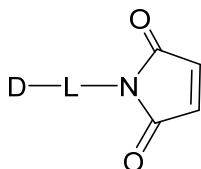


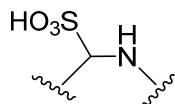
CLAIMS

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:

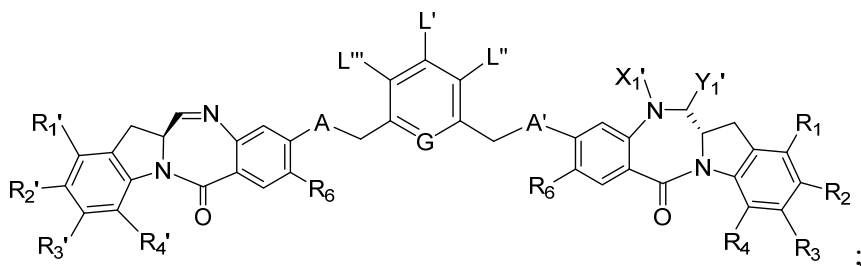


or a pharmaceutically acceptable salt thereof; and

(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:

D is represented by the following formula:

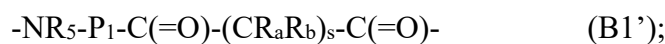


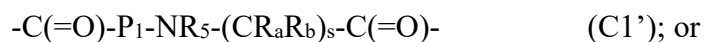
or a pharmaceutically acceptable salt thereof,

wherein:

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;

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

R₆ is -OMe;

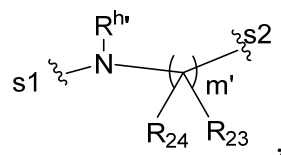
X₁' and Y₁' are both -H;

A and A' are -O-;

G is -CH- or -N-;

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

-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;

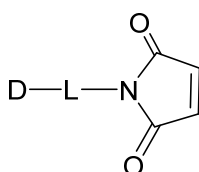
m' is an integer between 0 and 10; and

$R^{h'}$ is H or an optionally substituted alkyl.

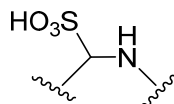
2. The method of claim 1, wherein the reaction of step (a) is carried out at a pH of 2.9 to 4.0, a pH of 2.9 to 3.7, a pH of 3.1 to 3.5, a pH of 3.2 to 3.4, or a pH of 3.3.

3. 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:

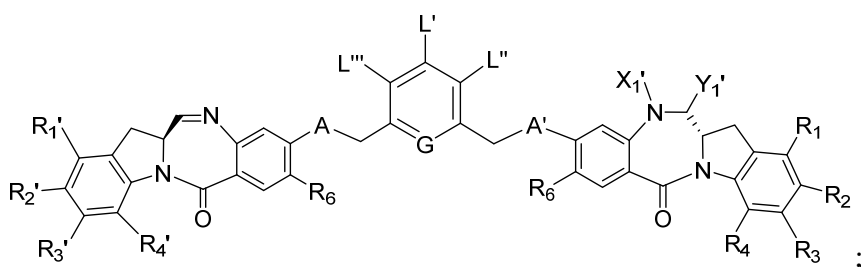


or a pharmaceutically acceptable salt thereof; and

(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:

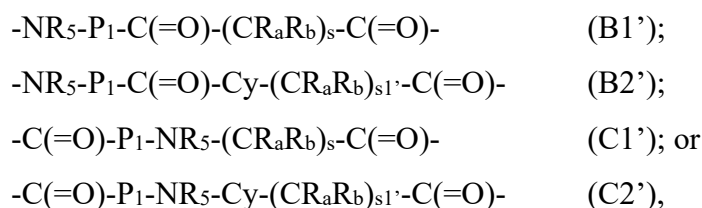


or a pharmaceutically acceptable salt thereof,

wherein:

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;

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

R₆ is -OMe;

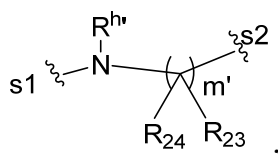
X₁' and Y₁' are both -H;

A and A' are -O-;

G is -CH- or -N-;

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

-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.

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

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.

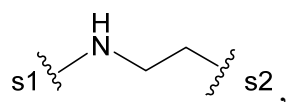
6. The method of any one of claims 1-5, wherein the reaction of step (a) is carried out in a mixture of dimethylacetamide (DMA) and water.

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

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

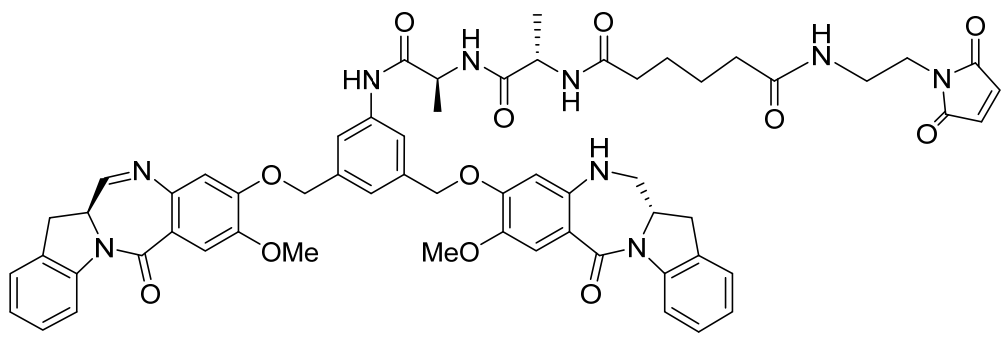
9. The method of any one of claims 1-8, wherein the reaction of step (b) is carried out at a pH of 4 to 9, a pH of 5 to 8.5, or a pH of 5.5 to 6.5.

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

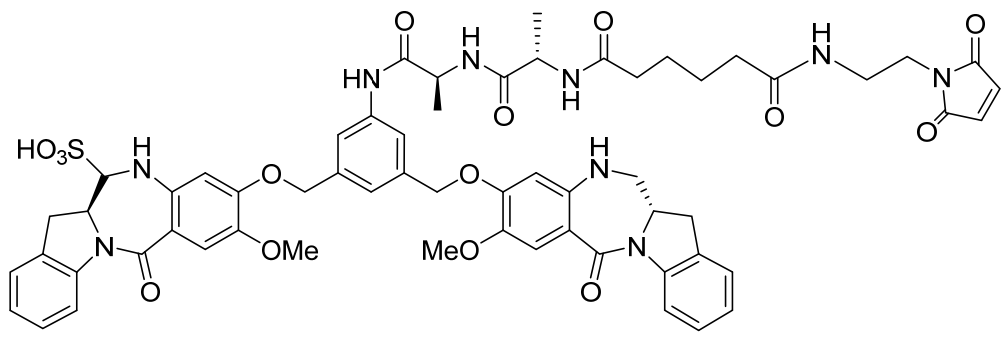


or a pharmaceutically acceptable salt thereof.

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

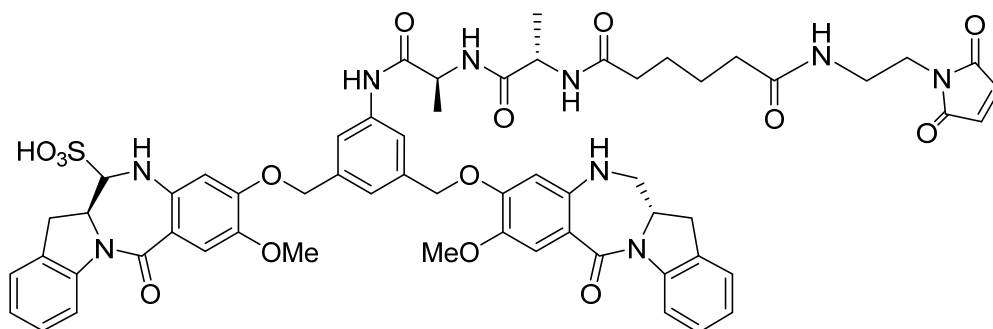


or a pharmaceutically acceptable salt thereof, and the modified cytotoxic agent is represented by the following formula:



or a pharmaceutically acceptable salt thereof.

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



or a sodium or potassium salt thereof.

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

