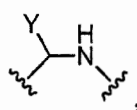


Claims:

1. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising reacting a modified cytotoxic compound with a modified CBA at a pH of about 4 to about 9, wherein:
 - a) the modified CBA comprises a residue of a bifunctional crosslinking agent bonded to the CBA, and the residue comprises the linking group and a thiol-reactive group; and
 - b) the modified cytotoxic compound comprises a thiol group, and a group represented by:

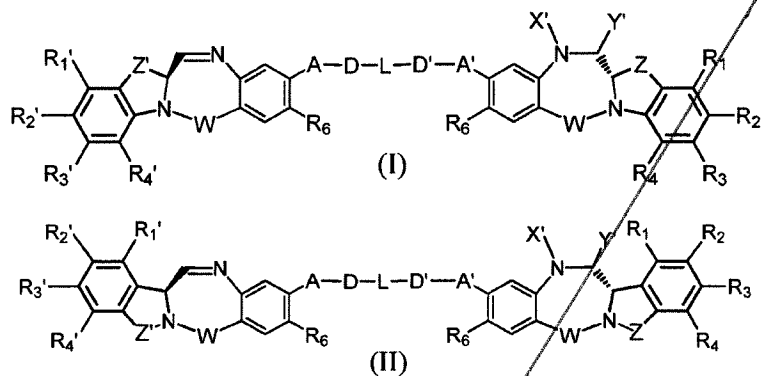


wherein:

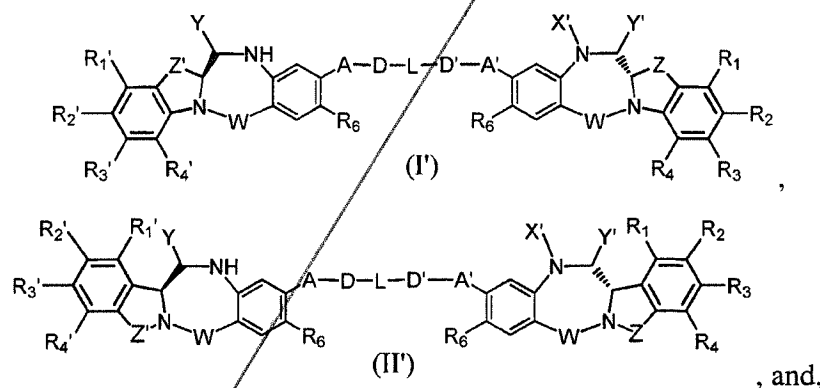
Y is a leaving group, and is a sulfite (HSO_3^- , HSO_2^- or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_2$, POS_3H_2 , PS_4H_2 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate ester ($\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, $\text{R}^i\text{S}-$, R^iSO , R^iSO_2 , R^iSO_3 , thiosulfate (HS_2O_3 or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite (HS_2O_4 or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{S})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NOH}$ or a salt formed with a cation), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2^-$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$) or a mixture thereof, wherein R^i is a linear or branched alkyl having 1 to 10 carbon atoms and is substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl.

2. The method of claim 1, wherein the modified cytotoxic compound is produced by reacting an imine-containing cytotoxic compound bearing the thiol group with an imine reactive reagent.

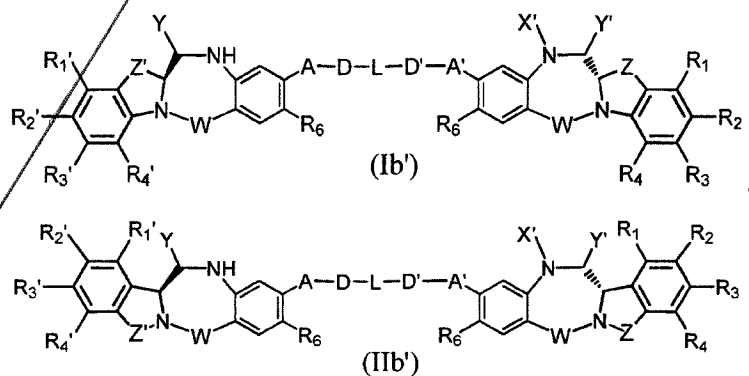
3. The method of claim 1 or 2, further comprising purifying the modified cytotoxic compound prior to reacting with the modified CBA.
4. The method of any one of claims 1-3, wherein:
- (1) the imine-containing cytotoxic compound is represented by one of the following formulae, or a pharmaceutically acceptable salt thereof:



- (2) the modified cytotoxic compound is represented by one of the following formulae, or a pharmaceutically acceptable salt thereof:



- (3) the modified cytotoxic compound and the linking group portion of the conjugate is represented by one of the following formulae:



wherein:

X' is selected from -H, an amine-protecting group, an optionally

substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃⁻M⁺, a sulfate -OSO₃⁻M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'';

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or

alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -
 $(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}^c$, and an optionally substituted 3-18-membered heterocyclic ring
 having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O , C=S , CH_2 , BH , SO and SO_2 ;

R_6 is $-\text{H}$, $-\text{R}$, $-\text{OR}$, $-\text{SR}$, $-\text{NR}'\text{R}''$, $-\text{NO}_2$, or, halogen;

Z and Z' are independently selected from $-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n\text{-CR}_7\text{R}_8\text{-}$
 $(\text{CH}_2)_{n'a}-$, $-(\text{CH}_2)_{n'}\text{-NR}_9\text{-(CH}_2)_{n'a}-$, $-(\text{CH}_2)_{n'}\text{-O-(CH}_2)_{n'a}-$ and $-(\text{CH}_2)_{n'}\text{-S-(CH}_2)_{n'a}-$;

n' and $n'a$ are the same or different, and are selected from 0, 1, 2 and 3;

R_7 and R_8 are the same or different, and are each independently selected
 from $-\text{H}$, $-\text{OH}$, $-\text{SH}$, $-\text{COOH}$, $-\text{NHR}'$, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$,
 an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally
 substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from $-\text{H}$, an optionally substituted linear,
 branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol
 unit $-(\text{OCH}_2\text{CH}_2)_n-$;

A and A' are the same or different, and are independently selected from $-\text{O}-$,
 oxo ($-\text{C(=O)}-$), $-\text{CRR}'\text{O}-$, $-\text{CRR}'-$, $-\text{S}-$, $-\text{CRR}'\text{S}-$, $-\text{N(R}_5)-$ and $-\text{CRR}'\text{N(R}_5)-$,

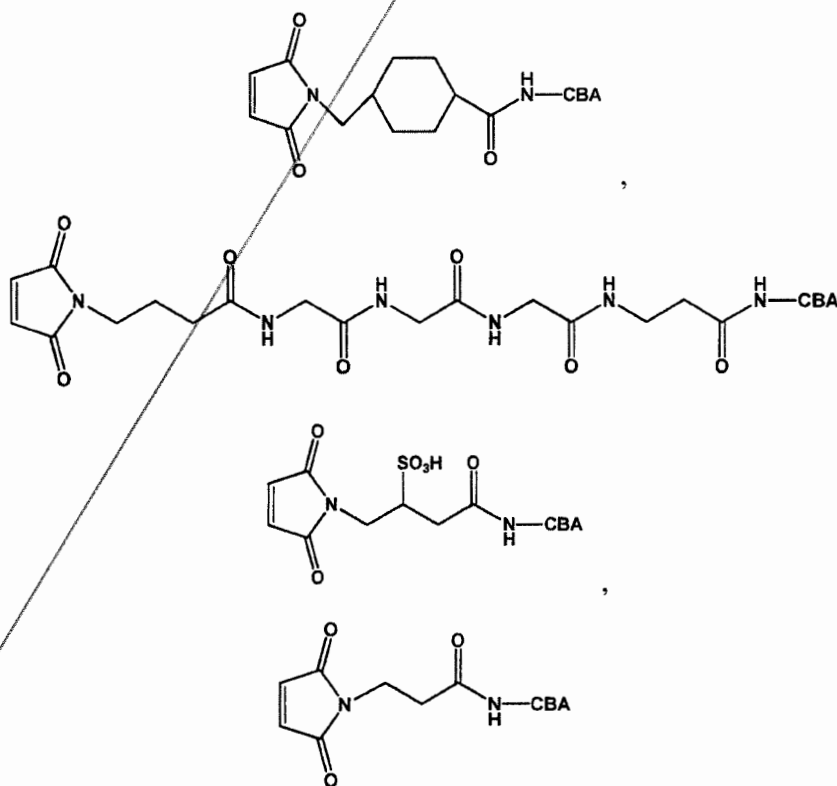
R_5 for each occurrence is independently $-\text{H}$ or an optionally substituted
 linear or branched alkyl having 1 to 10 carbon atoms;

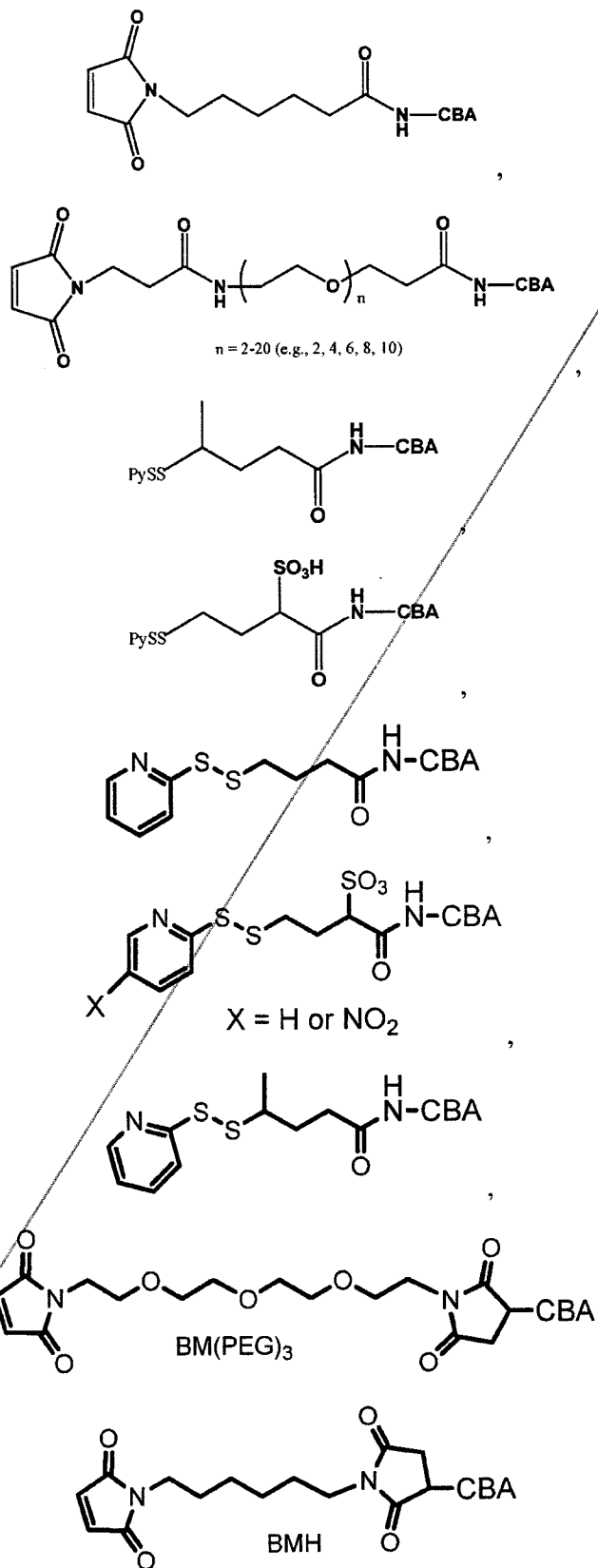
D and D' are the same or different, and are independently absent or
 selected from the group consisting of an optionally substituted linear, branched
 or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a
 peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$;

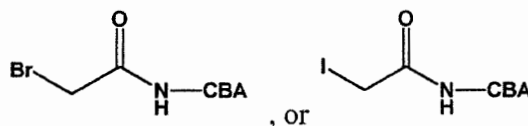
L is absent, or when present, comprises the thiol group, or is a
 polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$, a linear, branched or cyclic alkyl or
 alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered
 heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6
 heteroatoms independently selected from O, S, N and P, wherein the alkyl,
 alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted;

wherein at least one of X' , Y' , R_6 , R^c , R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' , R_4' , L
 (e.g., through an optionally substituted group), is bonded to the linking group in
 formulas (Ib') or (IIb').

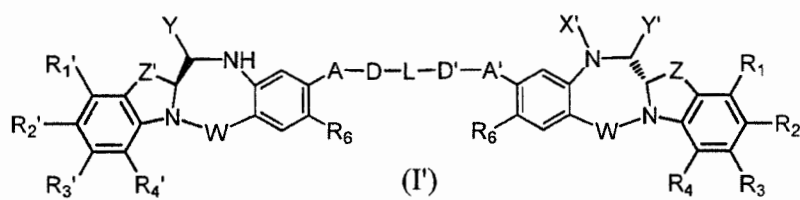
5. The method of any one of claims 1-4, wherein the modified CBA is prepared by reacting the CBA with the bifunctional crosslinking agent, said bifunctional crosslinking agent comprising the thiol-reactive group and a group reactive with the CBA, both bonded to the linking group.
6. The method of claim 5, wherein said group reactive with the CBA reacts with an amino group of the CBA (such as the amino group of a Lys sidechain), or with a thiol group of the CBA (such as the thiol group of a Cys sidechain).
7. The method of any one of claims 1-6, wherein the thiol-reactive group is selected from the group consisting of maleimido, vinylpyridine, vinyl sulfone, vinyl sulfonamide, a haloacetyl-based group and a disulfide group.
8. The method of any one of claims 1-6, wherein the thiol-reactive group is maleimido, haloacetamido or $-SSR^d$, wherein R^d is a linear or branched alkyl having 1 to 4 carbon atoms, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl, 2-nitropyridyl, 4-nitropyridyl, or 3-carboxy-4-nitropyridyl.
9. The method of any one of claims 1-8, wherein the modified CBA is:

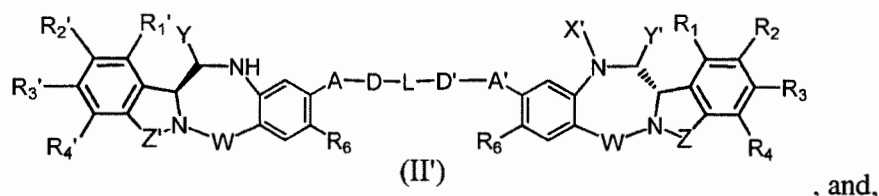






10. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising reacting the CBA with an imine-containing cytotoxic compound, an imine reactive reagent, and a bifunctional crosslinking agent comprising the linking group to form the conjugate.
11. The method of claim 10, wherein the CBA is contacted with the imine-containing cytotoxic compound and the imine reactive reagent to form a first mixture; and the first mixture is then contacted with the bifunctional crosslinking agent to form the conjugate.
12. The method of claim 11, wherein the bifunctional crosslinking agent is contacted with the first mixture immediately after the formation of the first mixture.
13. The method of any one of claims 10-12, further comprising purifying the conjugate.
14. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising:
 - a) reacting a modified cytotoxic compound with a bifunctional crosslinking agent comprising the linking group, a group reactive with the CBA (such as a thiol group, a maleimide group, a haloacetamide group, or an amine group), and a group reactive with the modified cytotoxic compound, to form a second modified cytotoxic compound covalently bonded to a residue of the bifunctional crosslinking agent, wherein the residue comprises the linking group and the group reactive with the CBA; wherein the modified cytotoxic compound is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:





wherein:

Y is a leaving group, and is a sulfite (HSO_3 , HSO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_2$, POS_3H_2 , PS_4H_2 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate ester ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, $\text{R}^i\text{S}-$, R^iSO , R^iSO_2 , R^iSO_3 , thiosulfate (HS_2O_3 or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite (HS_2O_4 or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{S})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NOH}$ or a salt formed with a cation), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2^-$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$) or a mixture thereof, wherein R^i is a linear or branched alkyl having 1 to 10 carbon atoms and is substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl;

X' is selected from $-\text{H}$, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from $-\text{H}$, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently

selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'';

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, and an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or, halogen;

Z and Z' are independently selected from $-(CH_2)_{n'}$, $-(CH_2)_{n'}-CR_7R_8-$, $(CH_2)_{na'}$, $-(CH_2)_{n'}-NR_9-(CH_2)_{na'}$, $-(CH_2)_{n'}-O-(CH_2)_{na'}$ and $-(CH_2)_{n'}-S-(CH_2)_{na'}$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R₇ and R₈ are the same or different, and are each independently selected from -H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit $-(OCH_2CH_2)_n-$,

an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

A and A' are the same or different, and are independently selected from -O-, oxo ($-C(=O)-$), $-CRR'O-$, $-CRR'-$, $-S-$, $-CRR'S-$, $-N(R_5)-$ and $-CRR'N(R_5)-$,

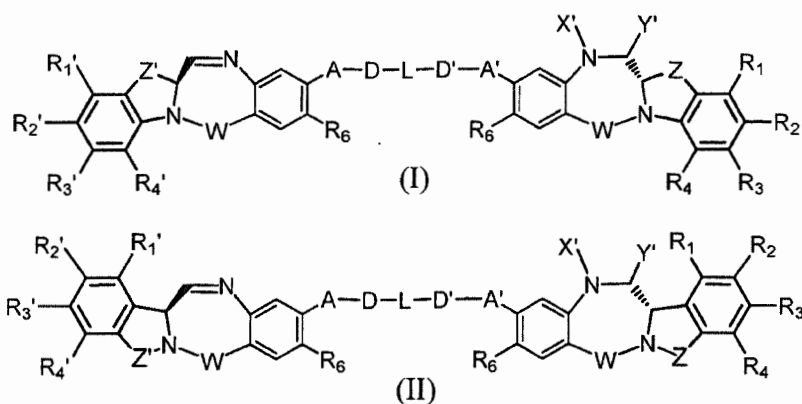
R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

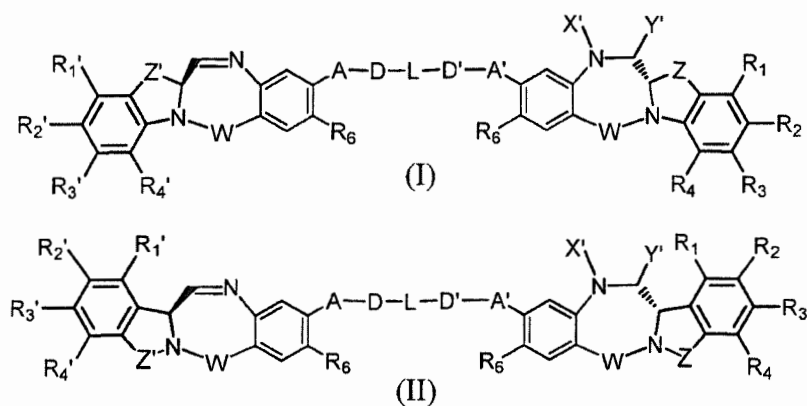
L is absent, or when present, comprises the thiol group, or is a polyethylene glycol unit $-(OCH_2CH_2)_n-$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl, alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted; and,

b) reacting the second modified cytotoxic compound with the CBA through the group reactive with the CBA, at a pH of about 4 to about 9, to form the conjugate.

15. The method of claim 14, wherein the group reactive with the CBA is a thiol group, a maleimide group, a haloacetamide group, or an amine group.
16. The method of claim 14, wherein the modified cytotoxic compound comprises a thiol group, and the group reactive with the modified cytotoxic compound is a thiol-reactive group.
17. The method of claim any one of claims 14-16, wherein the modified cytotoxic compound is produced by reacting an imine-containing cytotoxic compound bearing the thiol group of the following formula with an imine reactive reagent in a reaction mixture



18. The method of claim 17, further comprising purifying the modified cytotoxic compound prior to step a).
19. The method of any one of claims 14-18, further comprising purifying the second modified cytotoxic compound prior to step b).
20. The method of any one of claims 17-19, wherein the reaction mixture is stored frozen before the frozen mixture is thawed and step a) is carried out.
21. The method of any one of claims 17-20, further comprising storing the reaction mixture of step a) frozen before thawing and before step b) is carried out.
22. The method of any one of claims 14-21, wherein the bifunctional crosslinking agent is bis-maleimido-hexane or BMPEO.
23. The method of any one of claims 2-22, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas, or a pharmaceutically acceptable salt thereof:



wherein:

X' is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to

10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

$R_1, R_2, R_3, R_4, R_1', R_2', R_3'$ and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'' and the linking group;

R , for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -

$(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}^c$, and an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O , C=S , CH_2 , BH , SO and SO_2 ;

R_6 is $-\text{H}$, $-\text{R}$, $-\text{OR}$, $-\text{SR}$, $-\text{NR}'\text{R}''$, $-\text{NO}_2$, or halogen;

Z and Z' are independently selected from $-(\text{CH}_2)_{n'}$, $-(\text{CH}_2)_{n'}\text{-CR}_7\text{R}_8\text{-}(\text{CH}_2)_{na'}$, $-(\text{CH}_2)_{n'}\text{-NR}_9\text{-}(\text{CH}_2)_{na'}$, $-(\text{CH}_2)_{n'}\text{-O-}(\text{CH}_2)_{na'}$ and $-(\text{CH}_2)_{n'}\text{-S-}(\text{CH}_2)_{na'}$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R_7 and R_8 are the same or different, and are each independently selected from $-\text{H}$, $-\text{OH}$, $-\text{SH}$, $-\text{COOH}$, $-\text{NHR}'$, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n$, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from $-\text{H}$, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n$;

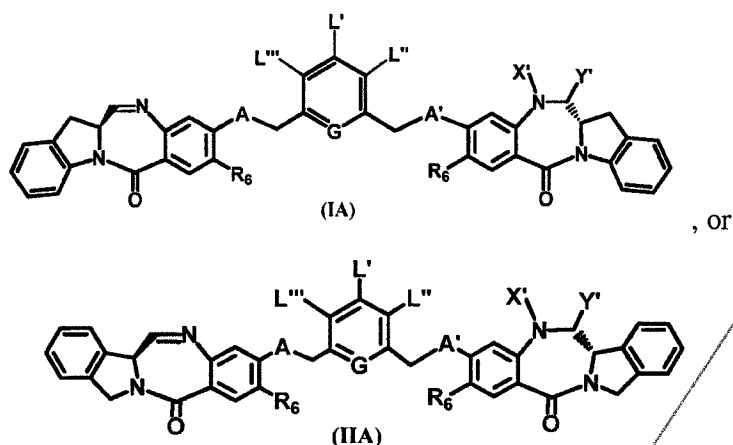
A and A' are the same or different, and are independently selected from $-\text{O-}$, $\text{oxo } (-\text{C}(=\text{O})-)$, $-\text{CRR}'\text{O-}$, $-\text{CRR}'$, $-\text{S-}$, $-\text{CRR}'\text{S-}$, $-\text{N}(\text{R}_5)-$ and $-\text{CRR}'\text{N}(\text{R}_5)-$,

R_5 for each occurrence is independently $-\text{H}$ or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n$;

L is absent, or when present, comprises the thiol group, and is a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl, alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted.

24. The method of claim 23, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas:



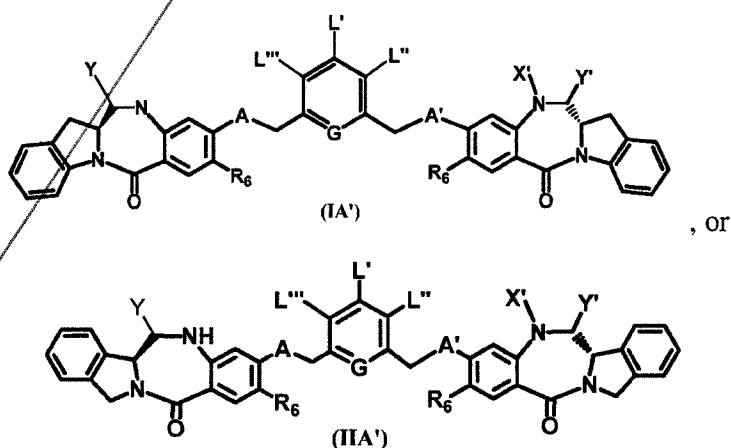
wherein:

L' , L'' , and L''' are the same or different, and are independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit - $(OCH_2CH_2)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M, a sulfate -OSO₃M, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'';

M is -H or a pharmaceutically acceptable cation; and,

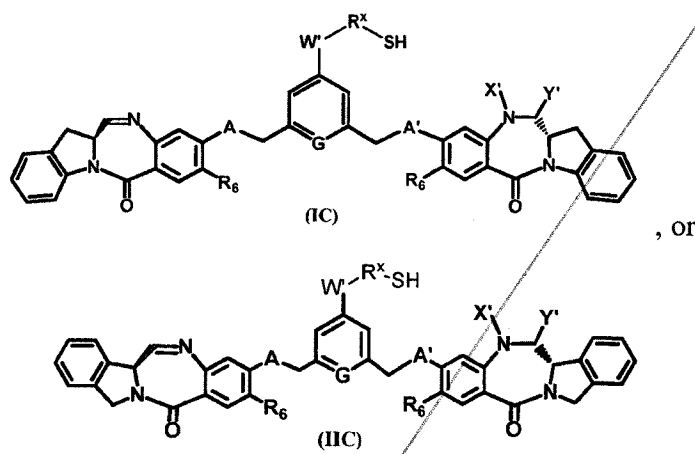
G is selected from -CH- or -N-.

25. The method of claim 24, the modified cytotoxic compound, when present, is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:



26. The method of claim 23, 24, or 25, wherein one of L' , L'' , or L''' bears the thiol group, while the others are -H.

27. The method of claim 26, wherein L' bears the thiol group, and L'' and L''' are -H.
28. The method of any of claims 23-27, wherein A and A' are both -O-; R₆ is -OMe; and G is -CH-.
29. The method of any one of claims 23-28, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas:



wherein:

W' is absent, or when present, is selected from -CR^eR^{e'}-, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO₂-, -CH₂-S-, -CH₂O-, -CH₂NR^e-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)-, -N(C(=O)R^e)-, -(O-CH₂-CH₂)_n-, -SS-, or -C(=O)-, or an amino acid, or a peptide having 2 to 8 amino acids;

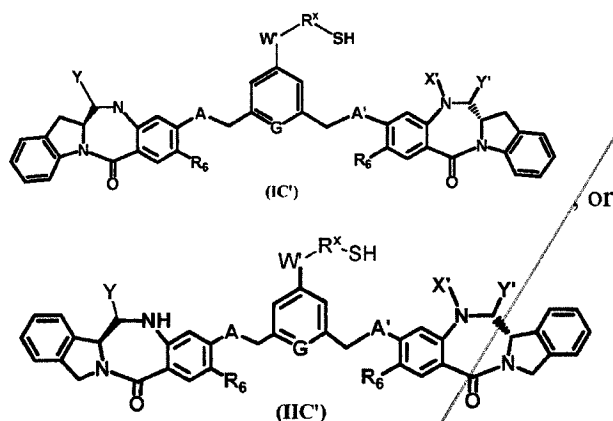
R^x is absent, or when present, is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an aryl bearing 6 to 10 carbon atoms or a 3- to 8-membered heterocyclic ring bearing 1 to 3 heteroatoms selected from O, N or S;

R^e and R^{e'} are the same or different, and are selected from -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms; preferably, R¹⁰¹ and R¹⁰² are each independently a linear or

branched alkyl having 1 to 6 carbon atoms;

n is an integer from 1 to 24.

30. The method of claim 29, wherein the modified cytotoxic compound, when present, is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:



31. The method of claim 29 or 30, wherein:

Y is selected from $-\text{SO}_3\text{M}$, $-\text{SO}_2\text{M}$ or $-\text{OSO}_3\text{M}$;

M is $-\text{H}$ or a pharmaceutically acceptable cation such as Na^+ or K^+ ;

X' is selected from the group consisting of $-\text{H}$, $-\text{OH}$, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, and an amine-protecting group;

Y' is selected from the group consisting of $-\text{H}$, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms;

A and A' are selected from $-\text{O}-$ and $-\text{S}-$;

W' is absent, or selected from $-\text{O}-$, $-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)-$, $-\text{S}-$ or $-\text{CH}_2-\text{S}-$, $-\text{CH}_2\text{NR}^e-$;

R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is $-\text{H}$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or $-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{R}^k$, wherein R^k is a $-\text{H}$, a linear, branched or cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (e.g., $-\text{NHR}^{101}$) or tertiary amino ($-\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R^{101}

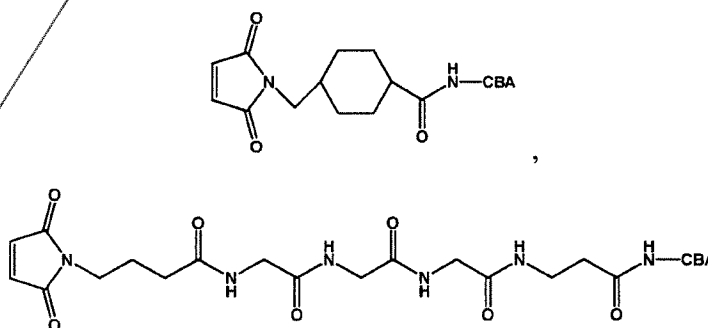
and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

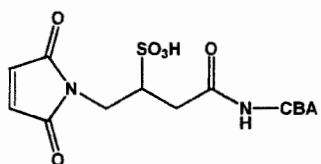
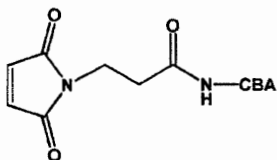
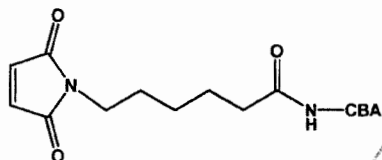
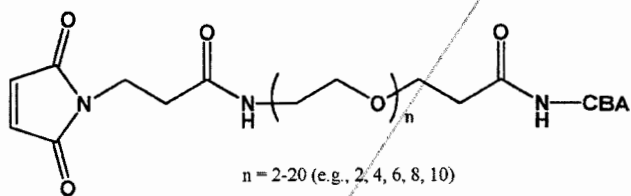
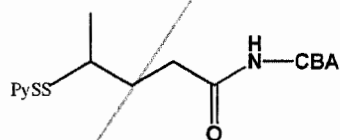
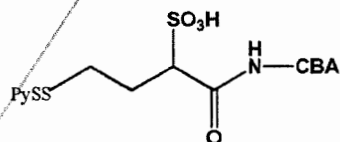
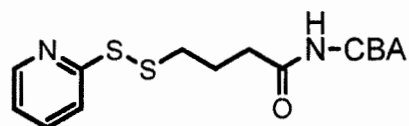
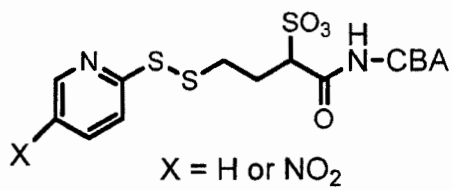
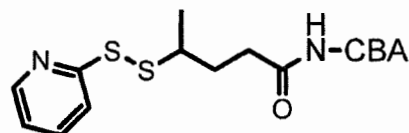
G is selected from -CH- or -N-.

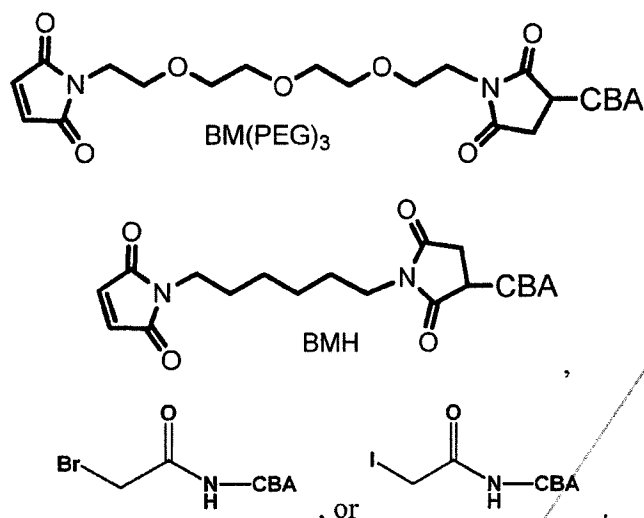
32. The method of any one of claims 29-31, wherein W' is -N(R^e)-
33. The method of claim 32, wherein R^e is -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms.
34. The method of claim 33, wherein R^k is -H or -Me, n is 4, and q is 2.
35. The method of claim 34, wherein R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
36. The method of claim 34, wherein R^x is -(CH₂)_p-(CR^fR^g)-, wherein R^f and R^g are each independently selected from -H or a linear or branched alkyl having 1 to 4 carbon atoms; and p is 0, 1, 2 or 3.
37. The method of claim 36, wherein R^f and R^g are the same or different, and are selected from -H and -Me; and p is 1.
38. The method of any one of claims 29-37, wherein:
 - Y is -SO₃M, -SO₂M, or a sulfate -OSO₃M; preferably -SO₃M;
 - M is -H or a pharmaceutically acceptable cation (*e.g.*, Na⁺);
 - X' and Y' are both -H;
 - A and A' are both -O-;
 - R₆ is -OMe; and
 - R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
39. The method of any one of claims 10-16 and 23-38, wherein the bifunctional crosslinking agent is:
 - a maleimido-based moiety selected from: N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), κ-maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid N-succinimidyl ester (GMBS), ε-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α-maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-

maleimidophenyl)-butyrate (SMPB), N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate; or, a haloacetyl-based moiety selected from: N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA), and N-succinimidyl 3-(bromoacetamido)propionate (SBAP), bis-maleimidopolyethyleneglycol (BMPEO), BM(PEO)₂, BM(PEO)₃, N-(β-maleimidopropoxy)succinimide ester (BMPS), 5-maleimidovaleric acid NHS, HBVS, 4-(4-N-maleimidophenyl)-butyric acid hydrazide•HCl (MPBH), Succinimidyl-(4-vinylsulfonyl)benzoate (SVSB), dithiobis-maleimidoethane (DTME), 1,4-bis-maleimidobutane (BMB), 1,4-bismaleimidyl-2,3-dihydroxybutane (BMDB), bis-maleimidohexane (BMH), bis-maleimidoethane (BMOE), sulfosuccinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (sulfo-SMCC), sulfosuccinimidyl(4-iodo-acetyl)aminobenzoate (sulfo-SIAB), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS), N-(γ-maleimidobutyloxy)sulfosuccinimide ester (sulfo-GMBS), N-(ε-maleimidocaproxyloxy)sulfosuccinimide ester (sulfo-EMCS), N-(κ-maleimidoundecanoyloxy)sulfosuccinimide ester (sulfo-KMUS), sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate (sulfo-SMPB), CX1-1, sulfo-Mal and PEG_n-Mal.

40. The method of any one of claims 23-38, wherein the bifunctional crosslinking agent is selected from the group consisting of SMCC, Sulfo-SMCC, BMPS, GMBS, SIA, SIAB, N-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate, bis-maleimidoethane or BMPEO.
41. The method of any one of claims 23-40, wherein the modified CBA, when present, is:

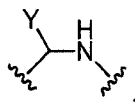






42. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising reacting a second modified cytotoxic compound with the CBA at a pH of about 4 to about 9, wherein the second modified cytotoxic compound comprises:

- a) a residue of a bifunctional crosslinking agent bonded to the cytotoxic compound, and the residue comprises the linking group and a reactive group selected from a reactive ester and a thiol-reactive group, and,
- b) a group represented by:

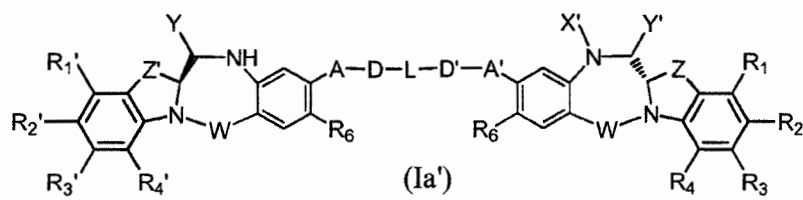


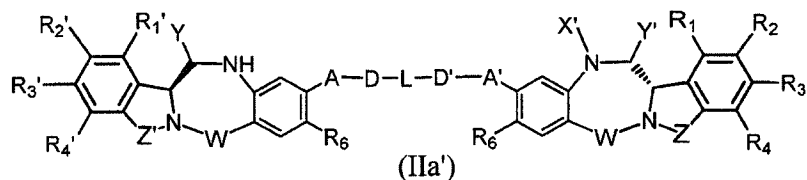
wherein:

Y is a leaving group, and is a sulfite (HSO_3 , HSO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_2$, POS_3H_2 , PS_4H_2 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate ester ($\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, $\text{R}^i\text{S}-$, R^iSO , R^iSO_2 , R^iSO_3 , thiosulfate (HS_2O_3 or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite (HS_2O_4 or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{S})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NOH}$ or a salt formed with a cation), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2^-$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$) or a mixture thereof, wherein R^i is a linear or branched alkyl having 1 to 10 carbon atoms and is

substituted with at least one substituent selected from $-N(R^j)_2$, $-CO_2H$, $-SO_3H$, and $-PO_3H$; R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl.

43. The method of claim 42, wherein the second modified cytotoxic compound is produced by reacting an imine reactive reagent with an imine-containing cytotoxic compound bearing the linking group and the reactive group.
44. The method of claim 42 or 43, further comprising purifying the second modified cytotoxic compound prior to reacting with the CBA.
45. The method of any one of claims 42-44, wherein the reactive ester is selected from the group consisting of N-hydroxysuccinimide ester, N-hydroxy sulfosuccinimide ester, nitrophenyl ester, dinitrophenyl ester, tetrafluorophenyl ester, sulfo-tetrafluorophenyl ester, and pentafluorophenyl ester.
46. The method of any one of claims 42-44, wherein the reactive ester is N-hydroxysuccinimide ester.
47. The method of any one of claims 42-44, wherein the thiol-reactive group is selected from the group consisting of maleimido, vinylpyridine, vinyl sulfone, vinyl sulfonamide, a haloacetyl-based group and a disulfide group.
48. The method of any one of claims 42-44, wherein the thiol-reactive group is maleimido, haloacetamido or $-SSR^d$, wherein R^d is a linear or branched alkyl having 1 to 4 carbon atoms, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl, 2-nitropyridyl, 4-nitropyridyl, or 3-carboxy-4-nitropyridyl.
49. The method of any one of claims 14-48, wherein the second modified cytotoxic compound is represented by any one of the following formulas:





or a pharmaceutically acceptable salt thereof, wherein:

Y is a sulfite (HSO_3 , HSO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_2$, POS_3H_2 , PS_4H_2 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate ester ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, $\text{R}^i\text{S}-$, R^iSO , R^iSO_2 , R^iSO_3 , thiosulfate (HS_2O_3 or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite (HS_2O_4 or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^{k'}) (\text{S})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^{k'}\text{C}(=\text{O})\text{NOH}$ or a salt formed with a cation), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2^-$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$) or a mixture thereof, wherein R^i is a linear or branched alkyl having 1 to 10 carbon atoms and is substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; $\text{R}^{k'}$ is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl;

X' is selected from $-\text{H}$, an amine-protecting group, the linking group with the reactive group bonded thereto, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from $-\text{H}$, an oxo group, the linking group with the reactive group bonded thereto, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl

ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms, or the linking group with the reactive group bonded thereto;

R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-R^c$, halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'', and the linking group with the reactive group bonded thereto;

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, halogen or the linking group with the reactive group bonded thereto;

Z and Z' are independently selected from $-(CH_2)_{n'}$, $-(CH_2)_{n'}-CR_7R_8-$, $(CH_2)_{na'}$, $-(CH_2)_{n'}-NR_9-(CH_2)_{na'}$, $-(CH_2)_{n'}-O-(CH_2)_{na'}$ and $-(CH_2)_{n'}-S-(CH_2)_{na'}$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R_7 and R_8 are the same or different, and are each independently selected from -H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit $-(OCH_2CH_2)_n-$, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

A and A' are the same or different, and are independently selected from -O-, oxo ($-C(=O)-$), -CRR'O-, -CRR'-, -S-, -CRR'S-, -NR₅ and -CRR'N(R₅)-;

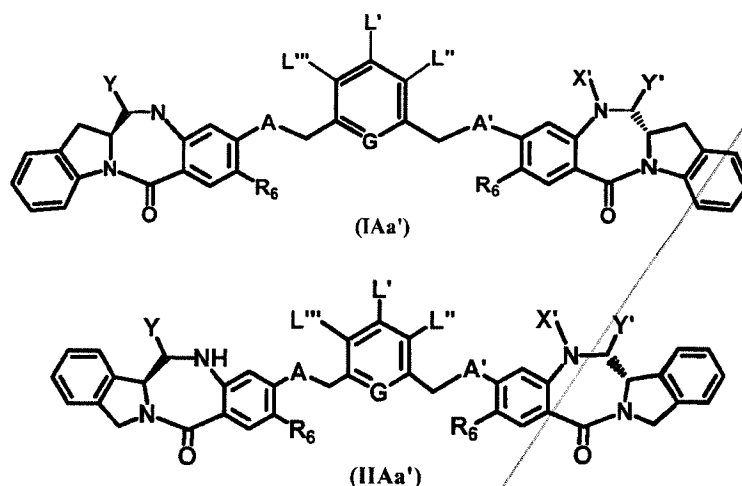
R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

L is absent, the linking group with the reactive group bonded thereto, a polyethylene glycol unit $-(OCH_2CH_2)_n-$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3 to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl or alkenyl is optionally substituted with the linking group with the reactive group bonded thereto; phenyl or heterocyclic or heteroaryl ring can be optionally substituted, wherein the substituent can be the linking group with the reactive group bonded thereto;

50. The method of claim 49, wherein L is absent, or is selected from an optionally substituted phenyl group and an optionally substituted pyridyl group, wherein the phenyl and the pyridyl group bears the linking group with the reactive group bonded thereto, or L is an amine group bearing the linking group with the reactive group bonded thereto (*i.e.*, -N(linking group)-), or L is a linear, branched or cyclic alkyl or alkenyl having from 1 to 6 carbon atoms and bearing the linking group with the reactive group bonded thereto.

51. The method of claim 49, wherein the cytotoxic compound bonded to the linking group with the reactive group attached thereto is represented by any one of the following formulas:



wherein:

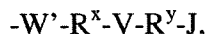
L' , L'' , and L''' are the same or different, and are independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit - $(OCH_2CH_2)_n-R^c$, halogen, guanidinium [$-NH(C=NH)NH_2$], -OR, -NR'R'', -NO₂, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M, a sulfate -OSO₃M, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'' and the linking group with the reactive group bonded thereto, provided only one of L' , L'' , and L''' is the linking group with the reactive group bonded thereto;

M is -H or a pharmaceutically acceptable cation; and,

G is selected from -CH- or -N-.

52. The method of claim 51, wherein one of L' , L'' , or L''' is the linking group with the reactive group bonded thereto, while the others are -H.
53. The method of claim 52, wherein L' is the linking group with the reactive group bonded thereto, and L'' and L''' are -H.
54. The method of claim 52 or 53, wherein A and A' are both -O-, R_6 is -OMe, and G is -CH-.

55. The method of claim 52, 53, or 54, wherein L' is represented by the following formula:



wherein:

W' and V are the same or different, and are each independently absent, or selected from -CR^eR^{e'}-, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO₂-, -CH₂-S-, -CH₂O-, -CH₂NR^e-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)-, -N(C(=O)R^e)-, -(O-CH₂-CH₂)_n-, -SS-, or -C(=O)-, or an amino acid, or a peptide having 2 to 8 amino acids;

R^x and R^y are the same or different, and are each independently absent or an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an aryl bearing 6 to 10 carbon atoms or a 3- to 8-membered heterocyclic ring bearing 1 to 3 heteroatoms selected from O, N or S;

R^e and R^{e'} are the same or different, and are selected from -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms; preferably, R¹⁰¹ and R¹⁰² are each independently a linear or branched alkyl having 1 to 6 carbon atoms;

n is an integer from 1 to 24;

J comprises the reactive group bonded thereto, and is selected from a maleimide, a haloacetamido, -SH, -SSR^d, -CH₂SH, -CH(Me)SH, -C(Me)₂SH, and -COE, wherein -COE represents a reactive ester selected from, but not limited to, N-hydroxysuccinimide ester, N-hydroxy sulfosuccinimide 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, and wherein R^{c1} is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms, and,

R^d is selected from phenyl, nitrophenyl (*e.g.*, 2 or 4-nitrophenyl),

dinitrophenyl (*e.g.*, 2 or 4-nitrophenyl), carboxynitrophenyl (*e.g.*, 3-carboxy-4-nitrophenyl), pyridyl or nitropyridyl (*e.g.*, 4-nitropyridyl).

56. The method of claim 55, wherein J is -SH, -SSR^d, a maleimide, or a N-hydroxysuccinimide ester.
57. The method of claim 55 or 56, wherein:
 - R^e is -H or -Me;
 - R^e is a linear or branched alkyl having 1 to 6 carbon atoms or -(CH₂-CH₂-O)_n-R^k;
 - n is an integer from 2 to 8; and
 - R^k is -H, -Me or -CH₂CH₂-NMe₂.
58. The method of any one of claims 55-57, wherein V is an amino acid or a peptide having 2 to 8 amino acids.
59. The method of claim 58, wherein V is valine-citrulline, gly-gly-gly, or ala-leu-ala-leu.
60. The method of claim 55, wherein:
 - W' is -O-, -N(R^e)- or -N(R^e)-C(=O)-;
 - R^e is -H, a linear or branched alkyl having 1 to 4 carbon atoms, or -(CH₂-CH₂-O)_n-R^k;
 - R^x is a linear or branched alkyl having 1 to 6 carbon atoms;
 - V is absent, -(O-CH₂-CH₂)_n-, -C(=O)-NH-, -S-, -NH-C(=O)-;
 - R^y is absent or a linear or branched alkyl having 1 to 4 carbon atoms; and
 - J is -SH, -SSR^d or -COE.
61. The method of claim 55, wherein:
 - W' is -O-, -N(R^e)- or -N(R^e)-C(=O)-;
 - R^e is -H, -Me, or -(CH₂-CH₂-O)_n-Me;
 - n is an integer from 2 to 6;
 - R^x is linear or branched alkyl bearing 1 to 6 carbon atoms;
 - V and R^y are absent; and
 - J is -COE.
62. The method of claim 60 or 61, wherein -COE is N-hydroxysuccinimide ester.

63. The method of claim 55, wherein L' is represented by the following formula:



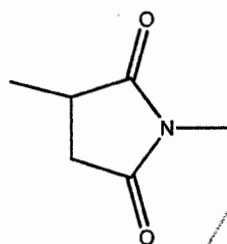
wherein:

R_1'' , R_2'' , and R_3'' are each independently -H or -Me;

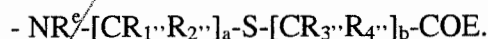
R_4'' is -H, -Me, -SO₃H, or -SO₃M⁺, wherein M⁺ is a pharmaceutically acceptable cation;

a is an integers from 0-2, b is an integer from 0-3; and,

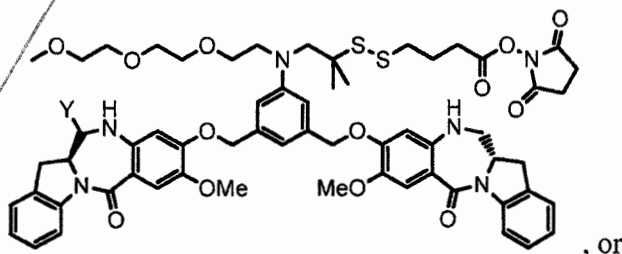
Cy is an optionally substituted 5-membered heterocyclic ring bearing an N heteroatom, preferably Cy is

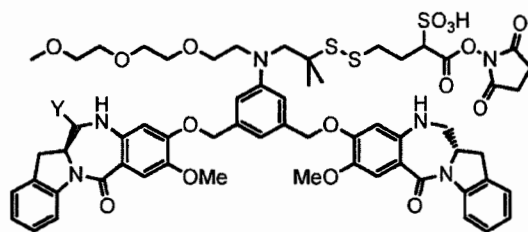


64. The method of claim 55 or 63, wherein W' is -N(R^e)-.
65. The method of claim 55, 63, or 64, wherein R^e is -(CH₂-CH₂-O)₂₋₆- R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms.
66. The method to claim 55, 63, 64, or 65, wherein V is -S- or -SS-.
67. The method of claim 55, 63, 64, 65, or 66, wherein L' is represented by the following formula:



68. The method to claim 55, 63, 64, 65, 66, or 67, wherein the cytotoxic compound bonded to the linking group with the reactive group attached thereto is:



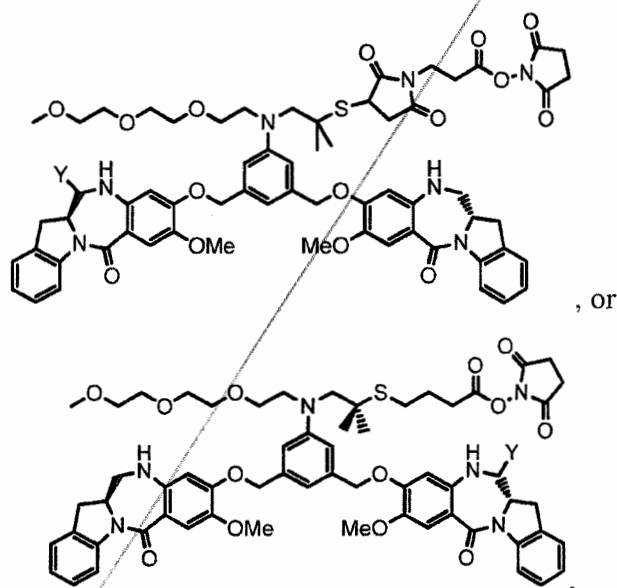


wherein Y is $-\text{SO}_3\text{M}$, and M is H or a pharmaceutically acceptable cation.

69. The method of claim 55, 63, 64, 65, or 66, wherein L' is represented by the following formula:

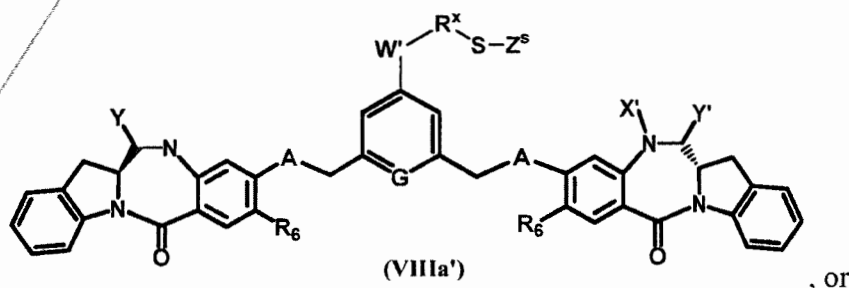


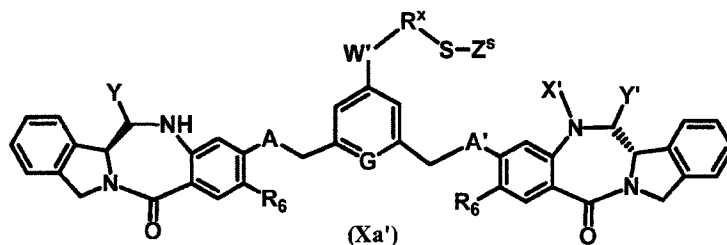
70. The method of claim 55, 63, 64, 65, 66, or 68, wherein the cytotoxic compound bonded to the linking group with the reactive group attached thereto is:



wherein Y is $-\text{SO}_3\text{M}$, and M is -H or a pharmaceutically acceptable cation.

71. The method of claim 49, 51, or 55, wherein the cytotoxic compound bonded to the linking group with the reactive group attached thereto is represented by any one of the following formulas:





wherein:

Y is selected from $-\text{SO}_3\text{M}$, $-\text{SO}_2\text{M}$ or $-\text{OSO}_3\text{M}$;

M is $-\text{H}$ or a pharmaceutically acceptable cation such as Na^+ or K^+ ;

X' is selected from the group consisting of $-\text{H}$, $-\text{OH}$, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, and an amine-protecting group;

Y' is selected from the group consisting of $-\text{H}$, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms;

A and A' are selected from $-\text{O}-$ and $-\text{S}-$;

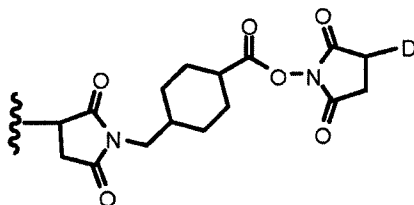
W' is absent, or selected from $-\text{O}-$, $-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)-$, $-\text{S}-$ or $-\text{CH}_2\text{S}-$, $-\text{CH}_2\text{NR}^e-$;

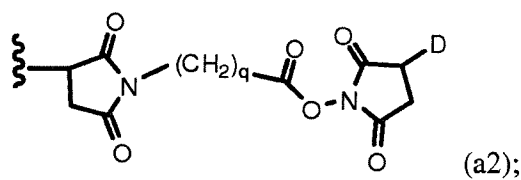
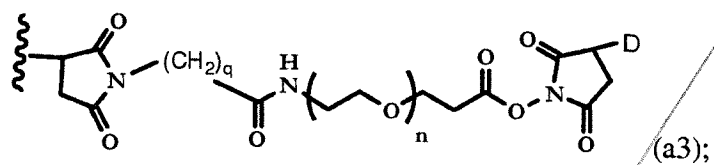
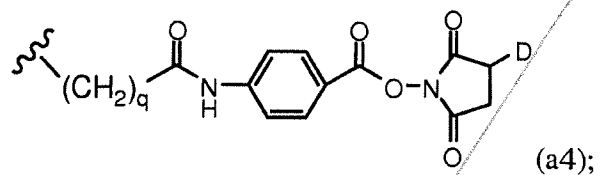
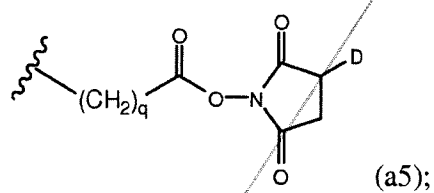
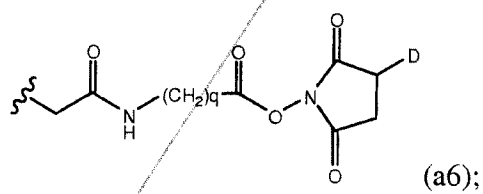
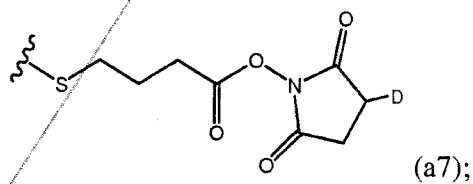
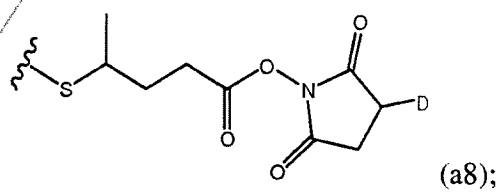
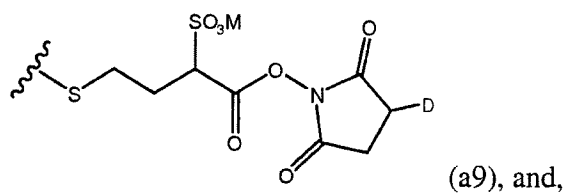
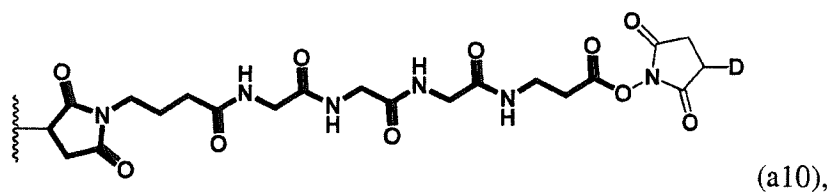
R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is $-\text{H}$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or $-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{R}^k$, wherein R^k is a $-\text{H}$, a linear, branched or cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, $-\text{NHR}^{101}$) or tertiary amino ($-\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

G is selected from $-\text{CH}-$ or $-\text{N}-$;

Z^s is $-\text{H}$, or is selected from any one of the following formulas:





wherein:

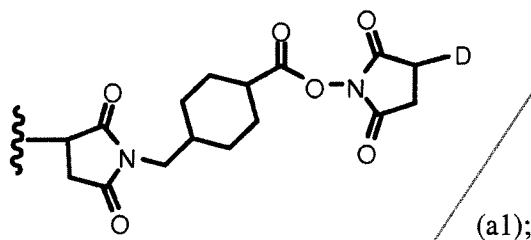
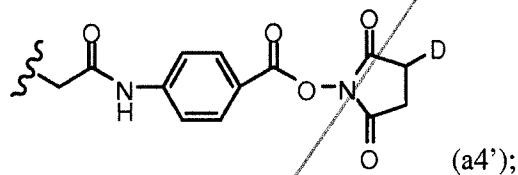
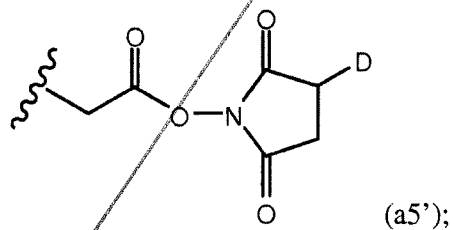
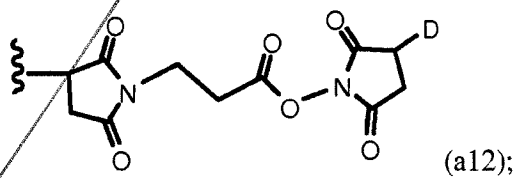
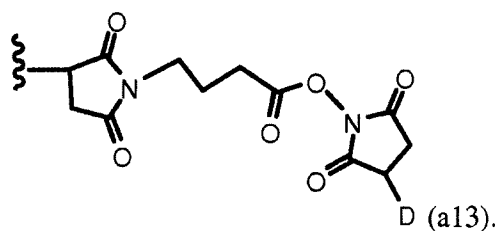
q is an integer from 1 to 5; preferably q is 2;

n is an integer from 2 to 6; preferably n is 4;

D is -H or -SO₃M;

M is -H or a pharmaceutically acceptable cation, such as Na⁺ or K⁺.

72. The method of claim 71, wherein Z^s is represented by any one of the following formulas:

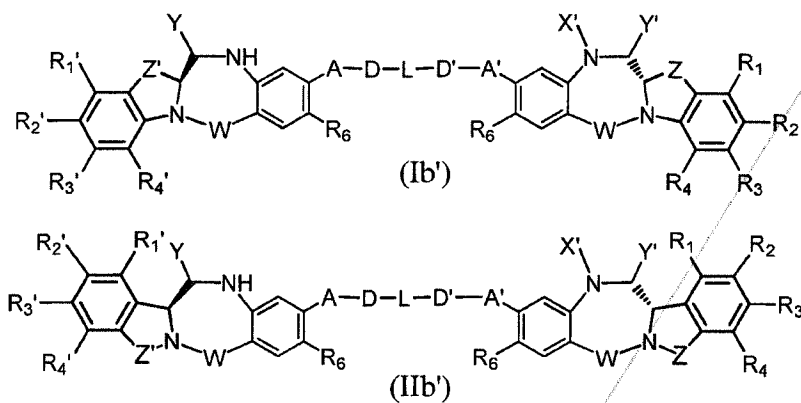


73. The method of claim 71 or 72, wherein W' is -N(R^e)-.
74. The method of claim 73, wherein R^e is -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms.
75. The method of claim 74, wherein R^k is -H or -Me, n is 4, and q is 2.

76. The method of claim 75, wherein R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
77. The method of claim 75, wherein R^x is $-(CH_2)_p-(CR^fR^g)-$, wherein R^f and R^g are each independently selected from -H or a linear or branched alkyl having 1 to 4 carbon atoms; and p is 0, 1, 2 or 3.
78. The method of claim 77, wherein R^f and R^g are the same or different, and are selected from -H and -Me; and p is 1.
79. The method of any one of claims 71-78, wherein:
Y is $-SO_3M$;
M is -H or a pharmaceutically acceptable cation (*e.g.*, Na^+);
 X' and Y' are both -H;
A and A' are both -O-;
 R_6 is -OMe; and
 R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
80. The method of any one of claims 49-78, wherein Y is selected from $-SO_3M$, $-SO_2M$ and a sulfate $-OSO_3M$.
81. The method of claim 80, wherein Y is $-SO_3M$.
82. The method of any one of claims 80-81, wherein M is -H, Na^+ or K^+ .
83. The method of any one of claims 49-82, wherein W, when present, is $C=O$.
84. The method of any one of claims 49-83, wherein Z and Z' , when present, are $-CH_2-$.
85. The method of any one of claims 49-84, wherein X' is selected from the group consisting of -H, -OH, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, the linking group with the reactive group bonded thereto, and an amine-protecting group.
86. The method of claim 85, wherein X' is -H, -OH, -Me or the linking group with the reactive group bonded thereto.
87. The method of claim 86, wherein X' is -H.

88. The method of any one of claims 49-87, wherein Y' is selected from the group consisting of -H, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms.
89. The method of claim 88, wherein Y' is -H or oxo.
90. The method of claim 89, wherein Y' is -H.
91. The method of any one of claims 49-90, wherein A and A' are the same or different, and are selected from -O-, -S-, -NR₅-, and oxo -(C=O)-.
92. The method of claim 91, wherein A and A' are the same or different, and are selected from -O- and -S-.
93. The method of claim 92, wherein A and A' are -O-.
94. The method of any one of claims 49-93, wherein D and D', when present, are the same or different, and are independently selected from a polyethylene glycol unit (-OCH₂CH₂)_n, wherein n is an integer from 1 to 24, an amino acid, a peptide bearing 2 to 6 amino acids, or a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, wherein the alkyl, alkenyl and alkynyl are optionally substituted with one or more substituents independently selected from the group consisting of halogen, -OR, -NR'COR'', -SR and -COR'.
95. The method of claim 94, wherein D and D' are linear or branched alkyl bearing 1 to 4 carbon atoms.
96. The method of claim 49, 50, 51, or 71, wherein:
W is C=O;
R₁, R₂, R₁', R₂', R₄ and R₄' are -H;
one of R₃, or R₃' is optionally the linking group with the reactive group bonded thereto and the other is -H;
R₆ is -OMe;
Z and Z' are -CH₂-;
X' is -H;
Y' is -H; and
A and A' are -O-.
97. The method of any one of claims 49-96, wherein the linking group with the reactive group attached thereto is any one in List 1.

98. The method of any one of claims 1-97, wherein the cytotoxic compound and the linking group portion of the conjugate is represented by any one of the following formulas:



or a pharmaceutically acceptable salt thereof, wherein:

Y is a leaving group, and is a sulfite (HSO_3 , HSO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_2$, POS_3H_2 , PS_4H_2 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate ester ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, $\text{R}^i\text{S}-$, R^iSO , R^iSO_2 , R^iSO_3 , thiosulfate (HS_2O_3 or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite (HS_2O_4 or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{S})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NOH}$ or a salt formed with a cation), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2^-$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$) or a mixture thereof, wherein R^i is a linear or branched alkyl having 1 to 10 carbon atoms and is substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl;

X' is selected from $-\text{H}$, an amine-protecting group, the linking group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms

independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, the linking group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms, or the linking group;

R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(OCH₂CH₂)_n-R^c, halogen, guanidinium [-NH(C=NH)NH₂], -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'' and the linking group;

M is -H or a pharmaceutically acceptable cation, such as Na⁺;

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^c, and an optionally substituted 3-18-membered heterocyclic ring

having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, halogen or the linking group;

Z and Z' are independently selected from -(CH₂)_n-, -(CH₂)_n-CR₇R₈-(CH₂)_{na'}-, -(CH₂)_n-NR₉-(CH₂)_{na'}-, -(CH₂)_n-O-(CH₂)_{na'}- and -(CH₂)_n-S-(CH₂)_{na'}-;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R₇ and R₈ are the same or different, and are each independently selected from -H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit -(OCH₂CH₂)_n-, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R₉ is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(OCH₂CH₂)_n-;

A and A' are the same or different, and are independently selected from -O-, oxo (-C(=O)-), -CRR'O-, -CRR'-, -S-, -CRR'S-, -N(R₅)- and -CRR'N(R₅)-,

R₅ for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit -(OCH₂CH₂)_n-;

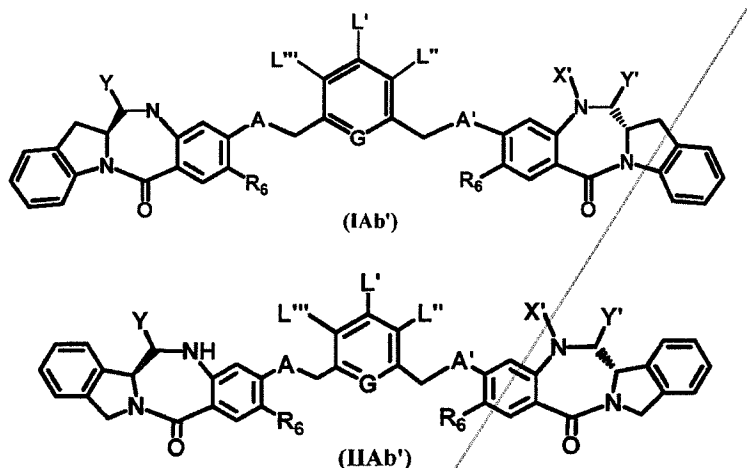
L is absent, the linking group, a polyethylene glycol unit -(OCH₂CH₂)_n-, an optionally substituted linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl or alkenyl is optionally substituted with the linking group; phenyl or heterocyclic or heteroaryl ring can be optionally substituted, wherein the substituent can comprise the linking group.

99. The method of claim 98, wherein Y is -SO₂M, -SO₃M, or -OSO₃M.

100. The method of claim 98 or 99, wherein L is absent, or is selected from an optionally substituted phenyl group and an optionally substituted pyridyl group, wherein the phenyl and the pyridyl group bears the linking group, or L is an

amine group bearing the linking group (*i.e.*, -N(linking group)-), or L is a linear, branched or cyclic alkyl or alkenyl having from 1 to 6 carbon atoms and bearing the linking group.

101. The method of claim 98 or 99, wherein the cytotoxic compound bonded to the linking group is represented by any one of the following formulas:



wherein:

L', L'', and L''' are the same or different, and are independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit - (OCH₂CH₂)_n-R^c, halogen, guanidinium [-NH(C=NH)NH₂], -OR, -NR'R'', -NO₂, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M, a sulfate -OSO₃M, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'' and the linking group, provided only one of L', L'', and L''' is the linking group; and G is selected from -CH- or -N-.

102. The method of claim 101, wherein one of L', L'', or L''' is the linking group, while the others are -H.
103. The method of claim 102, wherein L' is the linking group, and L'' and L''' are -H.
104. The method of claim 102 or 103, wherein A and A' are both -O-, R₆ is -OMe, and G is -CH-.
105. The method of claim 102, 103, or 104, wherein L' is represented by the following formula:



wherein:

W' and V are the same or different, and are each independently absent, or selected from -CR^eR^{e'}-, -O-, -O-C(=O)-, -C(=O)-O-, -S-, -SO-, -SO₂-, -CH₂-S-, -CH₂O-, -CH₂NR^e-, -O-(C=O)O-, -O-(C=O)N(R^e)-, -N(R^e)-, -N(R^e)-C(=O)-, -C(=O)-N(R^e)-, -N(R^e)-C(=O)O-, -N(C(=O)R^e)C(=O)-, -N(C(=O)R^e)-, -(O-CH₂-CH₂)_n-, -SS-, or -C(=O)-, or an amino acid, or a peptide having 2 to 8 amino acids;

R^x and R^y are the same or different, and are each independently absent or an optionally substituted linear, branched or cyclic alkyl, alkenyl, or alkynyl having 1 to 10 carbon atoms, an aryl bearing 6 to 10 carbon atoms or a 3- to 8-membered heterocyclic ring bearing 1 to 3 heteroatoms selected from O, N or S;

R^e and R^{e'} are the same or different, and are selected from -H, a linear, branched or cyclic alkyl, alkenyl, or alkynyl having 1 to 10 carbon atoms or -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms; preferably, R¹⁰¹ and R¹⁰² are each independently a linear or branched alkyl having 1 to 6 carbon atoms;

n is an integer from 1 to 24; and

J is covalently linked to the CBA, and is selected from a succinimide, a acetamido, -S-, -SS-, -CH₂S-, -CH(Me)S-, -C(Me)₂S-, -NR^{c1}-, -CH₂NR^{c1}-, -NR^{c1}N-, and -C(=O)-, wherein R^{c1} is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms.

106. The method of claim 105, wherein J is -S-, -SS-, a succinimide, or -C(=O)-.

107. The method of claim 105 or 106, wherein:

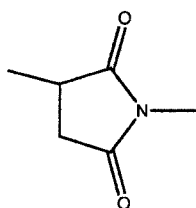
R^{e'} is -H or -Me;

R^e is a linear or branched alkyl having 1 to 6 carbon atoms or -(CH₂-CH₂-O)_n-R^k;

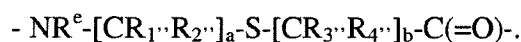
n is an integer from 2 to 8; and

R^k is -H, -Me or -CH₂CH₂-NMe₂.

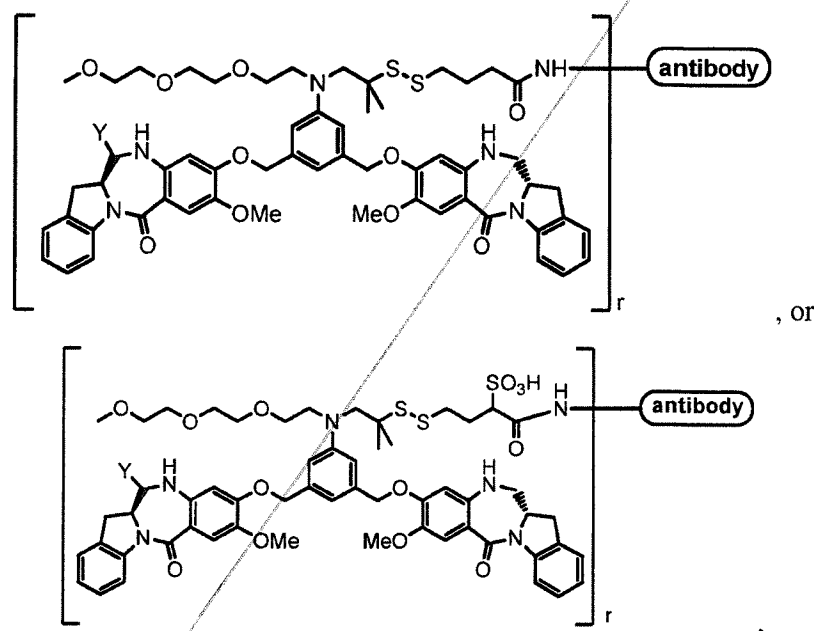
108. The method of any one of claims 105-107, wherein V is an amino acid or a peptide having 2 to 8 amino acids.
109. The method of claim 108, wherein V is valine-citrulline, gly-gly-gly, or ala-leu-ala-leu.
110. The method of claim 105, wherein:
W' is -O-, -N(R^e)- or -N(R^e)-C(=O)-;
R^e is -H, a linear or branched alkyl having 1 to 4 carbon atoms, or -(CH₂-CH₂-O)_n-R^k;
R^x is a linear or branched alkyl having 1 to 6 carbon atoms;
V is absent, -(O-CH₂-CH₂)_n-, -C(=O)-NH-, -S-, -NH-C(=O)-;
R^y is absent or a linear or branched alkyl having 1 to 4 carbon atoms; and
J is -S-, -SS-, or -C(=O)-.
111. The method of claim 105, wherein:
W' is -O-, -N(R^e)- or -N(R^e)-C(=O)-;
R^e is -H, -Me, or -(CH₂-CH₂-O)_n-Me;
n is an integer from 2 to 6;
R^x is linear or branched alkyl bearing 1 to 6 carbon atoms;
V and R^y are absent; and
J is -C(=O)-.
112. The method of claim 105, wherein L' is represented by the following formula:
-W'-[CR_{1''}R_{2''}]_a-V-[Cy]₀₋₁-[CR_{3''}R_{4''}]_b-C(=O)-,
wherein:
R_{1''}, R_{2''}, and R_{3''} are each independently -H or -Me;
R_{4''} is -H, -Me, -SO₃H, or -SO₃⁻M⁺, wherein M⁺ is a pharmaceutically acceptable cation;
a is an integers from 0-2, b is an integer from 0-3; and,
Cy is an optionally substituted 5-membered heterocyclic ring bearing an N heteroatom, preferably Cy is



113. The method of claim 105 or 112, wherein W' is $-N(R^e)-$.
114. The method of claim 105, 112, or 113, wherein R^e is $-(CH_2-CH_2-O)_{2-6}-R^k$, wherein R^k is a linear or branched alkyl having 1 to 6 carbon atoms.
115. The method to claim 105, 112, 113, or 114, wherein V is $-S-$ or $-SS-$.
116. The method of claim 105, 112, 113, 114, or 115, wherein L' is represented by the following formula:

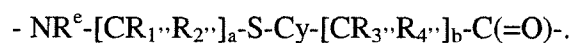


117. The method to claim 105, 112, 113, 114, or 115, wherein the conjugate is:

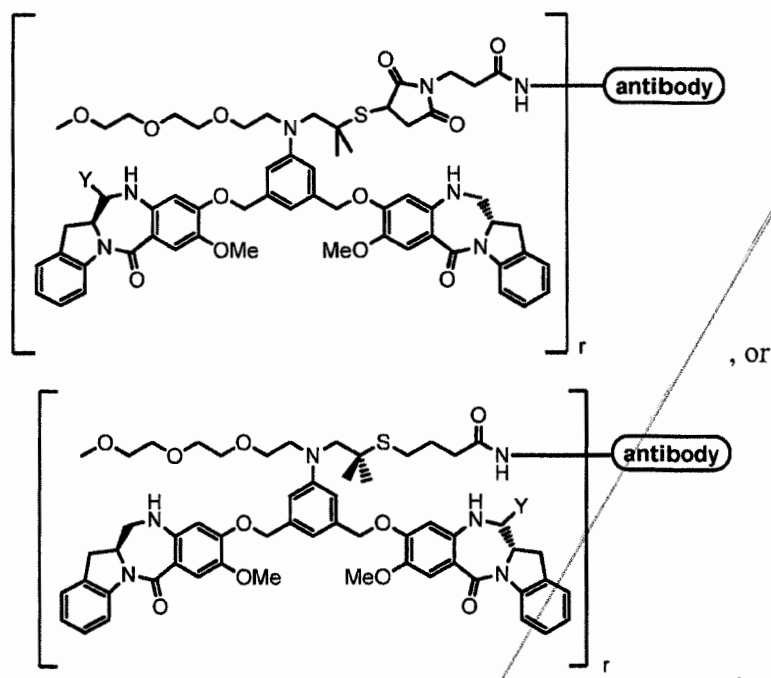


wherein r is an integer from 1 to 10, Y is $-SO_3M$, and M is $-H$ or a pharmaceutically acceptable cation.

118. The method of claim 105, 112, 113, 114, 115, 116, or 117, wherein the antibody is huMy9-6.
119. The method of claim 105, 112, 113, 114, or 115, wherein L' is represented by the following formula:

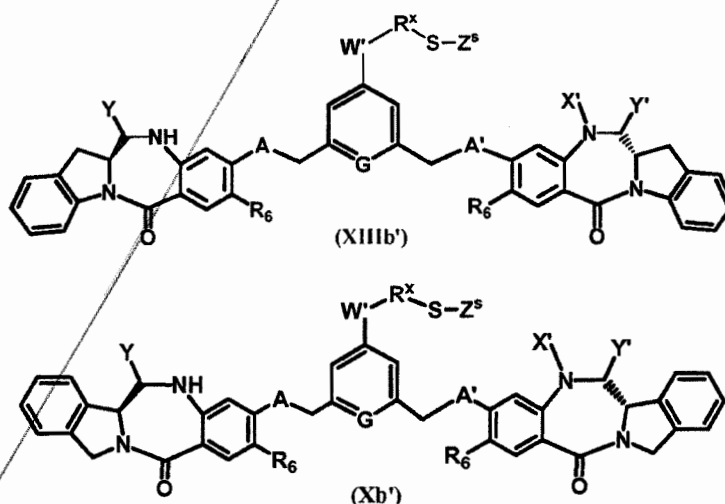


120. The method of claim 105, 112, 113, 114, 115, or 119, wherein the conjugate is:



wherein r is an integer from 1 to 10, Y is $-\text{SO}_3\text{M}$, and M is $-\text{H}$ or a pharmaceutically acceptable cation.

121. The method of claim 105, 112, 113, 114, 115, 119, or 120, wherein the antibody is huMy9-6.
122. The method of claim 98, 99, or 101, wherein the cytotoxic compound bonded to the linking group is represented by the following formula:



wherein:

W' is absent, or selected from $-\text{O}-$, $-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)-$, $-\text{S}-$, $-\text{CH}_2-\text{S}-$, or $-\text{CH}_2\text{NR}^e-$;

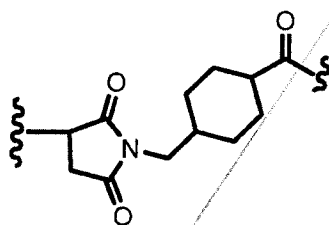
R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or $-(CH_2-CH_2-O)_n-R^k$, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, $-NHR^{101}$) or tertiary amino ($-NR^{101}R^{102}$) group or a 5 or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

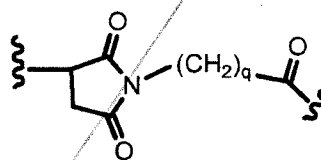
n is an integer from 2 to 6;

Z^s is linked to the CBA, and is selected from:

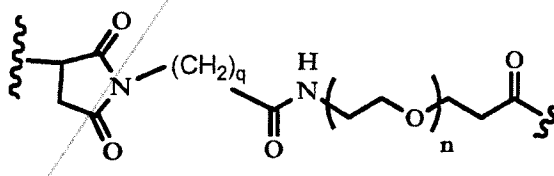
a bond;



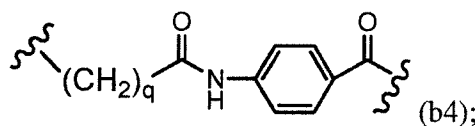
(b1);



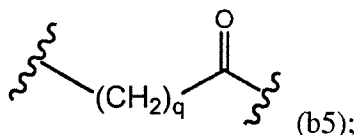
(b2);



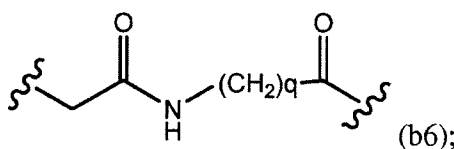
(b3);



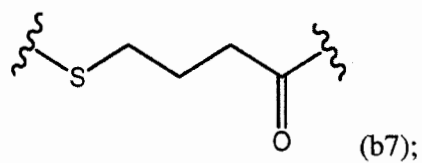
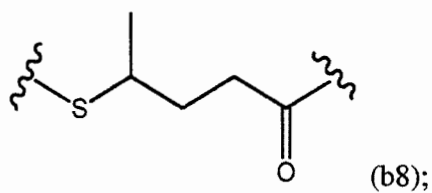
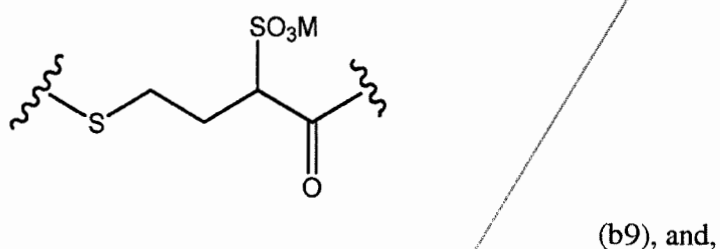
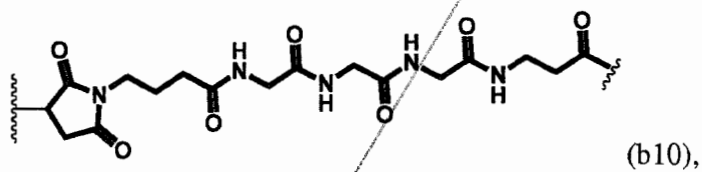
(b4);



(b5);



(b6);

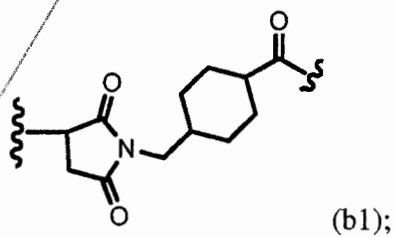
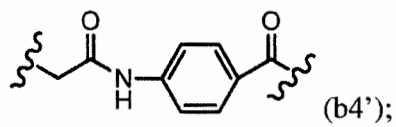
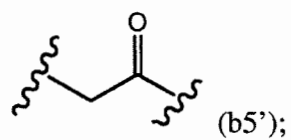
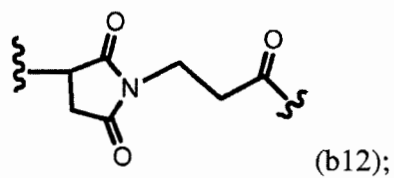


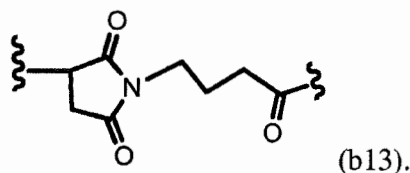
wherein:

q is an integer from 1 to 5; and,

M is -H or a pharmaceutically acceptable cation, such as Na⁺ or K⁺.

123. The method of claim 122, wherein Z^s is:





124. The method of claim 122 or 123, wherein W' is -N(R^e)-
125. The method of claim 124, wherein R^e is -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms.
126. The method of claim 125, wherein R^k is -H or -Me, n is 4, and q is 2.
127. The method of claim 126, wherein R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
128. The method of claim 126, wherein R^x is -(CH₂)_p-(CR^fR^g)-, wherein R^f and R^g are each independently selected from H or a linear or branched alkyl having 1 to 4 carbon atoms; and p is 0, 1, 2 or 3.
129. The method of claim 128, wherein R^f and R^g are the same or different, and are selected from H and Me; and p is 1.
130. The method of any one of claims 122-129, wherein:
 - Y is -SO₃M;
 - M is -H or a pharmaceutically acceptable cation (*e.g.*, Na⁺);
 - X' and Y' are both -H;
 - A and A' are both -O-;
 - R₆ is -OMe; and
 - R^x is a linear or branched alkyl having 1 to 6 carbon atoms.
131. The method of any one of claims 98-129, wherein Y is selected from -SO₃M, -SO₂M and a sulfate -OSO₃M.
132. The method of claim 131, wherein Y is -SO₃M.
133. The method of any one of claims 131-132, wherein M is -H, Na⁺ or K⁺.
134. The method of any one of claims 98-133, wherein W, when present, is C=O.
135. The method of any one of claims 98-134, wherein Z and Z', when present, are -CH₂-.

136. The method of any one of claims 98-135, wherein X' is selected from the group consisting of -H, -OH, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, the linking group, and an amine-protecting group.
137. The method of claim 136, wherein X' is -H, -OH, -Me or the linking group.
138. The method of claim 137, wherein X' is -H.
139. The method of any one of claims 98-138, wherein Y' is selected from the group consisting of -H, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms.
140. The method of claim 139, wherein Y' is -H or oxo.
141. The method of claim 140, wherein Y' is -H.
142. The method of any one of claims 98-141, wherein A and A' are the same or different, and are selected from -O-, -S-, -N(R₅)-, and oxo (C=O).
143. The method of claim 142, wherein A and A' are the same or different, and are selected from -O- and -S-.
144. The method of claim 143, wherein A and A' are -O-.
145. The method of any one of claims 98-144, wherein D and D', when present, are the same or different, and are independently selected from a polyethylene glycol unit (-OCH₂CH₂)_n, wherein n is an integer from 1 to 24, an amino acid, a peptide bearing 2 to 6 amino acids, or a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, wherein the alkyl, alkenyl and alkynyl are optionally substituted with one or more substituents independently selected from the group consisting of halogen, -OR, -NR'COR'', -SR and -COR'.
146. The method of claim 145, wherein D and D' are linear or branched alkyl bearing 1 to 4 carbon atoms.
147. The method of claim 98, 99, 101, or 122, wherein:
Y is -SO₃M;
M is -H or a pharmaceutically acceptable cation (such as Na⁺);
W is C=O;
R₁, R₂, R₁', R₂', R₄ and R₄' are -H;

one of R_3 , or R_3' is optionally the linking group and the other is -H;
 R_6 is -OMe;
 Z and Z' are $-CH_2-$;
 X' is -H;
 Y' is -H; and
 A and A' are -O-.

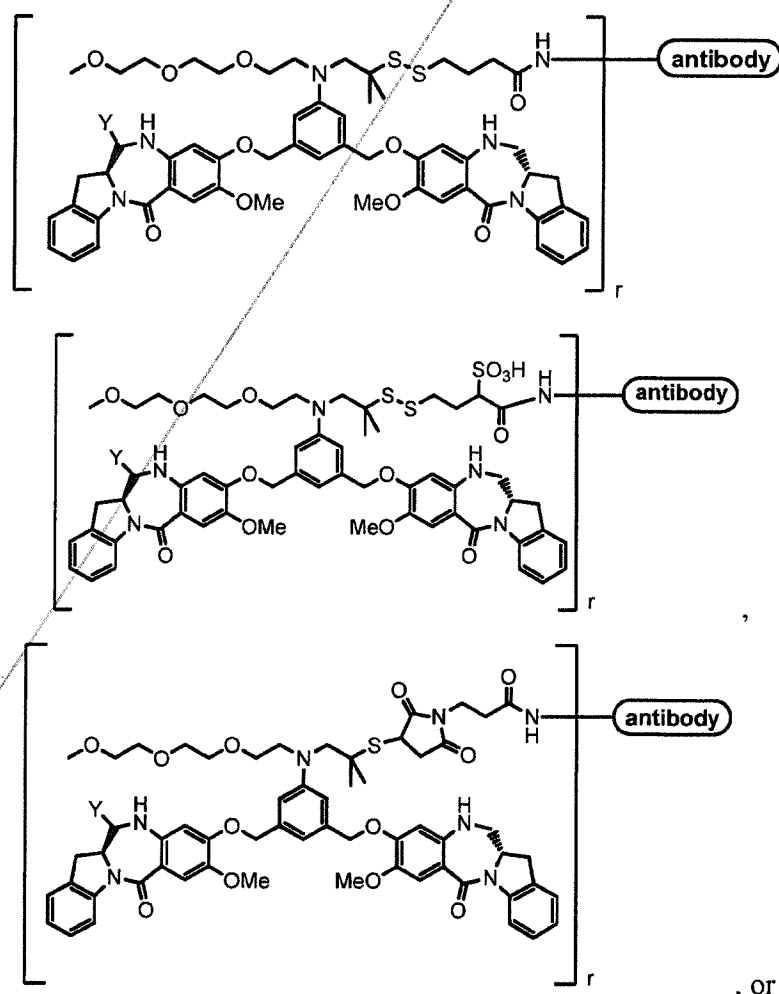
148. The method of any one of claims 98-147, wherein the conjugate comprises 1-10 cytotoxic compounds, each cytotoxic compound is linked to the CBA through the linking group, and each cytotoxic compound on the conjugate is the same.
149. The method of any one of claims 1-9 and 14-22, wherein the conjugate comprises 1-10 modified cytotoxic compounds, each modified cytotoxic compound is linked to the CBA through the linking group, and each modified cytotoxic compound on the conjugate is the same.
150. The method of any one of claims 98-149, wherein the cell-binding agent binds to target cells selected from tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melanocytes; cells expressing the CD4, CD6, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD44, CD56, EpCAM, CanAg, CALLA, or Her-2 antigens; Her-3 antigens; or cells expressing insulin growth factor receptor, epidermal growth factor receptor, and folate receptor.
151. The method of claim 150, 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, or a monoclonal antibody fragment that specifically binds the 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.
152. The method of claim 151, wherein the antibody is a resurfaced antibody, a resurfaced single chain antibody, or a resurfaced antibody fragment.
153. The method of claim 151, wherein the antibody is a monoclonal antibody, a single chain monoclonal antibody, or a monoclonal antibody fragment thereof.

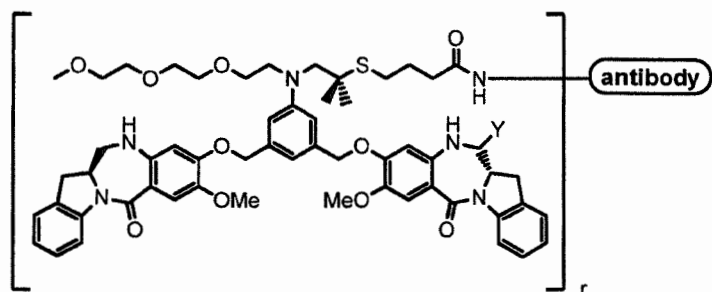
154. The method of claim 151, wherein the antibody is a humanized antibody, a humanized single chain antibody, or a humanized antibody fragment.
155. The method of any one of claims 2-154, wherein the imine reactive reagent is selected from the group consisting of sulfites (H_2SO_3 , H_2SO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_3$, POS_3H_3 , PS_4H_3 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate esters ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, R^iSH , R^iSOH , $\text{R}^i\text{SO}_2\text{H}$, $\text{R}^i\text{SO}_3\text{H}$), various amines (hydroxyl amine (*e.g.*, NH_2OH), hydrazine (*e.g.*, NH_2NH_2), $\text{NH}_2\text{O}-\text{R}^i$, $\text{R}^i\text{NH}-\text{R}^i$, NH_2-R^i), $\text{NH}_2-\text{CO}-\text{NH}_2$, $\text{NH}_2-\text{C}(=\text{S})-\text{NH}_2$), thiosulfate ($\text{H}_2\text{S}_2\text{O}_3$ or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite ($\text{H}_2\text{S}_2\text{O}_4$ or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{SH})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NHOH}$ or a salt formed with a cation), hydrazide ($\text{R}^k\text{CONHNH}_2$), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2\text{H}$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$), glycated nucleotide (such as GDP-mannose), fludarabine or a mixture thereof, wherein R^i and R^i are each independently a linear or branched alkyl having 1 to 10 carbon atoms and are substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i and R^i can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl.
156. The method of any one of claims 2-154, wherein the imine reactive reagent is selected from sulfites, hydroxylamine, hydrazine and urea.
157. The method of any one of claims 2-154, wherein the imine reactive reagent is NaHSO_3 or KHSO_3 .
158. The method of any one of claims 2-157, wherein about 0.1 to about 30 molar equivalents of the imine reactive reagent to the imine-containing cytotoxic compound is used.

159. The method of any one of claims 2-157, wherein about 1 to about 10 molar equivalents of the imine reactive reagent to the imine-containing cytotoxic compound is used.
160. The method of any one of claims 2-157, wherein about 3 to about 5 molar equivalents of the imine reactive reagent to the imine-containing cytotoxic compound is used.
161. The method of any one of claims 1-160, wherein the bifunctional crosslinking agent is:
 a maleimido-based moiety selected from: N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), κ -maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ -maleimidobutyric acid N-succinimidyl ester (GMBS), ϵ -maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α -maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB), N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate; or,
 a haloacetyl-based moiety selected from: N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA), and N-succinimidyl 3-(bromoacetamido)propionate (SBAP), bis-maleimidopolyethyleneglycol (BMPEO), BM(PEO)₂, BM(PEO)₃, N-(β -maleimidopropoxy)succinimide ester (BMPS), 5-maleimidovaleric acid NHS, HBVS, 4-(4-N-maleimidophenyl)-butyric acid hydrazide•HCl (MPBH), Succinimidyl-(4-vinylsulfonyl)benzoate (SVSB), dithiobis-maleimidoethane (DTME), 1,4-bis-maleimidobutane (BMB), 1,4-bismaleimidyl-2,3-dihydroxybutane (BMDB), bis-maleimidoethane (BMH), bis-maleimidoethane (BMOE), sulfosuccinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (sulfo-SMCC), sulfosuccinimidyl(4-iodo-acetyl)aminobenzoate (sulfo-SIAB), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS), N-(γ -maleimidobutryloxy)sulfosuccinimide ester (sulfo-GMBS), N-(ϵ -maleimidocaproyloxy)sulfosuccinimide ester (sulfo-EMCS), N-(κ -

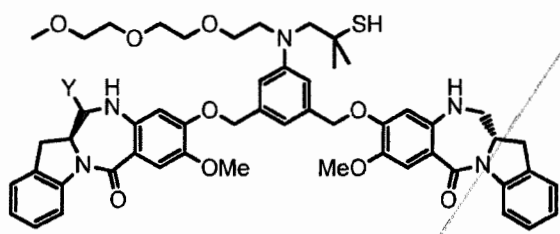
maleimidoundecanoyloxy)sulfosuccinimide ester (sulfo-KMUS), sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate (sulfo-SMPB), CX1-1, sulfo-Mal and PEG_n-Mal.

162. The method of any one of claims 1-161, wherein the bifunctional crosslinking agent is selected from the group consisting of SMCC, Sulfo-SMCC, BMPS, GMBS, SIA, SIAB, *N*-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate, bis-maleimido-hexane or BMPEO.
163. The method of any one of claims 1-162, wherein the conjugate is purified by tangential flow filtration, adsorptive chromatography, adsorptive filtration, selective precipitation, non-adsorptive filtration or combination thereof.
164. The method of any one of claims 1-162, wherein the conjugate is purified by tangential flow filtration and/or adsorptive chromatography.
165. A method for preparing a conjugate of the following formula:

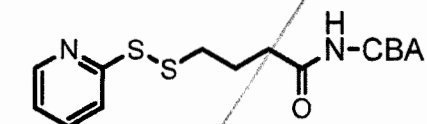
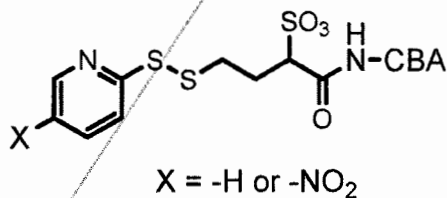
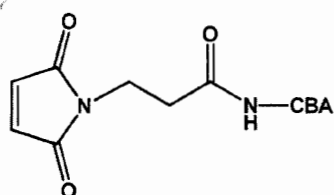
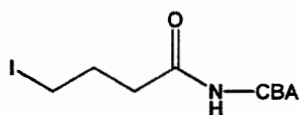




the method comprising reacting a modified cytotoxic compound of the following formula,



with a modified CBA of the following formula, respectively, at a pH of about 4 to about 9,



wherein:

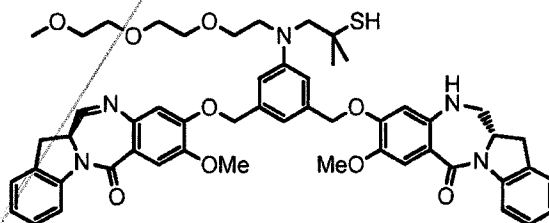
r is an integer from 1 to 10;

Y is a leaving group, and is a sulfite (HSO_3 , HSO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono-, di-, tri-, and tetra- thiophosphate (PO_3SH_3 ,

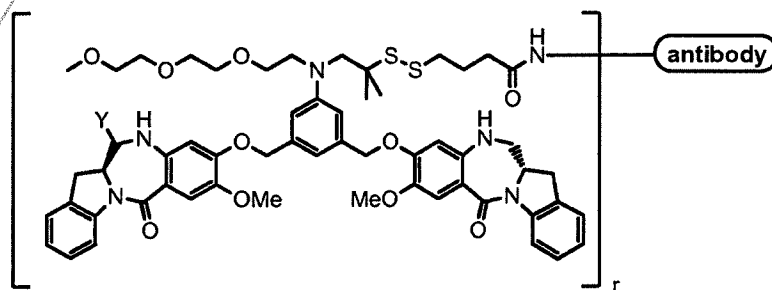
PO₂S₂H₂, POS₃H₂, PS₄H₂ or a salt of PO₃S³⁻, PO₂S₂³⁻, POS₃³⁻ or PS₄³⁻ formed with a cation), thio phosphate ester (RⁱO)₂PS(ORⁱ), RⁱS-, RⁱSO, RⁱSO₂, RⁱSO₃, thiosulfate (HS₂O₃ or a salt of S₂O₃²⁻ formed with a cation), dithionite (HS₂O₄ or a salt of S₂O₄²⁻ formed with a cation), phosphorodithioate (P(=S)(OR^{k'})(S)(OH) or a salt thereof formed with a cation), hydroxamic acid (R^{k'}C(=O)NOH or a salt formed with a cation), formaldehyde sulfoxylate (HOCH₂SO₂⁻ or a salt of HOCH₂SO₂⁻ formed with a cation, such as HOCH₂SO₂⁻Na⁺) or a mixture thereof, wherein Rⁱ is a linear or branched alkyl having 1 to 10 carbon atoms and is substituted with at least one substituent selected from -N(R^j)₂, -CO₂H, -SO₃H, and -PO₃H; Rⁱ can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^{k'} is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl; preferably Y is -SO₃M; and

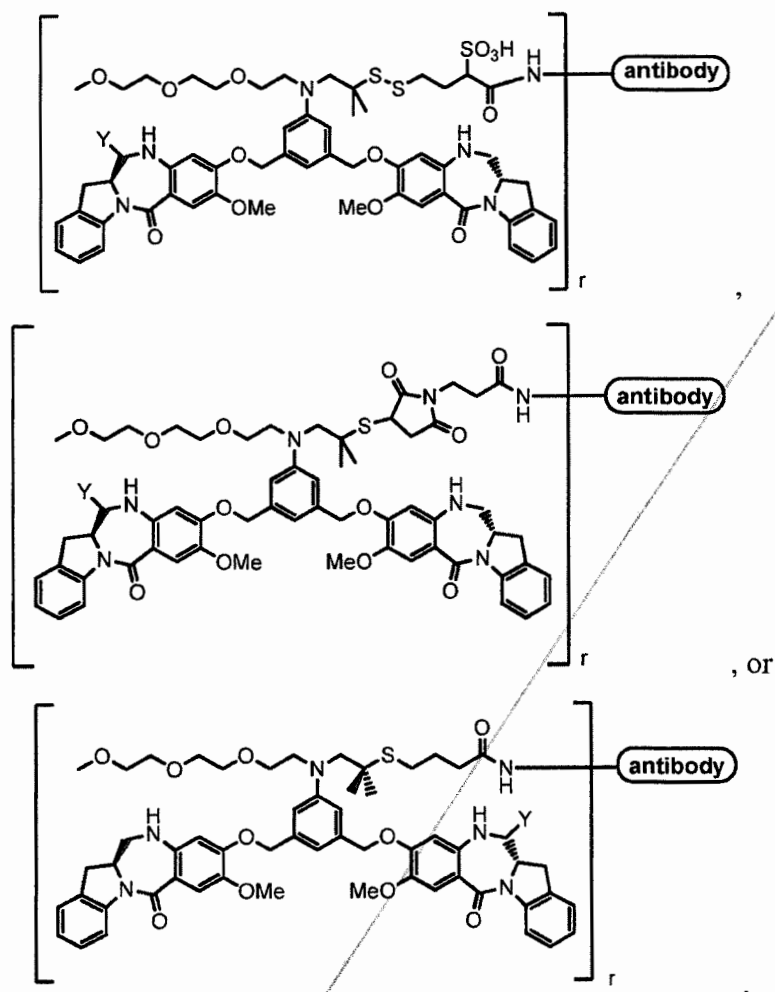
M is -H or a pharmaceutically acceptable cation.

166. The method of claim 165, wherein Y is -SO₃M; and M is -H or a pharmaceutically acceptable cation.
167. The method of claim 165 or 166, wherein the modified cytotoxic compound is produced by reacting an imine reactive reagent with an imine-containing cytotoxic compound of the following formula:



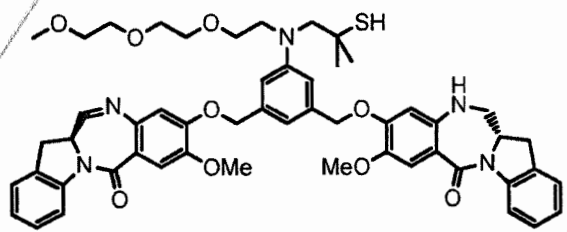
168. The method of any one of claims 165-167, wherein the CBA is huMy9-6.
169. A method for preparing a conjugate of the following formula:



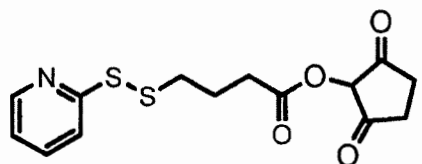


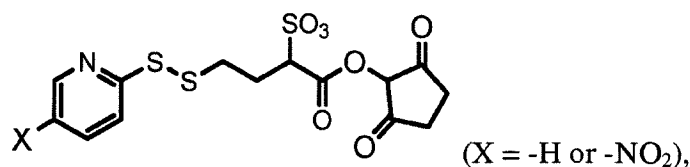
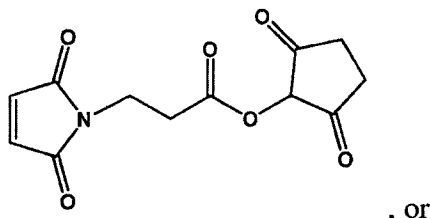
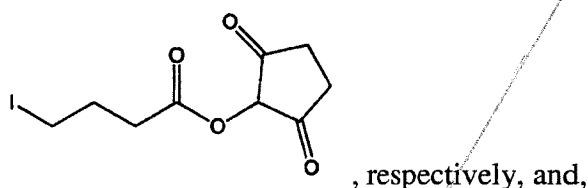
the method comprising reacting the CBA with an imine-containing cytotoxic compound, an imine reactive reagent, and a bifunctional crosslinking agent comprising the linking group to form the conjugate, wherein:

the imine-containing cytotoxic compound is:

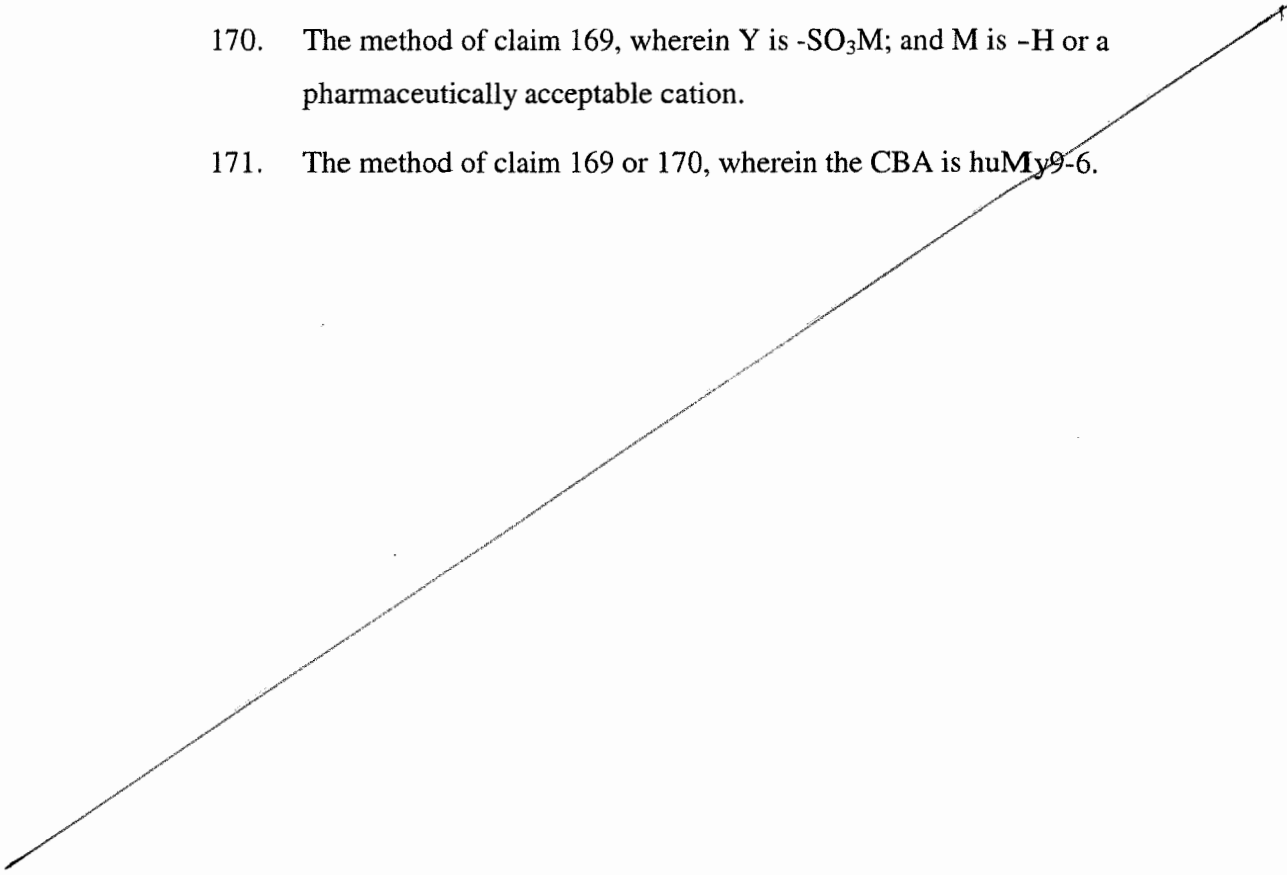


the bifunctional crosslinking agent is:



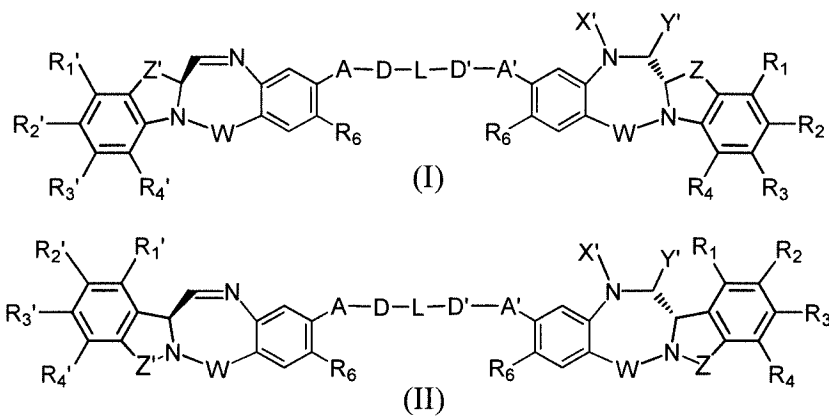


the imine reactive reagent is selected from: sulfites (H₂SO₃, H₂SO₂ or a salt of HSO₃⁻, SO₃²⁻ or HSO₂⁻ formed with a cation), metabisulfite (H₂S₂O₅ or a salt of S₂O₅²⁻ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO₃SH₃, PO₂S₂H₃, POS₃H₃, PS₄H₃ or a salt of PO₃S³⁻, PO₂S₂³⁻, POS₃³⁻ or PS₄³⁻ formed with a cation), thio phosphate esters ((RⁱO)₂PS(ORⁱ), RⁱSH, RⁱSOH, RⁱSO₂H, RⁱSO₃H), various amines (hydroxyl amine (*e.g.*, NH₂OH), hydrazine (*e.g.*, NH₂NH₂), NH₂O-Rⁱ, RⁱNH-Rⁱ, NH₂-Rⁱ), NH₂-CO-NH₂, NH₂-C(=S)-NH₂), thiosulfate (H₂S₂O₃ or a salt of S₂O₃²⁻ formed with a cation), dithionite (H₂S₂O₄ or a salt of S₂O₄²⁻ formed with a cation), phosphorodithioate (P(=S)(OR^k)(SH)(OH) or a salt thereof formed with a cation), hydroxamic acid (R^kC(=O)NHOH or a salt formed with a cation), hydrazide (R^kCONHNH₂), formaldehyde sulfoxylate (HOCH₂SO₂H or a salt of HOCH₂SO₂⁻ formed with a cation, such as HOCH₂SO₂⁻Na⁺), glycosylated nucleotide (such as GDP-mannose), fludarabine or a mixture thereof, wherein Rⁱ and Rⁱ are each independently a linear or branched alkyl having 1 to 10 carbon atoms and are substituted with at least one substituent selected from -N(R^j)₂, -CO₂H, -SO₃H, and -PO₃H; Rⁱ and Rⁱ can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl.

170. The method of claim 169, wherein Y is $-\text{SO}_3\text{M}$; and M is $-\text{H}$ or a pharmaceutically acceptable cation.
171. The method of claim 169 or 170, wherein the CBA is huMy9-6.
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CLAIMS

1. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising reacting a modified cytotoxic compound with a modified CBA at a pH of about 4 to about 9, wherein:
 - a) the modified CBA comprises a residue of a bifunctional crosslinking agent bonded to the CBA, and the residue comprises the linking group and a thiol-reactive group; and
 - b) the modified cytotoxic compound comprises a thiol group, and is produced by reacting an imine-containing cytotoxic compound bearing the thiol group with an imine reactive agent, and wherein the imine-containing cytotoxic compound is represented by one of the following formulae:



or a pharmaceutically acceptable salt thereof, wherein:

X' is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered

heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$, R^c , halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'';

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, and an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or, halogen;

Z and Z' are independently selected from $-(CH_2)_{n'}-$, $-(CH_2)_{n'}-CR_7R_8-(CH_2)_{na'}-$, $-(CH_2)_{n'}-NR_9-(CH_2)_{na'}-$, $-(CH_2)_{n'}-O-(CH_2)_{na'}-$ and $-(CH_2)_{n'}-S-(CH_2)_{na'}-$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R₇ and R₈ are the same or different, and are each independently selected from -

H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$;

A and A' are the same or different, and are independently selected from -O-, oxo $(-\text{C}(=\text{O})-)$, -CRR'O-, -CRR'-, -S-, -CRR'S-, $-\text{N}(\text{R}_5)-$ and $-\text{CRR}'\text{N}(\text{R}_5)-$,

R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

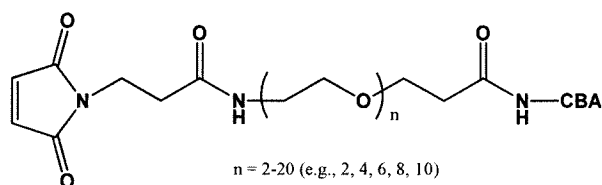
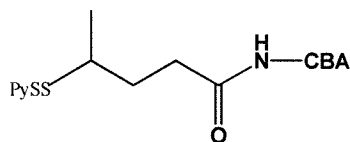
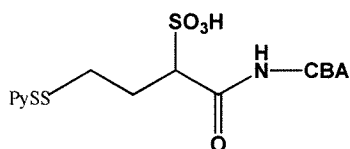
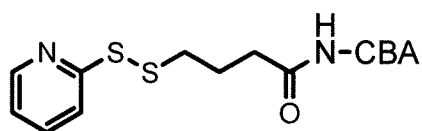
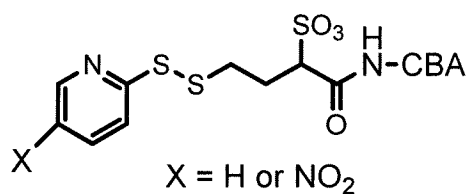
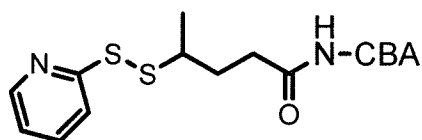
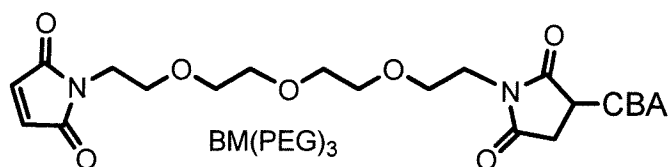
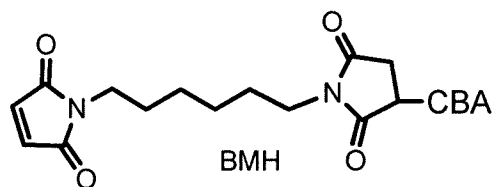
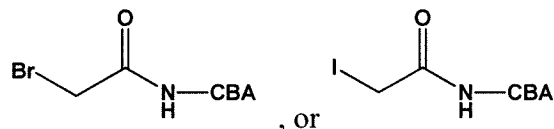
D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$; and

L is absent, or when present, comprises the thiol group, or is a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl, alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted; and

the imine reactive reagent is selected from the group consisting of sulfites (H_2SO_3 , H_2SO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_3$, POS_3H_3 , PS_4H_3 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate esters ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, R^iSH , R^iSOH , $\text{R}^i\text{SO}_2\text{H}$, $\text{R}^i\text{SO}_3\text{H}$), various amines (hydroxyl amine (*e.g.*, NH_2OH), hydrazine (*e.g.*, NH_2NH_2), $\text{NH}_2\text{O}-\text{R}^i$, $\text{R}^i\text{NH}-\text{R}^i$, NH_2-R^i), $\text{NH}_2-\text{CO}-\text{NH}_2$, $\text{NH}_2-\text{C}(=\text{S})-\text{NH}_2$, thiosulfate ($\text{H}_2\text{S}_2\text{O}_3$ or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite ($\text{H}_2\text{S}_2\text{O}_4$ or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{SH})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NHOH}$ or a salt formed with a cation), hydrazide ($\text{R}^k\text{CONHNH}_2$), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2\text{H}$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$), glycated nucleotide (such as GDP-

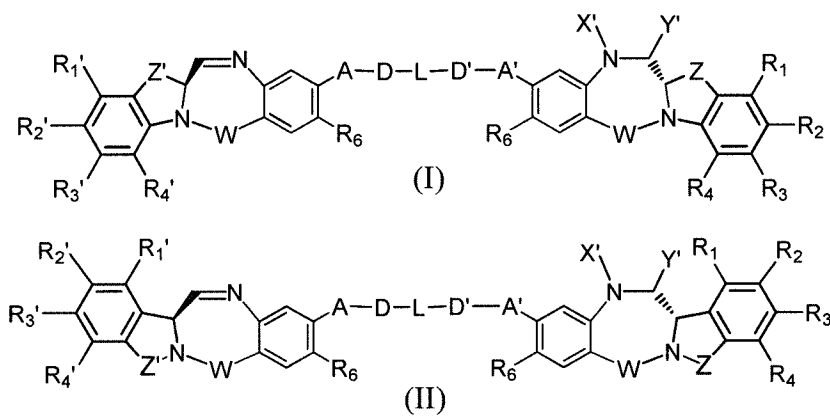
2. The method of claim 1, wherein the thiol-reactive group is maleimido, haloacetamido or -SSR^d, wherein R^d is a linear or branched alkyl having 1 to 4 carbon atoms, phenyl, nitrophenyl, dinitrophenyl, carboxynitrophenyl, pyridyl, 2-nitropyridyl, 4-nitropyridyl, or 3-carboxy-4-nitropyridyl.
3. The method of claim 1 or 2, wherein the modified CBA is:





4. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated

to a cytotoxic compound with a linking group, the method comprising reacting the CBA with an imine-containing cytotoxic compound, an imine reactive reagent, and a bifunctional crosslinking agent comprising the linking group to form the conjugate, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas, or a pharmaceutically acceptable salt thereof:



wherein:

X' is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$

R^c, halogen, guanidinium [-NH(C=NH)NH₂], -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃⁻M⁺, a sulfate -OSO₃⁻M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'' and the linking group;

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(CH₂CH₂O)_n-R^c, and an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or halogen;

Z and Z' are independently selected from -(CH₂)_{n'}-, -(CH₂)_{n'}-CR₇R₈-(CH₂)_{na'}-, -(CH₂)_{n'}-NR₉-(CH₂)_{na'}-, -(CH₂)_{n'}-O-(CH₂)_{na'}- and -(CH₂)_{n'}-S-(CH₂)_{na'}-;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R₇ and R₈ are the same or different, and are each independently selected from -H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit -(OCH₂CH₂)_n-, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R₉ is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -(OCH₂CH₂)_n-;

A and A' are the same or different, and are independently selected from -O-, oxo (-C(=O)-), -CRR'O-, -CRR'-, -S-, -CRR'S-, -N(R₅)- and -CRR'N(R₅)-,

R₅ for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit (-OCH₂CH₂)_n-; and

L is absent, or when present, comprises the thiol group, and is a polyethylene glycol unit (-OCH₂CH₂)_n-, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl, alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted; and

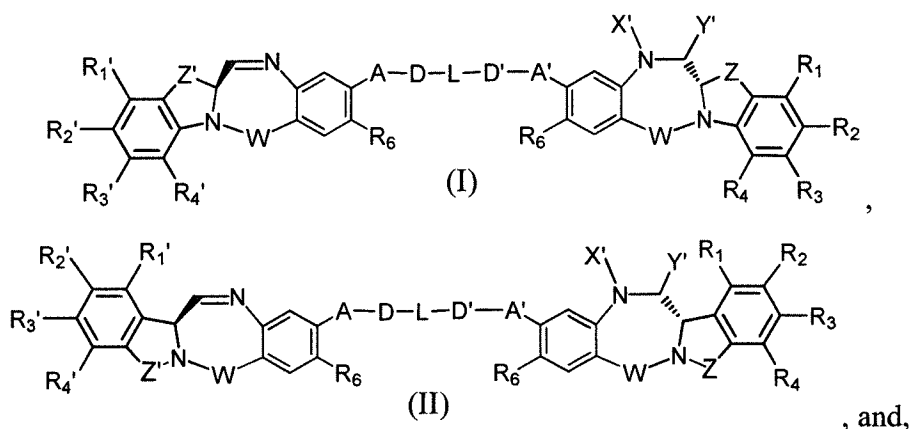
the imine reactive reagent is selected from the group consisting of sulfites (H₂SO₃, H₂SO₂ or a salt of HSO₃⁻, SO₃²⁻ or HSO₂⁻ formed with a cation), metabisulfite (H₂S₂O₅ or a salt of S₂O₅²⁻ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO₃SH₃, PO₂S₂H₃, POS₃H₃, PS₄H₃ or a salt of PO₃S³⁻, PO₂S₂³⁻, POS₃³⁻ or PS₄³⁻ formed with a cation), thio phosphate esters ((RⁱO)₂PS(ORⁱ), RⁱSH, RⁱSOH, RⁱSO₂H, RⁱSO₃H), various amines (hydroxyl amine (*e.g.*, NH₂OH), hydrazine (*e.g.*, NH₂NH₂), NH₂O-Rⁱ, RⁱNH-Rⁱ, NH₂-Rⁱ), NH₂-CO-NH₂, NH₂-C(=S)-NH₂'), thiosulfate (H₂S₂O₃ or a salt of S₂O₃²⁻ formed with a cation), dithionite (H₂S₂O₄ or a salt of S₂O₄²⁻ formed with a cation), phosphorodithioate (P(=S)(OR^k)(SH)(OH) or a salt thereof formed with a cation), hydroxamic acid (R^kC(=O)NHOH or a salt formed with a cation), hydrazide (R^kCONHNH₂), formaldehyde sulfoxylate (HOCH₂SO₂H or a salt of HOCH₂SO₂⁻ formed with a cation, such as HOCH₂SO₂⁻Na⁺), glycosylated nucleotide (such as GDP-mannose), fludarabine or a mixture thereof, wherein Rⁱ and R^{i'} are each independently a linear or branched alkyl having 1 to 10 carbon atoms and are substituted with at least one substituent selected from -N(R^j)₂, -CO₂H, -SO₃H, and -PO₃H; Rⁱ and R^{i'} can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl.

5. The method of claim 4, wherein the CBA is contacted with the imine-containing cytotoxic compound and the imine reactive reagent to form a first mixture; and the first

mixture is then contacted with the bifunctional crosslinking agent to form the conjugate.

6. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising:
- reacting a modified cytotoxic compound with a bifunctional crosslinking agent comprising the linking group, a group reactive with the CBA (such as a thiol group, a maleimide group, a haloacetamide group, or an amine group), and a group reactive with the modified cytotoxic compound, to form a second modified cytotoxic compound covalently bonded to a residue of the bifunctional crosslinking agent, wherein the residue comprises the linking group and the group reactive with the CBA;

wherein the modified cytotoxic compound is produced by reacting an imine-containing cytotoxic compound with an imine reactive reagent, wherein the imine-containing cytotoxic compound is represented by one of the following formulas, or a pharmaceutically acceptable salt thereof:



wherein:

X' is selected from -H, an amine-protecting group, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, an optionally substituted linear, branched

or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;

R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$, R^c , halogen, guanidinium $[-NH(C=NH)NH_2]$, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -SR, a sulfoxide represented by -SOR', a sulfone represented by -SO₂R', a sulfonate -SO₃M⁺, a sulfate -OSO₃M⁺, a sulfonamide represented by -SO₂NR'R'', cyano, an azido, -COR', -OCOR', -OCONR'R'';

R, for each occurrence, is independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from -H, -OH, -OR, -NHR, -NR₂, -COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, and an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from C=O, C=S, CH₂, BH, SO and SO₂;

R₆ is -H, -R, -OR, -SR, -NR'R'', -NO₂, or, halogen;

Z and Z' are independently selected from $-(CH_2)_n-$, $-(CH_2)_n-CR_7R_8-(CH_2)_{n_a}-$, $-(CH_2)_n-NR_9-(CH_2)_{n_a}-$, $-(CH_2)_n-O-(CH_2)_{n_a}-$ and $-(CH_2)_n-S-(CH_2)_{n_a}-$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R_7 and R_8 are the same or different, and are each independently selected from -H, -OH, -SH, -COOH, -NHR', a polyethylene glycol unit $-(OCH_2CH_2)_n-$, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from -H, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

A and A' are the same or different, and are independently selected from -O-, oxo $(-C(=O)-)$, -CRR'O-, -CRR'-, -S-, -CRR'S-, -N(R₅)- and -CRR'N(R₅)-,

R_5 for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $-(OCH_2CH_2)_n-$;

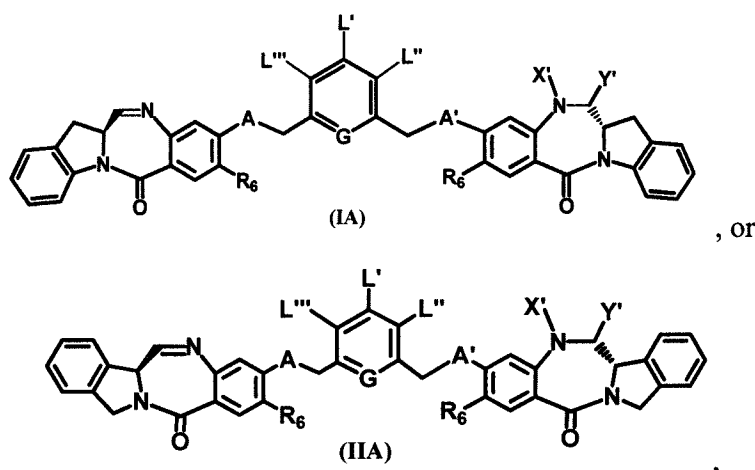
L is absent, or when present, comprises the thiol group, or is a polyethylene glycol unit $-(OCH_2CH_2)_n-$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3- to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl, alkenyl, phenyl, or heterocyclic or heteroaryl ring is optionally substituted; and

the imine reactive reagent is selected from the group consisting of sulfites (H_2SO_3 , H_2SO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($H_2S_2O_5$ or a salt of $S_2O_5^{2-}$ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO_3SH_3 , $PO_2S_2H_3$, POS_3H_3 , PS_4H_3 or a salt of PO_3S^{3-} , $PO_2S_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate esters $((R^iO)_2PS(OR^i)$, R^iSH , R^iSOH , R^iSO_2H , R^iSO_3H), various amines (hydroxyl amine (*e.g.*, NH_2OH), hydrazine (*e.g.*, NH_2NH_2), NH_2O-R^i , R^iNH-R^i , NH_2-R^i), $NH_2-CO-NH_2$, $NH_2-C(=S)-NH_2$, thiosulfate ($H_2S_2O_3$ or a salt of $S_2O_3^{2-}$ formed with a cation), dithionite ($H_2S_2O_4$ or a salt of $S_2O_4^{2-}$ formed with a cation), phosphorodithioate $(P(=S)(OR^k)(SH)(OH)$ or a salt thereof formed with a cation), hydroxamic acid $(R^kC(=O)NHOH$ or a salt formed with a cation), hydrazide

($R^k\text{CONHNH}_2$), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2\text{H}$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$), glycosylated nucleotide (such as GDP-mannose), fludarabine or a mixture thereof, wherein R^i and $R^{i'}$ are each independently a linear or branched alkyl having 1 to 10 carbon atoms and are substituted with at least one substituent selected from $-\text{N}(R^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i and $R^{i'}$ can be further optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl; and,

b) reacting the second modified cytotoxic compound with the CBA through the group reactive with the CBA, at a pH of about 4 to about 9, to form the conjugate.

7. The method of claim 6, wherein the modified cytotoxic compound comprises a thiol group, and the group reactive with the modified cytotoxic compound is a thiol-reactive group.
8. The method of claim 6, further comprising purifying the modified cytotoxic compound prior to step a).
9. The method of any one of claims 1-8, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas:



wherein:

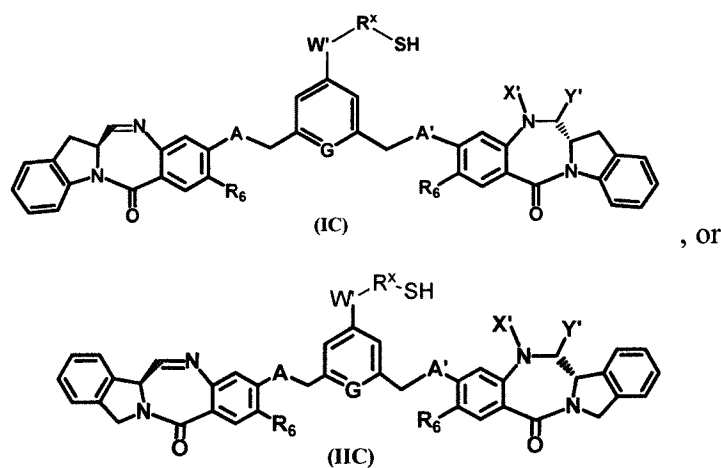
L' , L'' , and L''' are the same or different, and are independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-\text{R}^c$, halogen,

guanidinium $[-\text{NH}(\text{C}=\text{NH})\text{NH}_2]$, $-\text{OR}$, $-\text{NR}'\text{R}''$, $-\text{NO}_2$, $-\text{NR}'\text{COR}''$, $-\text{SR}$, a sulfoxide represented by $-\text{SOR}'$, a sulfone represented by $-\text{SO}_2\text{R}'$, a sulfonate $-\text{SO}_3\text{M}$, a sulfate $-\text{OSO}_3\text{M}$, a sulfonamide represented by $-\text{SO}_2\text{NR}'\text{R}''$, cyano, an azido, $-\text{COR}'$, $-\text{OCOR}'$, $-\text{OCONR}'\text{R}''$;

M is $-\text{H}$ or a pharmaceutically acceptable cation; and,

G is selected from $-\text{CH}-$ or $-\text{N}-$.

10. The method of any one of claims 1-9, wherein the imine-containing cytotoxic compound is represented by any one of the following formulas:



wherein:

W' is absent, or when present, is selected from $-\text{CR}^e\text{R}^{e'}$ -, $-\text{O}-$, $-\text{O}-\text{C}(=\text{O})-$, $-\text{C}(=\text{O})-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{CH}_2-\text{S}-$, $-\text{CH}_2\text{O}-$, $-\text{CH}_2\text{NR}^e-$, $-\text{O}-\text{C}(=\text{O})\text{O}-$, $-\text{O}-\text{C}(=\text{O})\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})-$, $-\text{C}(=\text{O})-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})\text{O}-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)\text{C}(=\text{O})-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)-$, $-(\text{O}-\text{CH}_2-\text{CH}_2)_n-$, $-\text{SS}-$, or $-\text{C}(=\text{O})-$, or an amino acid, or a peptide having 2 to 8 amino acids;

R^x is absent, or when present, is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an aryl bearing 6 to 10 carbon atoms or a 3- to 8-membered heterocyclic ring bearing 1 to 3 heteroatoms selected from O, N or S;

R^e and $\text{R}^{e'}$ are the same or different, and are selected from $-\text{H}$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or $-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{R}^k$, wherein R^k is a $-\text{H}$, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, $-\text{NHR}^{101}$) or tertiary amino ($-\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine,

wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms; preferably, R^{101} and R^{102} are each independently a linear or branched alkyl having 1 to 6 carbon atoms;

n is an integer from 1 to 24.

11. The method of claim 10, wherein:

X' is selected from the group consisting of -H, -OH, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, and an amine-protecting group;

Y' is selected from the group consisting of -H, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms;

A and A' are selected from -O- and -S-;

W' is absent, or selected from -O-, -N(R^e)-, -N(R^e)-C(=O)-, -N(C(=O)R^e)-, -S- or -CH₂-S-, -CH₂NR^e-;

R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is -H, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (e.g., -NHR¹⁰¹) or tertiary amino (-NR¹⁰¹R¹⁰²) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

G is selected from -CH- or -N-.

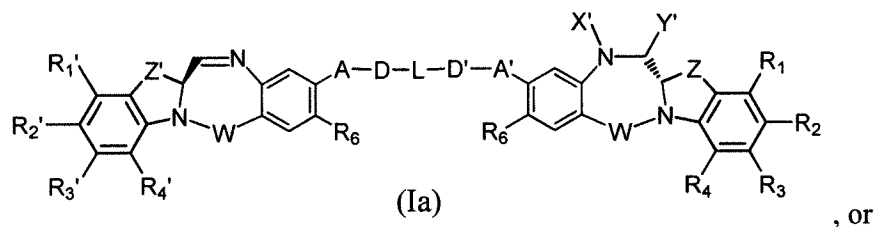
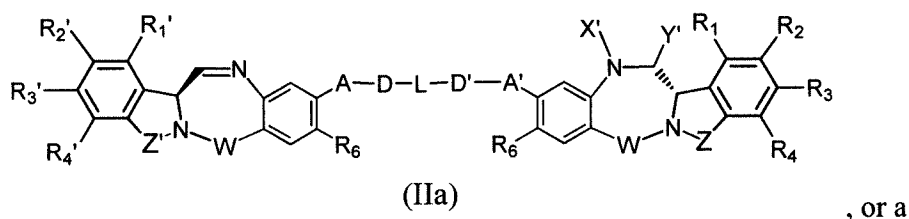
12. The method of claim 10 or 11, wherein W' is -N(R^e)-; R^e is -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms; R^x is -(CH₂)_p-(CR^fR^g)-, wherein R^f and R^g are each independently selected from -H or a linear or branched alkyl having 1 to 4 carbon atoms; and p is 0, 1, 2 or 3
13. The method of claim 12, wherein R^f and R^g are the same or different, and are selected from -H and -Me; and p is 1.
14. The method of any one of claims 1-13, wherein the bifunctional crosslinking agent is:

a maleimido-based moiety selected from: N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), κ -maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ -maleimidobutyric acid N-succinimidyl ester (GMBS), ϵ -maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimido benzoyl-N-hydroxysuccinimide ester (MBS), N-(α -maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB), N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate; or,

a haloacetyl-based moiety selected from: N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA), and N-succinimidyl 3-(bromoacetamido)propionate (SBAP), bis-maleimidopolyethyleneglycol (BMPEO), BM(PEO)₂, BM(PEO)₃, N-(β -maleimidopropoxy)succinimide ester (BMPS), 5-maleimidovaleric acid NHS, HBVS, 4-(4-N-maleimidophenyl)-butyric acid hydrazide·HCl (MPBH), Succinimidyl-(4-vinylsulfonyl)benzoate (SVSB), dithiobis-maleimidoethane (DTME), 1,4-bis-maleimidobutane (BMB), 1,4-bismaleimidyl-2,3-dihydroxybutane (BMDB), bis-maleimidohexane (BMH), bis-maleimidoethane (BMOE), sulfosuccinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (sulfo-SMCC), sulfosuccinimidyl(4-iodo-acetyl)aminobenzoate (sulfo-SIAB), m-maleimido benzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS), N-(γ -maleimidobutyloxy)sulfosuccinimide ester (sulfo-GMBS), N-(ϵ -maleimidocaproxy)sulfosuccinimide ester (sulfo-EMCS), N-(κ -maleimidoundecanoyloxy)sulfosuccinimide ester (sulfo-KMUS), sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate (sulfo-SMPB), CX1-1, sulfo-Mal and PEG_n-Mal.

15. A method for preparing a conjugate comprising a cell-binding agent (CBA) conjugated to a cytotoxic compound with a linking group, the method comprising reacting a second modified cytotoxic compound with the CBA at a pH of about 4 to about 9, wherein the second modified cytotoxic compound comprises: a residue of a bifunctional crosslinking agent bonded to the cytotoxic compound, and the residue comprises the linking group and a reactive group selected from a reactive ester and a thiol-reactive group, and, is

produced by reacting an imine reactive reagent with an imine-containing cytotoxic compound bearing the linking group and the reactive group and wherein the imine-containing cytotoxic compound is represented by one of the following formulae:



pharmaceutically acceptable salt thereof,
wherein:

X' is selected from -H, an amine-protecting group, the linking group with the reactive group bonded thereto, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit - (CH₂CH₂O)_n-R^c, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

Y' is selected from -H, an oxo group, the linking group with the reactive group bonded thereto, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted 6- to 18-membered aryl, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms;

R^c is -H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms, or the linking group with the reactive group bonded thereto;

R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are each independently selected from the group consisting of -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl

or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(OCH_2CH_2)_n-$, R^c , halogen, guanidinium $[-NH(C=NH)NH_2]$, $-OR$, $-NR'R''$, $-NO_2$, $-NCO$, $-NR'COR''$, $-SR$, a sulfoxide represented by $-SOR'$, a sulfone represented by $-SO_2R'$, a sulfonate $-SO_3^-M^+$, a sulfate $-OSO_3^-M^+$, a sulfonamide represented by $-SO_2NR'R''$, cyano, an azido, $-COR'$, $-OCOR'$, $-OCONR'R''$, and the linking group with the reactive group bonded thereto;

R , for each occurrence, is independently selected from the group consisting of $-H$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, an optionally substituted aryl having 6 to 18 carbon atoms, an optionally substituted 5- to 18-membered heteroaryl ring containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N and P;

R' and R'' are each independently selected from $-H$, $-OH$, $-OR$, $-NHR$, $-NR_2$, $-COR$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^c$, and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

n is an integer from 1 to 24;

W is selected from $C=O$, $C=S$, CH_2 , BH , SO and SO_2 ;

R_6 is $-H$, $-R$, $-OR$, $-SR$, $-NR'R''$, $-NO_2$, halogen or the linking group with the reactive group bonded thereto;

Z and Z' are independently selected from $-(CH_2)_{n'}-$, $-(CH_2)_{n'}-CR_7R_8-(CH_2)_{na'}-$, $-(CH_2)_{n'}-NR_9-(CH_2)_{na'}-$, $-(CH_2)_{n'}-O-(CH_2)_{na'}-$ and $-(CH_2)_{n'}-S-(CH_2)_{na'}-$;

n' and na' are the same or different, and are selected from 0, 1, 2 and 3;

R_7 and R_8 are the same or different, and are each independently selected from $-H$, $-OH$, $-SH$, $-COOH$, $-NHR'$, a polyethylene glycol unit $-(OCH_2CH_2)_n-$, an amino acid, a peptide unit bearing 2 to 6 amino acids, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms;

R_9 is independently selected from $-H$, an optionally substituted linear, branched or cyclic alkyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -

$(\text{OCH}_2\text{CH}_2)_n^-$;

A and A' are the same or different, and are independently selected from -O-, oxo (-C(=O)-), -CRR'O-, -CRR'-, -S-, -CRR'S-, -NR₅ and -CRR'N(R₅)-;

R₅ for each occurrence is independently -H or an optionally substituted linear or branched alkyl having 1 to 10 carbon atoms;

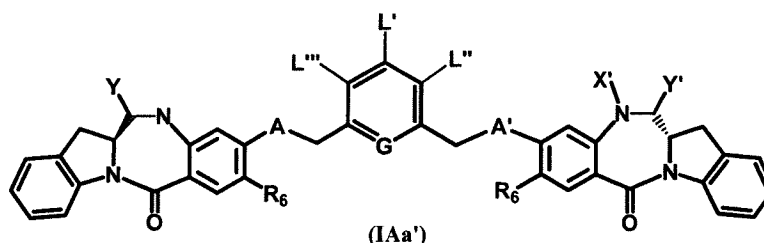
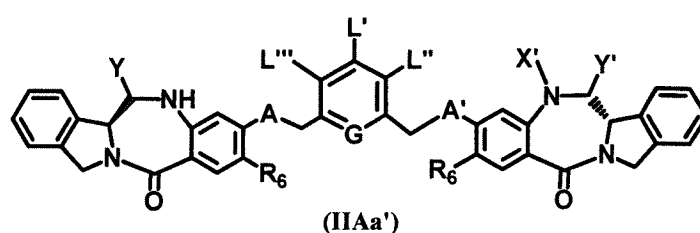
D and D' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $(-\text{OCH}_2\text{CH}_2)_n^-$;

L is absent, the linking group with the reactive group bonded thereto, a polyethylene glycol unit $(-\text{OCH}_2\text{CH}_2)_n^-$, a linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, a phenyl group, a 3 to 18-membered heterocyclic ring or a 5- to 18-membered heteroaryl ring having 1 to 6 heteroatoms independently selected from O, S, N and P, wherein the alkyl or alkenyl is optionally substituted with the linking group with the reactive group bonded thereto; phenyl or heterocyclic or heteroaryl ring can be optionally substituted, wherein the substituent can be the linking group with the reactive group bonded thereto; and

the imine reactive reagent is selected from the group consisting of sulfites (H_2SO_3 , H_2SO_2 or a salt of HSO_3^- , SO_3^{2-} or HSO_2^- formed with a cation), metabisulfite ($\text{H}_2\text{S}_2\text{O}_5$ or a salt of $\text{S}_2\text{O}_5^{2-}$ formed with a cation), mono, di, tri, and tetra- thiophosphates (PO_3SH_3 , $\text{PO}_2\text{S}_2\text{H}_3$, POS_3H_3 , PS_4H_3 or a salt of PO_3S^{3-} , $\text{PO}_2\text{S}_2^{3-}$, POS_3^{3-} or PS_4^{3-} formed with a cation), thio phosphate esters ($(\text{R}^i\text{O})_2\text{PS}(\text{OR}^i)$, R^iSH , R^iSOH , $\text{R}^i\text{SO}_2\text{H}$, $\text{R}^i\text{SO}_3\text{H}$), various amines (hydroxyl amine (*e.g.*, NH_2OH), hydrazine (*e.g.*, NH_2NH_2), $\text{NH}_2\text{O}-\text{R}^i$, $\text{R}^i\text{NH}-\text{R}^i$, NH_2-R^i), $\text{NH}_2-\text{CO}-\text{NH}_2$, $\text{NH}_2-\text{C}(=\text{S})-\text{NH}_2$, thiosulfate ($\text{H}_2\text{S}_2\text{O}_3$ or a salt of $\text{S}_2\text{O}_3^{2-}$ formed with a cation), dithionite ($\text{H}_2\text{S}_2\text{O}_4$ or a salt of $\text{S}_2\text{O}_4^{2-}$ formed with a cation), phosphorodithioate ($\text{P}(=\text{S})(\text{OR}^k)(\text{SH})(\text{OH})$ or a salt thereof formed with a cation), hydroxamic acid ($\text{R}^k\text{C}(=\text{O})\text{NHOH}$ or a salt formed with a cation), hydrazide ($\text{R}^k\text{CONHNH}_2$), formaldehyde sulfoxylate ($\text{HOCH}_2\text{SO}_2\text{H}$ or a salt of $\text{HOCH}_2\text{SO}_2^-$ formed with a cation, such as $\text{HOCH}_2\text{SO}_2^-\text{Na}^+$), glycosylated nucleotide (such as GDP-mannose), fludarabine or a mixture thereof, wherein R^i and $\text{R}^{i'}$ are each independently a linear or branched alkyl having 1 to 10 carbon atoms and are substituted with at least one substituent selected from $-\text{N}(\text{R}^j)_2$, $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, and $-\text{PO}_3\text{H}$; R^i and $\text{R}^{i'}$ can be further

optionally substituted with a substituent for an alkyl described herein; R^j is a linear or branched alkyl having 1 to 6 carbon atoms; R^k is a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, aryl, heterocyclyl or heteroaryl

16. The method of claim 15, wherein the second modified cytotoxic compound is represented by any one of the following formulas:



wherein:

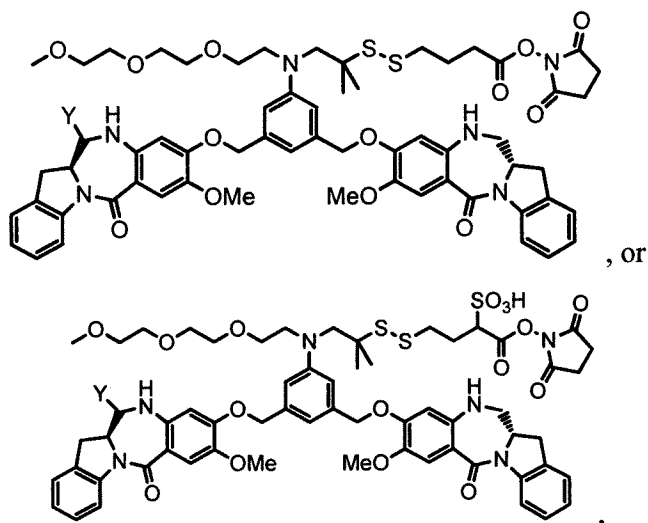
Y is $-\text{SO}_3\text{M}$, $-\text{SO}_2\text{M}$ or $-\text{OSO}_3\text{M}$;

L' , L'' , and L''' are the same or different, and are independently selected from -H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(\text{OCH}_2\text{CH}_2)_n-\text{R}^c$, halogen, guanidinium $[-\text{NH}(\text{C}=\text{NH})\text{NH}_2]$, $-\text{OR}$, $-\text{NR}'\text{R}''$, $-\text{NO}_2$, $-\text{NR}'\text{COR}''$, $-\text{SR}$, a sulfoxide represented by $-\text{SOR}'$, a sulfone represented by $-\text{SO}_2\text{R}'$, a sulfonate $-\text{SO}_3\text{M}$, a sulfate $-\text{OSO}_3\text{M}$, a sulfonamide represented by $-\text{SO}_2\text{NR}'\text{R}''$, cyano, an azido, $-\text{COR}'$, $-\text{OCOR}'$, $-\text{OCONR}'\text{R}''$ and the linking group with the reactive group bonded thereto, provided only one of L' , L'' , and L''' is the linking group with the reactive group bonded thereto;

M is -H or a pharmaceutically acceptable cation; and,

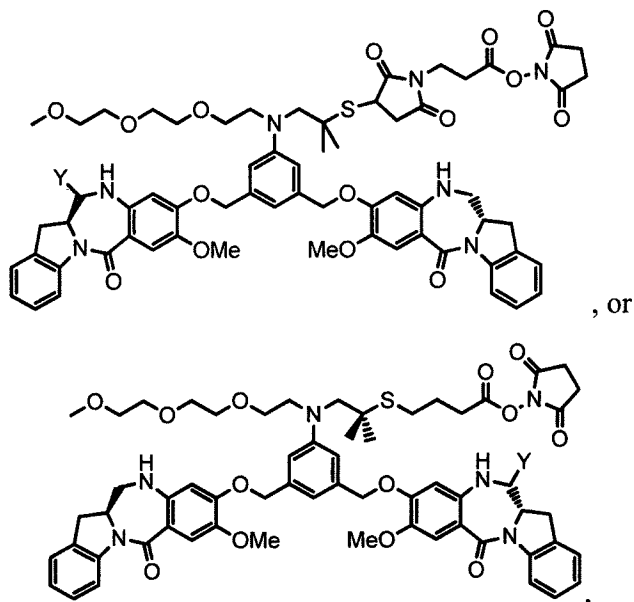
G is selected from $-\text{CH}-$ or $-\text{N}-$.

17. The method to claim 15, wherein the second modified cytotoxic compound is:



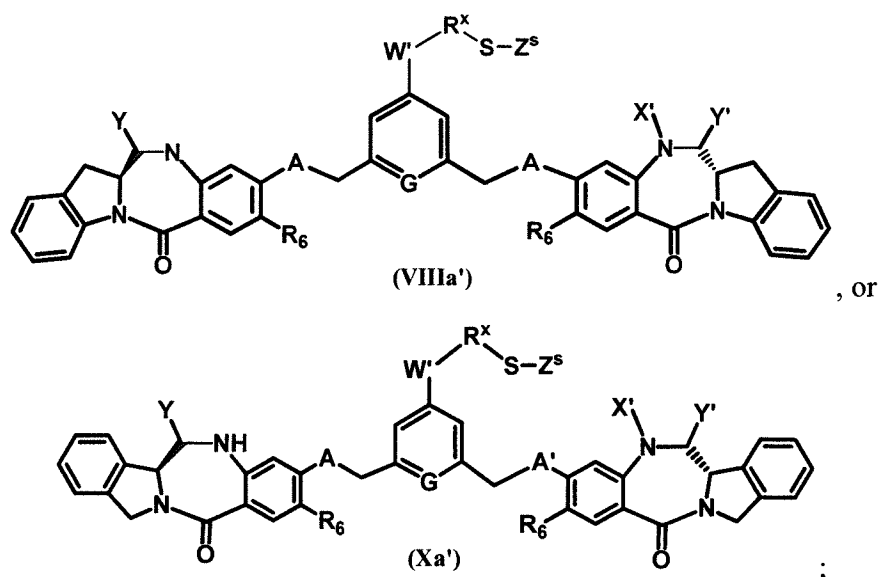
wherein Y is $-\text{SO}_3\text{M}$, and M is H or a pharmaceutically acceptable cation.

18. The method of claim 15, wherein the second modified cytotoxic compound is:



wherein Y is $-\text{SO}_3\text{M}$, and M is -H or a pharmaceutically acceptable cation.

19. The method of claim 15, wherein the second modified cytotoxic compound is represented by any one of the following formulas:



wherein:

Y is selected from $-\text{SO}_3\text{M}$, $-\text{SO}_2\text{M}$ or $-\text{OSO}_3\text{M}$;

M is $-\text{H}$ or a pharmaceutically acceptable cation such as Na^+ or K^+ ;

X' is selected from the group consisting of $-\text{H}$, $-\text{OH}$, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, phenyl, and an amine-protecting group;

Y' is selected from the group consisting of $-\text{H}$, an oxo group, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms;

A and A' are selected from $-\text{O}-$ and $-\text{S}-$;

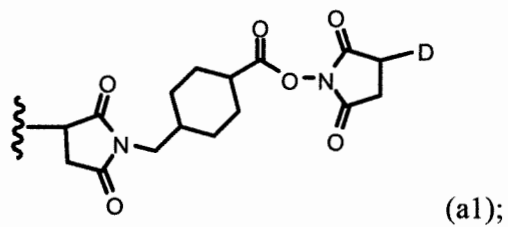
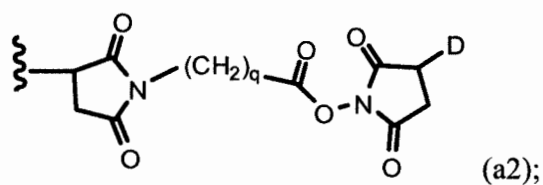
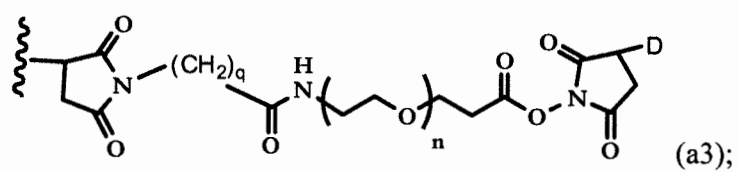
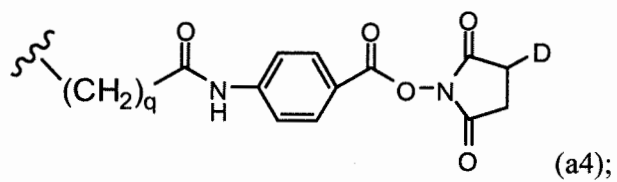
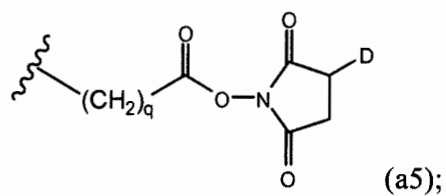
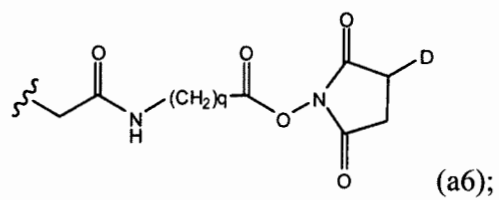
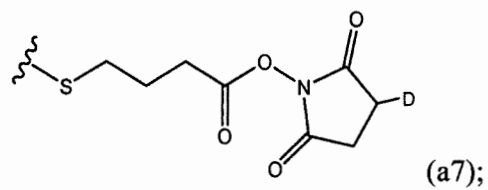
W' is absent, or selected from $-\text{O}-$, $-\text{N}(\text{R}^e)-$, $-\text{N}(\text{R}^e)-\text{C}(=\text{O})-$, $-\text{N}(\text{C}(=\text{O})\text{R}^e)-$, $-\text{S}-$ or $-\text{CH}_2-\text{S}-$, $-\text{CH}_2\text{NR}^e-$;

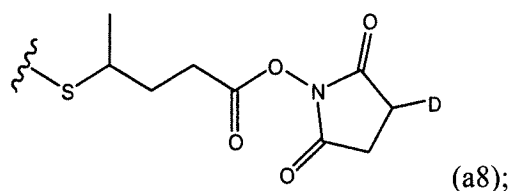
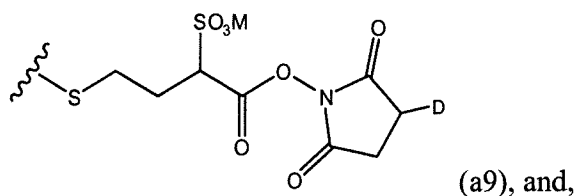
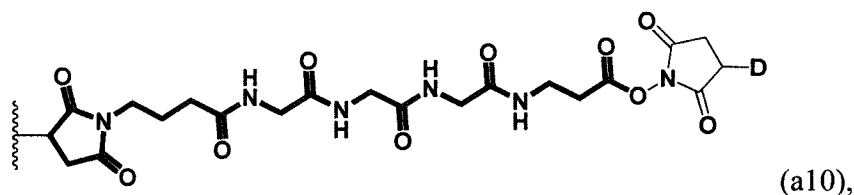
R^x is absent or selected from a linear, branched or cyclic alkyl having 1 to 10 carbon atoms;

R^e is $-\text{H}$, a linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms or $-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{R}^k$, wherein R^k is a $-\text{H}$, a linear, branched cyclic alkyl having 1 to 6 carbon atoms, optionally bearing a secondary amino (*e.g.*, $-\text{NHR}^{101}$) or tertiary amino ($-\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, such as piperidine or morpholine, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

G is selected from -CH- or -N-;

Z^s is -H, or is selected from any one of the following formulas:





wherein:

q is an integer from 1 to 5; preferably q is 2;

n is an integer from 2 to 6; preferably n is 4;

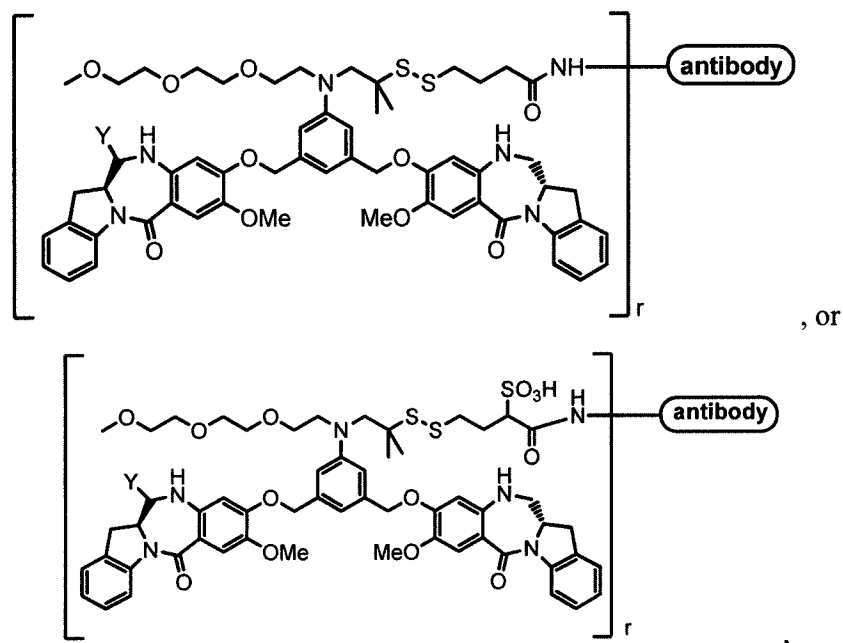
D is -H or -SO₃M;

M is -H or a pharmaceutically acceptable cation, such as Na⁺ or K⁺.

20. The method of claim 19, wherein W' is -N(R^e)-; R^e is -(CH₂-CH₂-O)_n-R^k, wherein R^k is a -H, a linear, branched cyclic alkyl having 1 to 6 carbon atoms; and R^x is -(CH₂)_p-(CR^fR^g)-, wherein R^f and R^g are each independently selected from -H or a linear or branched alkyl having 1 to 4 carbon atoms; and p is 0, 1, 2 or 3.
21. The method of claim 20, wherein R^f and R^g are the same or different, and are selected from -H and -Me; and p is 1.
22. The method of any one of claims 1-16 and 19-21, wherein:
 - W, when present, is C=O;
 - R₁, R₂, R₁', R₂', R₄ and R₄', when present, are -H;
 - one of R₃, or R₃', when present, is optionally the linking group with the reactive group bonded thereto and the other is -H;
 - R₆ is -OMe;
 - Z and Z' are -CH₂-;
 - X' is -H;
 - Y' is -H; and

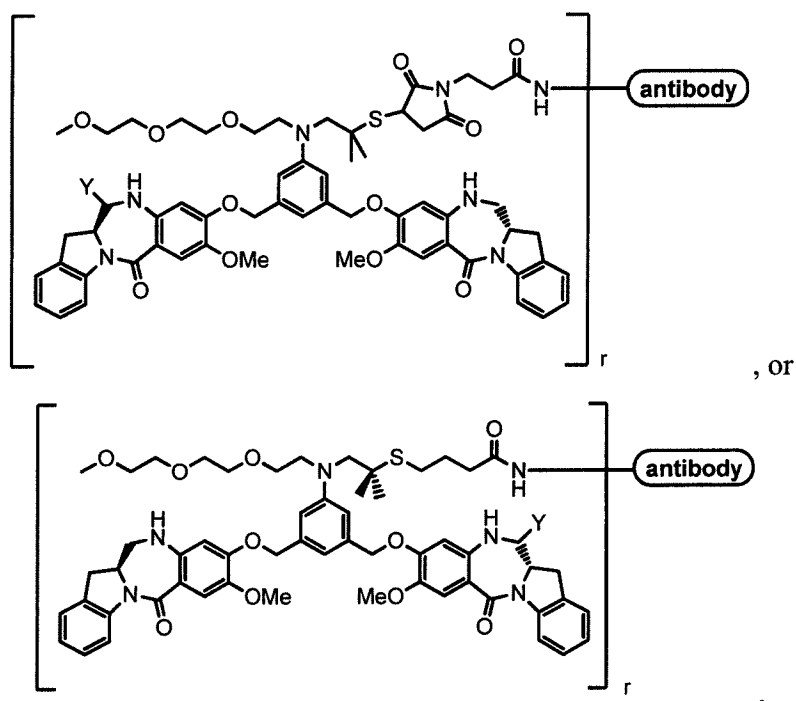
A and A' are -O-.

23. The method of any one of claims 1-17 and 19-22, wherein the conjugate is:



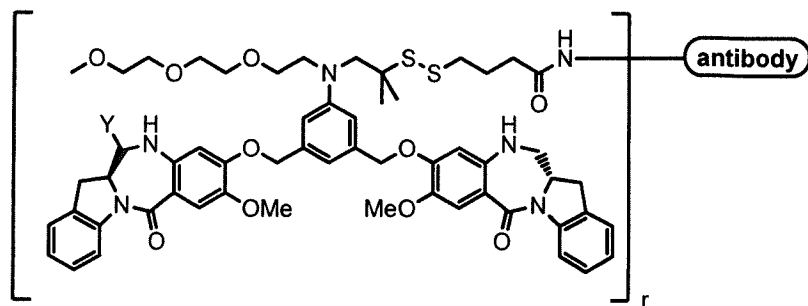
wherein r is an integer from 1 to 10, Y is $-\text{SO}_3\text{M}$, and M is $-\text{H}$ or a pharmaceutically acceptable cation.

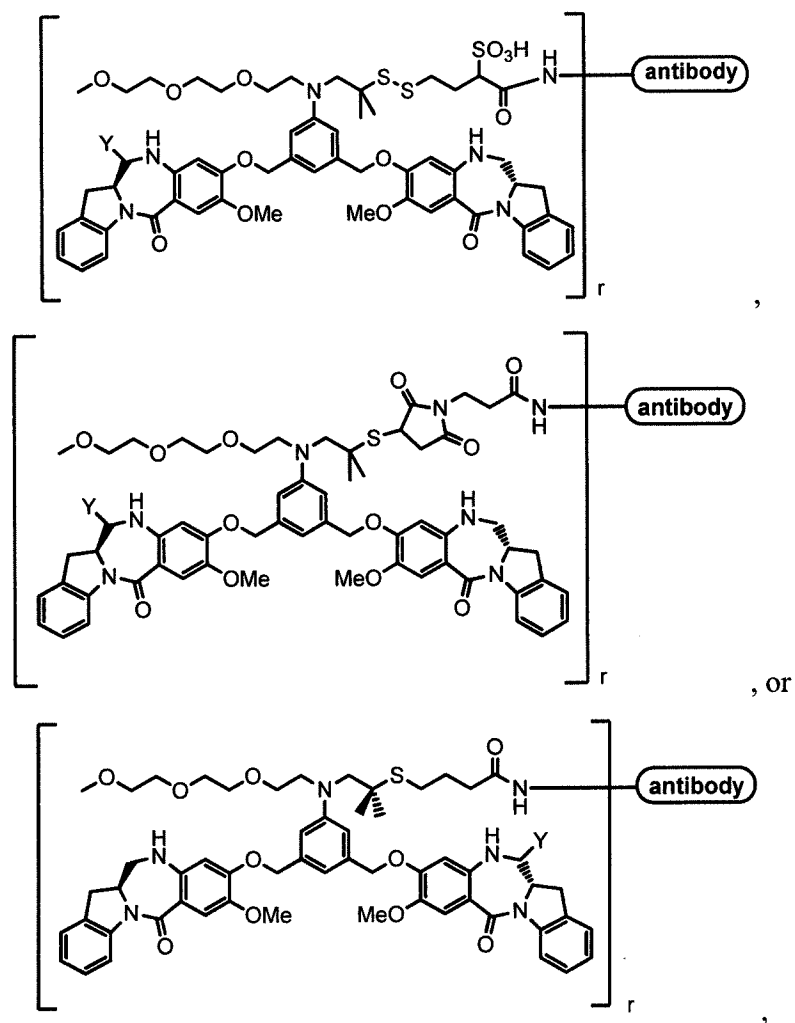
24. The method of any one of claims 1-16 and 18-22, wherein the conjugate is:



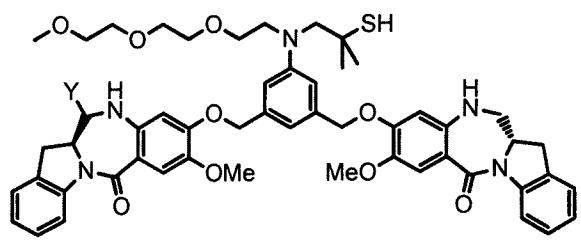
wherein r is an integer from 1 to 10, Y is $-\text{SO}_3\text{M}$, and M is $-\text{H}$ or a pharmaceutically acceptable cation.

25. The method of any one of claims 1-24, wherein the cell-binding agent binds to target cells selected from tumor cells, virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells, activated cells, myeloid cells, activated T-cells, B cells, or melanocytes; cells expressing the CD4, CD6, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD44, CD56, EpCAM, CanAg, CALLA, or Her-2 antigens; Her-3 antigens; or cells expressing insulin growth factor receptor, epidermal growth factor receptor, and folate receptor.
26. The method of claim 25, 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, or a monoclonal antibody fragment that specifically binds the 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 resurfaced antibody, a resurfaced single chain antibody or a resurfaced antibody fragment thereof, a humanized antibody, a humanized single chain antibody, or a humanized antibody fragment thereof, a lymphokine, a hormone, a vitamin, a growth factor, a colony stimulating factor, or a nutrient-transport molecule.
27. The method of any one of claims 1-26, wherein the imine reactive reagent is selected from sulfites, hydroxylamine, hydrazine and urea.
28. The method of any one of claims 1-26, wherein the imine reactive reagent is NaHSO_3 or KHSO_3 .
29. A method for preparing a conjugate of the following formula:

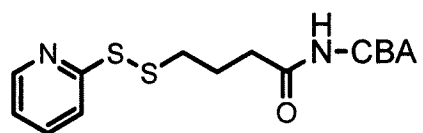


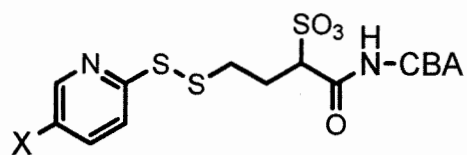


the method comprising reacting a modified cytotoxic compound of the following formula,

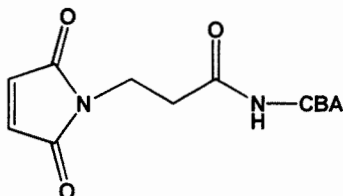
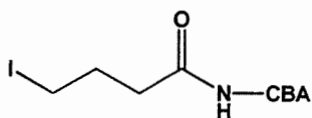


with a modified CBA of the following formula, respectively, at a pH of about 4 to about 9,





$X = -H \text{ or } -NO_2$



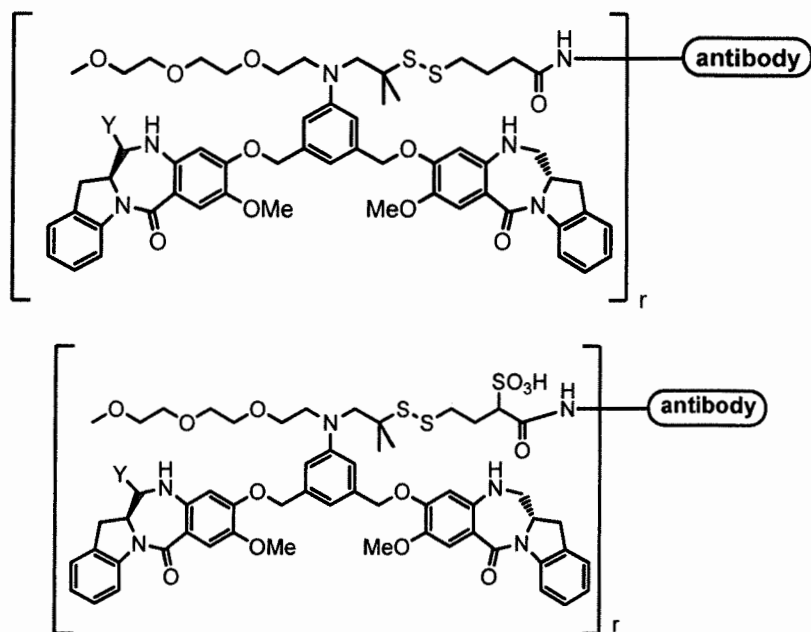
wherein:

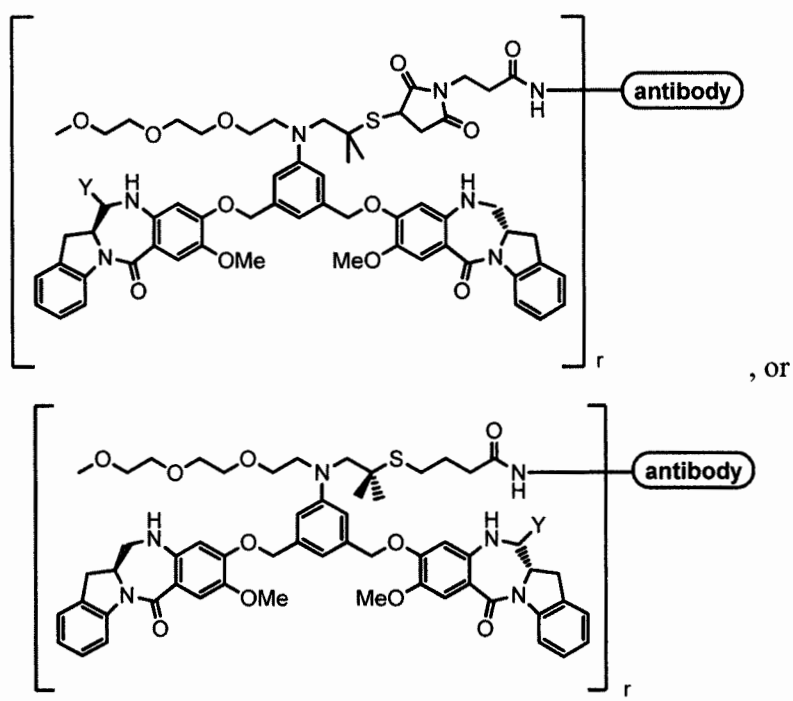
r is an integer from 1 to 10;

Y is $-SO_3M$; and

M is $-H$ or a pharmaceutically acceptable cation.

30. A method for preparing a conjugate of the following formula:

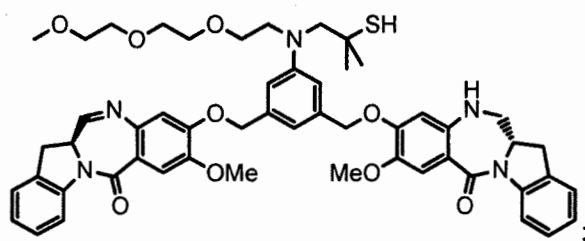




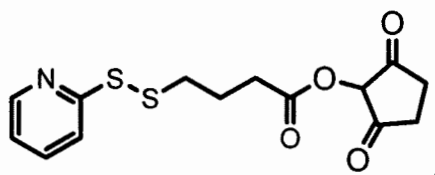
the method comprising reacting the CBA with an imine-containing cytotoxic compound, an imine reactive reagent, and a bifunctional crosslinking agent comprising the linking group to form the conjugate, wherein:

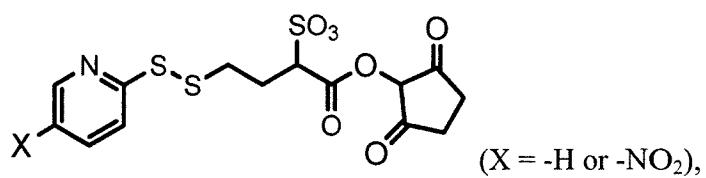
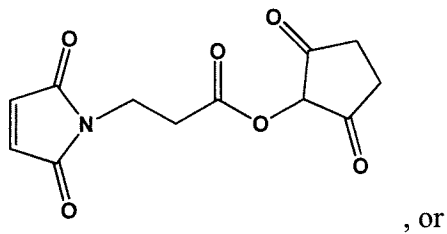
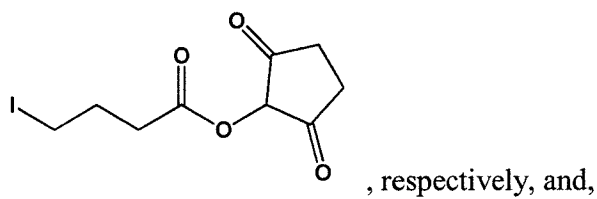
Y is $-\text{SO}_3\text{M}$; and M is $-\text{H}$ or a pharmaceutically acceptable cation;

the imine-containing cytotoxic compound is:



the bifunctional crosslinking agent is:





the imine reactive reagent is a sulfite.

31. The method of any one of claims 1-30, wherein the CBA is huMy9-6.