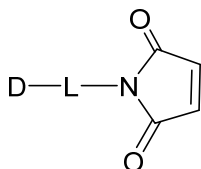


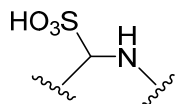
CLAIMS

1. A method of preparing a cell-binding agent-cytotoxic agent conjugate comprising the steps of:

(a) reacting an imine-moiety in an imine-containing cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with sulfur dioxide, a bisulfite salt or a metabisulfite salt in an aqueous solution at a pH of 2.5 to 4.5 to form a modified cytotoxic agent comprising a modified imine moiety represented by the following formula:

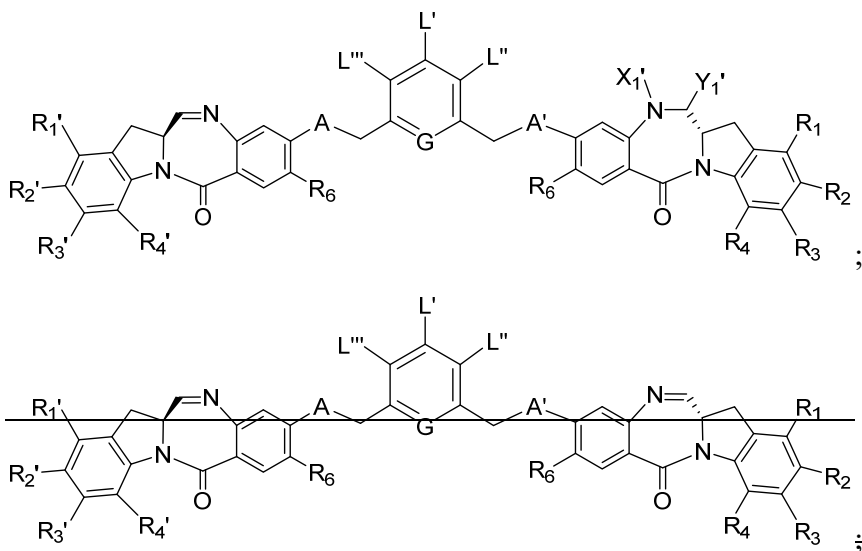


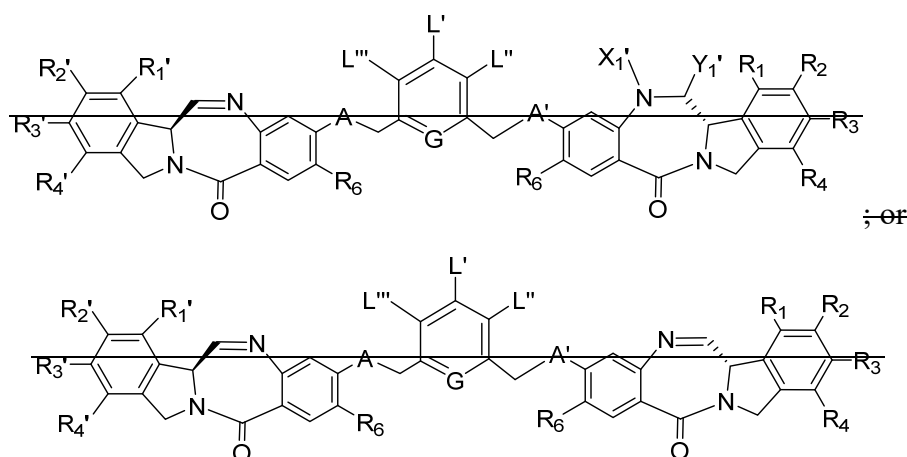
or a pharmaceutically acceptable salt thereof; and

(b) reacting the modified cytotoxic agent with a cell-binding agent to form the cell-binding agent-cytotoxic agent conjugate,

wherein the reaction of step (a) is carried out in a mixture of an organic solvent and water and in the presence of a buffer solution, and wherein D is an imine-containing benzodiazepine compound; and L is a linker,
wherein:

(4) D is represented by the following formula:

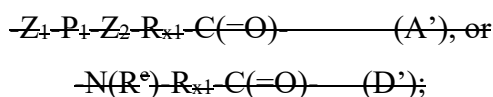




or a pharmaceutically acceptable salt thereof,

wherein:

one of L' , L'' , and L''' is represented by the following formula:

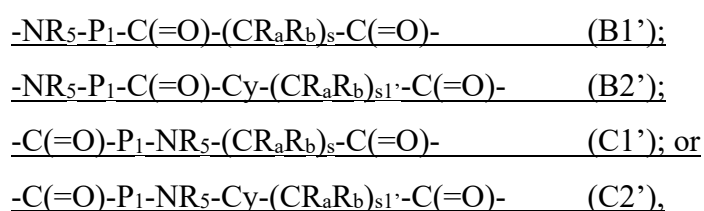


and the other two are each independently selected from $-H$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-$, R^e , halogen, guanidinium $[NH(C=NH)NH_2]$, $-OR$, $-NR'R''$, $-NO_2$, $-NR'COR''$, $-SR$, $-SOR'$, $-SO_2R'$, $-SO_3H$, $-OSO_3H$, $-SO_2NR'R''$, cyano, an azido, $-COR'$, $-OCOR'$, and $-OCONR'R''$;

one of the Z_1 and Z_2 is $-C(=O)-$, and the other is $-NR_5-$;

L'' and L''' are both $-H$;

L' is represented by the following formula:



wherein:

R_a and R_b , for each occurrence, are each independently $-H$, (C_1-C_3) alkyl or a charged substituent or an ionizable group Q , wherein Q is i) $-SO_3H$, $-Z'-SO_3H$, $-OPO_3H_2$, $-Z'-OPO_3H_2$, $-PO_3H_2$, $-Z'-PO_3H_2$, $-CO_2H$, $-Z'-CO_2H$, $-NR_{11}R_{12}$, or $-Z'-NR_{11}R_{12}$, or a pharmaceutically acceptable salt thereof; or, ii) $-N^+R_{14}R_{15}R_{16}X^-$ or $-Z'-N^+R_{14}R_{15}R_{16}X^-$; wherein Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R_{11} and R_{12} , for each occurrence, are independently H or an optionally substituted alkyl; and R_{14} to R_{16} are each independently an optionally substituted

alkyl; and X⁻ is a pharmaceutically acceptable anion;

s is an integer from 1 to 6;

s1' is 0 or an integer from 1 to 6;

Cy is a cyclic alkyl having 5 or 6 ring carbon atoms optionally substituted with halogen, -OH, (C₁-C₃)alkyl, (C₁-C₃)alkoxy, or halo(C₁-C₃)alkyl;

P₁ is an amino acid residue or a peptide containing between 2 to 20 amino acid residues;

~~R_{n+1} is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 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., NHR⁺¹⁰¹) or tertiary amino (-NR⁺¹⁰¹R⁺¹⁰²) group or a 5 or 6 membered nitrogen containing heterocycle, wherein R⁺¹⁰¹ and R⁺¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;~~

~~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^e, 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^e, and an optionally substituted 3 to 18 membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;~~

~~R^e is H or an optionally substituted linear or branched alkyl having 1 to 4 carbon atoms;~~

~~n is an integer from 1 to 24;~~

~~X₁' 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 (CH₂CH₂O)_n-R^e, 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;~~

~~Y₁' 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;~~

~~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 -(CH₂CH₂O)_n-R^e, halogen, guanidinium [NH(C=NH)NH₂], OR, NR'R'', NO₂, NCO, NR'COR'', SR, SOR', SO₂R', SO₃H, OSO₃H, SO₂NR'R'', cyano, an azido, COR', OCOR', and OCONR'R'';~~

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

~~R₁, R₂, R₃, R₄, R₁', R₂', R₃' and R₄' are all -H;~~

~~R₆ is -OMe;~~

~~X₁' and Y₁' are both -H;~~

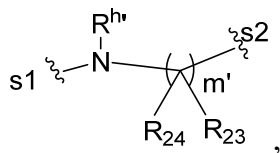
~~A and A' are -O-;~~

~~G is -CH- or -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); and~~

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

~~-L- is represented by the following structural formula:~~



~~or a pharmaceutically acceptable salt thereof,~~

~~wherein:~~

~~s1 is the site covalently linked to D;~~

~~s2 is the site covalently linked to the maleimide group;~~

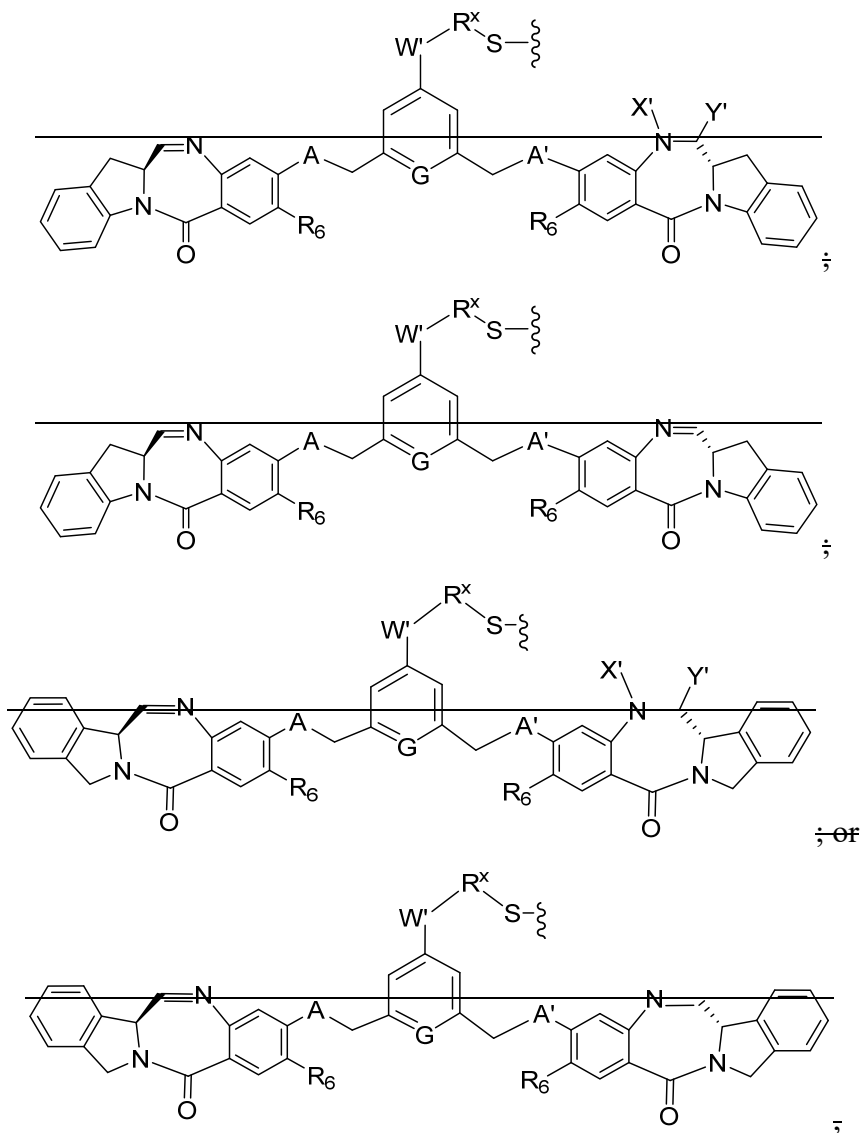
~~R₂₃ and R₂₄, for each occurrence, are independently H or an optionally~~

substituted alkyl;

m' is an integer between 0 and 10; and

R^h is H or an optionally substituted alkyl

~~(2) D is represented by the following formula:~~



~~or a pharmaceutically acceptable salt thereof,~~

~~—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 $-CH_2-S-$, $-CH_2NR^e-$;

— R^* 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, 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 1 to 24;

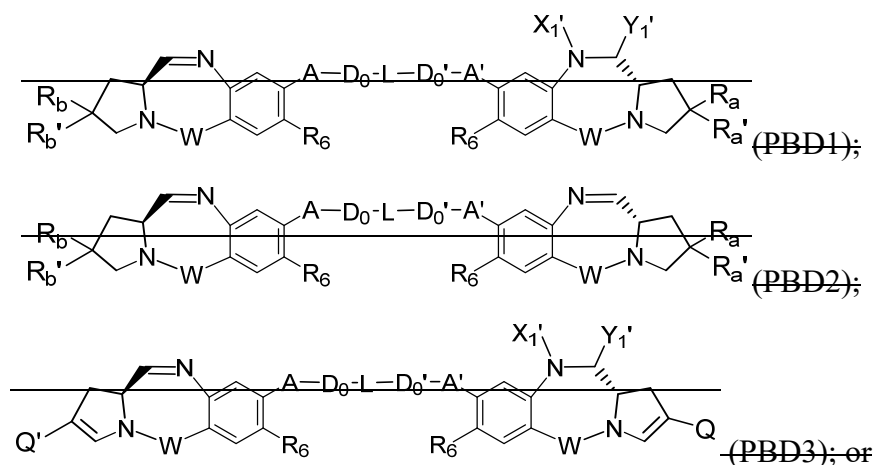
— G is selected from $-CH-$ or $-N-$;

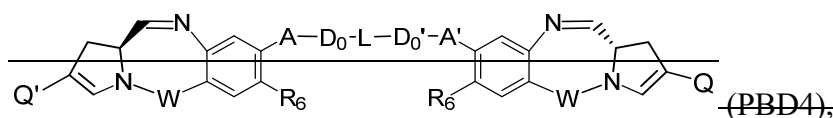
— R_6 is H , R , OR , SR , $NR'R''$, NO_2 , or halogen; and

— R is H , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $-(CH_2CH_2O)_n-R^e$, wherein n is an integer from 1 to 24, and R^e is a linear or branched alkyl having 1 to 4 carbon atoms;

— R' and R'' are each independently selected from H , OH , OR , NRR^z , COR , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted aryl having from 6 to 18 carbon atoms, an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms selected from O , S , N and P , a PEG group $-(CH_2CH_2O)_n-R^e$, and R^z is H , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $-(CH_2CH_2O)_n-R^e$; or

(3) D is represented by the following formula:





or a pharmaceutically acceptable salt thereof,

—wherein:

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

—X₁' 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 (CH₂CH₂O)_n-R^e, 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;

—Y₁' 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;

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

—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, 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^e, 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^e, and an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

—— R^e 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;

—— n is an integer from 1 to 24;

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

—— D_0 and D_0' 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, a linker, a polyethylene glycol unit $(OCH_2CH_2)_n$, an optionally substituted linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, an optionally substituted phenyl group, an optionally substituted 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;

—— R_a , R_a' , R_b and R_b' are the same or different, and are independently selected from the group consisting of H, halide, or an optionally substituted branched, linear or cyclic alkyl having 1 to 10 carbon atoms; or R_a and R_a' and/or R_b and R_b' together form a double bond containing group $=B$ and $=B'$ respectively;

—— $=B$ and $=B'$ are the same or different and independently selected from an optionally substituted branched or linear alkenyl or a carbonyl group;

—— Q is Q_1-Ar-Q_2 ;

—— Q' is $Q_1'-Ar'-Q_2'$;

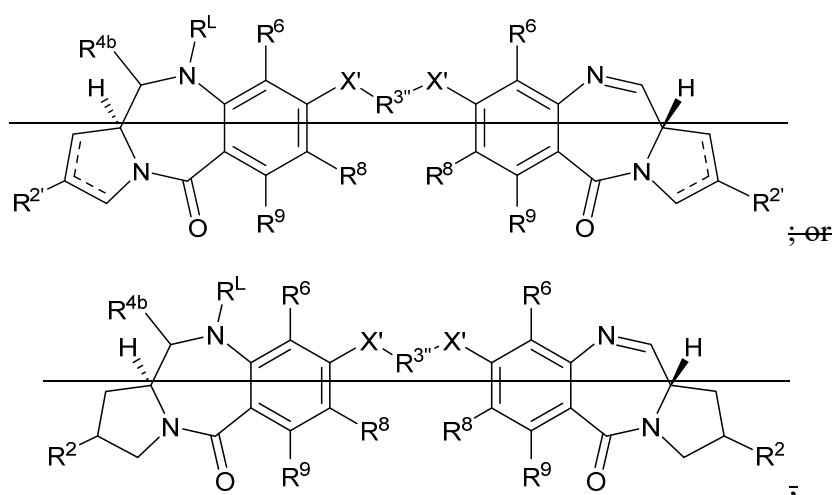
—— Q_1 and Q_1' are each independently absent, a linear, branched or cyclic alkyl from 1 to 6 carbon atoms or a $CH=CH$ unit;

—— Ar and Ar' are each independently absent, or represent an aryl group;

—— Q_2 and Q_2' are each independently selected from H, a linker, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-R^e-(OCH_2CH_2)_n-R^e$, or a substituent selected from a halogen, guanidinium $[NH(C=NH)NH_2]$, R, OR, $NR'R''$, NO_2 , NCO, $NR'COR''$, $NR'(C=O)OR''$, SR, a sulfoxide represented by SOR' , a sulfone represented by SO_2R' , a sulfonate SO_3M , a sulfate OSO_3M , a sulfonamide represented by $SO_2NR'R''$, cyano, an azido, COR' , $OCOR'$ or $OCONR'R''$; and

—— R^e is absent or selected from linear or branched alkyl, alkenyl or alkynyl having 1 to 5 carbon atoms; or

(4) the imine-containing cytotoxic agent is represented by the following formula:



or a pharmaceutically acceptable salt thereof,

wherein:

- the dotted lines indicate the optional presence of a double bond;
- R^{32} is a C_{3-12} alkylene group;
- each X' , for each occurrence, is independently O , S or $N(H)$;
- each R^2 is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, CO_2R^{1+} , COR^{1+} , or halo, or both R^2 taken together, are $=O$, $=CH_2$, $=CH-R^*$, or $=C(R^*)_2$;
- each R^{22} is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, CO_2R^{1+} , COR^{1+} or halo;
- R^{4b} is a leaving group selected from OR^{6+} , $OCOR^{4+}$, $OCOOR^{4+}$, $OCONR^{4+}R^{5+}$, $NR^{4+}R^{5+}$, $NR^{4+}COR^{5+}$, $NR^{4+}NR^{4+}R^{5+}$, an optionally substituted 5- or 6-membered nitrogen-containing heterocycle (e.g., piperidine, tetrahydropyrrole, pyrazole, morpholine), a guanidinium represented by $NR^{4+}(C=NH)NR^{4+}R^{5+}$, an amino acid, or a peptide represented by $NR^{6+}COP^2$, wherein P^2 is an amino acid or a polypeptide containing between 2 to 20 amino acid units, SR^{6+} , SOR^{4+} , SO_2M , SO_3M , OSO_3M , halogen, cyano and an azido;
- R^+ is linker bearing a maleimide moiety that can form a covalent bond with a cell binding agent (CBA);
- R^6 and R^9 are independently selected from H , R^{1+} , OH , OR^{1+} , SH , SR^{1+} , NH_2 , NHR^{1+} , $NR^{1+}R^{32}$, NO_2 , Me_3Sn and halo; and,
- R^{1+} and R^{32} are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl or C_{5-20} aryl groups, and optionally in relation to the group $NR^{1+}R^{32}$, R^{1+} and R^{32} together with the nitrogen atom to which they are attached form an

~~optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; or any pair of adjacent groups from R⁶ to R⁹ together form a group -O-(CH₂)_p-O-, where p is 1 or 2;~~

~~—— R⁴ and R⁵ are each independently selected~~

~~from -H-, -OH-, -OR⁶-, -NHR⁶-, -NR⁶-, -COR⁶-, 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^b-, or an optionally substituted 3-18 membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N or P;~~

~~—— R⁶-, 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^b-, 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, or sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N or P;~~

~~—— R^a is independently selected from -R¹-, -CO₂R¹-, -COR¹-, -CHO-, -CO₂H-, or halo;~~

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

~~—— M is H or a pharmaceutically acceptable cation; and~~

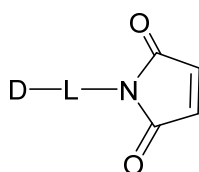
~~—— n is an integer from 1 to 24.~~

2. The method of claim 1, wherein the reaction of step (a) is carried out at a pH of 2.9 to 4.0, a pH of 2.9 to 3.7, a pH of 3.1 to 3.5, a pH of 3.2 to 3.4, or a pH of 3.3.

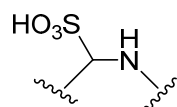
3. ~~The method of claim 1 or 2, wherein the reaction of step (a) is carried out in the presence of a buffer solution.~~

4. A method of preparing a cell-binding agent-cytotoxic agent conjugate comprising the steps of:

(a) reacting an imine-moiety in an imine-containing cytotoxic agent represented by the following formula:



or a pharmaceutically acceptable salt thereof, with sulfur dioxide, a bisulfite salt or a metabisulfite salt in an aqueous solution in the absence of a buffer to form a modified cytotoxic agent comprising a modified imine moiety represented by the following formula:

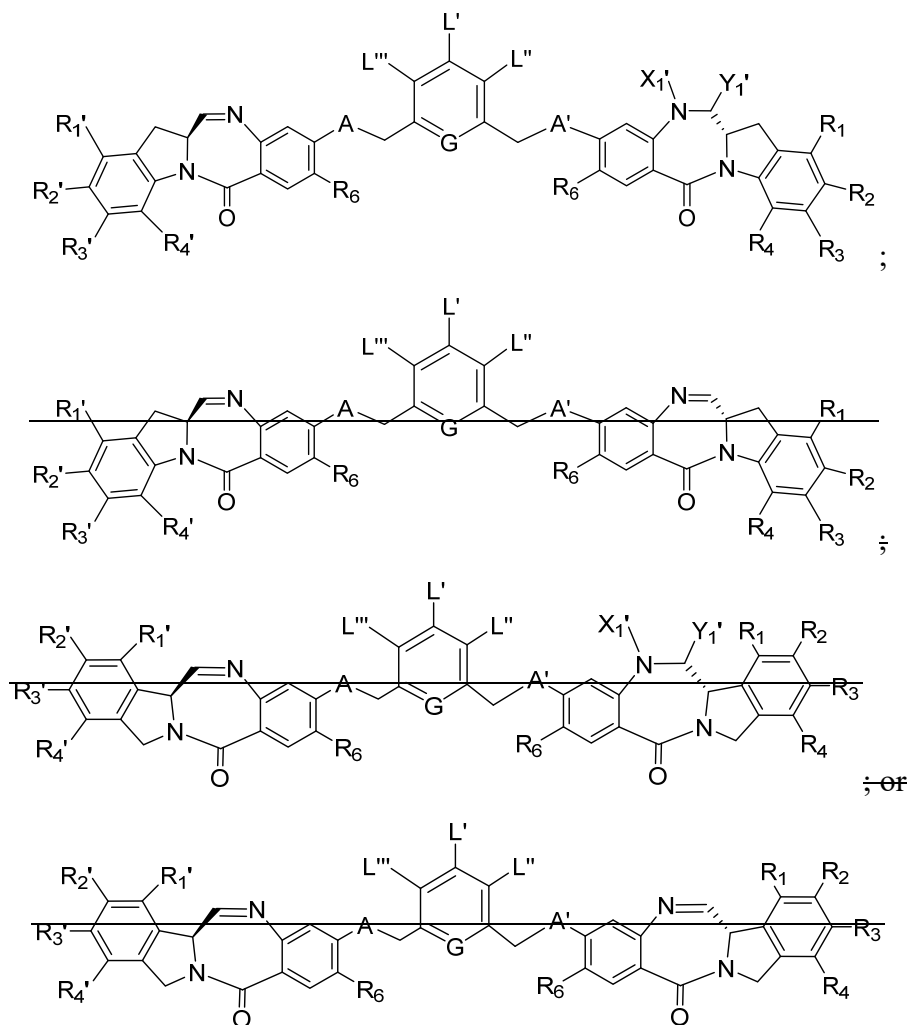


or a pharmaceutically acceptable salt thereof; and

(b) reacting the modified cytotoxic agent with a cell-binding agent to form the cell-binding agent-cytotoxic agent conjugate,

wherein the reaction of step (a) is carried out in a mixture of an organic solvent and water, and wherein D is an imine-containing benzodiazepine compound; and L is a linker, wherein:

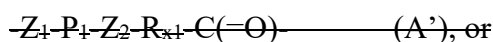
(+) D is represented by the following formula:



or a pharmaceutically acceptable salt thereof,

wherein:

one of L', L'', and L''' is represented by the following formula:

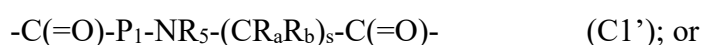
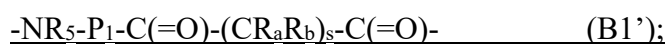


and the other two are each independently selected from ~~H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit (CH₂CH₂O)_n, R^e, halogen, guanidinium [NH(C=NH)NH₂], OR, NR'R'', NO₂, NR'COR'', SR, SOR', SO₂R', SO₃H, OSO₃H, SO₂NR'R'', cyano, an azido, COR', OCOR', and OCONR'R'';~~

~~one of the Z₄ and Z₂ is C(=O), and the other is NR₅;~~

L' and L'' are both H;

L' is represented by the following formula:



wherein:

R_a and R_b, for each occurrence, are each independently H, (C₁-C₃)alkyl or a charged substituent or an ionizable group Q, wherein Q is i) -SO₃H, -Z'-SO₃H, -OPO₃H₂, -Z'-OPO₃H₂, -PO₃H₂, -Z'-PO₃H₂, -CO₂H, -Z'-CO₂H, -NR₁₁R₁₂, or -Z'-NR₁₁R₁₂, or a pharmaceutically acceptable salt thereof; or, ii) -N⁺R₁₄R₁₅R₁₆X⁻ or -Z'-N⁺R₁₄R₁₅R₁₆X⁻;
wherein Z' is an optionally substituted alkylene, an optionally substituted cycloalkylene or an optionally substituted phenylene; R₁₁ and R₁₂, for each occurrence, are independently H or an optionally substituted alkyl; and R₁₄ to R₁₆ are each independently an optionally substituted alkyl; and X⁻ is a pharmaceutically acceptable anion;

s is an integer from 1 to 6;

s1' is 0 or an integer from 1 to 6;

Cy is a cyclic alkyl having 5 or 6 ring carbon atoms optionally substituted with halogen, -OH, (C₁-C₃)alkyl, (C₁-C₃)alkoxy, or halo(C₁-C₃)alkyl;

P₁ is an amino acid residue or a peptide containing between 2 to 20 amino acid residues;

R_K+ is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 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.*, NHR^{101}) or tertiary amino ($\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

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

—— R^e is H or an optionally substituted linear or branched alkyl having 1 to 4 carbon atoms;

—— n is an integer from 1 to 24;

—— X_1' 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, 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_1' 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_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 $-(CH_2CH_2O)_n-$, R^e , halogen, guanidinium $[-NH(C=NH)NH_2]$, ~~OR, $NR'R''$, NO_2 , NCO , $NR'COR''$, SR , SOR' , SO_2R' , SO_3H , OSO_3H , $SO_2NR'R''$, cyano, an azido, COR' , $OCOR'$, and $OCONR'R''$;~~

~~R_6 is H, R, OR, SR, $NR'R''$, NO_2 , or halogen;~~

$R_1, R_2, R_3, R_4, R_1', R_2', R_3'$ and R_4' are all -H;

R_6 is -OMe;

X_1' and Y_1' are both -H;

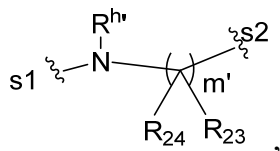
A and A' are -O-;

G is -CH- or -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 $CRR'N(R5)$;~~ and

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

-L- is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof,

wherein:

$s1$ is the site covalently linked to D;

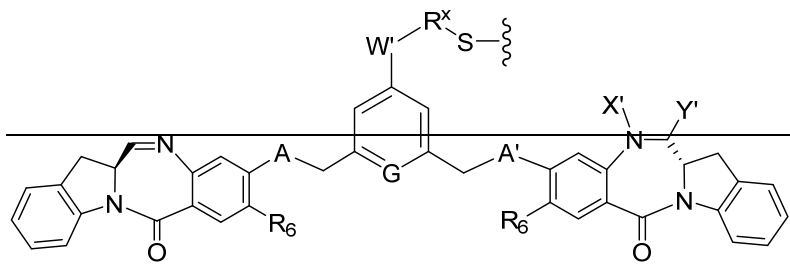
$s2$ is the site covalently linked to the maleimide group;

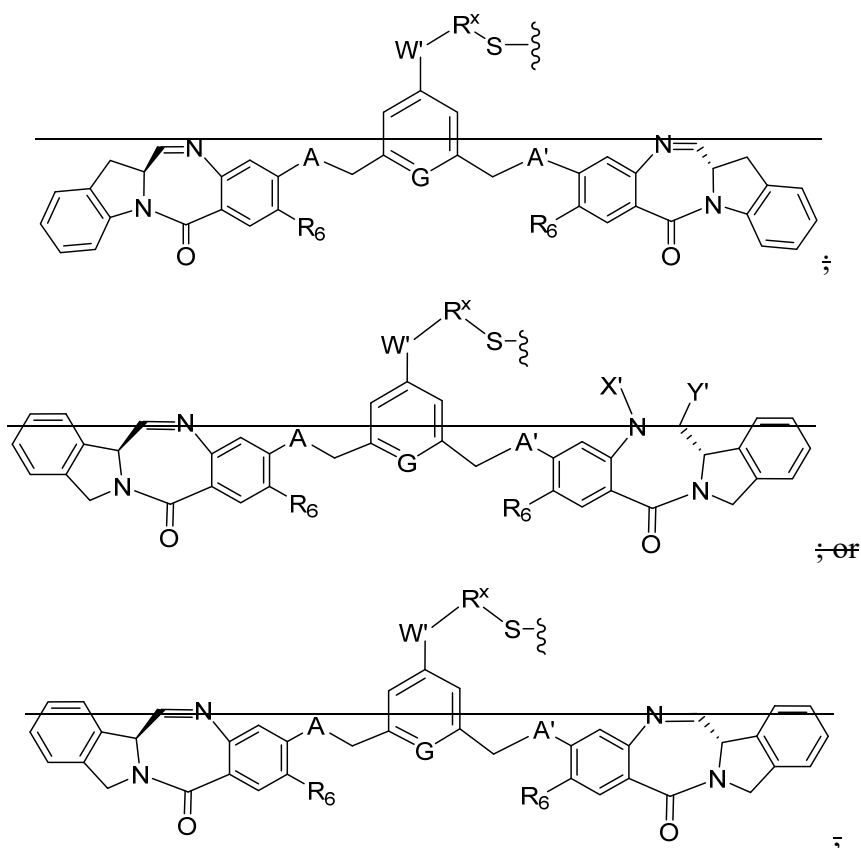
R_{23} and R_{24} , for each occurrence, are independently H or an optionally substituted alkyl;

m' is an integer between 0 and 10; and

R^h is H or an optionally substituted alkyl

(2) ~~D is represented by the following formula:~~





or a pharmaceutically acceptable salt thereof,

—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 CH_2S , CH_2NR^e ;

— R^* 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 $(CH_2CH_2O)_nR^k$, 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, 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 1 to 24;

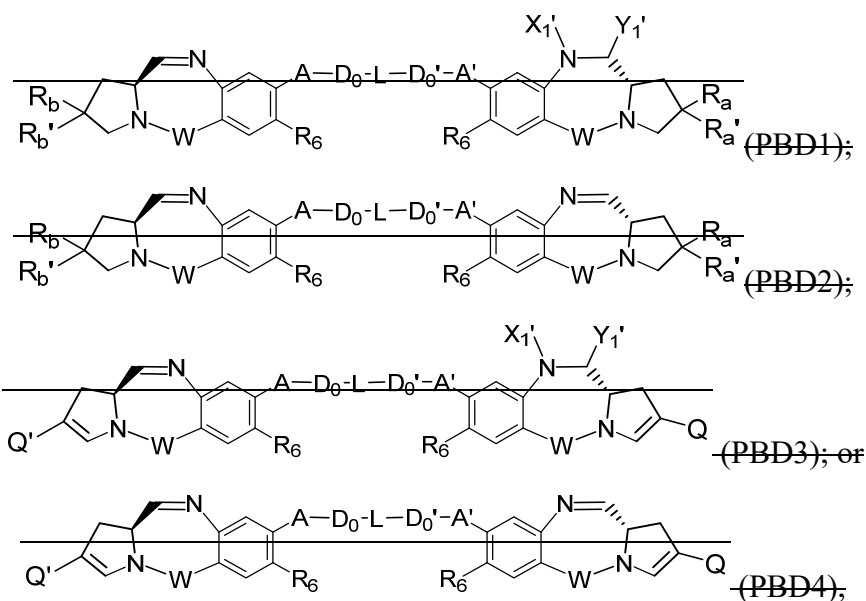
— G is selected from CH or N ;

— R_6 is H , R , OR , SR , $\text{NR}'\text{R}''$, NO_2 , or halogen; and

— R is H , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, wherein n is an integer from 1 to 24, and R^e is a linear or branched alkyl having 1 to 4 carbon atoms;

— R' and R'' are each independently selected from H , OH , OR , $\text{NRR}^{\varepsilon'}$, COR , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted aryl having from 6 to 18 carbon atoms, an optionally substituted 3 to 18-membered heterocyclic ring having 1 to 6 heteroatoms selected from O , S , N and P , a PEG group $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, and $\text{R}^{\varepsilon'}$ is H , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$; or

(3) D is represented by the following formula:



or a pharmaceutically acceptable salt thereof,

— wherein:

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

— $\text{X}_{1'}$ 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, 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;

——— $Y_{1'}$ 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_6 is H, R, OR, SR, $NR'R''$, NO_2 , or halogen;

——— A and A' are the same or different, and are independently selected from O, oxo ($C(=O)-$), $CRR'O$, CRR' , S, $CRR'S$, NR_5 and $CRR'N(R_5)$;

——— 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^e , 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^e , and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;

——— R^e 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;

——— n is an integer from 1 to 24;

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

——— D_0 and D_0' 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, a linker, a polyethylene glycol unit $(OCH_2CH_2)_n$, an optionally

~~— R_a , R_a' , R_b and R_b' are the same or different, and are independently selected from the group consisting of H, halide, or an optionally substituted branched, linear or cyclic alkyl having 1 to 10 carbon atoms; or R_a and R_a' and/or R_b and R_b' together form a double bond containing group =B and =B' respectively;~~

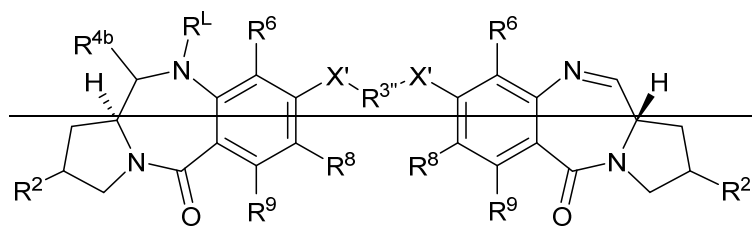
_____Q is Q₁-Ar-Q₂;

— Q₁ and Q₁' are each independently absent, a linear, branched or cyclic alkyl from 1 to 6 carbon atoms or a $\text{CH}=\text{CH}$ unit;

—Q₂ and Q₂' are each independently selected from —H, a linker, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit —R^e—(OCH₂CH₂)_n—R^e, or a substituent selected from a halogen, guanidinium [—NH(C=NH)NH₂], R, OR, NR'R'', NO₂, NCO, —NR'COR'', NR'(C=O)OR'', 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' or —CONR'R''; and

~~(4) the imine-containing cytotoxic agent is represented by the following formula:~~





or a pharmaceutically acceptable salt thereof,

wherein:

- the dotted lines indicate the optional presence of a double bond;
- $R^{3''}$ is a C_{3-12} alkylene group;
- each X' , for each occurrence, is independently O , S or $N(H)$;
- each R^2 is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, CO_2R^{1+} , COR^{1+} , or halo, or both R^2 taken together, are $=O$, $=CH_2$, $=CH-R^a$, or $=C(R^a)_2$;
- each $R^{2'}$ is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, CO_2R^{1+} , COR^{1+} or halo;
- R^{4b} is a leaving group selected from OR^{6+} , $OCOR^{4+}$, $OCOO R^{4+}$, $OCONR^{4+}R^{5+}$, $NR^{4+}R^{5+}$, $NR^{4+}COR^{5+}$, $NR^{4+}NR^{4+}R^{5+}$, an optionally substituted 5- or 6-membered nitrogen-containing heterocycle (e.g., piperidine, tetrahydropyrrole, pyrazole, morpholine), a guanidinium represented by $NR^{4+}(C=NH)NR^{4+}R^{5+}$, an amino acid, or a peptide represented by $NR^{6+}COP'$, wherein P' is an amino acid or a polypeptide containing between 2 to 20 amino acid units, SR^{6+} , SOR^{4+} , SO_2M , SO_3M , OSO_3M , halogen, cyano and an azido;
- R^L is linker bearing a maleimide moiety that can form a covalent bond with a cell binding agent (CBA);
- R^6 and R^9 are independently selected from H , R^{1+} , OH , OR^{1+} , SH , SR^{1+} , NH_2 , NHR^{1+} , $NR^{1+}R^{3+}$, NO_2 , Me_3Sn and halo; and,
- R^{1+} and R^{3+} are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl or C_{5-20} aryl groups, and optionally in relation to the group $NR^{1+}R^{3+}$, R^{1+} and R^{3+} together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; or any pair of adjacent groups from R^6 to R^9 together form a group $O-(CH_2)_p-O$, where p is 1 or 2;
- R^{4+} and R^{5+} are each independently selected from H , OH , OR^{6+} , NHR^{6+} , NR^{6+}_2 , COR^{6+} , an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^b$, or an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O , S , N or P ;

~~—— R⁶, 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^b, 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, or sulfur, and an optionally substituted 3 to 18 membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N or P;~~

~~—— R^{*} is independently selected from R¹, CO₂R¹, COR¹, CHO, CO₂H, or halo;~~

~~—— R^b is H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;~~

~~—— M is H or a pharmaceutically acceptable cation; and~~

~~—— n is an integer from 1 to 24.~~

5. ~~4.~~ The method of any one of claims 1-~~3~~ 4, wherein

0.5 to 5 equivalents of the bisulfite salt or 0.25 to 2.5 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent;

0.8 to 2.0 equivalents of the bisulfite salt or 0.4 to 1.0 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent;

1.1 to 1.6 equivalents of the bisulfite salt or 0.55 to 0.8 equivalents of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent; or

1.4 equivalents of the bisulfite salt or 0.7 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

6. ~~5.~~ The method of claim 1, wherein the reaction of step (a) is carried out at

(i) a pH of 2.9 to 3.7 and 1.0 to 1.8 equivalents of the bisulfite salt or 0.5 to 0.9 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent;

(ii) a pH of 3.1 to 3.5 and 1.1 to 1.6 equivalents of the bisulfite salt or 0.55 to 0.8 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent; or

(iii) a pH of 3.3 and 1.4 equivalents of the bisulfite salt or 0.7 equivalent of the metabisulfite salt is reacted with 1 equivalent of the imine-containing cytotoxic agent.

7. ~~The method of any one of claims 1-6, wherein the reaction of step (a) is carried out in~~

~~a mixture of an organic solvent and water.~~

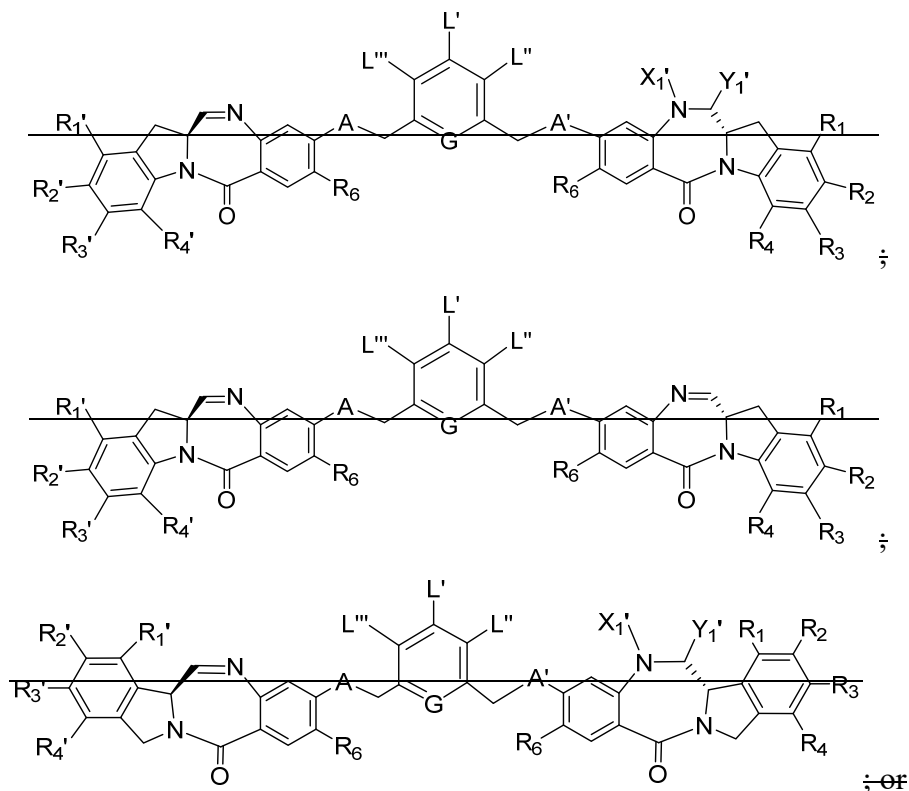
8 ~~6~~. The method of any one of claims 1-5 ~~7~~, wherein the reaction of step (a) is carried out in a mixture of dimethylacetamide (DMA) and water.

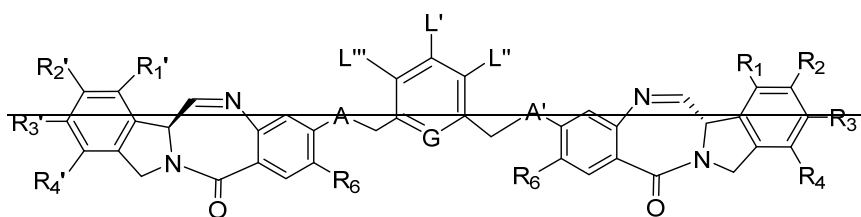
9 ~~7~~. The method of any one of claims 1-~~6~~ 8, wherein in step (a), the imine-containing cytotoxic agent is reacted with sodium bisulfite or sodium metabisulfite.

~~10~~ 8. The method of any one of claims 1-~~7~~ 9, wherein the modified cytotoxic agent is not purified before reacting with the cell-binding agent in step (b).

~~11~~ 9. The method of any one of claims 1-~~8~~ 10, wherein the reaction of step (b) is carried out at a pH of 4 to 9, a pH of 5 to 8.5, or a pH of 5.5 to 6.5.

~~12. The method of any one of claims 1-11, wherein D is represented by the following formula:~~

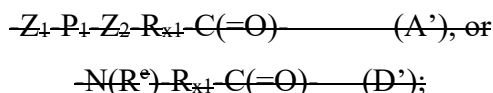




or a pharmaceutically acceptable salt thereof,

wherein:

— one of L', L'', and L''' is represented by the following formula:



and the other two are each 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 $(\text{CH}_2\text{CH}_2\text{O})_n$, R^e , halogen, guanidinium $[\text{NH}(\text{C}=\text{NH})\text{NH}_2]$, OR, $\text{NR}'\text{R}''$, NO_2 , $\text{NR}'\text{COR}''$, SR, SOR' , $\text{SO}_2\text{R}'$, SO_3H , OSO_3H , $\text{SO}_2\text{NR}'\text{R}''$, cyano, an azido, COR' , OCOR' , and $\text{OCONR}'\text{R}''$;

— one of the Z_1 and Z_2 is C(=O) , and the other is NR_5 ;

— P_1 is an amino acid residue or a peptide containing between 2 to 20 amino acid residues;

— R_{x+1} is an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^k$, 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 ($\text{NR}^{101}\text{R}^{102}$) group or a 5- or 6-membered nitrogen containing heterocycle, wherein R^{101} and R^{102} are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;

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

~~—— R^e is H or an optionally substituted linear or branched alkyl having 1 to 4 carbon atoms;~~

~~—— n is an integer from 1 to 24;~~

~~—— $\text{X}_{1'}$ 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, 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;~~

~~—— $\text{Y}_{1'}$ 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;~~

~~—— $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_{1'}, \text{R}_{2'}, \text{R}_{3'}$ and $\text{R}_{4'}$ 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{R}^e$, halogen, guanidinium $[\text{NH}(\text{C}=\text{NH})\text{NH}_2]$, OR, $\text{NR}'\text{R}''$, NO_2 , NCO , $\text{NR}'\text{COR}''$, SR , SOR' , $\text{SO}_2\text{R}'$, SO_3H , OSO_3H , $\text{SO}_2\text{NR}'\text{R}''$, cyano, an azido, COR' , OCOR' , and $\text{OCONR}'\text{R}''$;~~

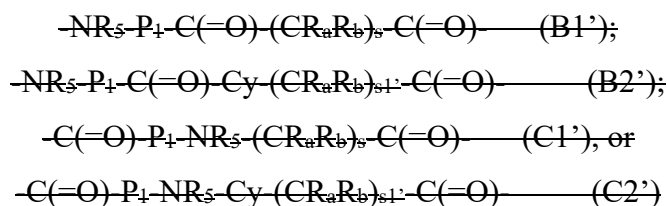
~~—— R_6 is H, R, OR, SR, $\text{NR}'\text{R}''$, NO_2 , or halogen;~~

~~—— G is CH or N;~~

~~—— A and A' are the same or different, and are independently selected from O, oxo ($\text{C}(=\text{O})$), $\text{CRR}'\text{O}$, CRR' , S, $\text{CRR}'\text{S}$, NR_5 and $\text{CRR}'\text{N}(\text{R}_5)$; and~~

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

13. The method of claim 12, wherein L'' and L''' are both H; and L' is represented by the following formula:



wherein:

— R_a and R_b , for each occurrence, are each independently H , $(C_1\text{--}C_3)$ alkyl or a charged substituent or an ionizable group Q ;

— s is an integer from 1 to 6;

— $s1'$ is 0 or an integer from 1 to 6; and

— Cy is a cyclic alkyl having 5 or 6 ring carbon atoms optionally substituted with halogen, OH , $(C_1\text{--}C_3)$ alkyl, $(C_1\text{--}C_3)$ alkoxy, or halo $(C_1\text{--}C_3)$ alkyl.

14. — The method of claim 12 or 13,

wherein:

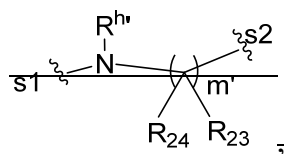
— R_1 , R_2 , R_3 , R_4 , R_1' , R_2' , R_3' and R_4' are all H ;

— R_6 is OMe ;

— X_1' and Y_1' are both H ; and

— A and A' are O .

15. — The method of any one of claims 1–14, wherein L is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof,

wherein:

— $s1$ is the site covalently linked to D ;

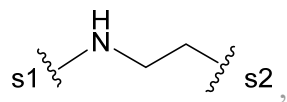
— $s2$ is the site covalently linked to the maleimide group;

— R_{23} and R_{24} , for each occurrence, are independently H or an optionally substituted alkyl;

— m' is an integer between 0 and 10; and

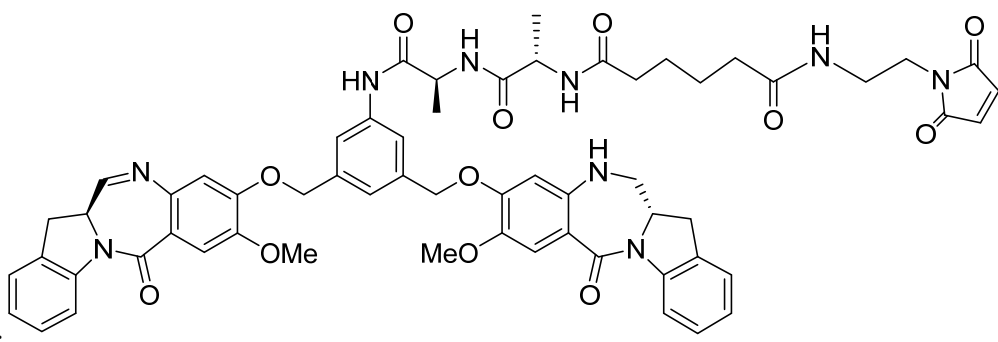
— R^{h2} is H or an optionally substituted alkyl.

10. The method of any one of claims 1-9, wherein L is represented by the following structural formula:

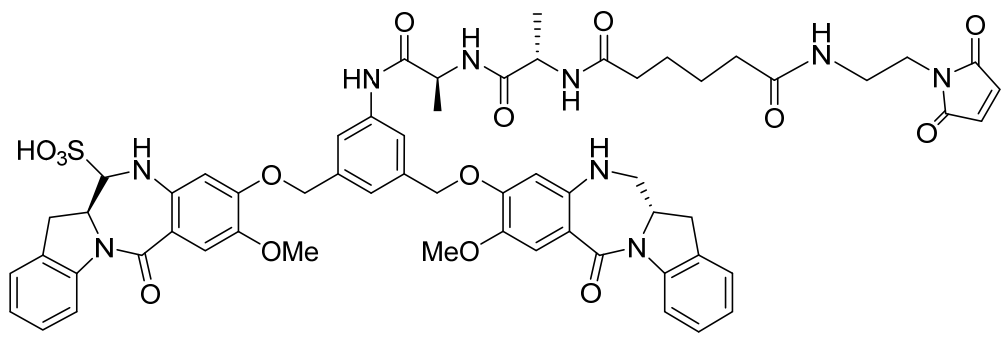


or a pharmaceutically acceptable salt thereof.

11. The method of any one of claims 1-10, wherein the imine-containing cytotoxic agent is represented by the following formula:

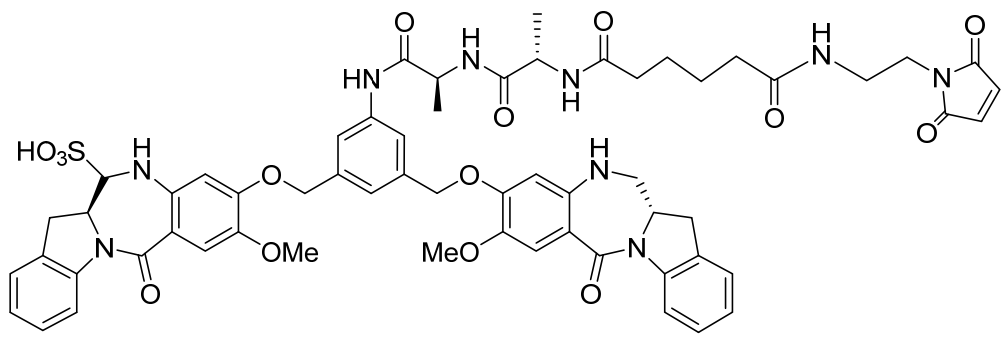


or a pharmaceutically acceptable salt thereof, and the modified cytotoxic agent is represented by the following formula:

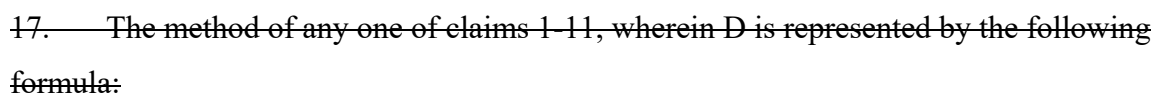


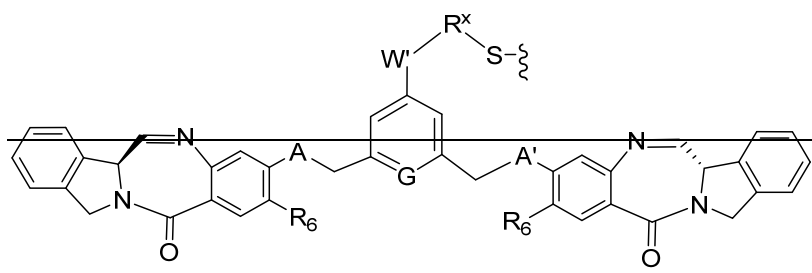
or a pharmaceutically acceptable salt thereof.

12. The method of claim 11, wherein the modified cytotoxic agent is represented by the following formula:



13. The method of claim 12, wherein the modified cytotoxic agent is represented by the following formula:





or a pharmaceutically acceptable salt thereof,

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 CH₂S, CH₂NR^e;~~

— ~~R^{*} 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 (CH₂CH₂O)_nR^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, wherein R¹⁰¹ and R¹⁰² are each independently a linear, branched, or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms;~~

— ~~n is an integer from 1 to 24;~~

— ~~G is selected from CH or N;~~

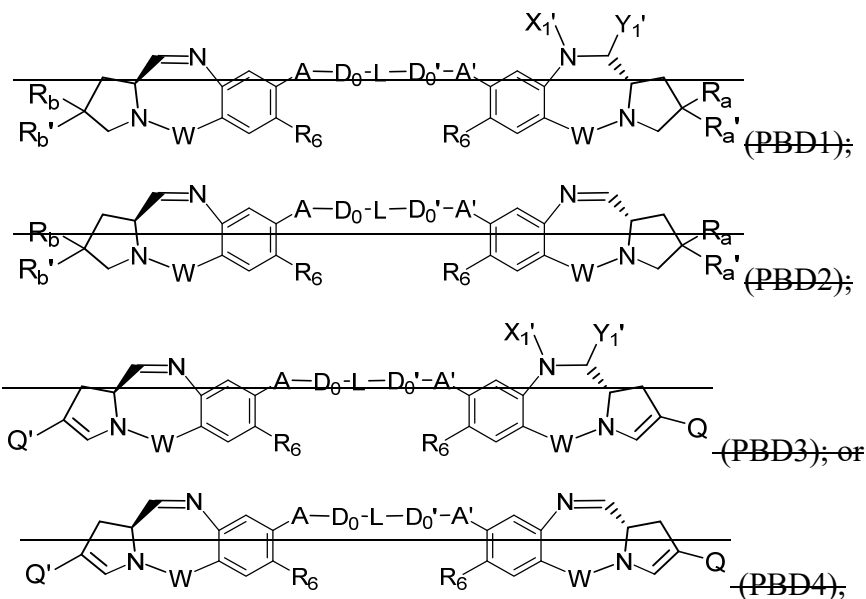
— ~~R₆ is H, R, OR, SR, NR'R'', NO₂, or halogen; and~~

— ~~R is H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group (CH₂CH₂O)_nR^e, wherein n is an integer from 1 to 24, and R^e is a linear or branched alkyl having 1 to 4 carbon atoms;~~

— ~~R' and R'' are each independently selected from H, OH, OR, NRR^e, COR, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, an optionally substituted aryl having from 6 to 18 carbon atoms, an optionally~~

substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms selected from O, S, N and P, a PEG group $(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}^e$, and R^{e2} is H, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms or a PEG group $(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}^e$.

18. The method of any one of claims 1-11, wherein D is represented by the following formula:



or a pharmaceutically acceptable salt thereof,

wherein:

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

— $\text{X}_{1'}$ 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 $(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}^e$, 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;

— $\text{Y}_{1'}$ 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_6 is H, R, OR, SR, $NR'R''$, NO_2 , or halogen;~~

~~———— A and A' are the same or different, and are independently selected from O, oxo ($C(=O)$), $CRR'O$, CRR' , S, $CRR'S$, NR_5 and $CRR'N(R_5)$;~~

~~———— 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^e , 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^e , and an optionally substituted 3- to 18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N and P;~~

~~———— R^e 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;~~

~~———— n is an integer from 1 to 24;~~

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

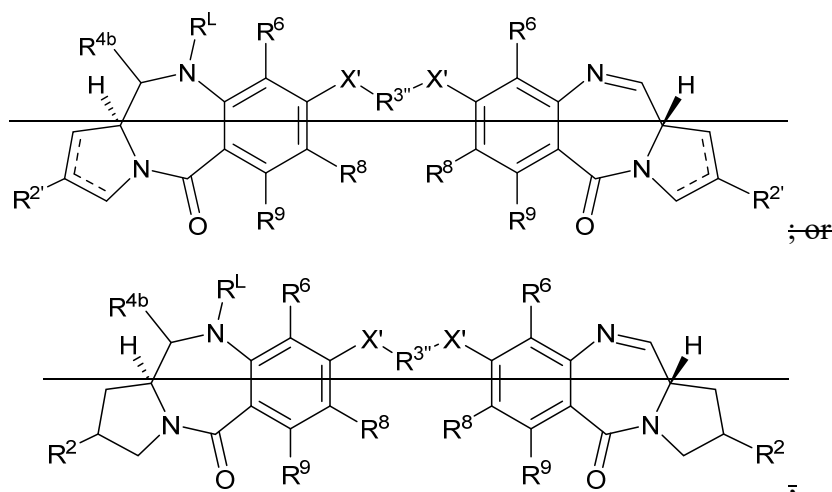
~~———— D_6 and D_6' are the same or different, and are independently absent or selected from the group consisting of an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having 1 to 10 carbon atoms, an amino acid, a peptide bearing 2 to 6 amino acids, and a polyethylene glycol unit $(OCH_2CH_2)_r$;~~

~~———— L is absent, a linker, a polyethylene glycol unit $(OCH_2CH_2)_n$, an optionally substituted linear, branched or cyclic alkyl or alkenyl having 1 to 10 carbon atoms, an optionally substituted phenyl group, an optionally substituted 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;~~

~~———— R_a , $R_{a'}$, R_b and $R_{b'}$ are the same or different, and are independently selected from the group consisting of H, halide, or an optionally substituted branched, linear or cyclic alkyl having 1 to 10 carbon atoms; or R_a and $R_{a'}$ and/or R_b and $R_{b'}$ together form a double bond containing group $=B$ and $=B'$ respectively;~~

~~—B and B' are the same or different and independently selected from an optionally substituted branched or linear alkenyl or a carbonyl group;~~
~~—Q is Q₁-Ar-Q₂;~~
~~—Q' is Q₁'-Ar'-Q₂';~~
~~—Q₁ and Q₁' are each independently absent, a linear, branched or cyclic alkyl from 1 to 6 carbon atoms or a -CH=CH unit;~~
~~—Ar and Ar' are each independently absent, or represent an aryl group;~~
~~—Q₂ and Q₂' are each independently selected from -H, a linker, a substituted or unsubstituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit -R^e-(OCH₂CH₂)_n-R^e, or a substituent selected from a halogen, guanidinium [NH(C=NH)NH₂], -R, -OR, -NR'R'', -NO₂, -NCO, -NR'COR'', -NR'(C=O)OR'', -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' or -OCONR'R''; and~~
~~—R^e is absent or selected from linear or branched alkyl, alkenyl or alkynyl having 1 to 5 carbon atoms.~~

19. ~~The method of any one of claims 1-11, wherein the imine-containing cytotoxic agent is represented by the following formula:~~



~~or a pharmaceutically acceptable salt thereof,~~

~~wherein:~~

~~—the dotted lines indicate the optional presence of a double bond;~~
~~—R^{3''} is a C₃₋₁₂ alkylene group,~~
~~—each X', for each occurrence, is independently -O-, -S- or -N(H)-;~~

~~each R^2 is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, $-CO_2R^{1+}$, $-COR^{1+}$, or halo, or both R^2 taken together, are $=O$, $=CH_2$, $=CH-R^a$, or $=C(R^a)_2$;~~
~~each R^{2+} is independently selected from H , OH , CN , R^{1+} , OR^{1+} , $O-SO_2-R^{1+}$, $-CO_2R^{1+}$, $-COR^{1+}$ or halo;~~
 ~~R^{4b} is a leaving group selected from OR^{6+} , $OCOR^{4+}$, $OCOOR^{4+}$, $OCONR^{4+}R^{5+}$, $-NR^{4+}R^{5+}$, $-NR^{4+}COR^{5+}$, $-NR^{4+}NR^{4+}R^{5+}$, an optionally substituted 5- or 6-membered nitrogen-containing heterocycle (e.g., piperidine, tetrahydropyrrole, pyrazole, morpholine), a guanidinium represented by $-NR^{4+}(C=NH)NR^{4+}R^{5+}$, an amino acid, or a peptide represented by $-NR^{6+}COP^+$, wherein P^+ is an amino acid or a polypeptide containing between 2 to 20 amino acid units, SR^{6+} , SOR^{4+} , SO_2M , SO_3M , OSO_3M , halogen, cyano and an azido;~~
 ~~R^L is linker bearing a maleimide moiety that can form a covalent bond with a cell binding agent (CBA);~~
 ~~R^6 and R^9 are independently selected from H , R^{1+} , OH , OR^{1+} , SH , SR^{1+} , NH_2 , NHR^{1+} , $NR^{1+}R^{3+}$, NO_2 , Me_3Sn and halo; and,~~
 ~~R^{1+} and R^{3+} are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl or C_{5-20} aryl groups, and optionally in relation to the group $-NR^{1+}R^{3+}$, R^{1+} and R^{3+} together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; or any pair of adjacent groups from R^6 to R^9 together form a group $-O-(CH_2)_p-O-$, where p is 1 or 2;~~
 ~~R^{4+} and R^{5+} are each independently selected from H , OH , OR^{6+} , NHR^{6+} , NR^{6+}_2 , $-COR^{6+}$, an optionally substituted linear, branched or cyclic alkyl, alkenyl or alkynyl having from 1 to 10 carbon atoms, a polyethylene glycol unit $-(CH_2CH_2O)_n-R^b$, or an optionally substituted 3-18-membered heterocyclic ring having 1 to 6 heteroatoms independently selected from O, S, N or P;~~
 ~~R^{6+} , 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 $-(CH_2CH_2O)_n-R^b$, 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, or sulfur, and an optionally substituted 3- to 18-membered heterocyclic ring containing 1 to 6 heteroatoms independently selected from O, S, N or P;~~
 ~~R^a is independently selected from R^{1+} , $-CO_2R^{1+}$, $-COR^{1+}$, CHO , CO_2H , or halo;~~
 ~~R^b is H or a substituted or unsubstituted linear or branched alkyl having 1 to 4 carbon atoms;~~

- ~~M is H or a pharmaceutically acceptable cation; and~~
- ~~n is an integer from 1 to 24.~~