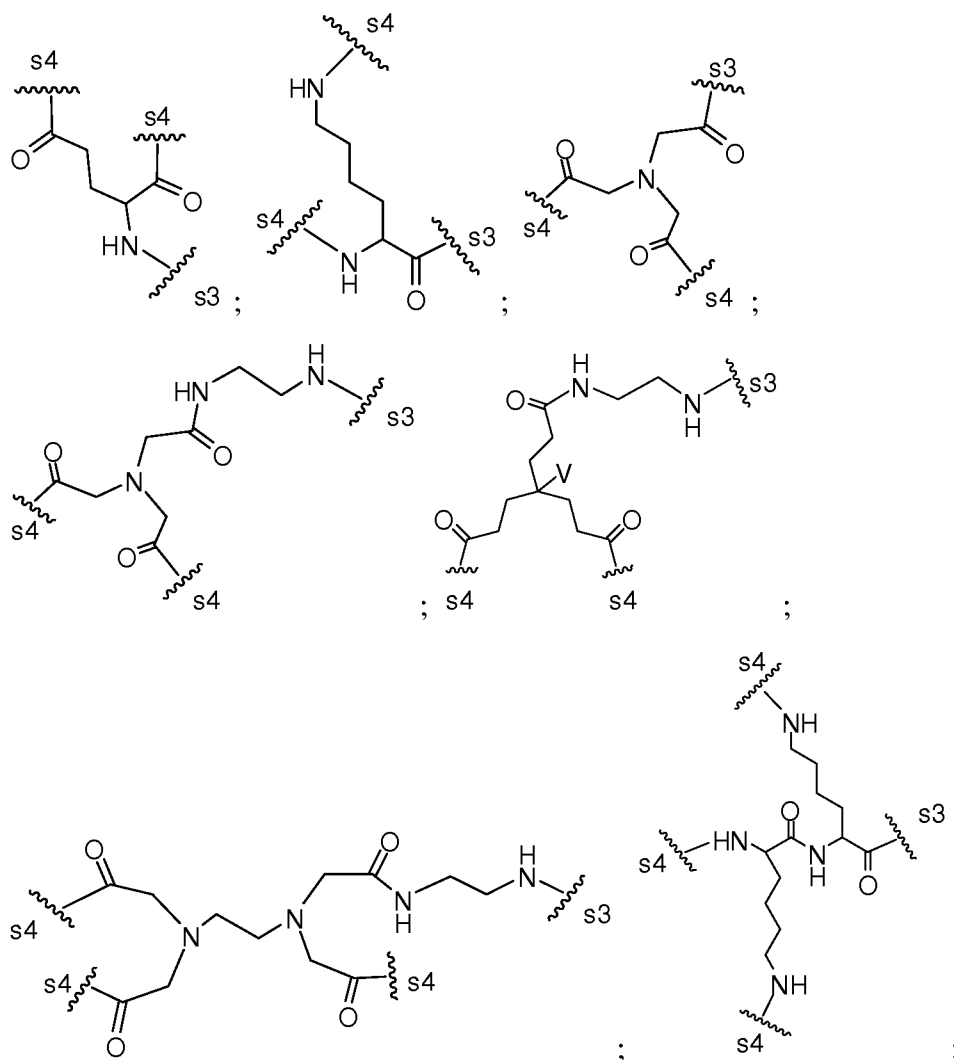
R_{12} is H, an optionally substituted alkyl;

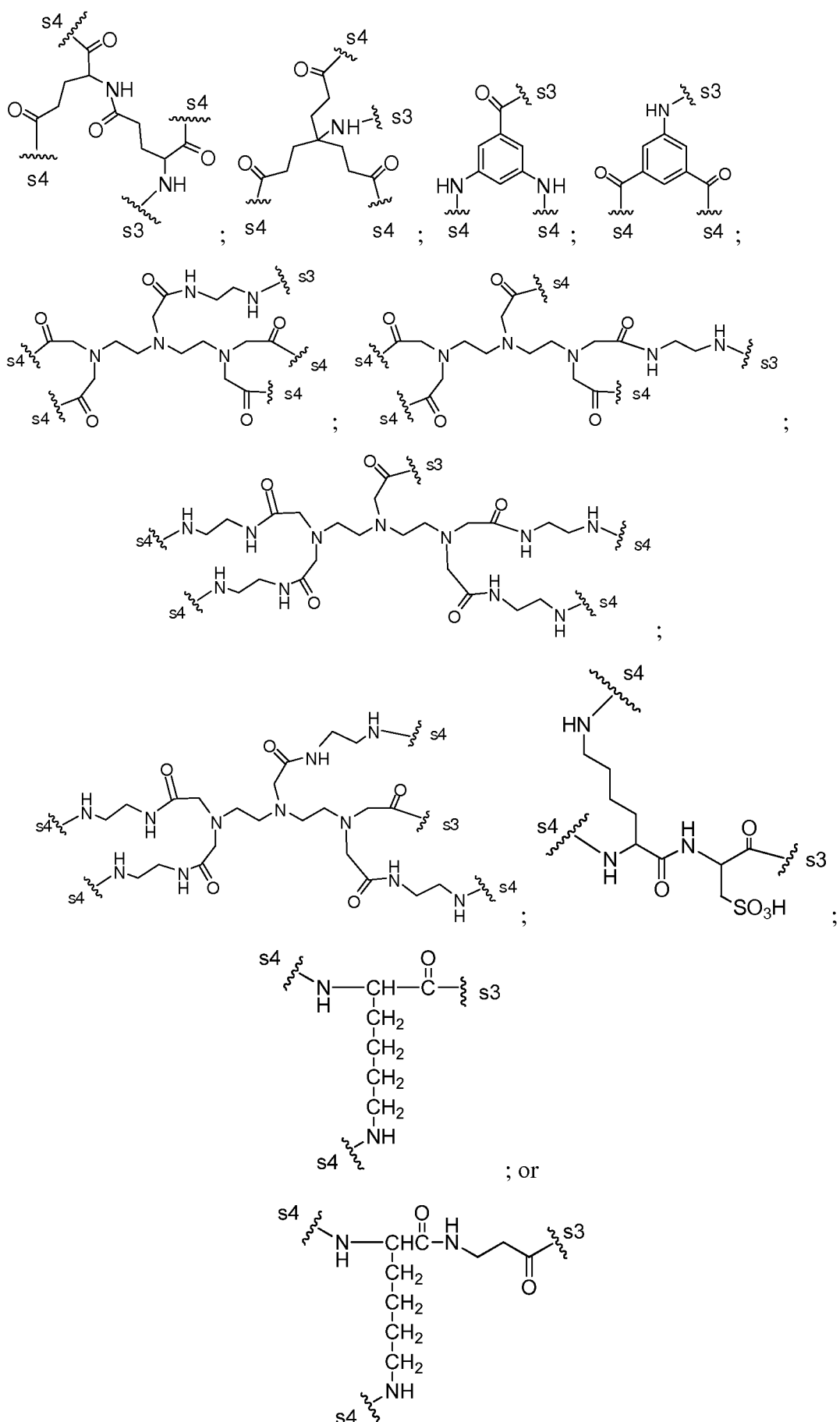
Q is H, a charged substituent or an ionizable group;

s3 is the site covalently linked the group L_1 ; and

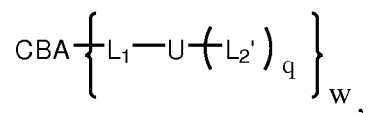
s4 is the site covalently linked to the group L_2 .

94. The linker compound of claim 93, wherein R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} are all H.
95. The linker compound of claim 93 or 94, wherein n, n', and n'' are independently an integer from 1 to 5.
96. The linker compound of claim 93, wherein U is represented by the following formula:





97. The linker compound of any one of claims 93-96, wherein V is H or NO₂.
98. A modified cell binding agent represented by the following formula, or a salt thereof:



wherein:

L₁ is a spacer;

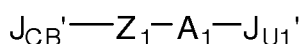
U is a branched scaffold;

L₂', for each occurrence, is independently a spacer attached to a reactive functional group that can form a covalent bond with a cytotoxic agent;

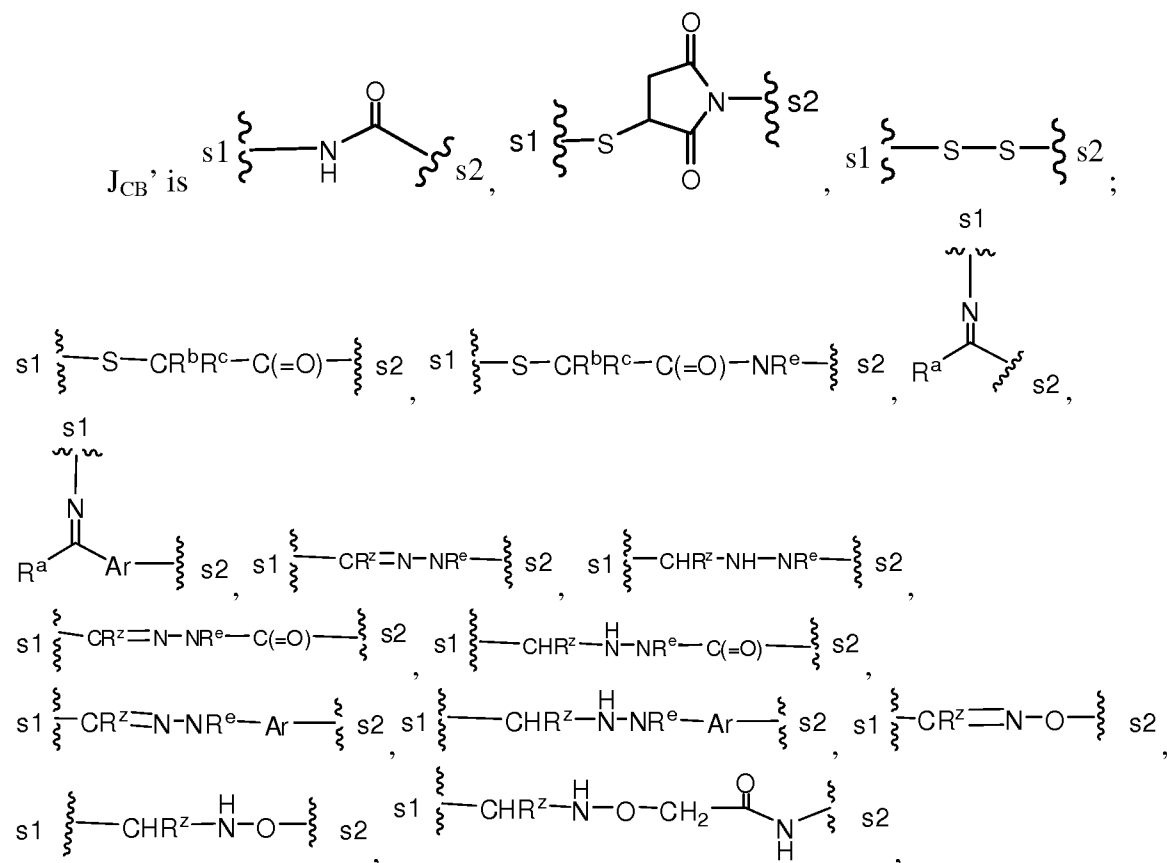
q is an integer from 2 to 5; and

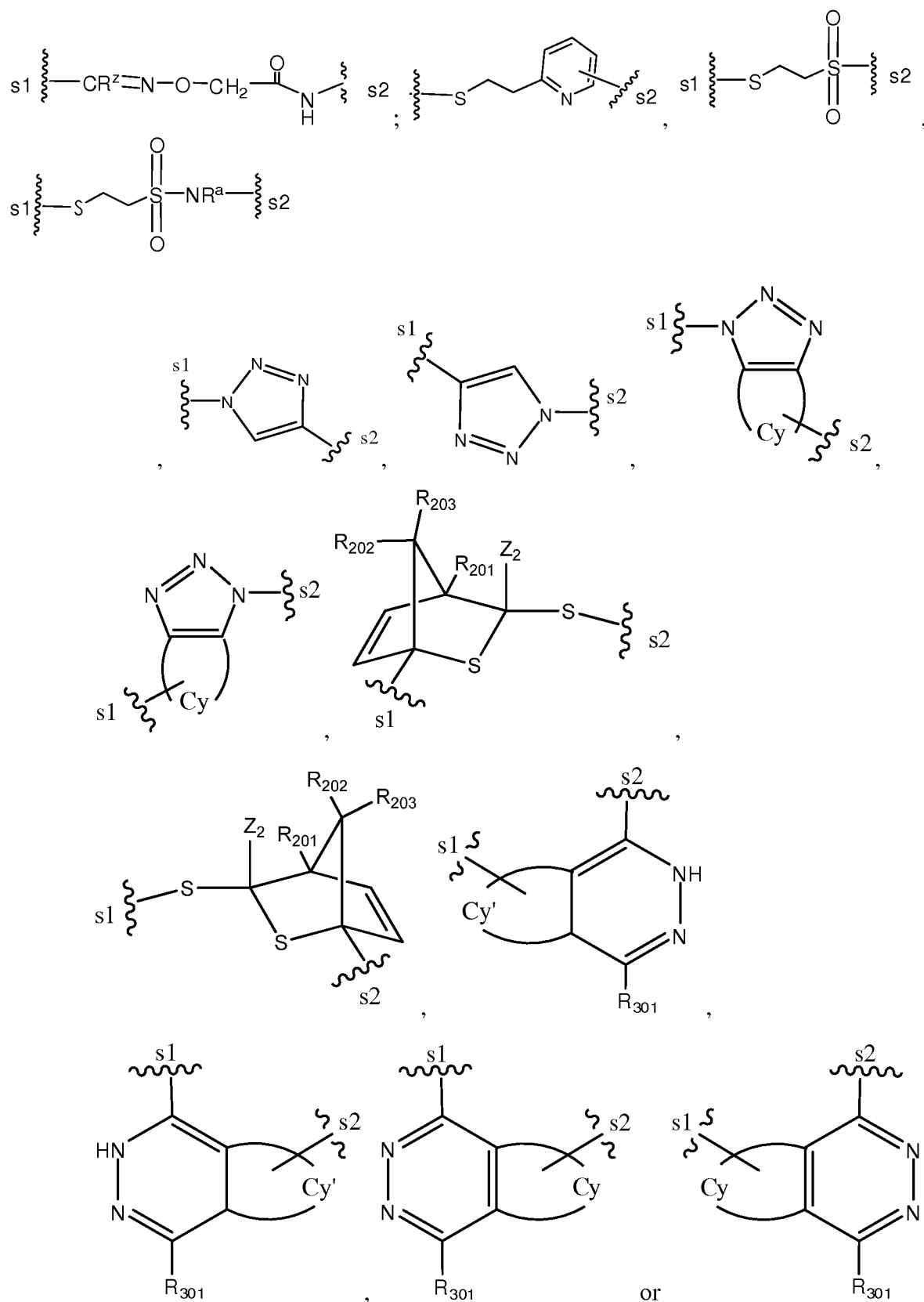
w is an integer between 1 and 10.

99. The modified cell binding agent of claim 98, wherein L₁ is represented by the following formula:

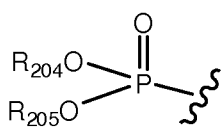


wherein:





in which s1 is the site covalently linked to the CBA, s2 is the site covalently linked to the group U, Ar is an optionally substituted arylene or an optionally substituted heteroarylene, Cy is an optionally substituted carbocyclic ring or an optionally substituted heterocyclic ring, R₂₀₁, R₂₀₂ and R₂₀₃ each are independently H or an

optionally substituted alkyl; Z₂ is pyridyl or , wherein R₂₀₄ and R₂₀₅ are each independently optionally substituted alkyl, R₃₀₁ is H or optionally substituted alkyl; and Cy' is the non-alkene residue of an optionally substituted strained cycloalkene or an optionally substituted strained heterocycloalkene;

Z₁ is -NR₉-C(=O)- or -C(=O)-NR₉- or absent,

A₁ is absent, an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, -(CH₂-CH₂-O)_{p1}-(CR^bR^c)_{p1'}- or -(CR^bR^c)_{p1'}-(O-CH₂-CH₂)_{p1}-,

J_{U1}' is absent, -NR₉- or -C(=O)-

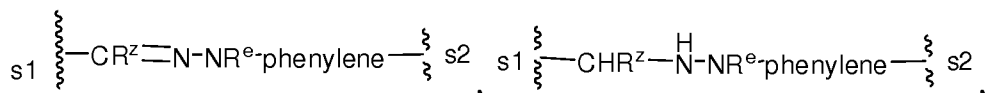
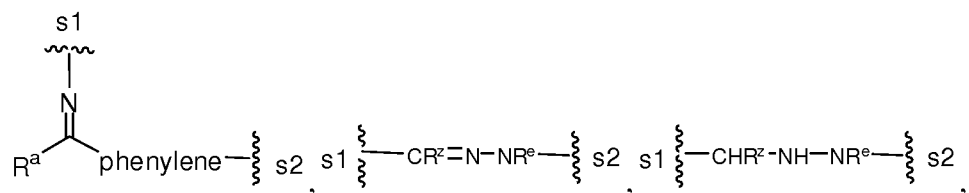
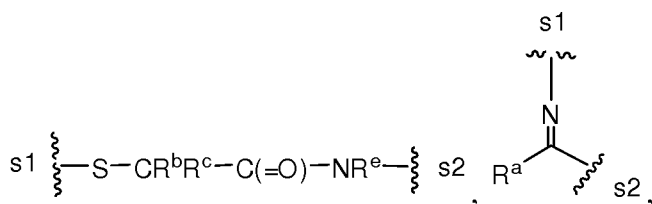
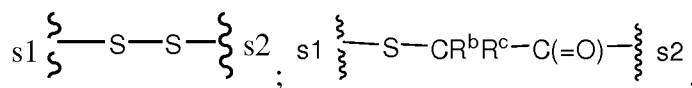
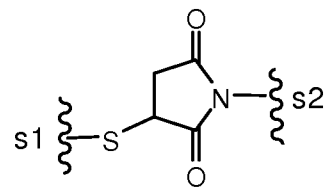
p₁ is an integer from 1 to 1000;

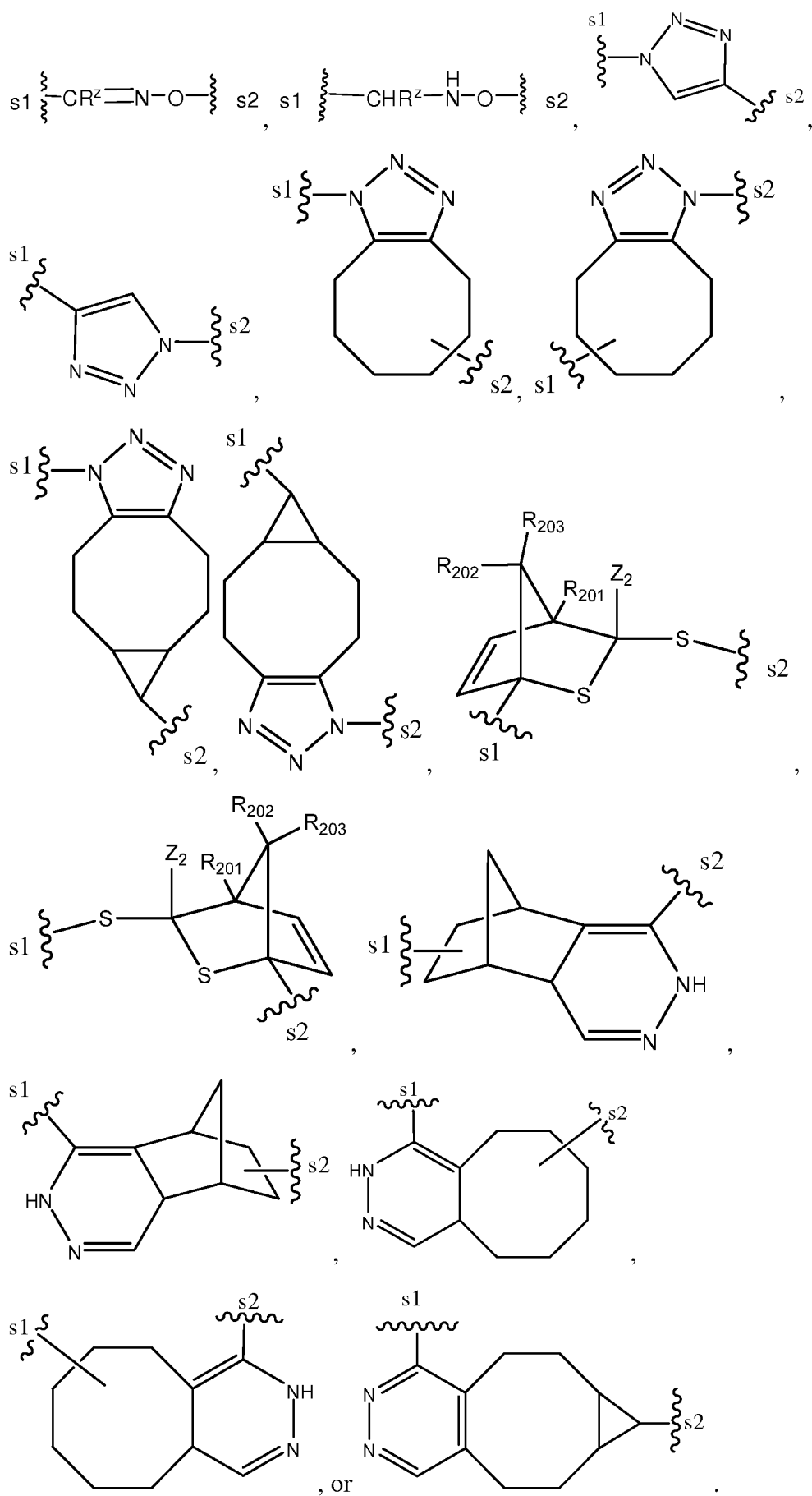
p₁' is an integer from 0 to 10;

R₉ is H or an optionally substituted alkyl; and

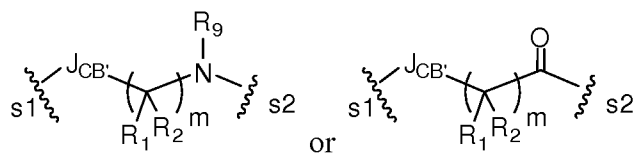
R^a, R^b, R^c, R^e and R^z, for each occurrence, are independently H or an optionally substituted alkyl.

100. The modified cell binding agent of claim 99, wherein J_{CB}' is



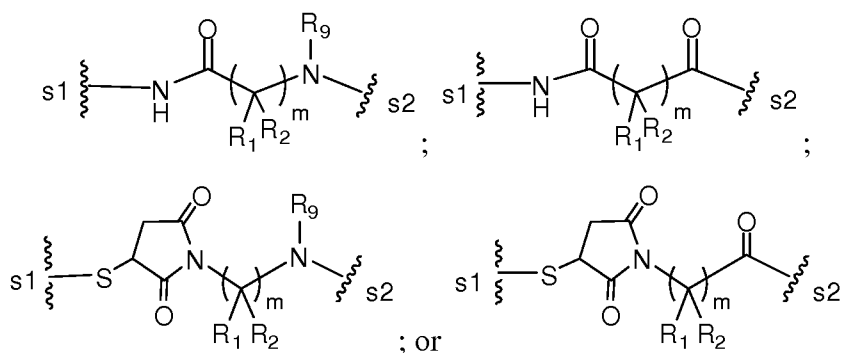


101. The modified cell binding agent of any of the claims 98-100, wherein L_1 is represented by the following formula:



wherein R_1 and R_2 , for each occurrence, is H, an optionally substituted alkyl, a charged substituent or an ionizable group; and m is an integer from 1 to 10.

102. The modified cell binding agent of any one of claims 98-101, wherein L_1 is represented by the following formula:



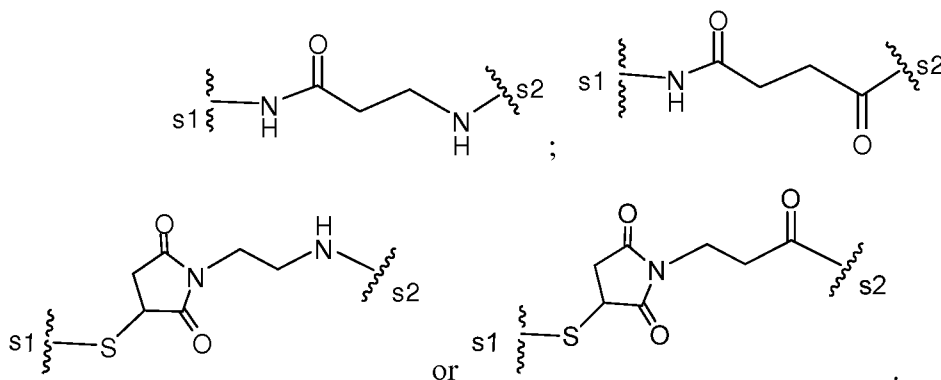
wherein:

R_1 and R_2 , for each occurrence, are each independently H or an alkyl;

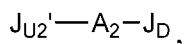
R_9 is H or an alkyl; and

m is an integer from 1 to 5.

103. The modified cell binding agent of claim 101 or 102, wherein R_1 and R_2 are both H; and R_9 is H.
104. The modified cell binding agent of claim 103, wherein L_1 is represented by the following formula:



105. The modified cell binding agent of any one of claims 98-104, wherein L_2' is represented by the following formula:

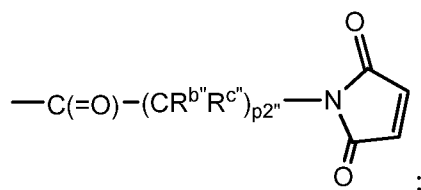


wherein:

J_{U2}' is $-C(=O)-$, $-NR_9-$ or absent;

A_2 is an optionally substituted alkylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkylalkylene, or $[XX]_{1-10}$;

J_D is $-C(=O)-X''$, $-C(=O)-(CR^{b'}R^{c'})_{p2}-C(=O)-X''$, $-C(=O)-(CH_2-CH_2-O)_{p2}-(CR^{b'}R^{c'})_{p2}-SZ$, $-SZ$, absent, $-C(=O)-(CR^{b''}R^{c''})_{p2''}-SZ$ or



X'' is $-OH$ or an carboxylic acid activating group;

Z is $-H$ or $-SR^d$;

R^d is alkyl, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl or nitropyridyl;

$[XX]$, for each occurrence, is independently an amino acid residue;

$R^{b'}$ and $R^{c'}$, for each occurrence, are independently H or an optionally substituted alkyl;

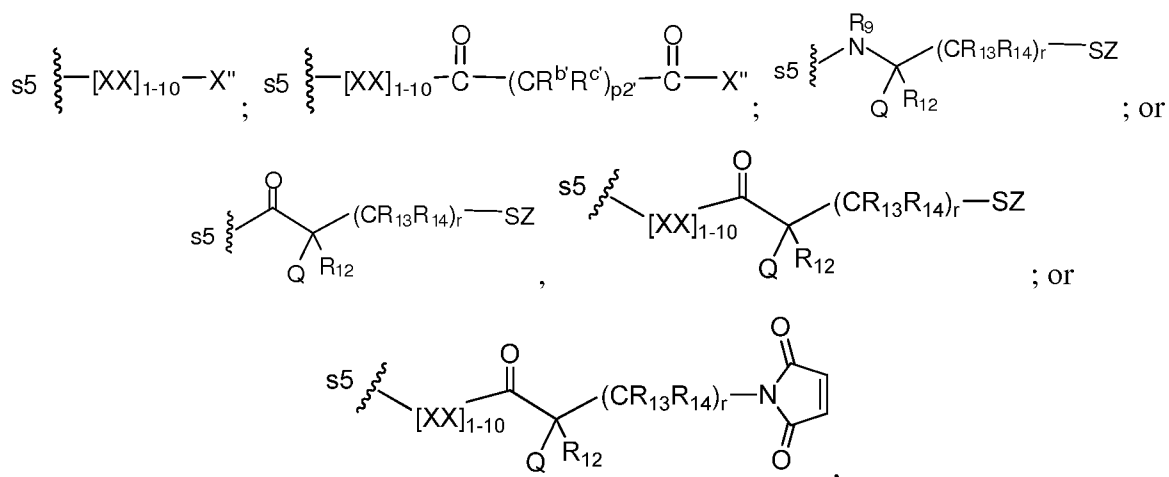
$R^{b''}$ and $R^{c''}$, for each occurrence, are independently H, an optionally substituted alkyl or a charged substituent or an ionizable group;

p_2 is an integer from 1 to 1000 ;

p_2' is an integer from 1 to 10; and

p_2'' is an integer from 1 to 10 (specifically 1 to 5).

106. The modified cell binding agent of claim 105, wherein L_2' is represented by the following formula:



wherein:

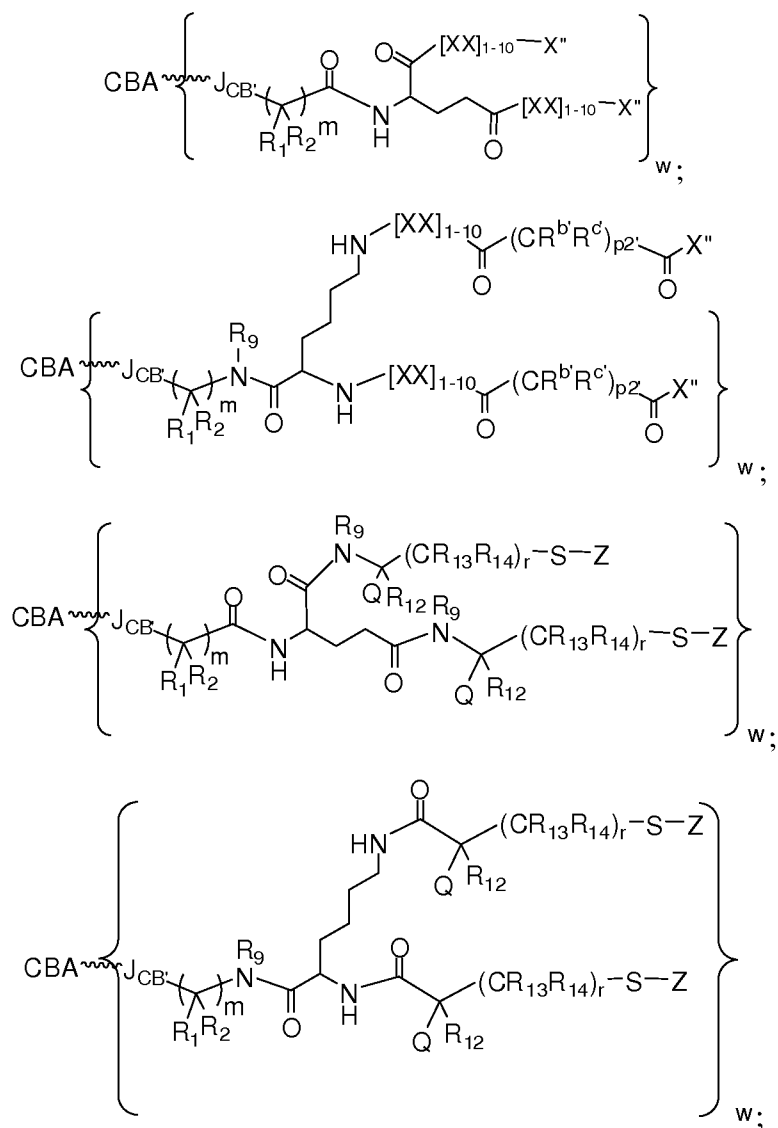
R₁₂, R₁₃, and R₁₄, for each occurrence, are each independently H, an optionally substituted alkyl;

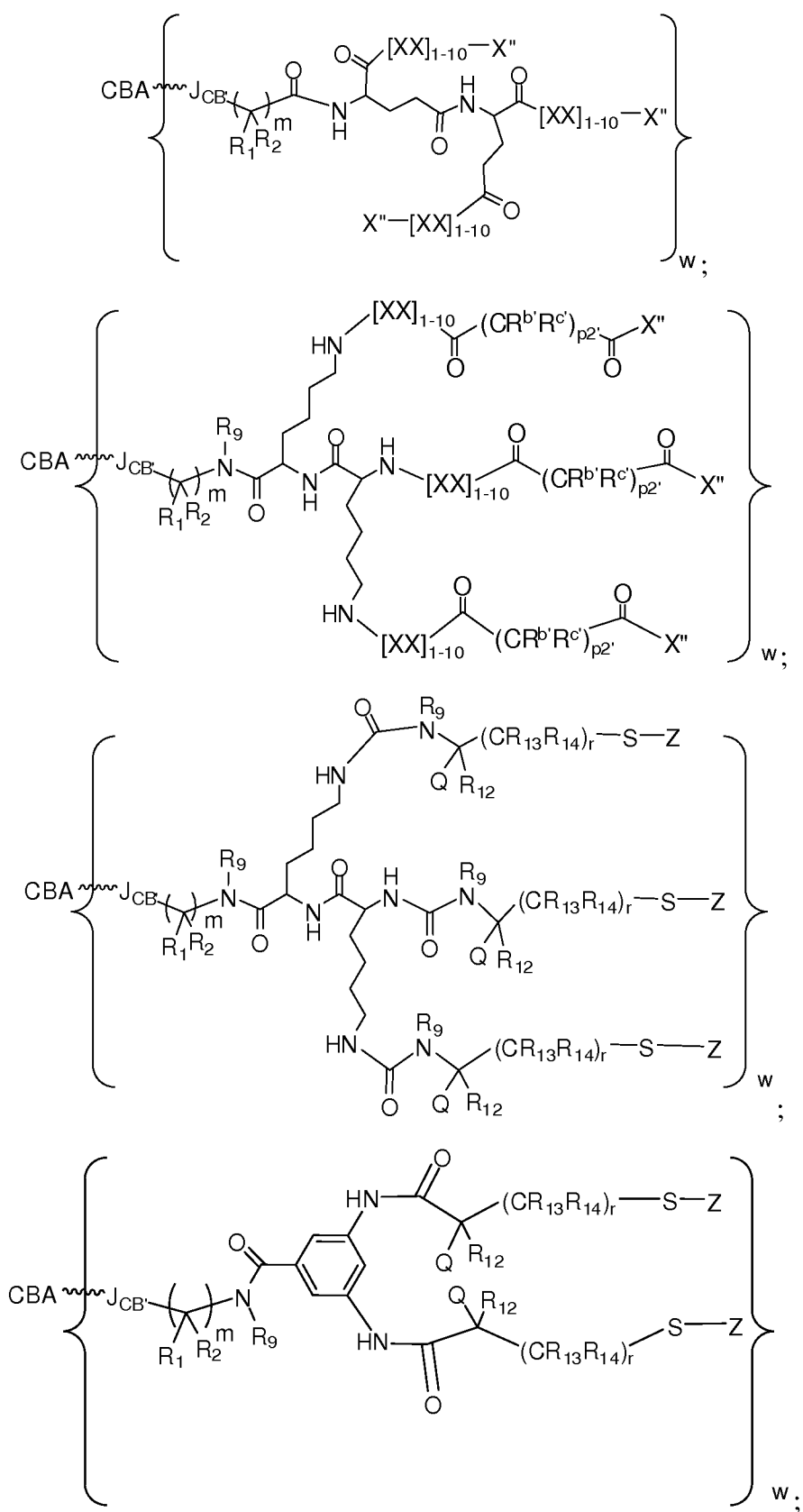
Q is H, a charged substituent or an ionizable group;

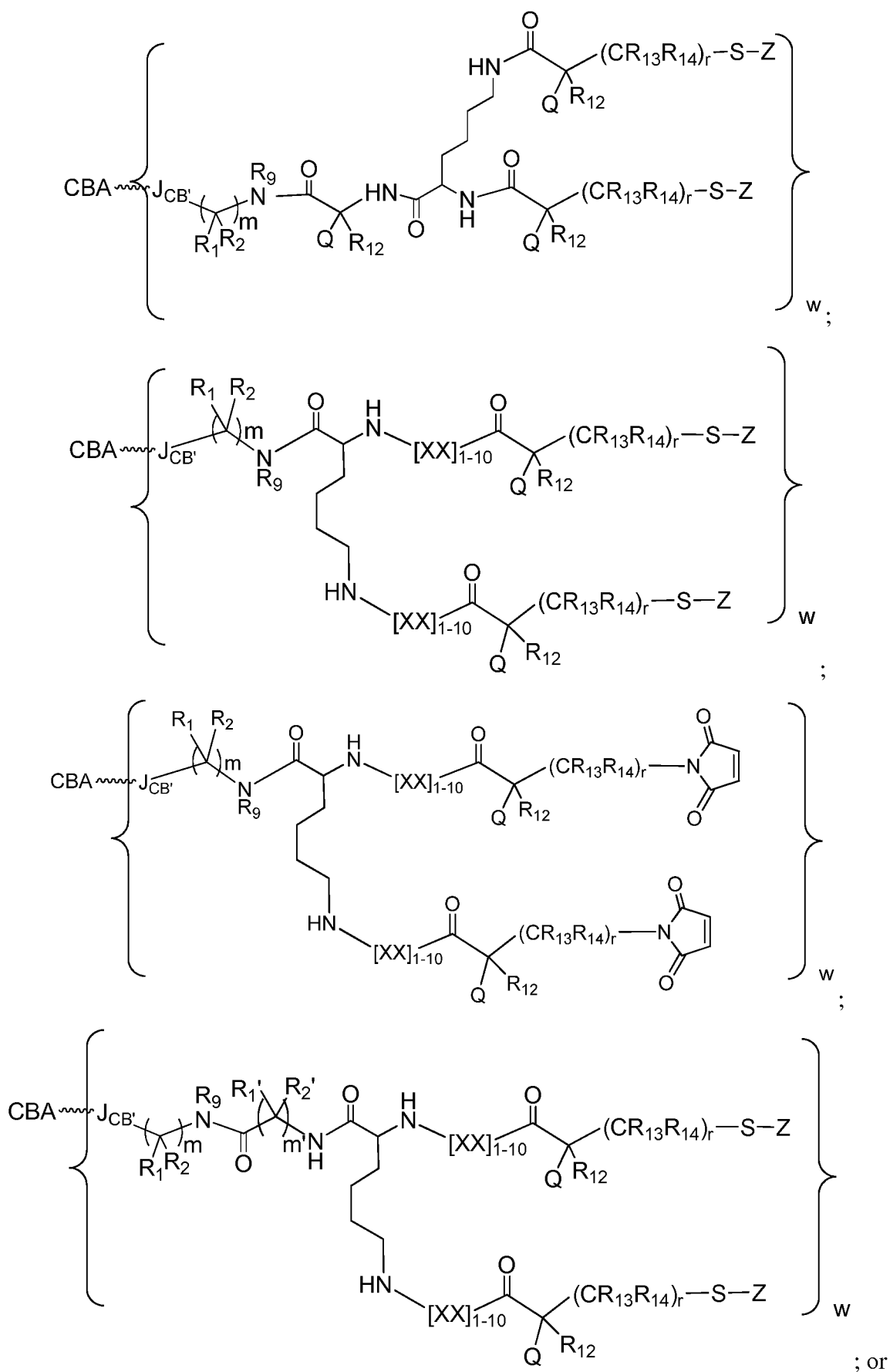
r is an integer from 0 to 10; and

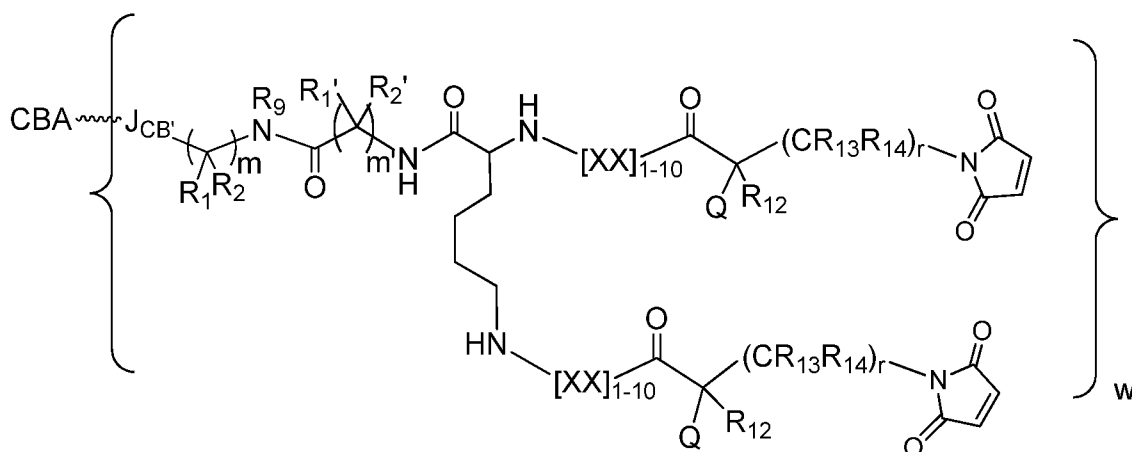
s5 is the site covalently linked to the group U.

107. The modified cell binding agent of claim 105 or 106, wherein X'' together with the adjacent -C(=O)- group is a reactive ester group; Z is SR^d ; and R^d is a pyridyl or nitropyridyl.
108. The modified cell binding agent of any one of claims 105-107, wherein the modified cell binding agent is represented by the following formula, or a salt thereof:





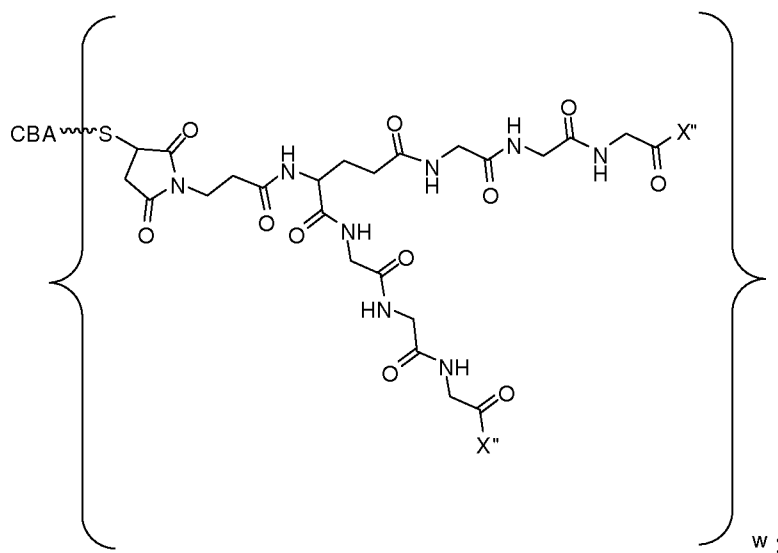


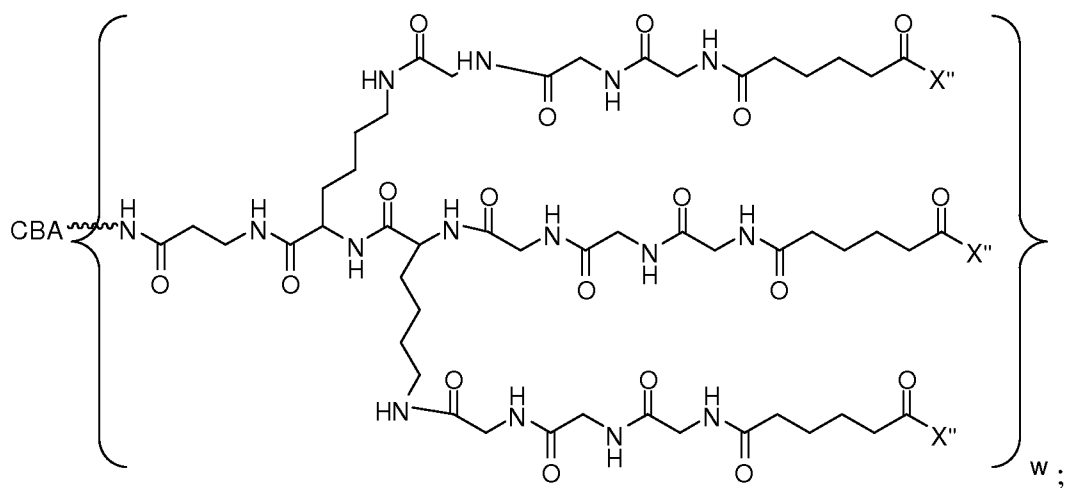
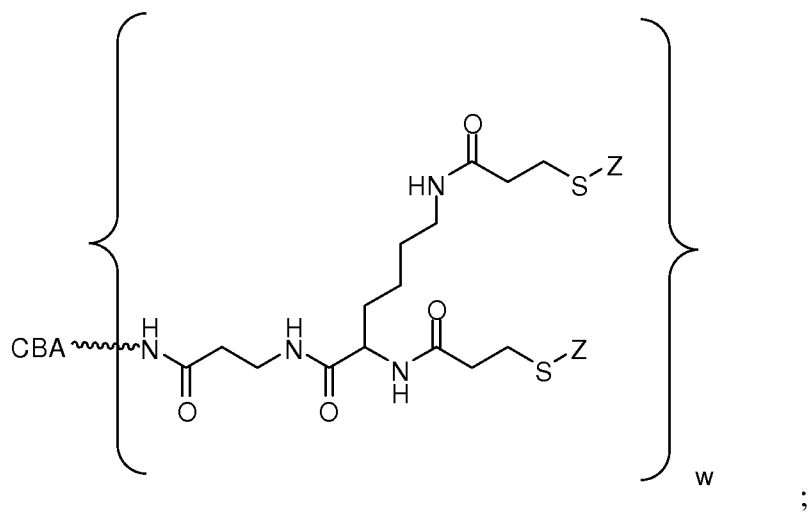
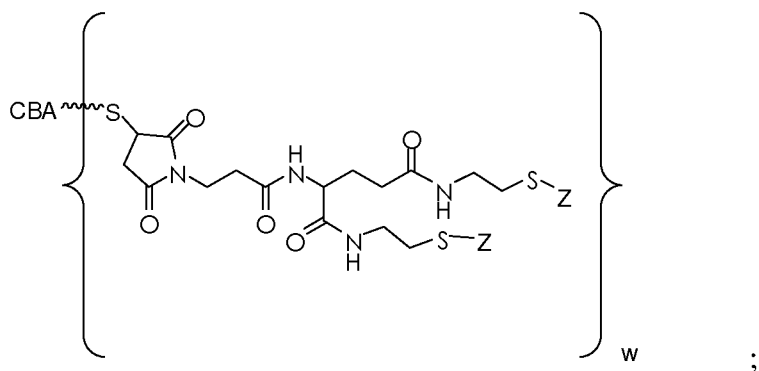
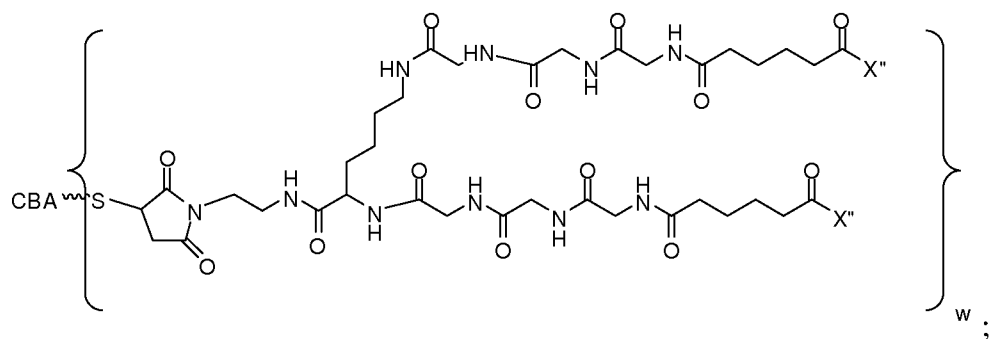


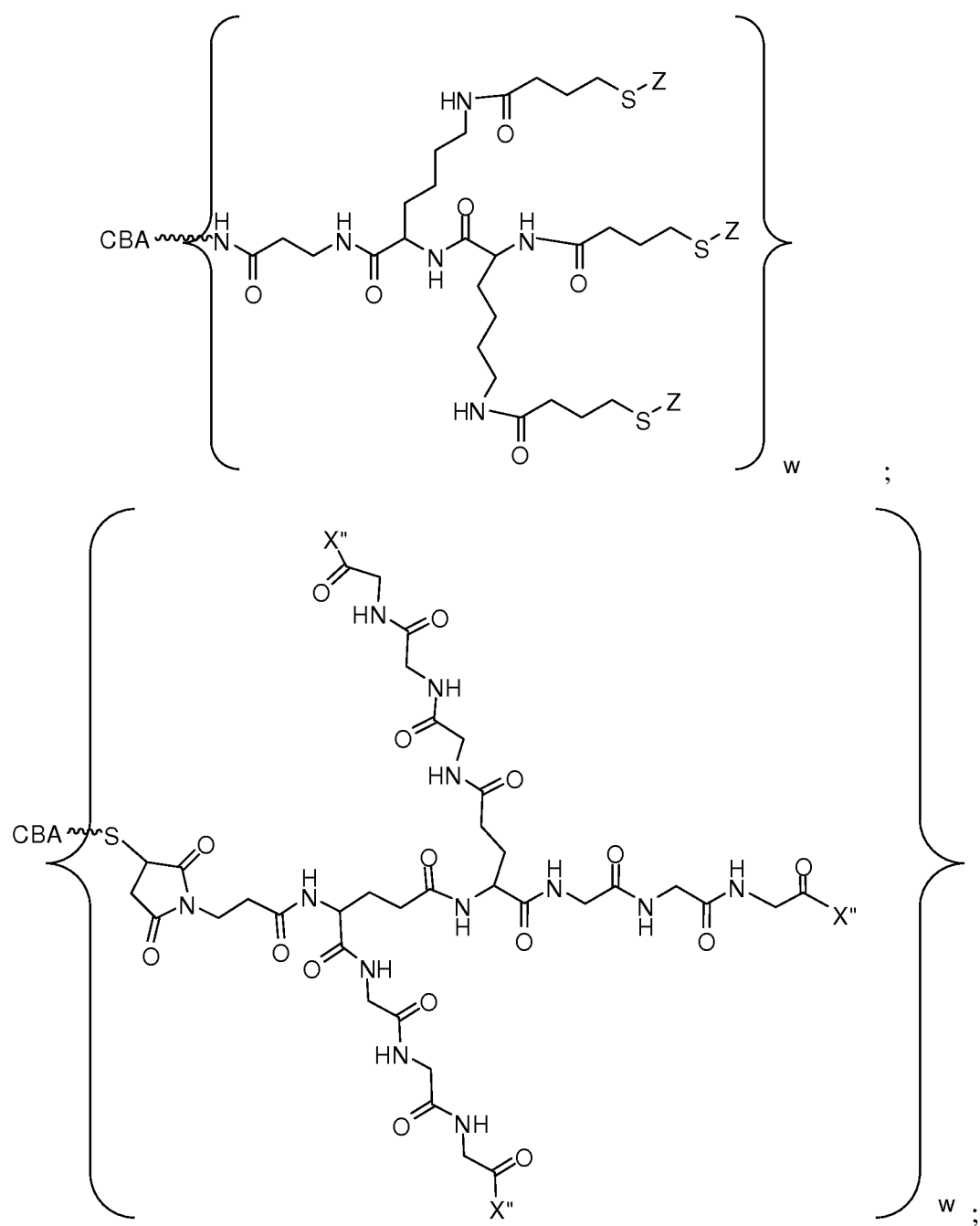
109. The modified cell binding agent of any one of claims 106-108, wherein Q is i) H; ii) –SO₃H, –Z'–SO₃H, –OPO₃H₂, –Z'–OPO₃H₂, –PO₃H₂, –Z'–PO₃H₂, –CO₂H, –Z'–CO₂H, –NR₁₀₁R₁₀₂, –Z'–NR₁₀₁R₁₀₂, or a pharmaceutically acceptable salt thereof; or iii) –N⁺R₁₀₃R₁₀₄R₁₀₅X[–] or –Z'–N⁺R₁₀₃R₁₀₄R₁₀₅X[–]; Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R₁₀₁ and R₁₀₂, are each independently H or an optionally substituted alkyl; R₁₀₃ to R₁₀₅ are each independently an optionally substituted alkyl; and X[–] is a pharmaceutically acceptable anion.
110. The modified cell binding agent of claim 109, wherein R₁₂ is H; and Q is H, SO₃H or a pharmaceutically acceptable salt thereof.
111. The modified cell binding agent of any one of claims 106 -110, wherein R^{b'} and R^{c'} are both H; and p2' is an integer from 1 to 5.
112. The modified cell binding agent of any one of claims 105-111, wherein R₁₃ and R₁₄ are both H; and r is 1 to 5.
113. The modified cell binding agent of any one of claims 105-112, wherein [XX]₁₋₁₀ is [XX]₂₋₅.
114. The modified cell binding agent of any one of claims 105-113, wherein [XX] is the residue of an amino acid independently selected from the group consisting of: histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline, serine, tyrosine, N-methyl-histidine, N-methyl-alanine, N-methyl-isoleucine, N-methyl-arginine, N-methyl-leucine, N-methyl-asparagine, N-methyl-lysine, N-methyl-aspartic acid, N-methyl-methionine, N-methyl-cysteine, N-methyl-phenylalanine, N-methyl-glutamic acid, N-methyl-threonine, N-methyl-glutamine, N-methyl-tryptophan, N-methyl-glycine, N-methyl-valine, N-methyl-

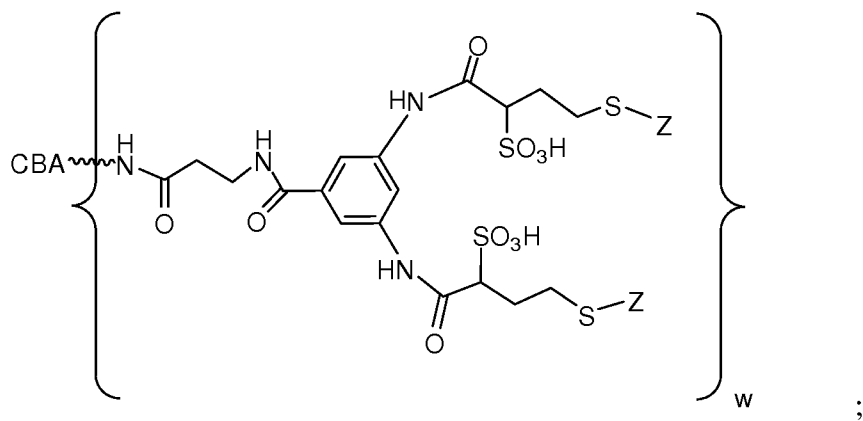
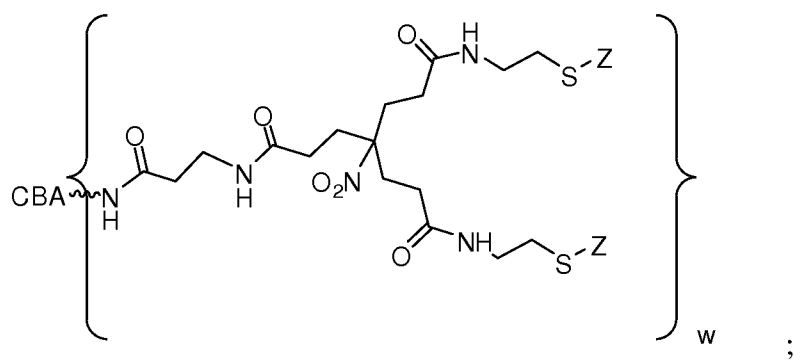
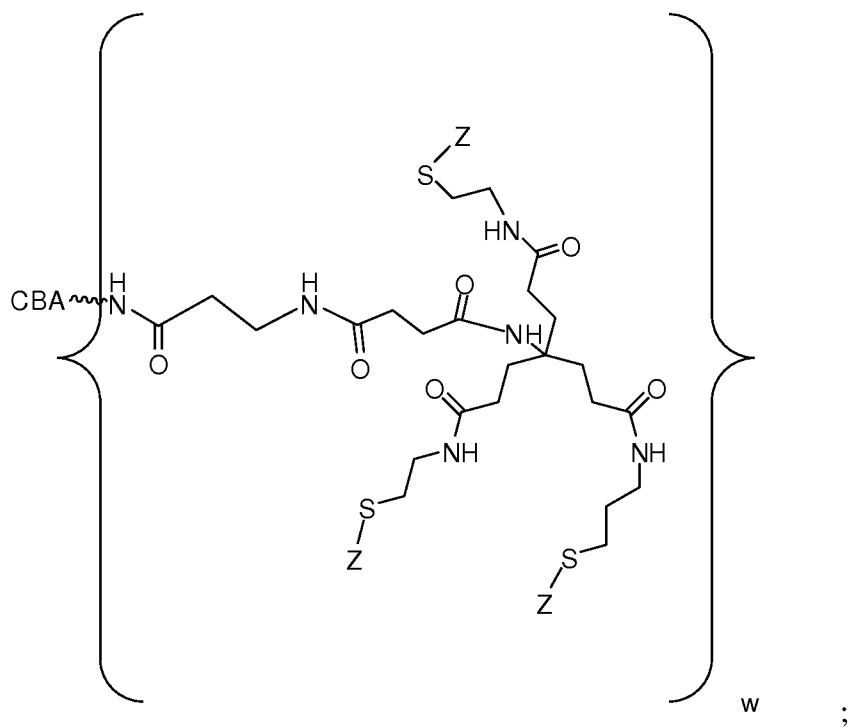
proline, N-methyl-serine, N-methyl-tyrosine, hydroxyproline, γ -carboxyglutamate, selenocysteine, O-phosphoserine, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, citrulline, ornithine, cysteine sulfonic acid, cysteine sulfinic acid, 3-aminoalanine, 3-dimethylaminoalanine, 2-amino-4-(dimethylamino)butanoic acid, 2,4-diaminobutanoic acid, 2-amino-6-(dimethylamino)hexanoic acid, 2-amino-5-(dimethylamino)pentanoic acid, and β -alanine, each independently as an L or D isomer.

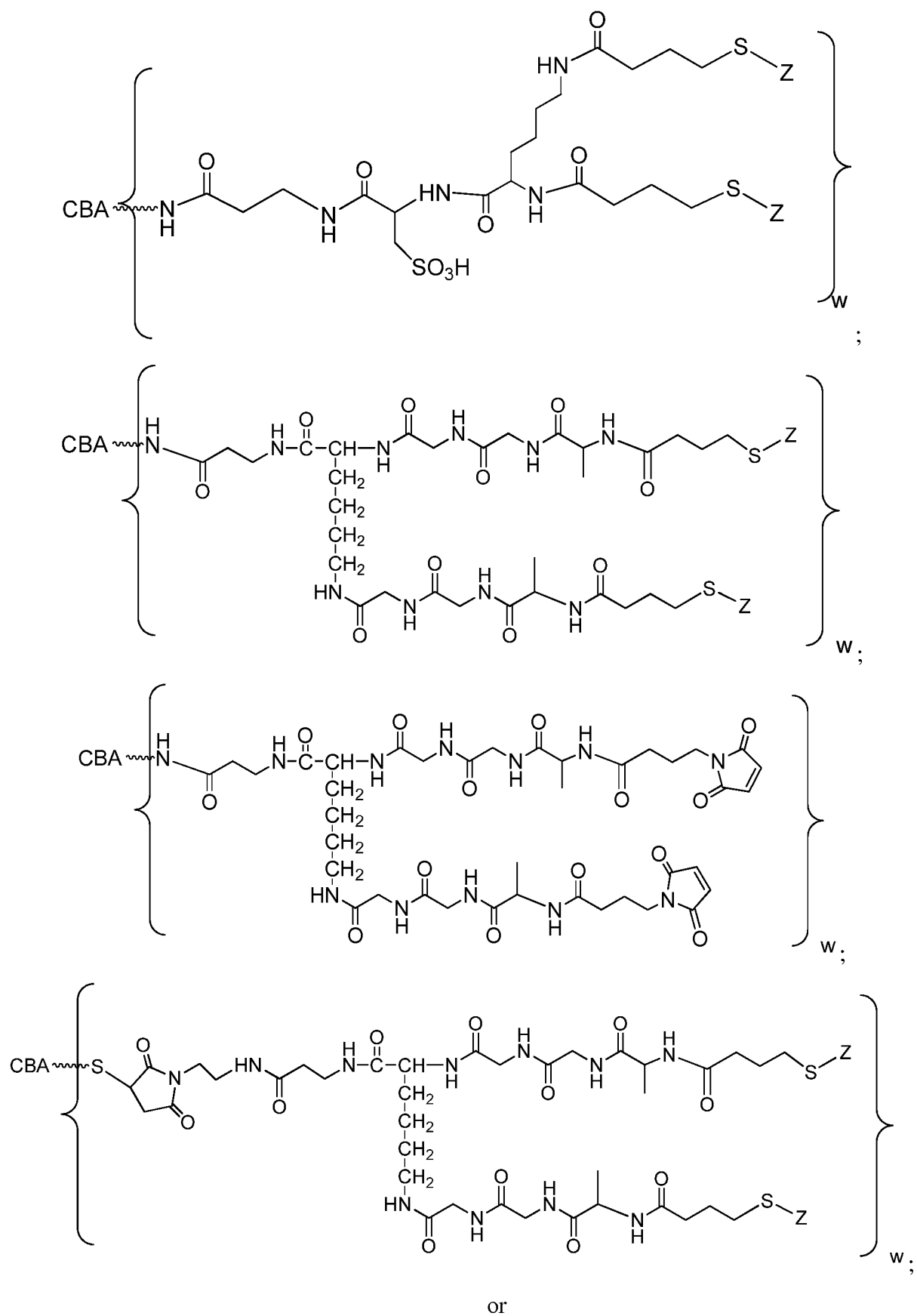
115. The modified cell binding agent of any one of claims 105-113 wherein [XX]₁₋₁₀ is selected from the group consisting of: Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Lle-Cit, Trp, Cit, Phe-Ala, Phe-N⁹-tosyl-Arg, Phe-N⁹-nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Leu-Ala-Leu, β -Ala-Leu-Ala-Leu, Gly-Phe-Leu-Gly, Val-Arg, Arg-Val, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, and D-Ala-D-Ala, Gly-Gly-Gly, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Val-Cit, and Ala-Val-Ala.
116. The modified cell binding agent of any one of claims 105-113, wherein [XX]₁₋₁₀ is Gly-Gly-Gly, Gly-Gly-Ala, Val-Ala, Glu-Ala, or Glu(OMe)-Ala.
117. The modified cell binding agent of claim 105, wherein the modified cell binding agent is represented by one of the following formulas, or a salt thereof:

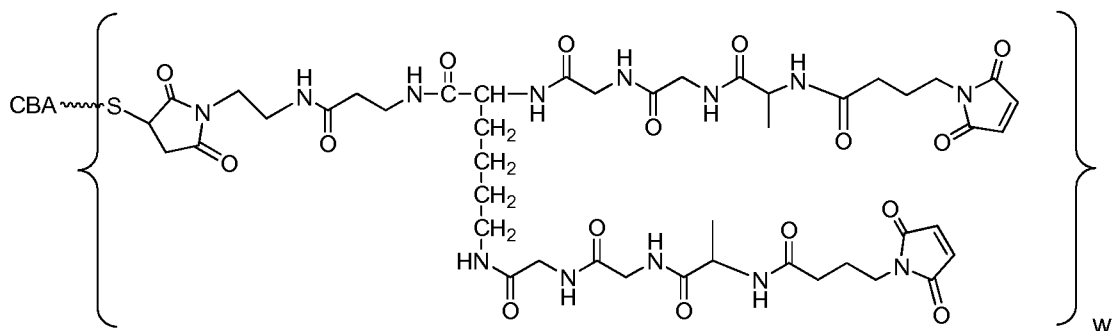




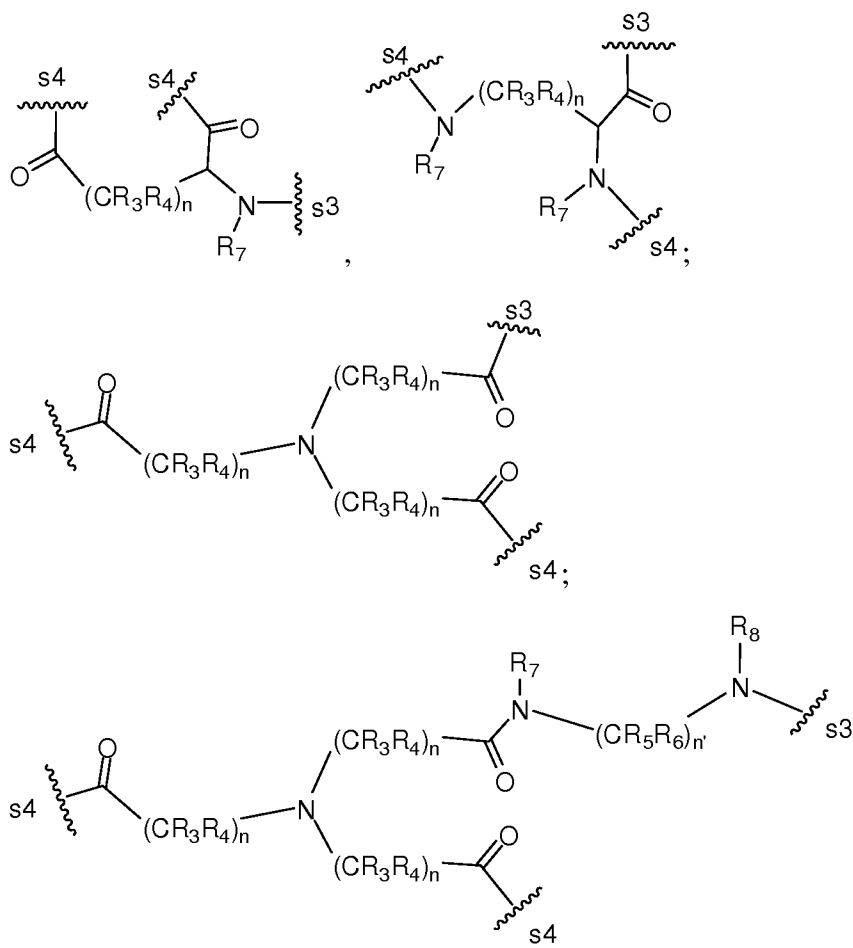


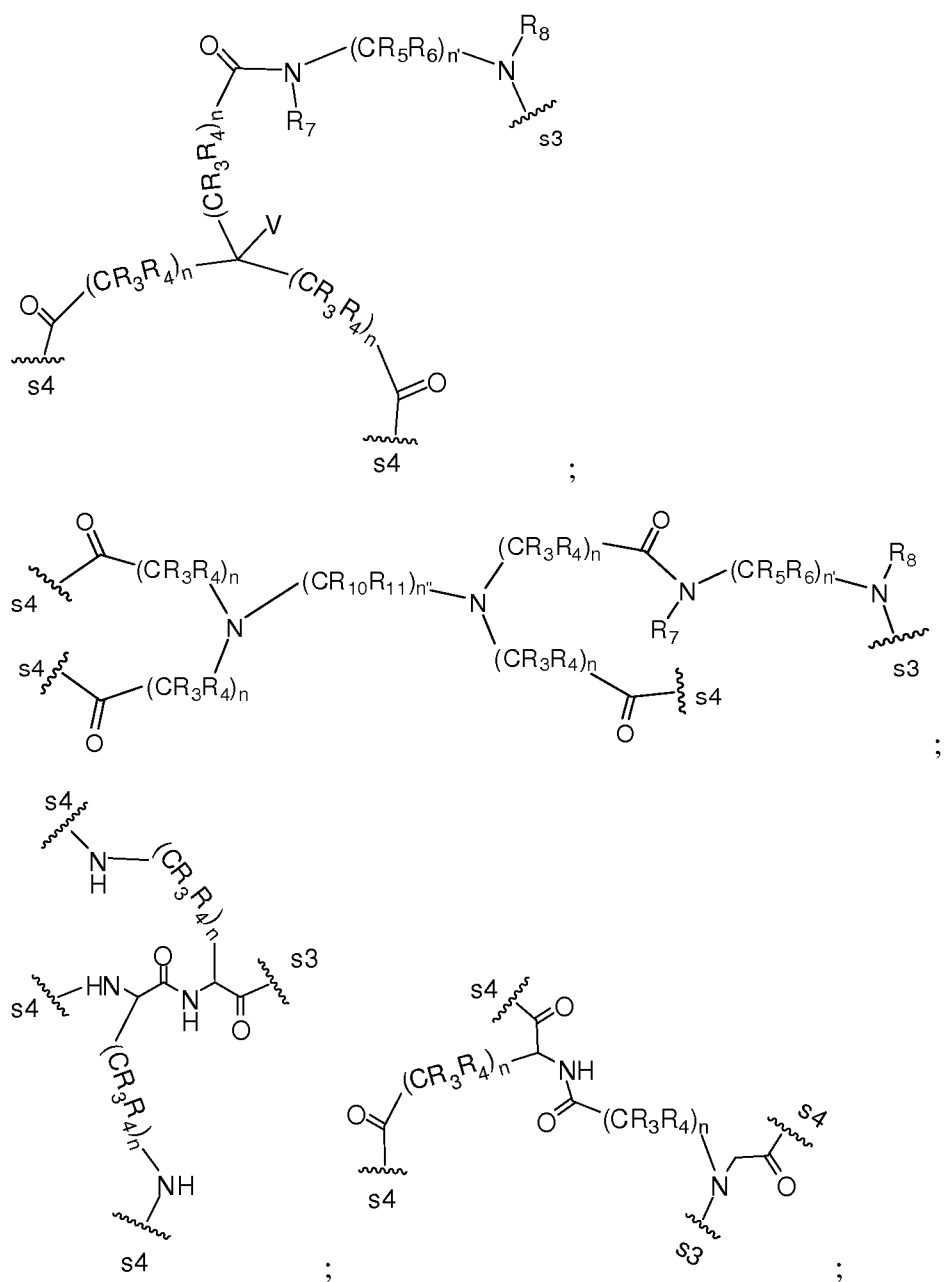


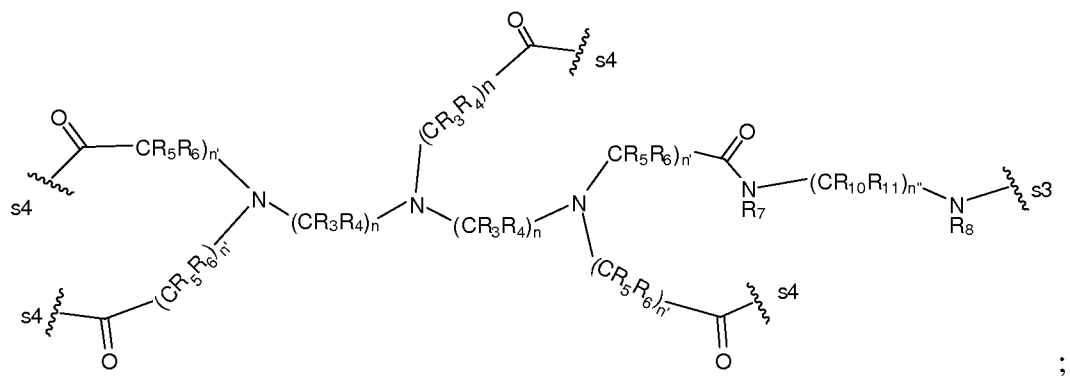
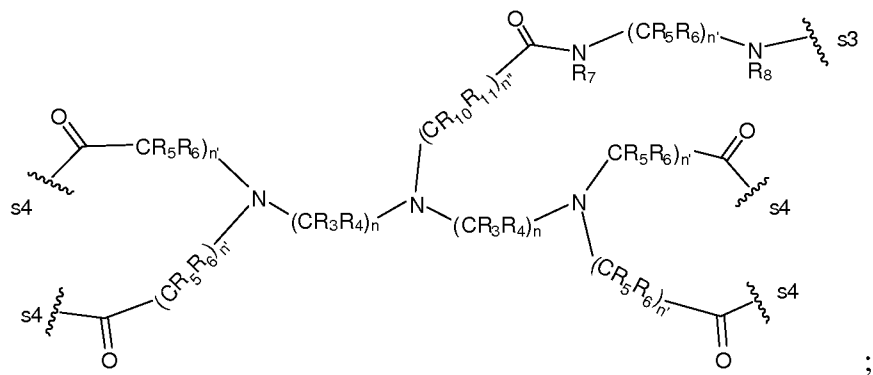
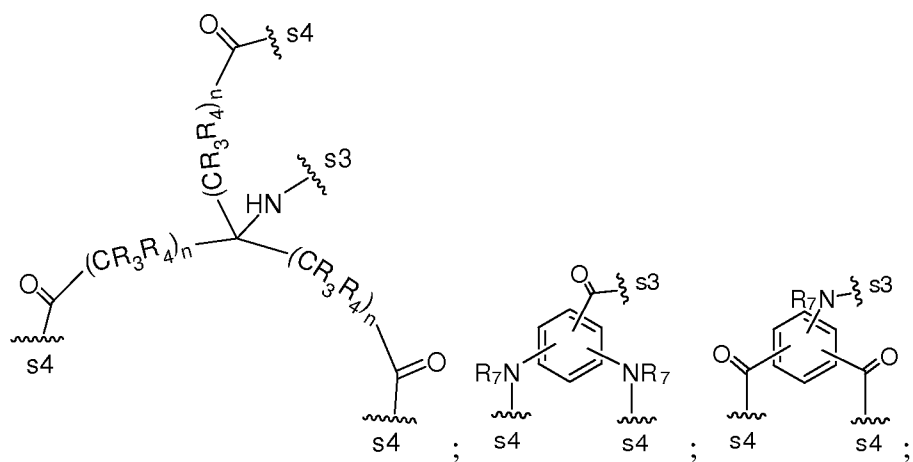


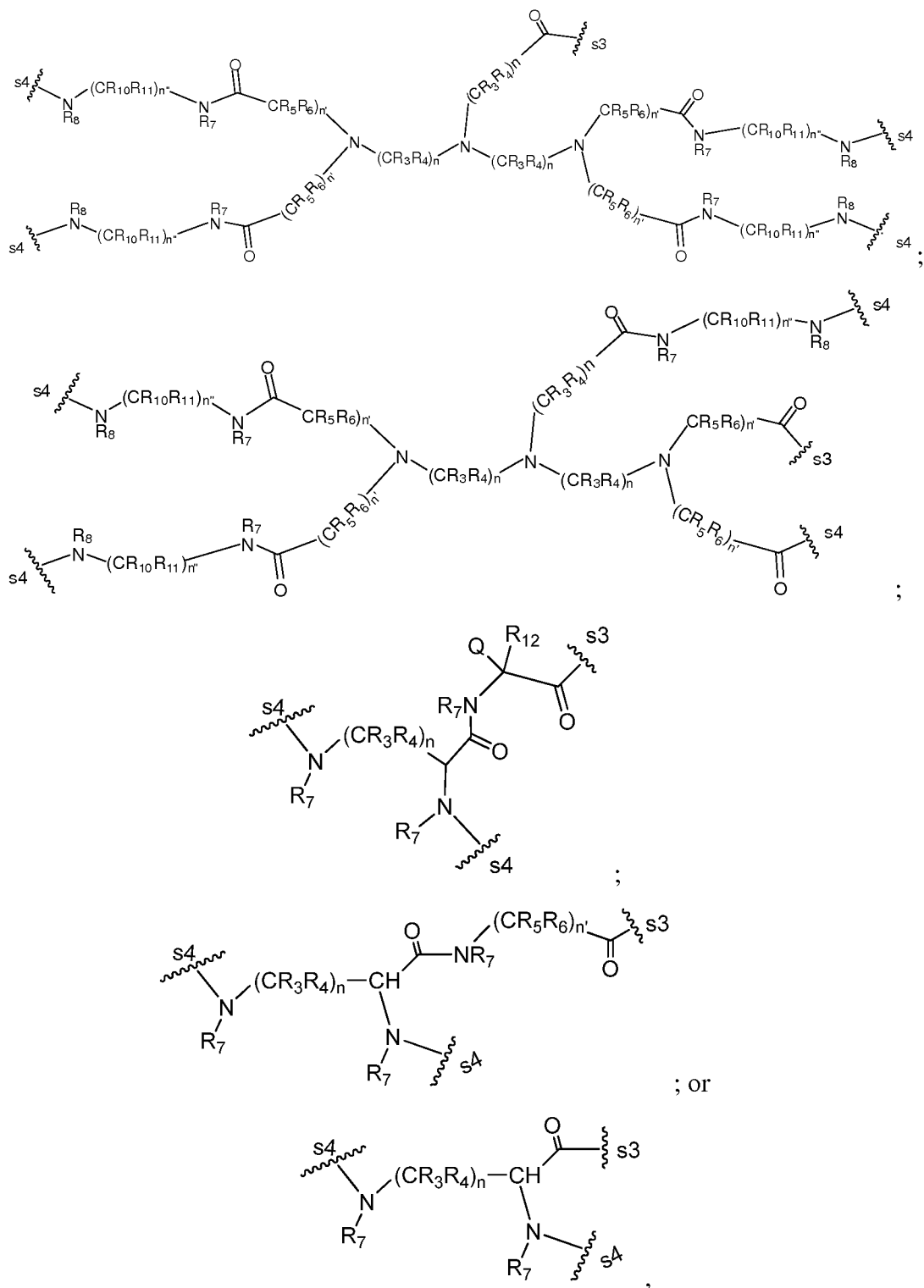


118. The modified cell binding agent of claim 117, wherein X'' together with the adjacent $-(C=O)-$ is a reactive ester; and Z is SR^d , wherein R^d is pyridyl or nitropyridyl.
119. The modified cell binding agent of any one of claims 98-107 and 109-116, wherein U is represented by the following formula:









wherein:

$R_3, R_4, R_5, R_6, R_7, R_8, R_{10}$ and R_{11} , for each occurrence, are independently H or an optionally substituted alkyl;

$n, n',$ and n'' are independently an integer from 1 to 10;

V is H, an optionally substituted alkyl, NO_2 , or $-\text{NH}-\text{C}(=\text{O})-\text{R}^{301}$;

R^{301} is H or an optionally substituted alkyl;

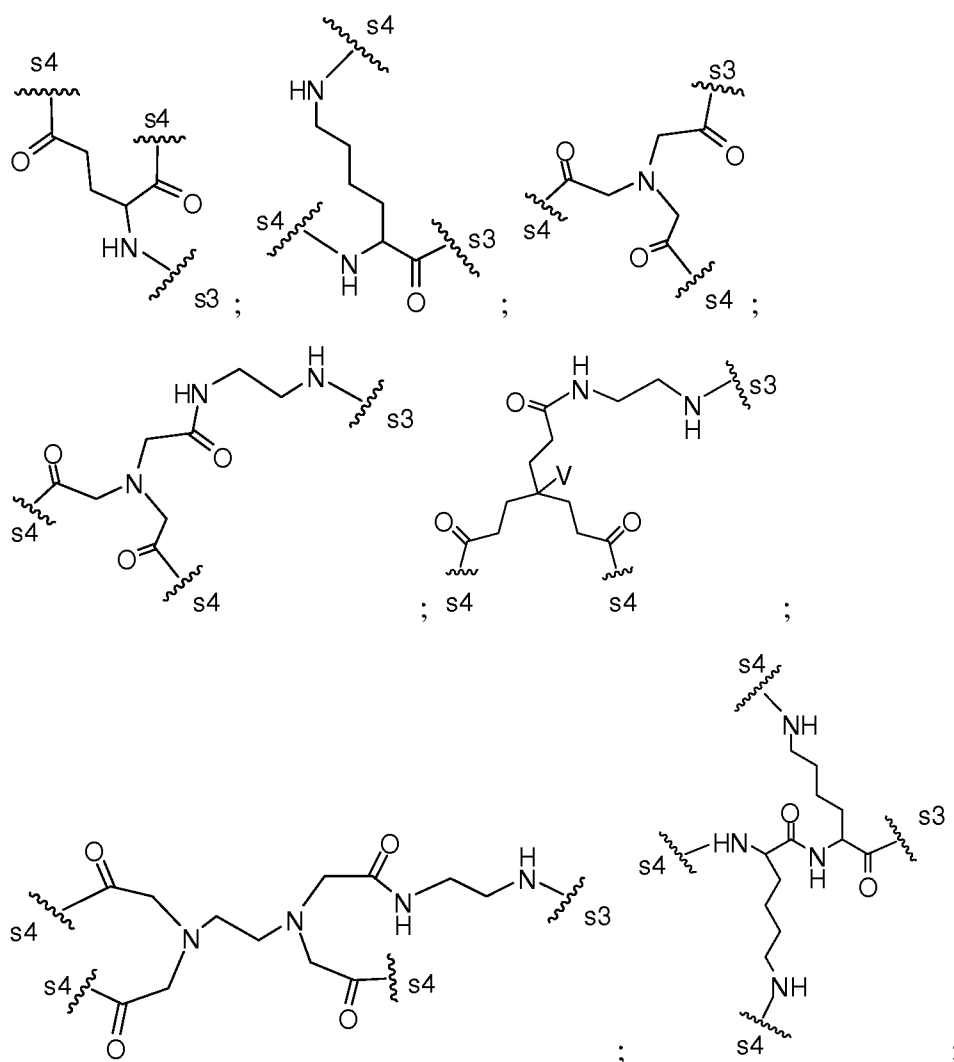
R_{12} is H, an optionally substituted alkyl;

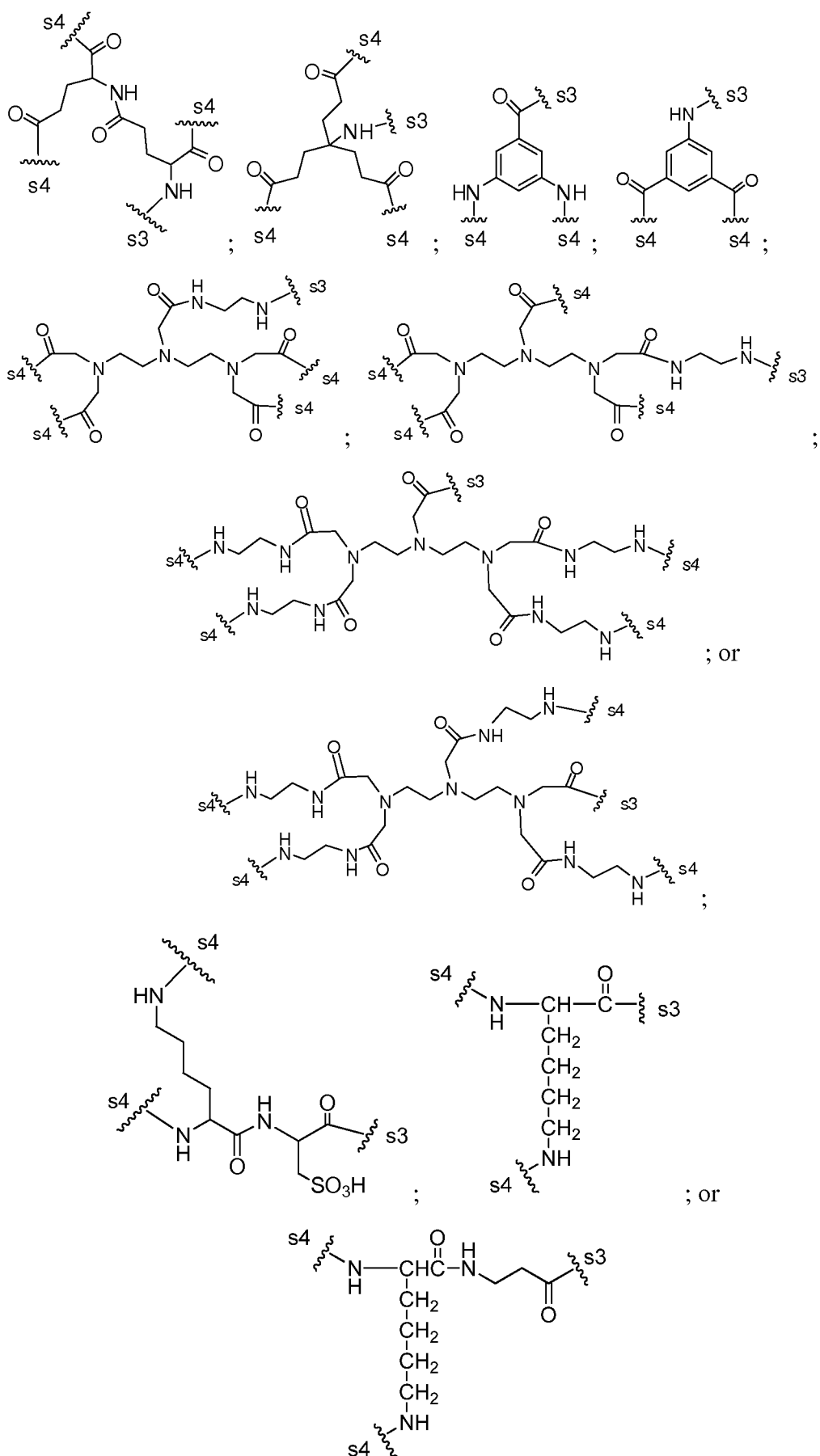
Q is H, a charged substituent or an ionizable group;

s3 is the site covalently linked the group L_1 ; and

s4 is the site covalently linked to the group L_2 .

120. The modified cell binding agent of claim 119, wherein R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} and R_{11} are all H.
121. The modified cell binding agent of claim 119 or 120, wherein n, n', and n'' are independently an integer from 1 to 5.
122. The modified cell binding agent of claim 119, wherein U is represented by the following formula:





123. The modified cell binding agent of any one of claims 119 - 122, wherein V is H or NO₂.
124. A pharmaceutical composition comprising the conjugate of any one of claims 1 - 39 or the compound of any one of claims 40 - 71; and a pharmaceutically acceptable carrier.
125. A method of inhibiting abnormal cell growth or treating a proliferative disorder, an autoimmune disorder, destructive bone disorder, infectious disease, viral disease, fibrotic disease, neurodegenerative disorder, pancreatitis or kidney disease in a mammal comprising administering to said mammal a therapeutically effective amount of the conjugate of any one of claims 1 - 39, the compound of any one of claims 40 - 71, or the composition of claim 124; and, an optionally, a second therapeutic agent.
126. The method of claim 125, wherein said second therapeutic agent is administered to said mammal sequentially or consecutively.
127. The method of claim 125 or 126 wherein the method is for treating a condition selected from the group consisting of cancer, rheumatoid arthritis, multiple sclerosis, graft versus host disease, transplant rejection, lupus, myositis, infection, and immune deficiency.
128. The method of claim 127, wherein the condition is cancer.
129. The method of claim 128, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, brain cancer, prostate cancer, kidney cancer, pancreatic cancer, ovarian cancer, head and neck cancer, melanoma, colorectal cancer, gastric cancer, squamous cancer, small-cell lung cancer, non small-cell lung cancer, testicular cancer, Merkel cell carcinoma, glioblastoma, neuroblastoma, a cancer of a lymphatic organ, and a hematological malignancy.

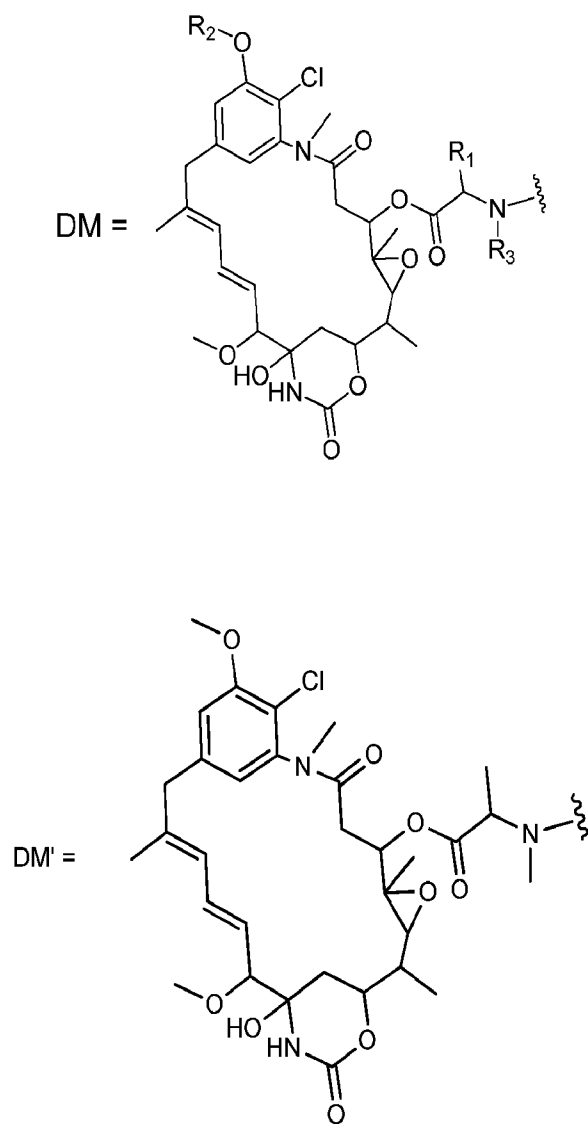


Figure 1

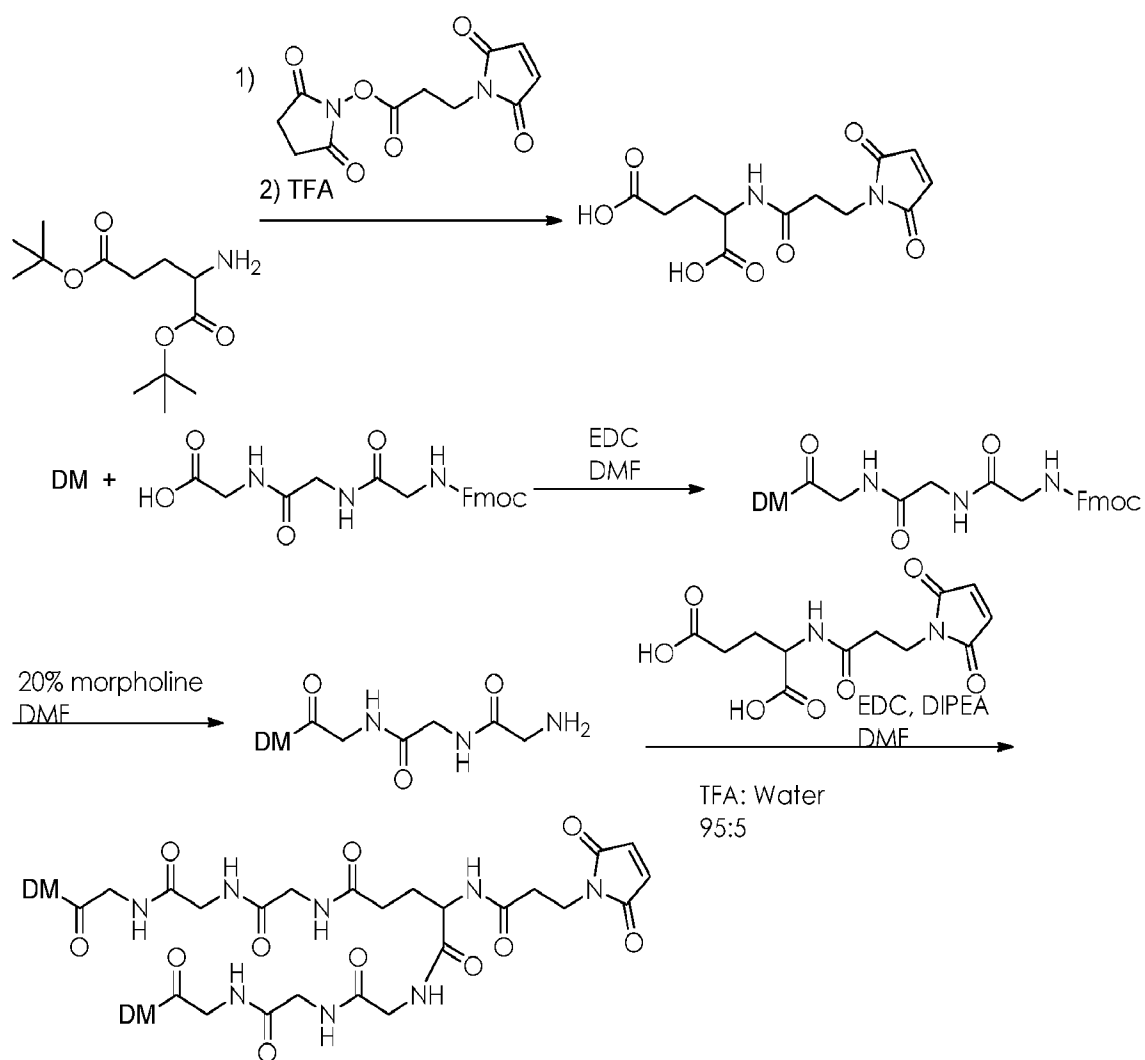


Figure 2

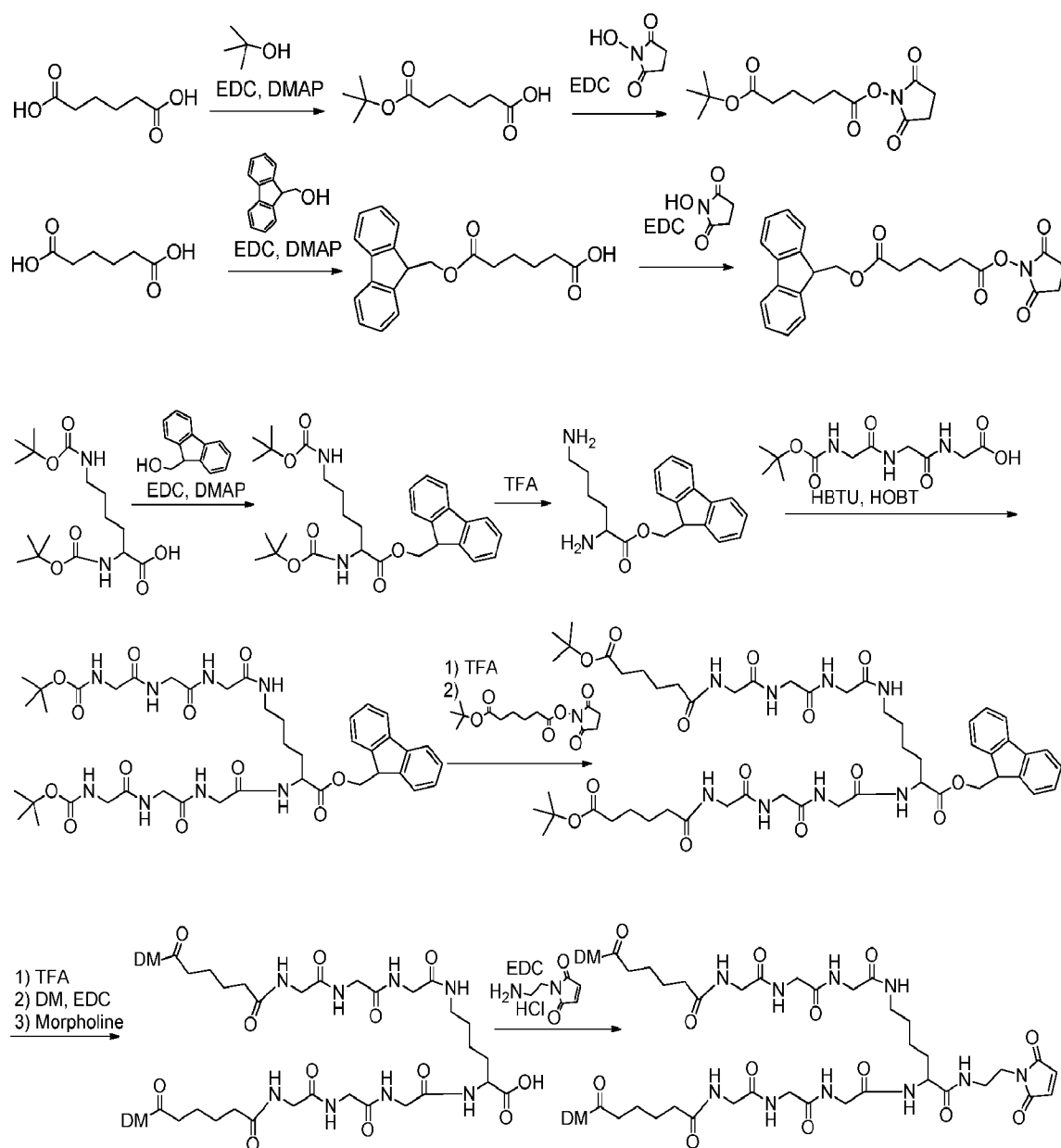


Figure 3

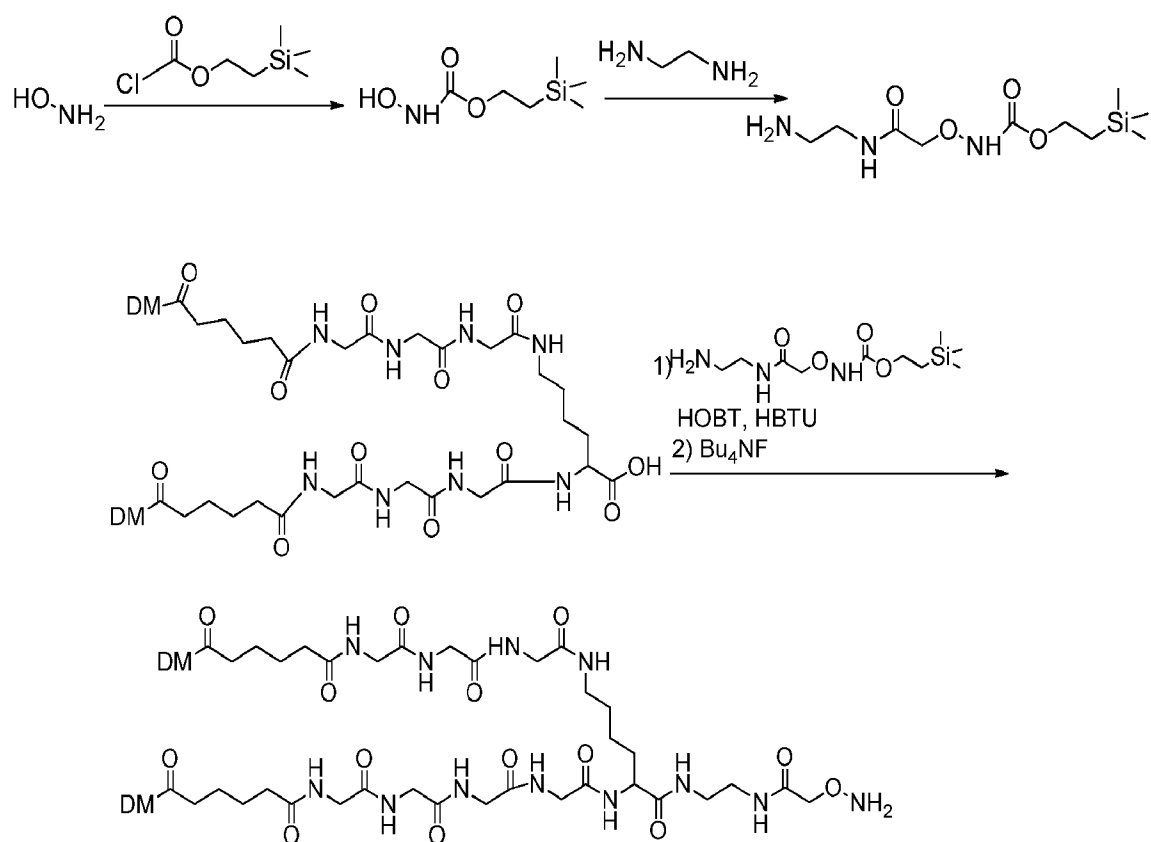
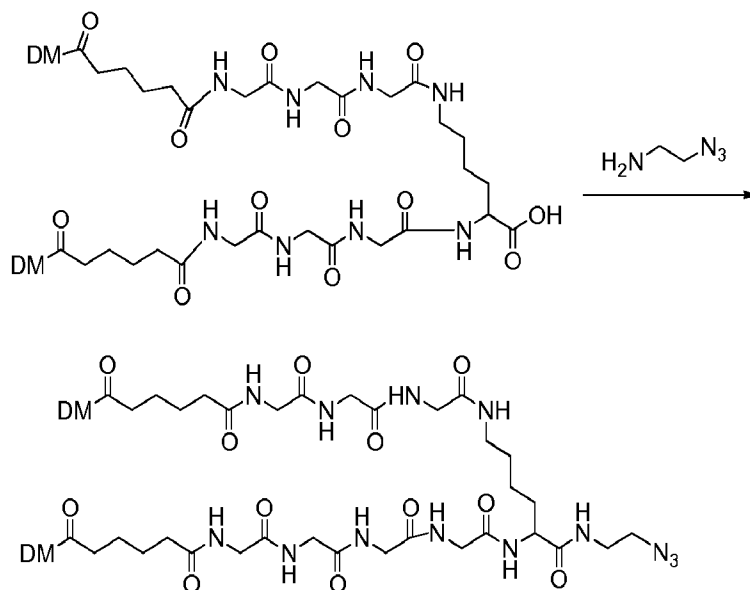


Figure 4

**Figure 5**

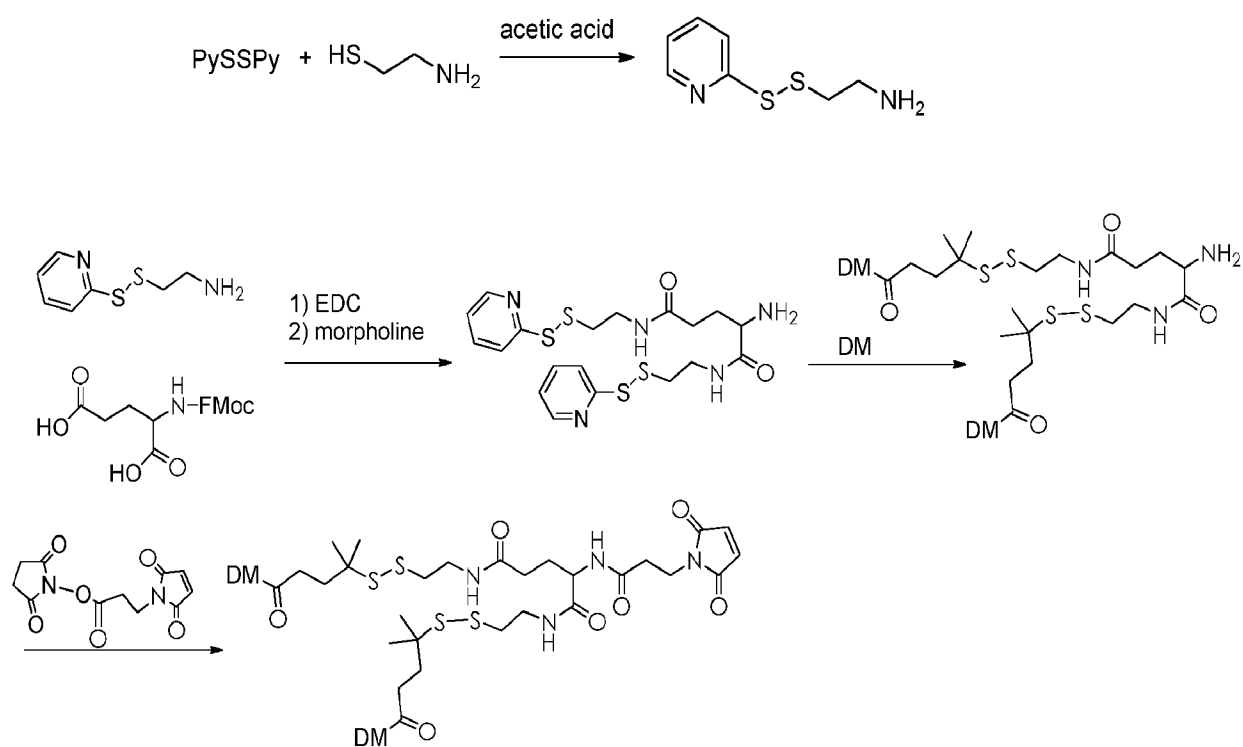


Figure 6

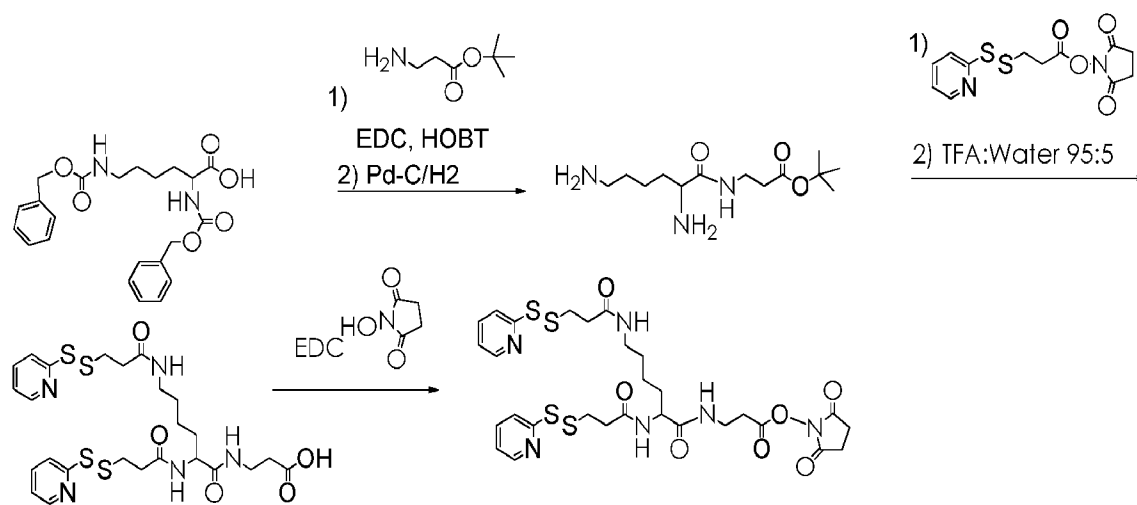


Figure 7

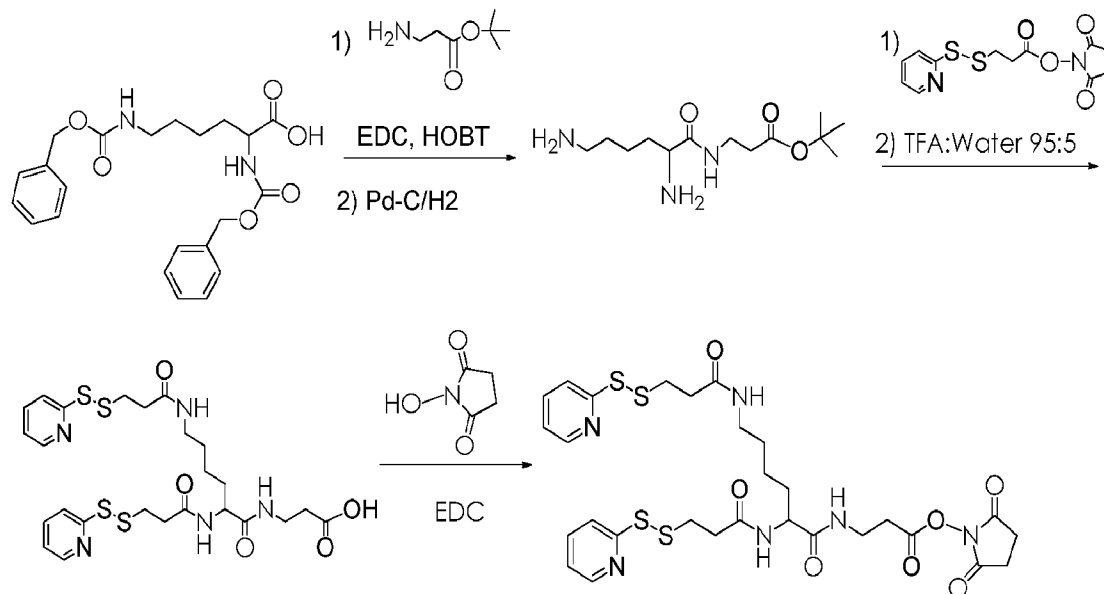


Figure 8

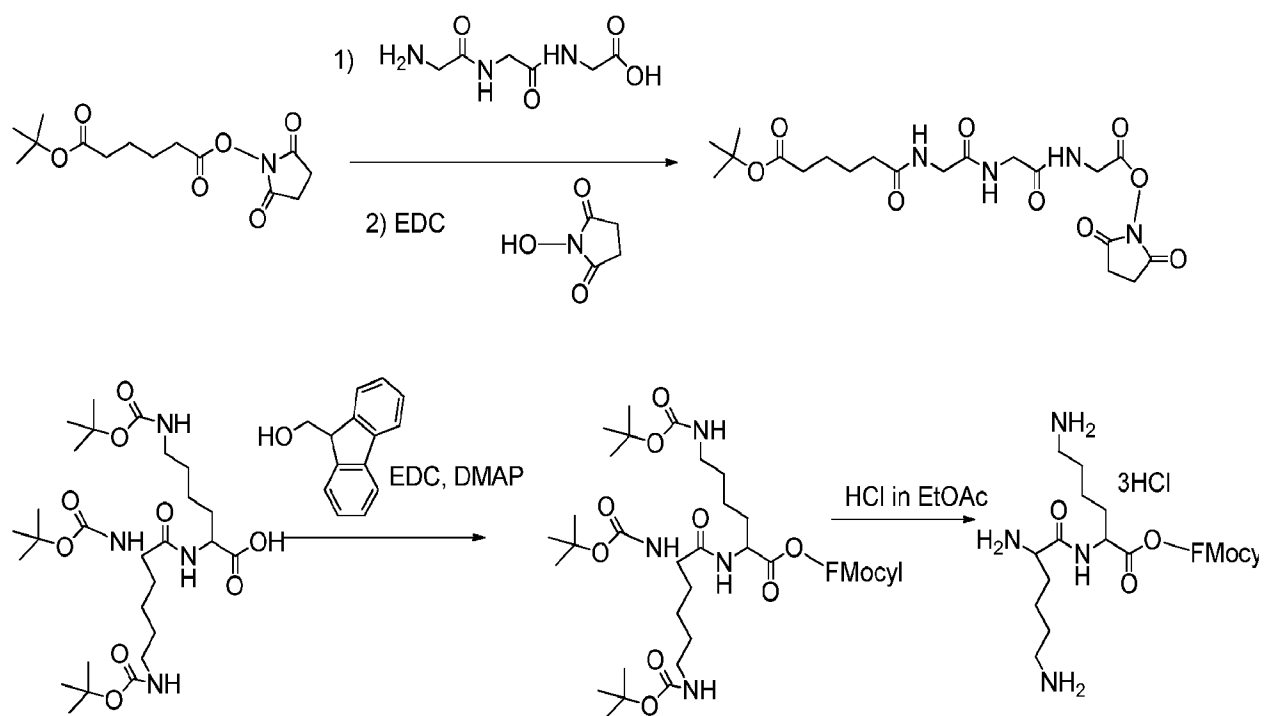


Figure 9A

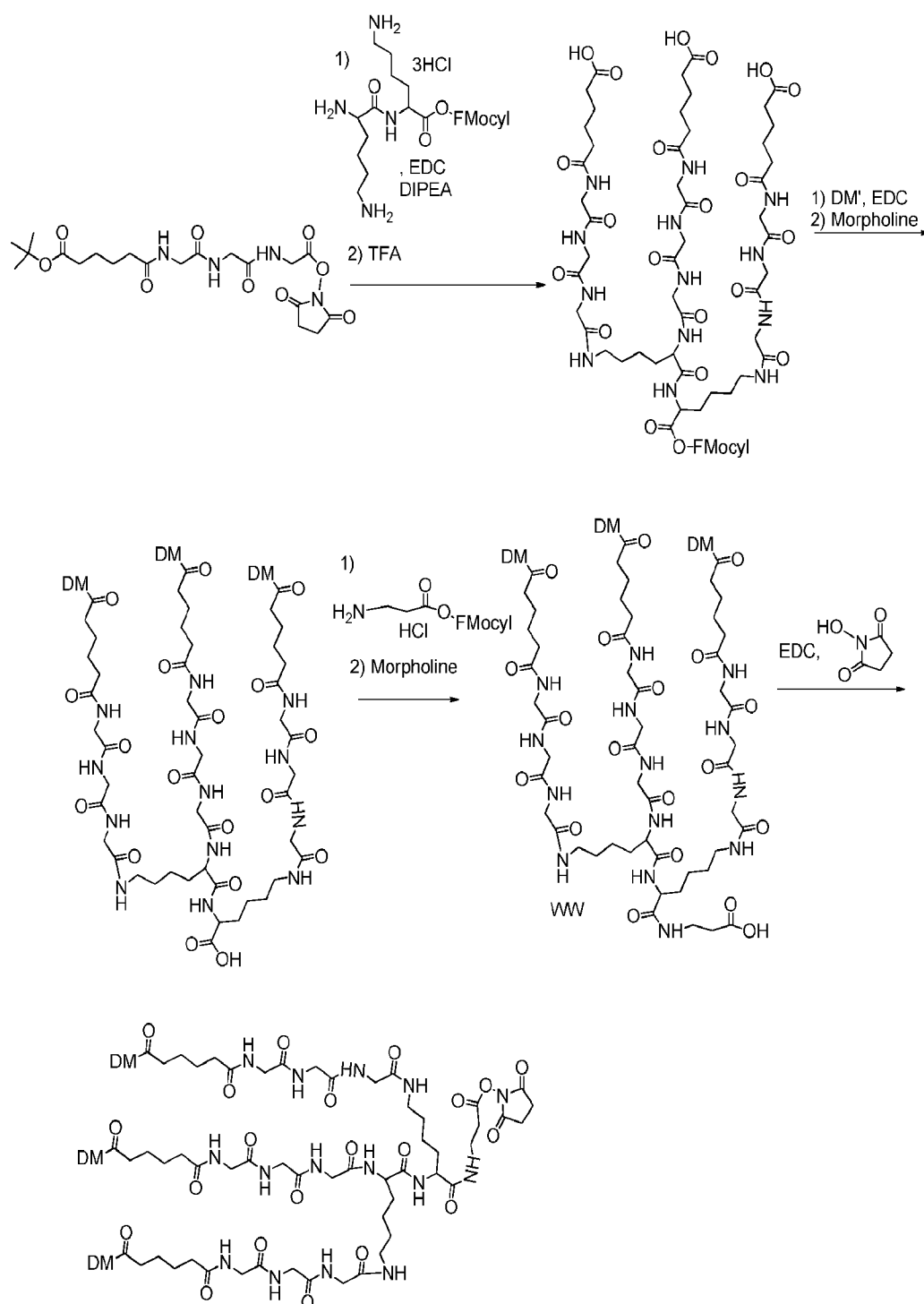


Figure 9B

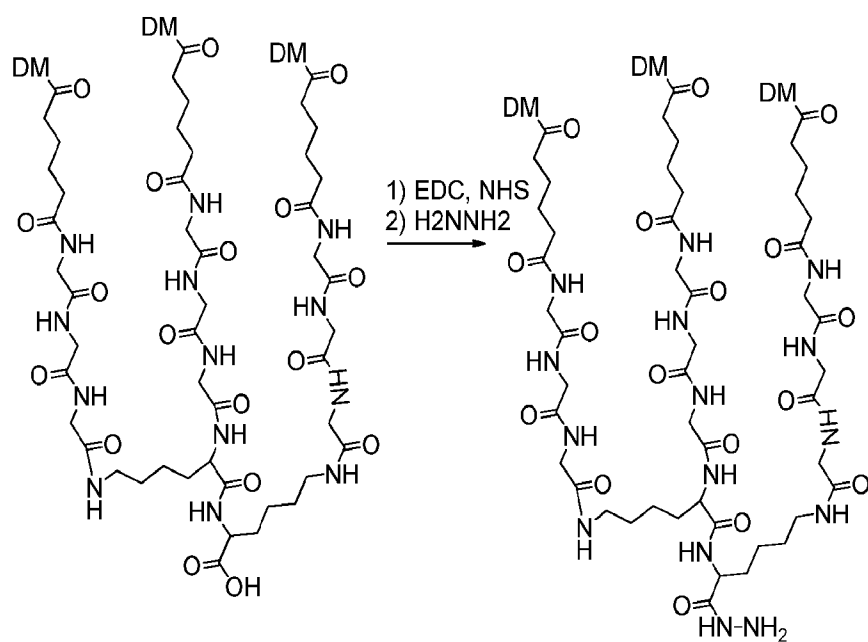


Figure 10

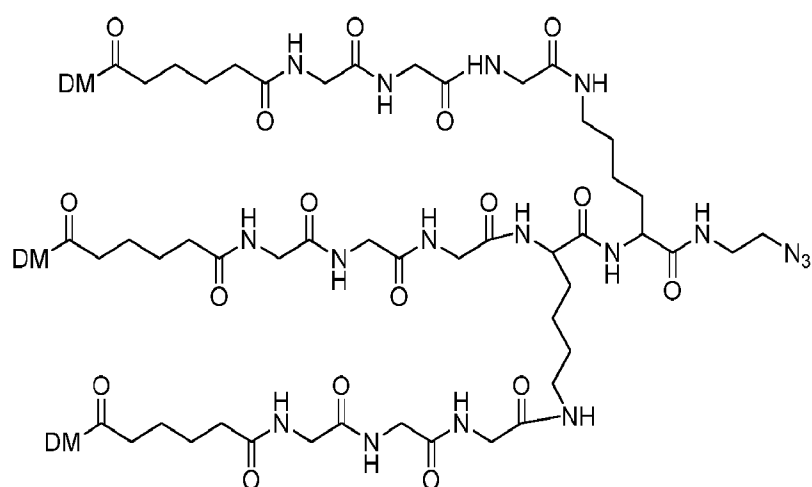


Figure 11

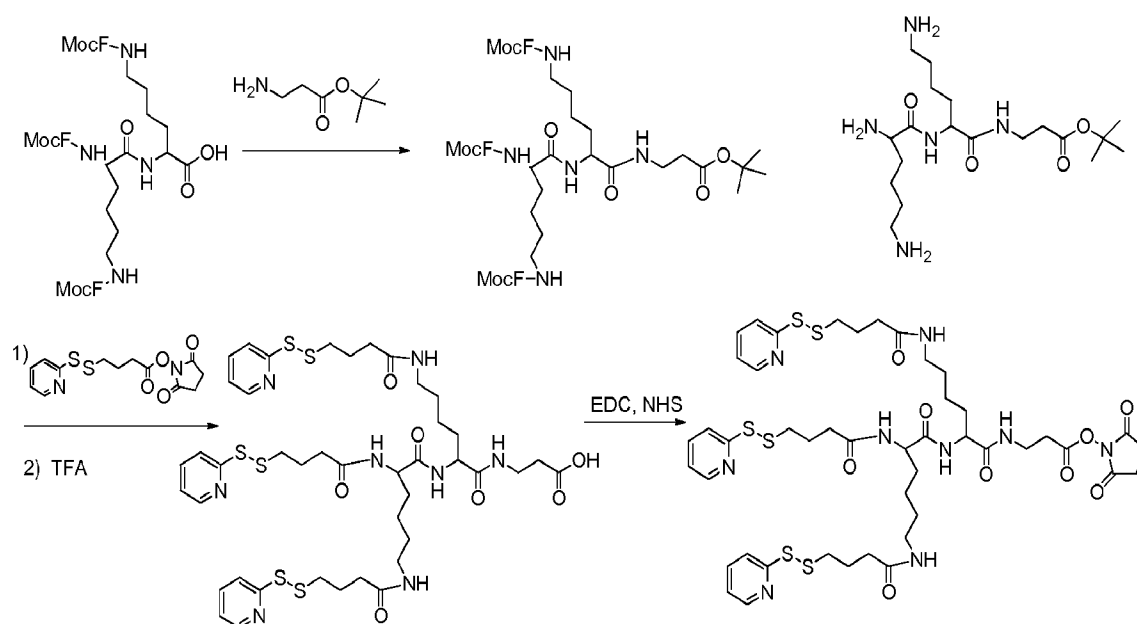


Figure 12

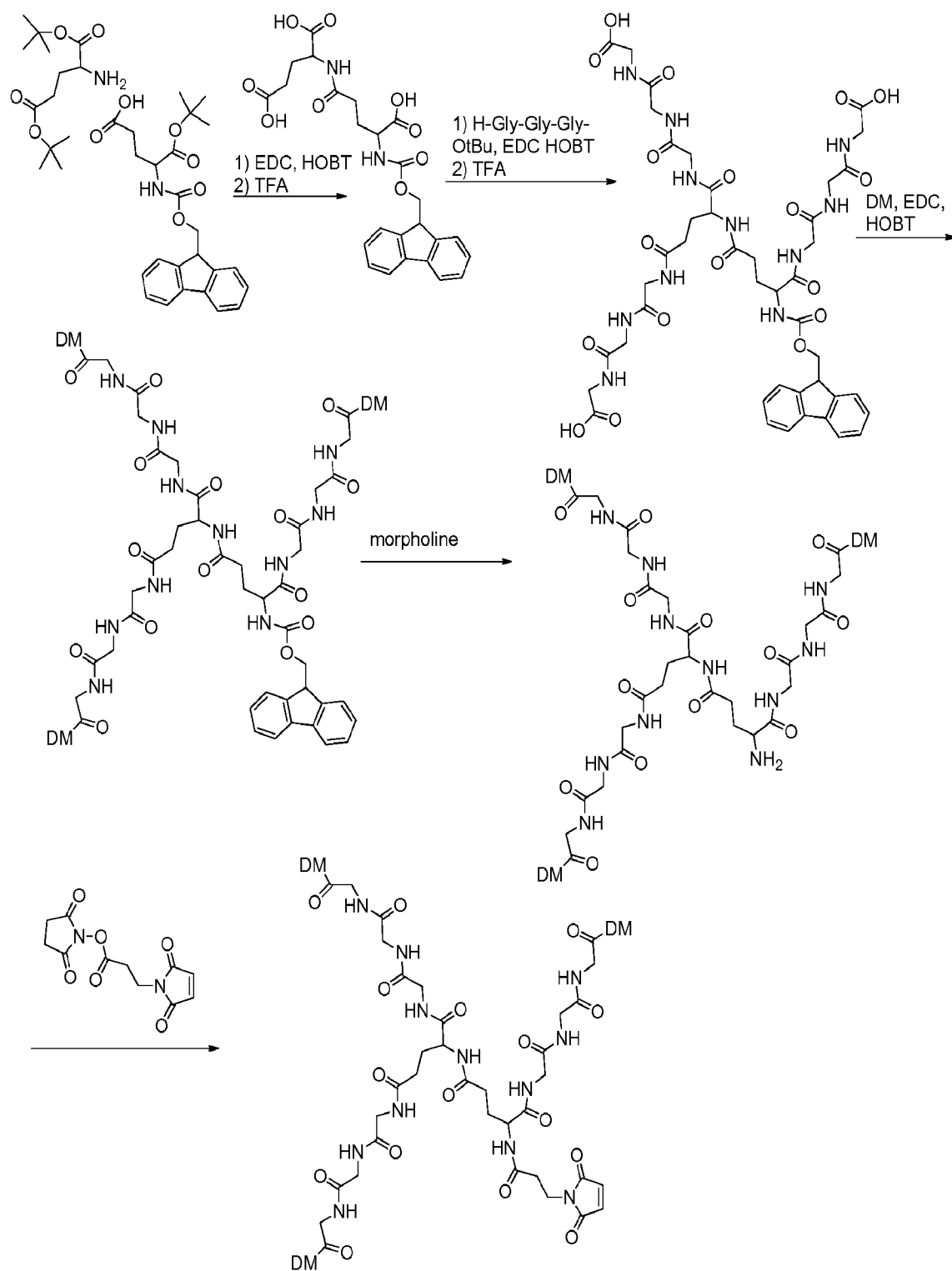


Figure 13

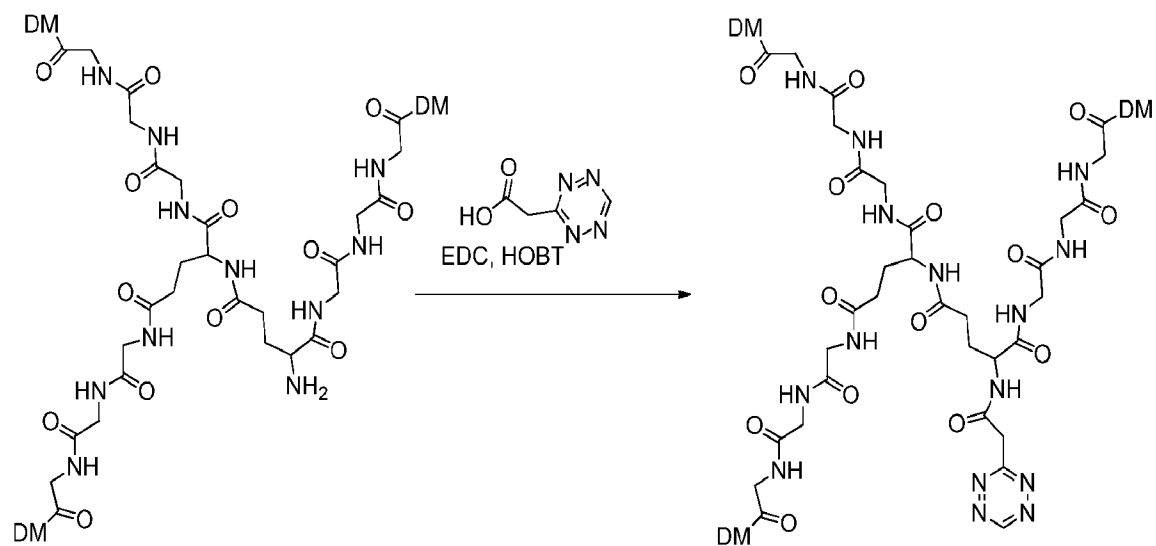


Figure 14

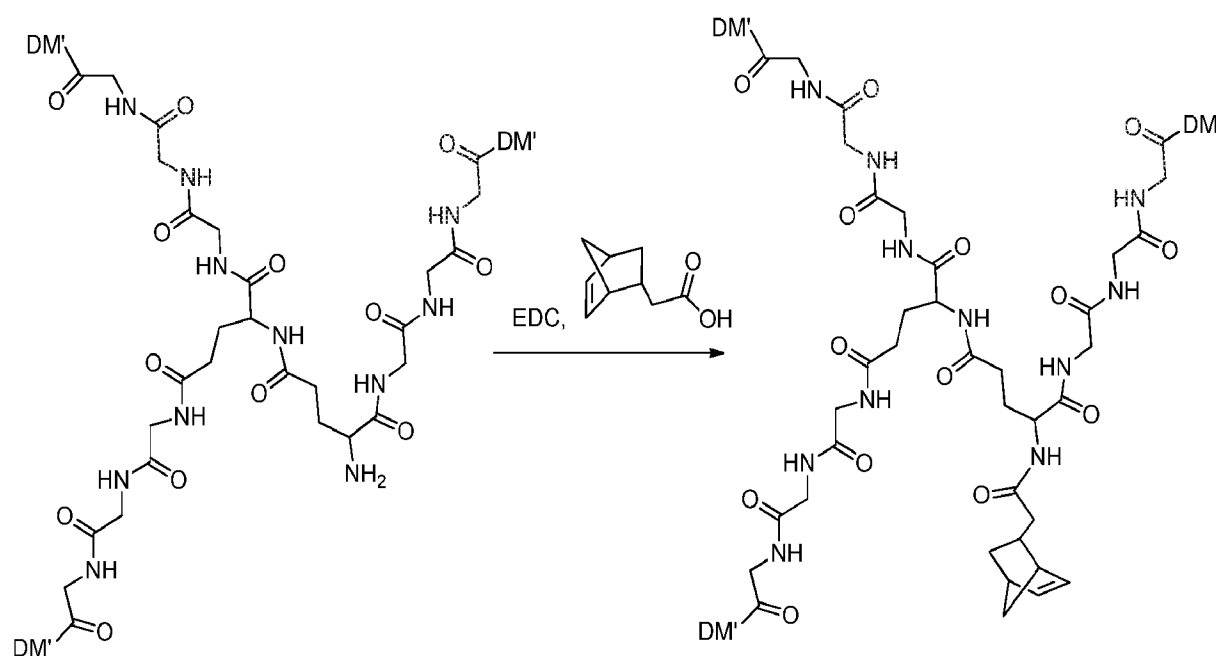


Figure 15

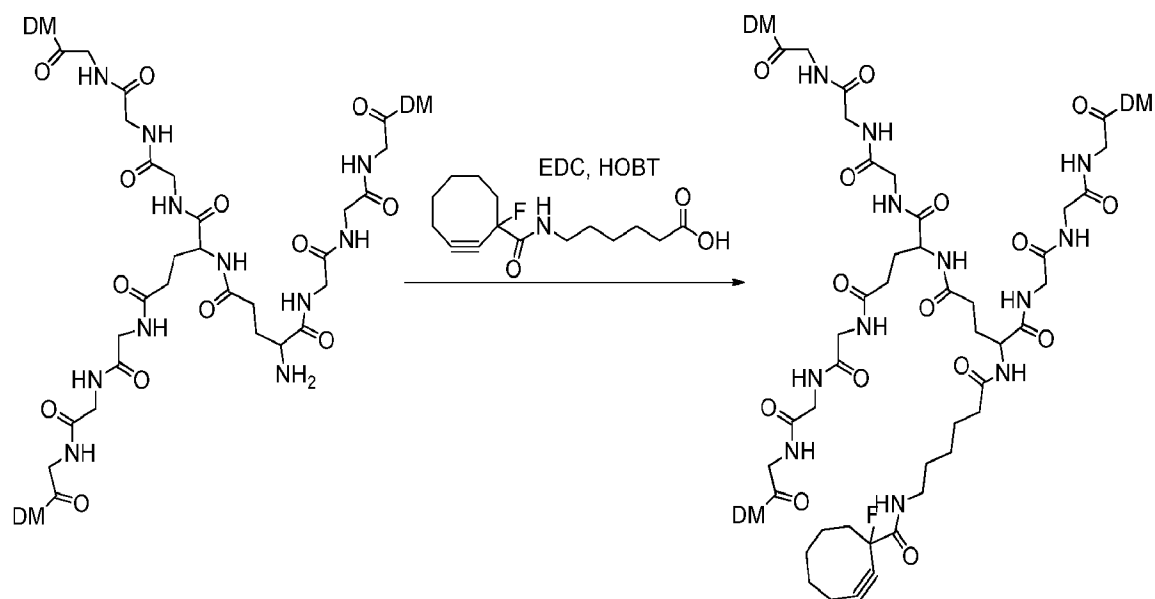


Figure 16

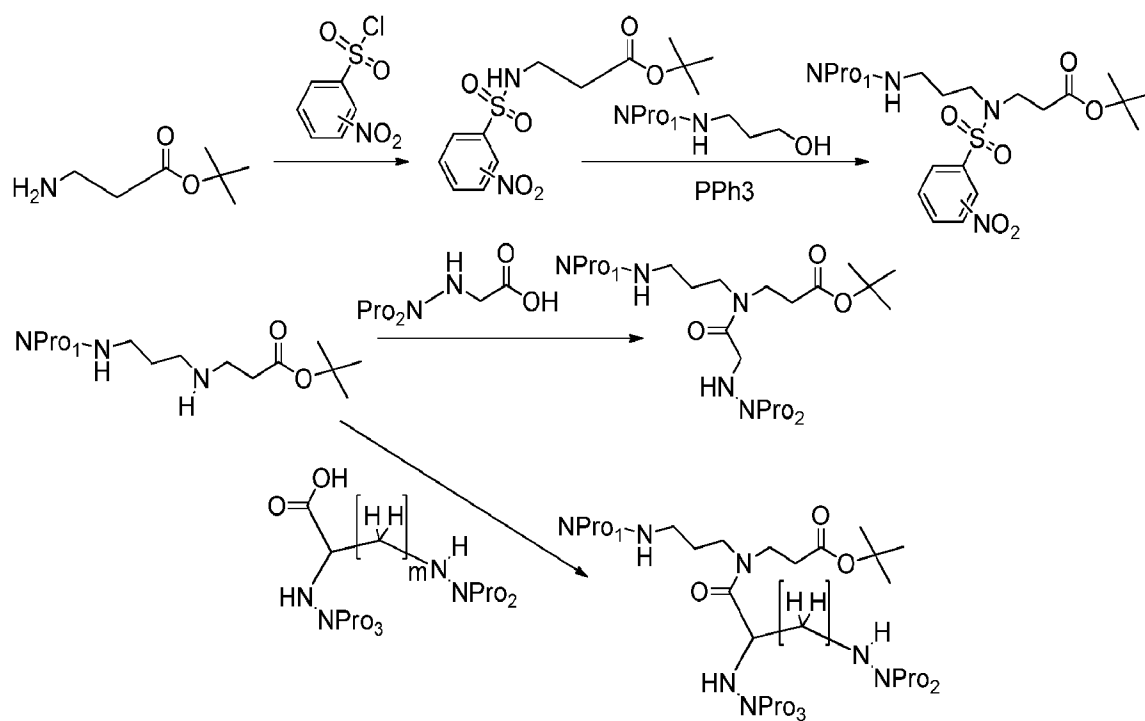


Figure 17

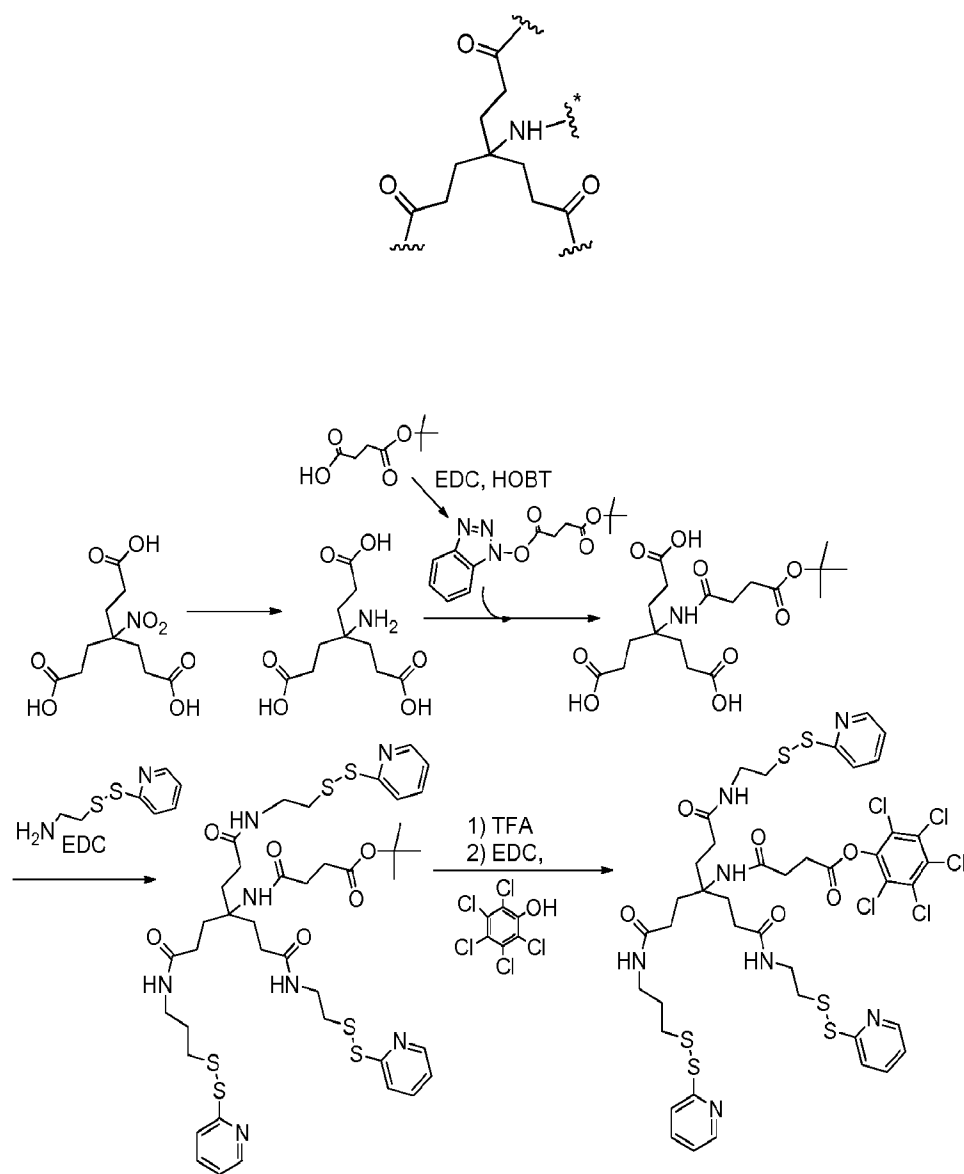


Figure 18

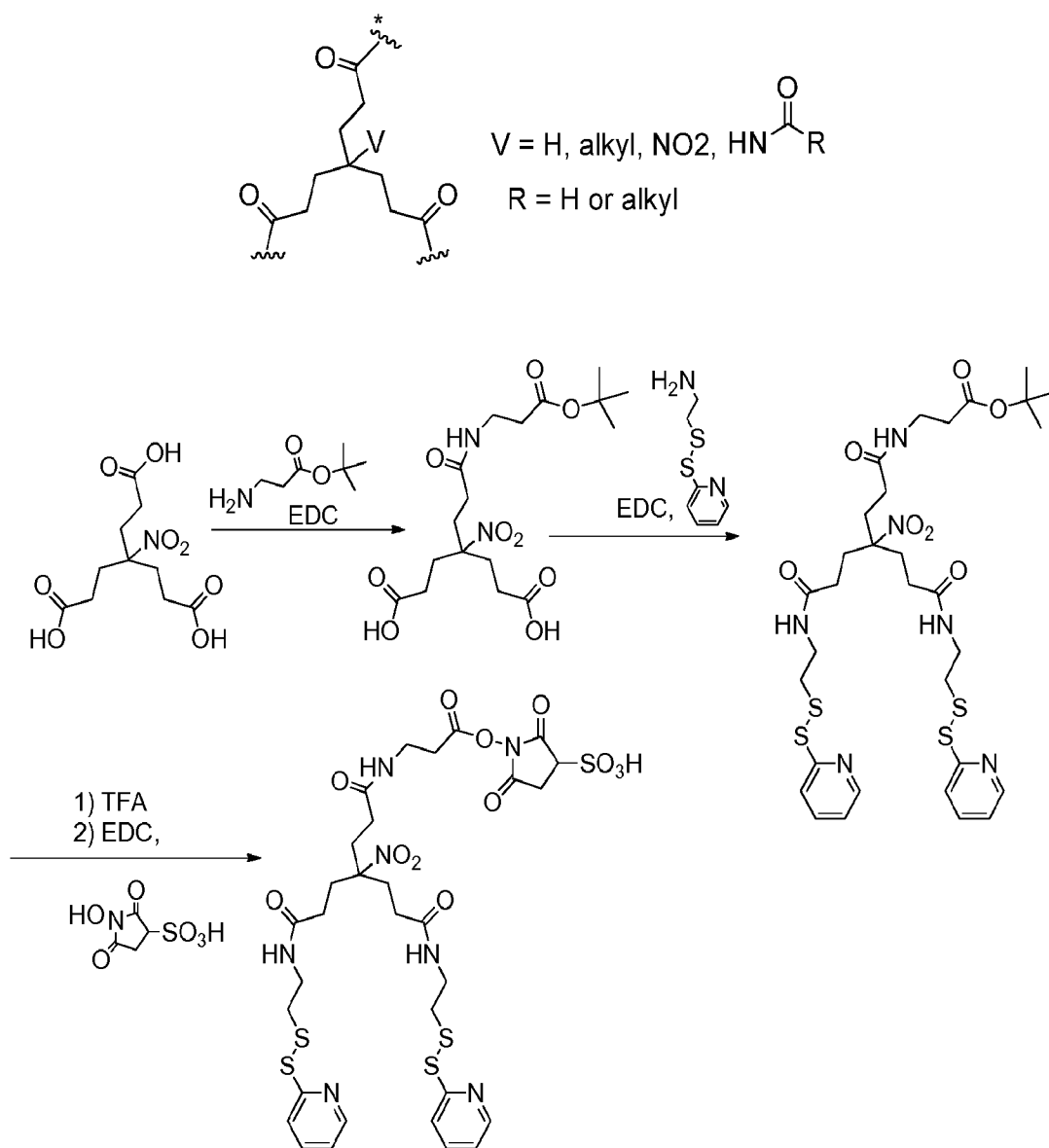


Figure 19

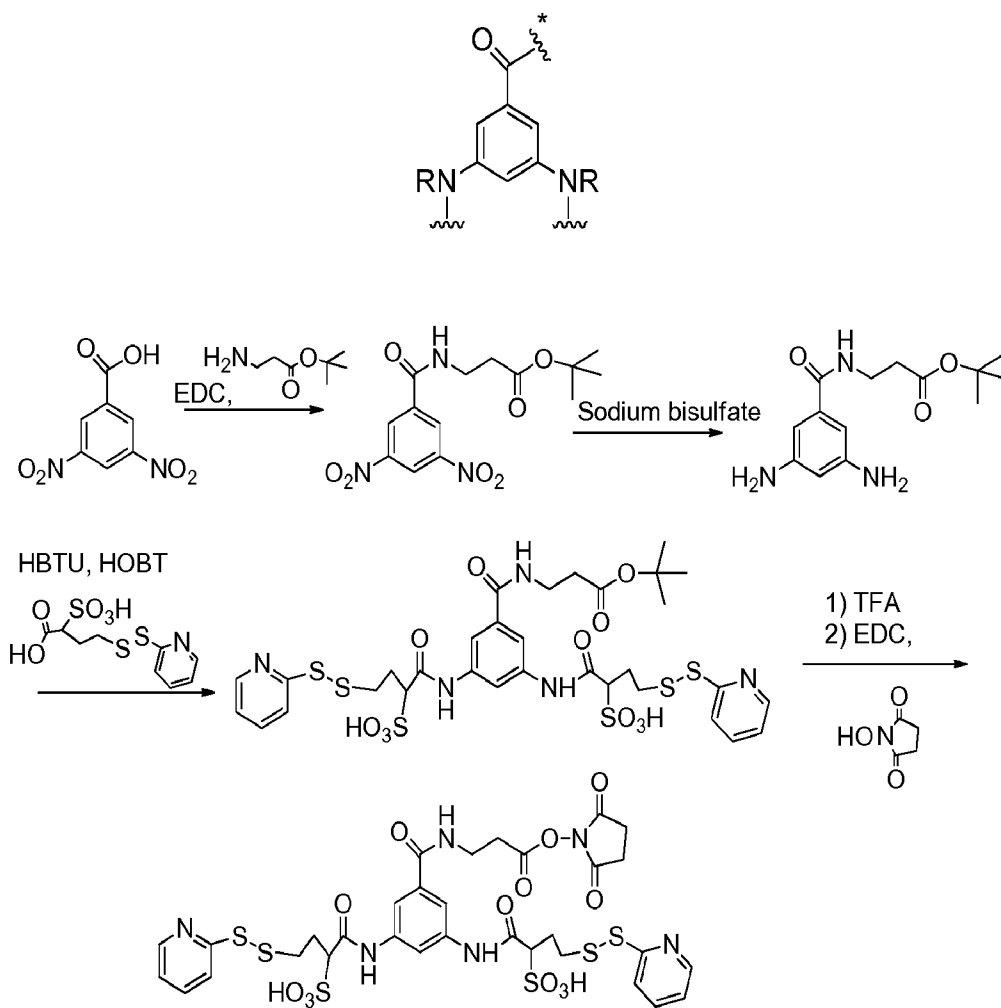


Figure 20

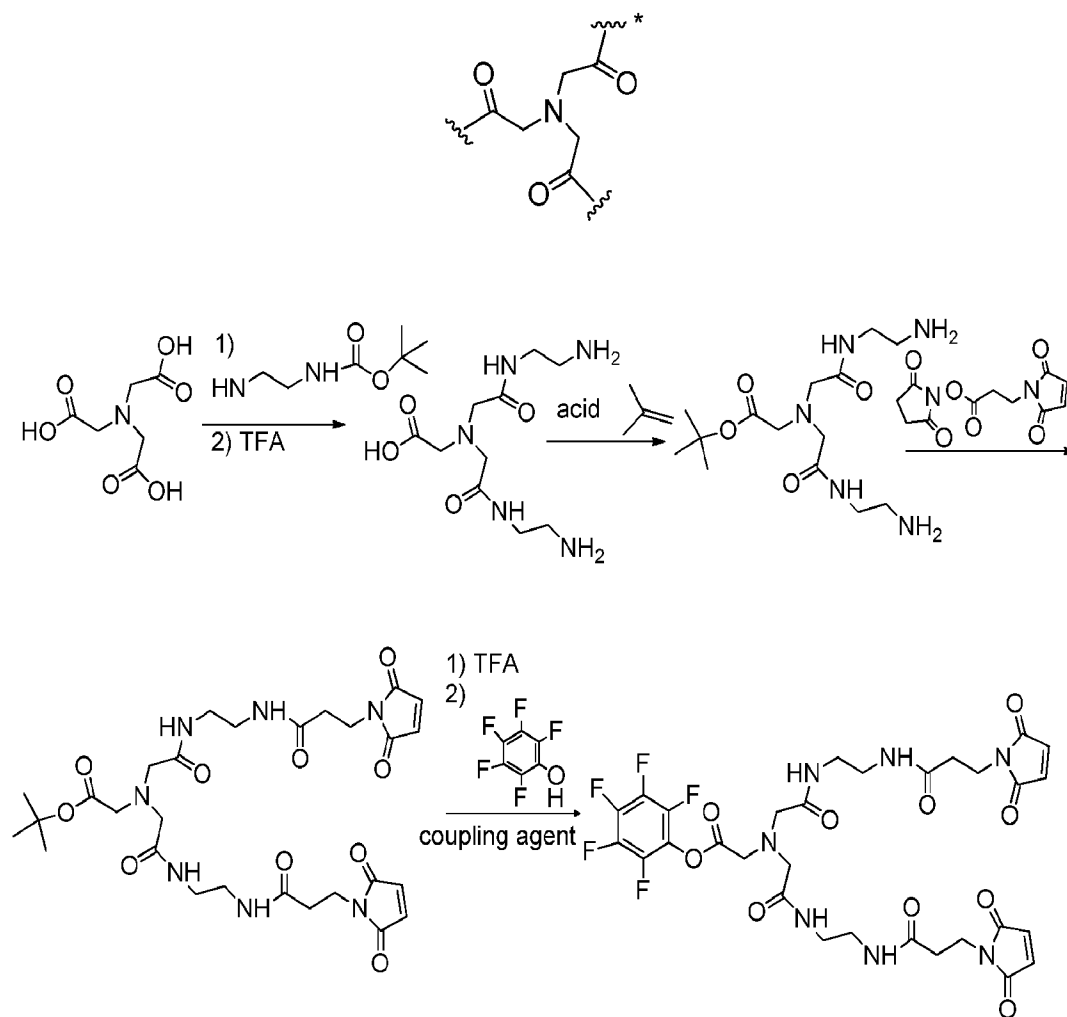


Figure 21

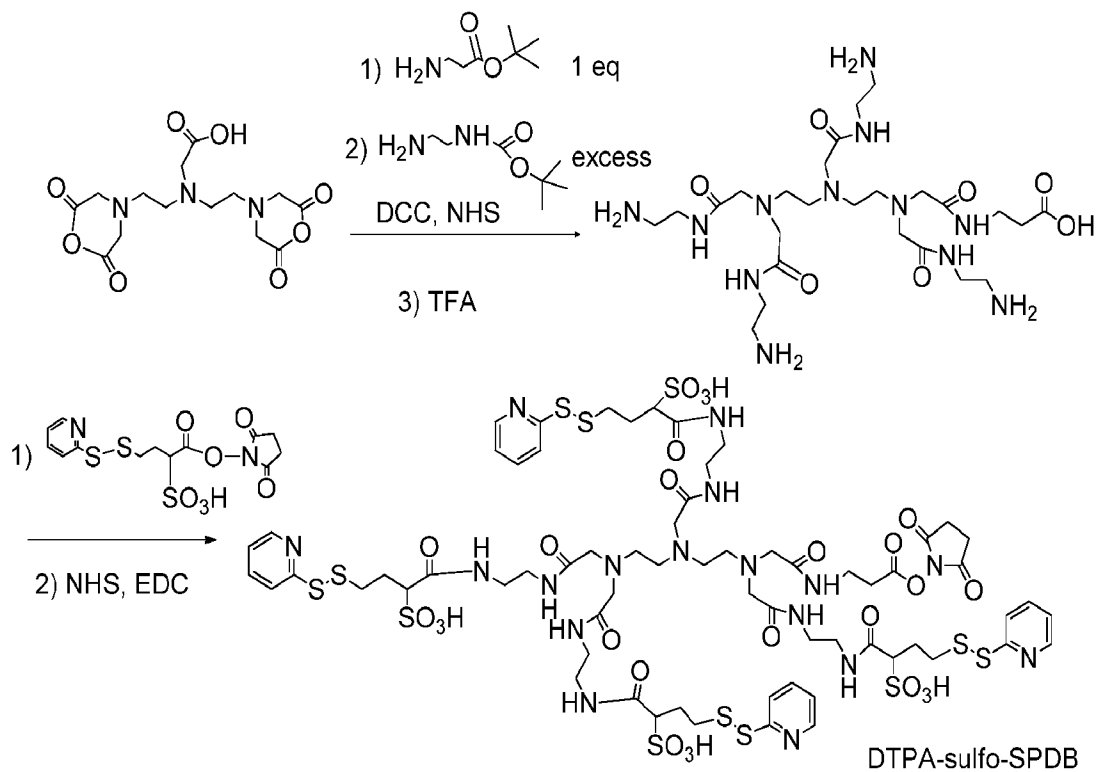


Figure 22

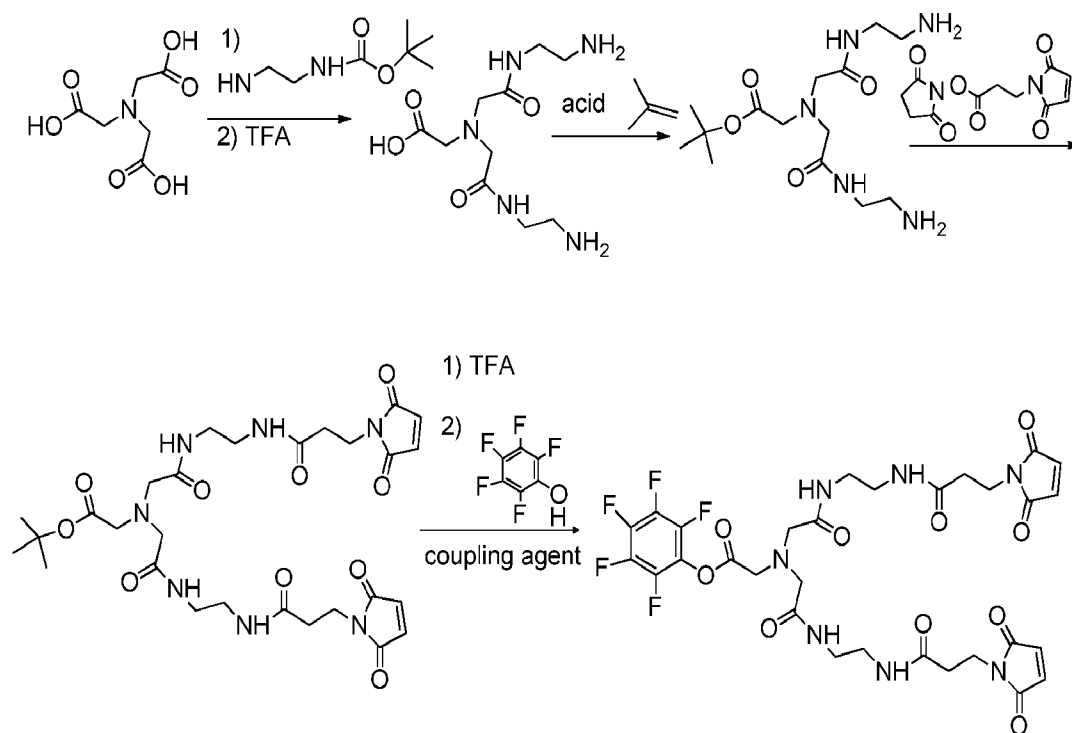


Figure 23

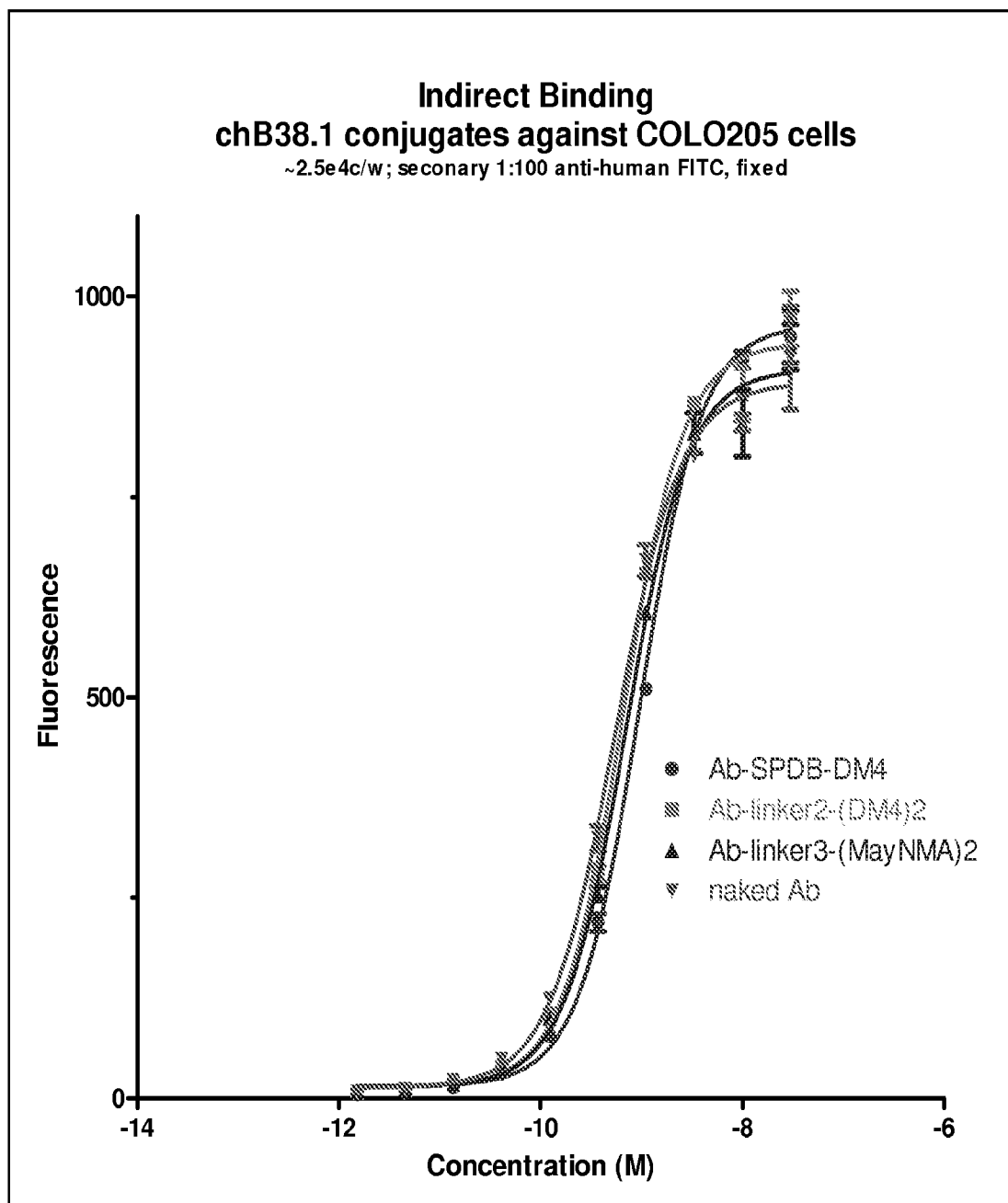


Figure 24

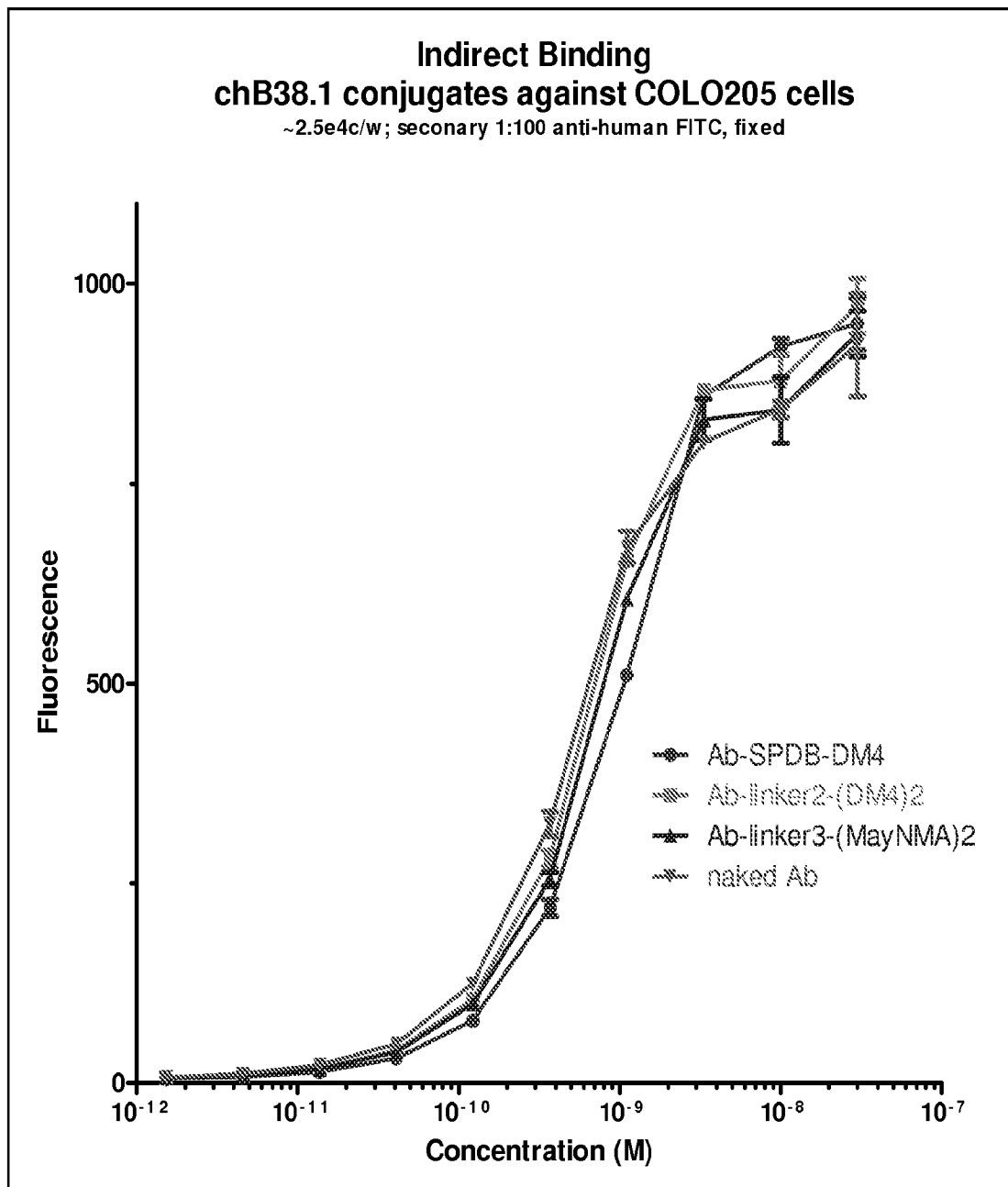


Figure 25

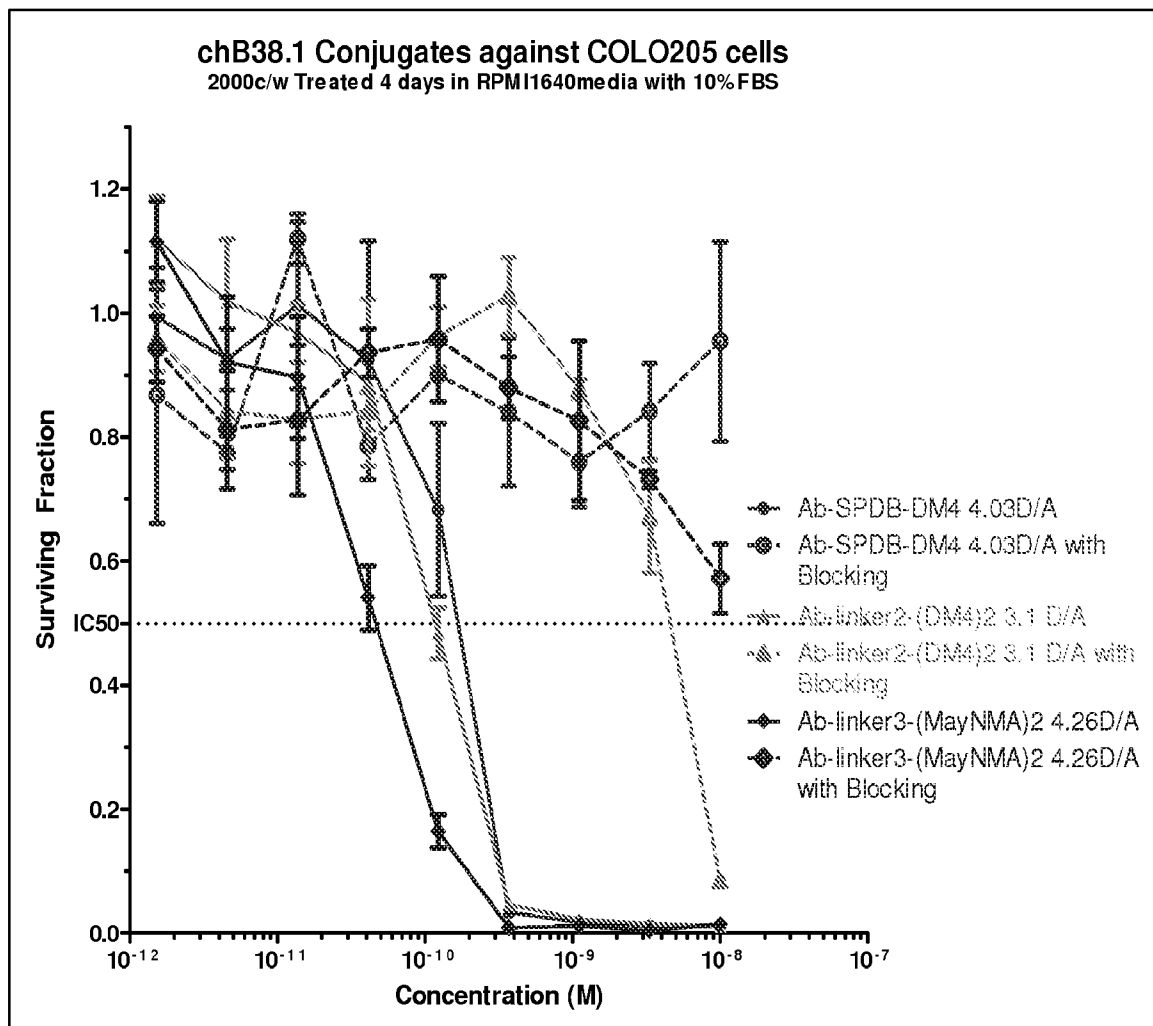


Figure 26

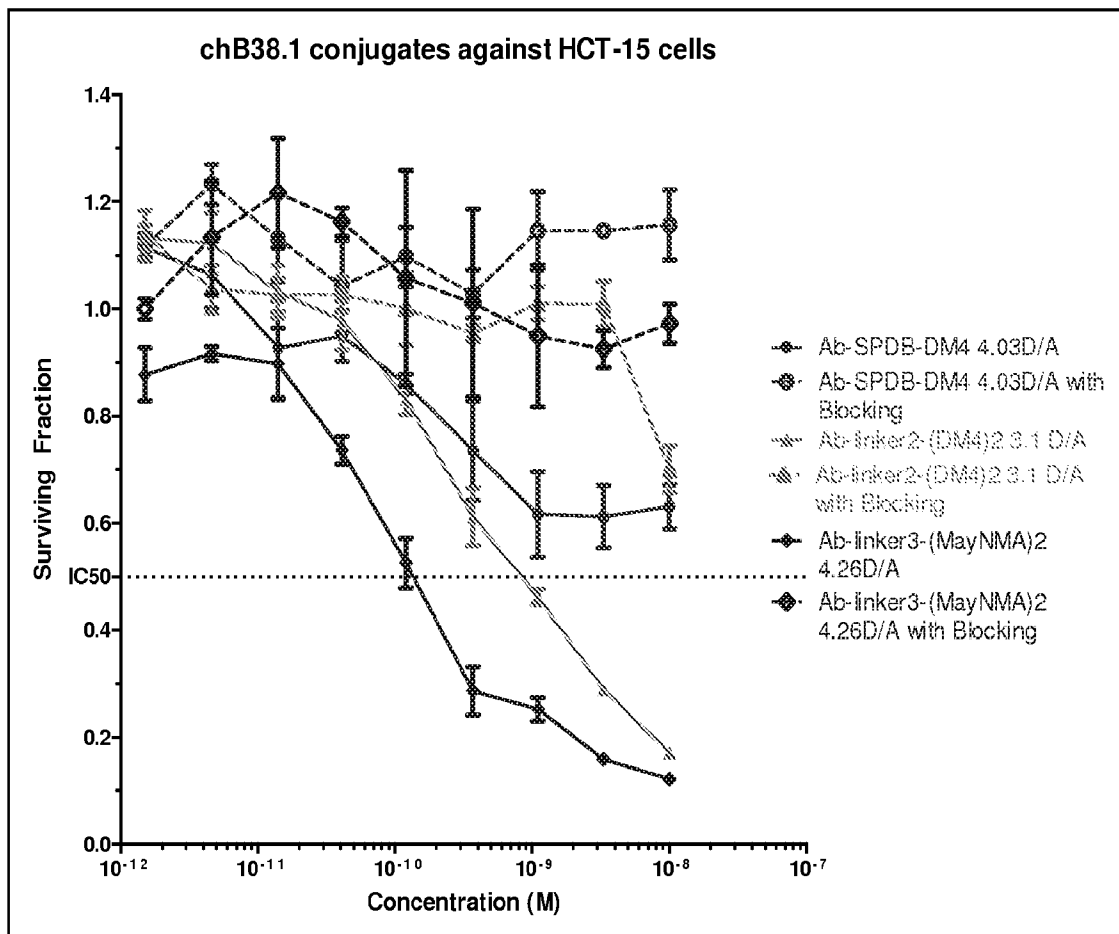


Figure 27

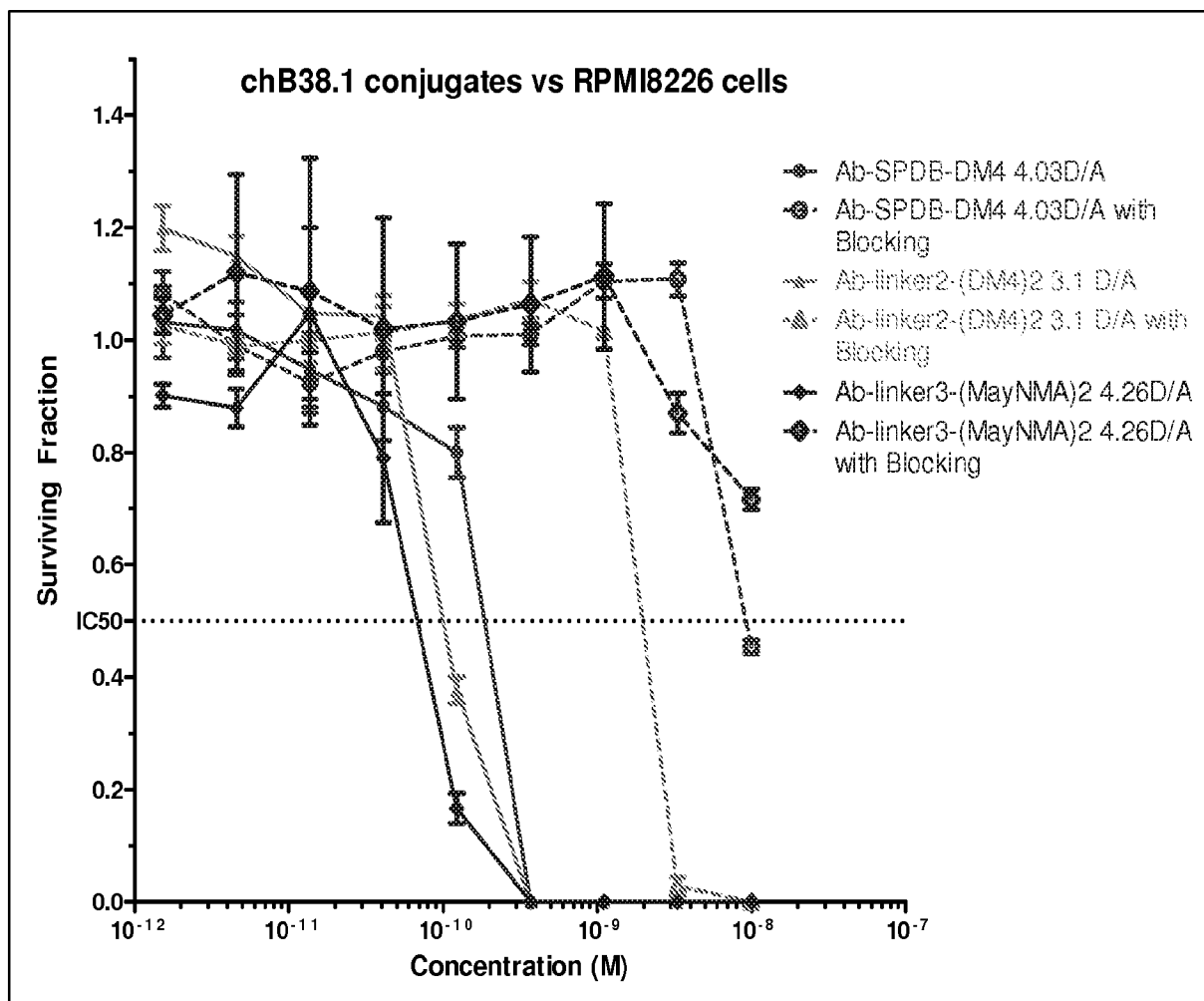


Figure 28

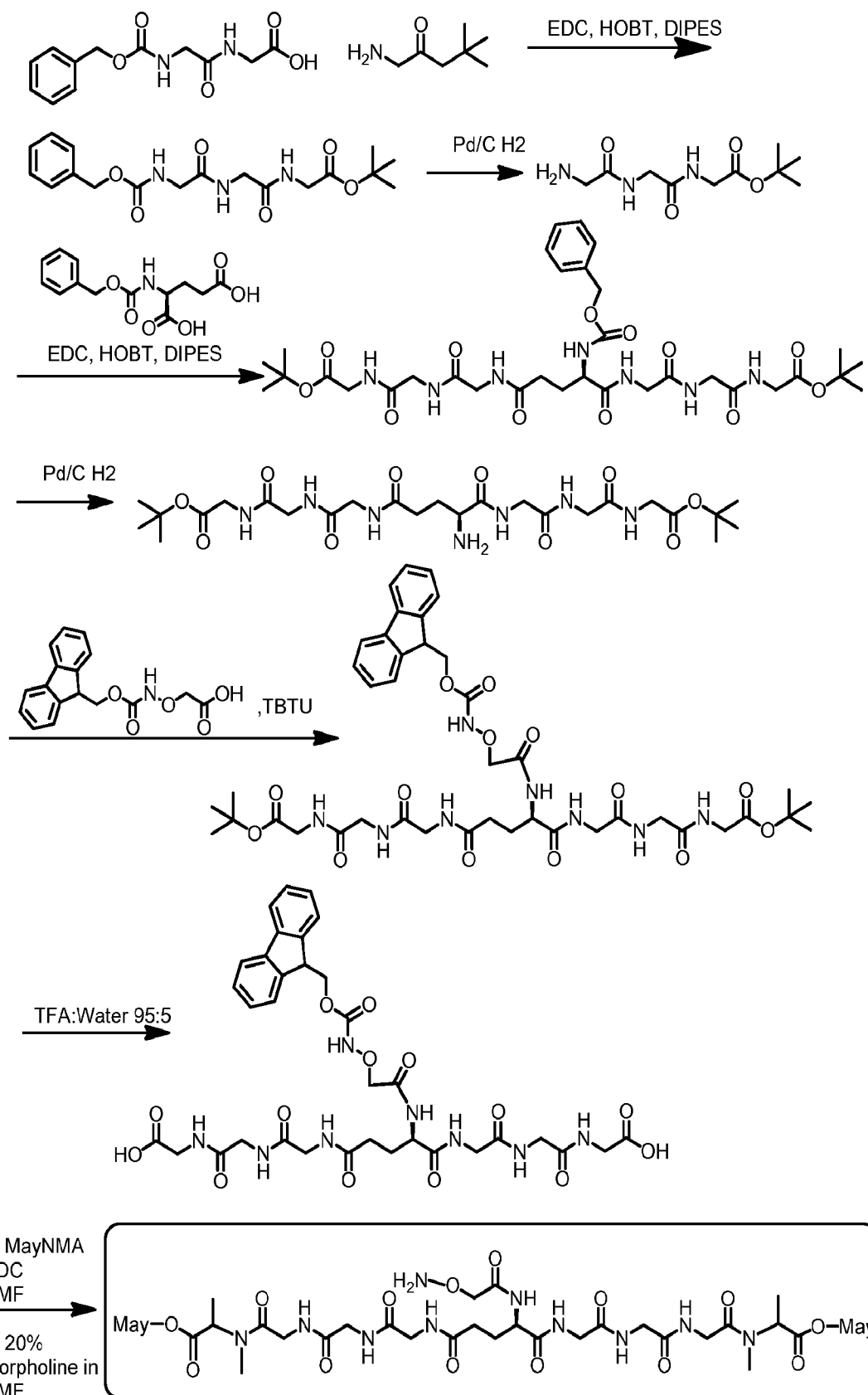


Figure 29A

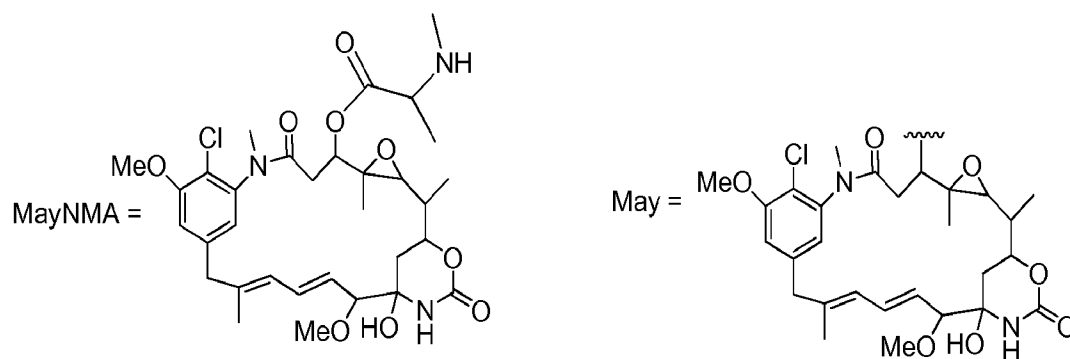


Figure 29B

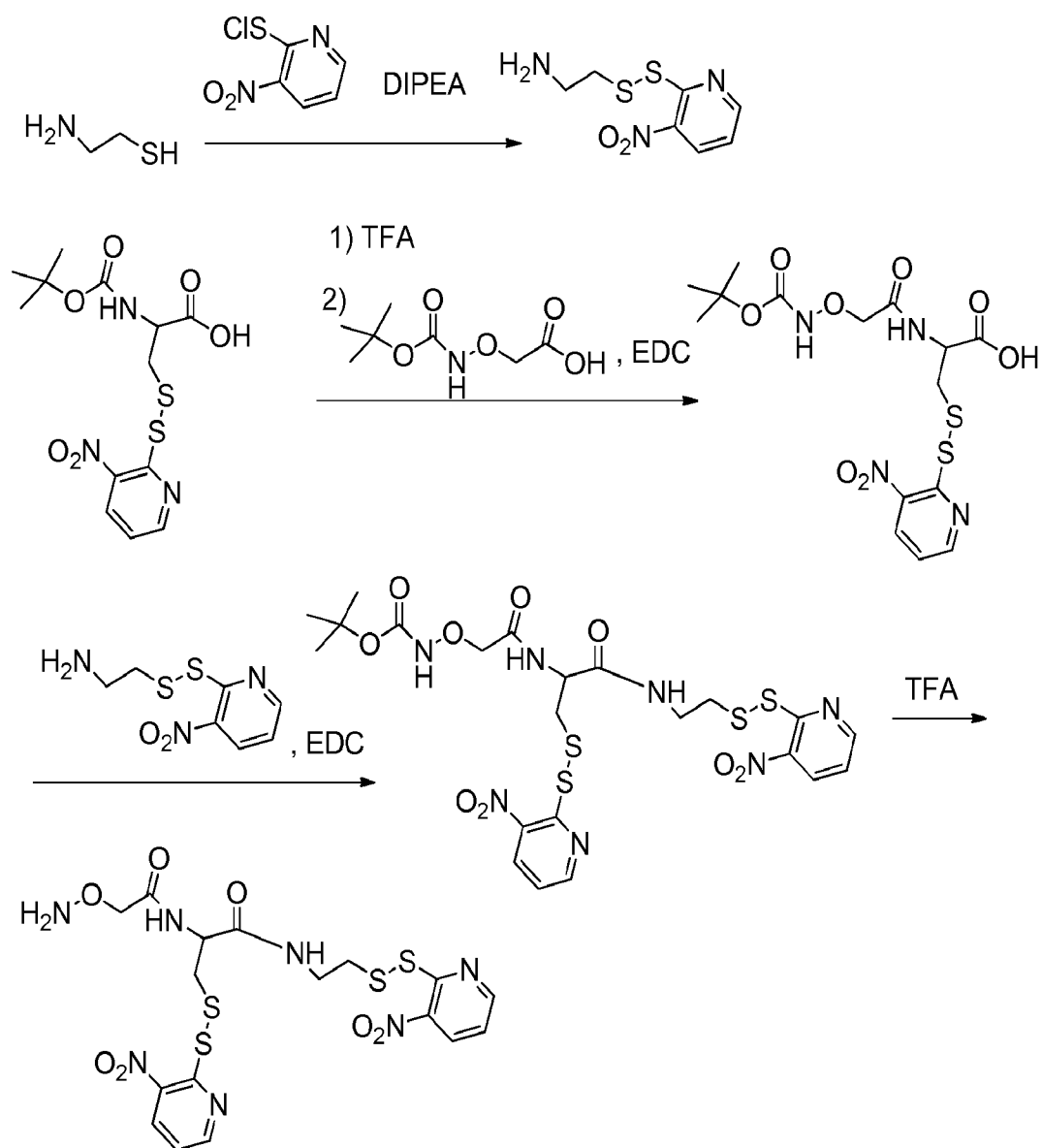


Figure 30