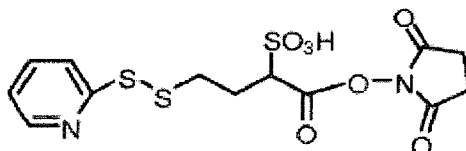


CLAIMS

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

(a) reacting a cytotoxic agent with a bifunctional crosslinking reagent represented by the following structural formula:



or a salt thereof, in a buffer solution comprising a buffering agent to provide a first mixture comprising a cytotoxic agent-linker compound, wherein the buffer solution has high buffer capacity and the molar ratio of the buffering agent to the bifunctional crosslinking reagent is 2:1 to 8:1;

(b) reacting the cytotoxic agent-linker compound in the first mixture obtained from step (a) with a cell-binding agent in a solution having a pH of 4 to 9 to provide a second mixture comprising the cell-binding agent cytotoxic agent conjugate, wherein the cell-binding agent is an antibody.

2. The process of claim 1, wherein the cell-binding agent is prone to fragmentation.
3. The process of claim 1, wherein the cytotoxic agent is a maytansinoid.
4. The process of claim 3, wherein the cytotoxic agent is DM4.
5. The process of claim 1, wherein molar ratio of the buffering agent to the bifunctional crosslinking reagent is 4:1 to 6:1.
6. The process of claim 5, wherein the molar ratio of the buffering agent to the bifunctional crosslinking reagent is 5:1.
7. The process of claim 1, wherein the buffer solution has a pH of 4 to 9, 7.5 to 8.5, 7.9 to 8.5, 8.0 to 8.4 or 8.1 to 8.3.

8. The process of claim 7, wherein the buffer solution has a pH of 8.2.
9. The process of claim 1, wherein the buffering agent is selected from the group consisting of a citrate buffer, an acetate buffer, a succinate buffer and a phosphate buffer or the buffering agent is selected from the group consisting of HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid)), POPSO (piperazine-1,4-bis-(2-hydroxy-propane-sulfonic acid) dehydrate), HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), EPPS (4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), MES (2-(N-morpholino)ethanesulfonic acid) and a combination thereof.
10. The process of claim 1, wherein the buffer solution further comprises sodium chloride.
11. The process of claim 1, wherein the buffer solution further comprises an organic solvent.
12. The process of claim 1, wherein molar excess amount of the cytotoxic agent relative to the bifunctional crosslinking reagent is used in the reaction of step (a).
13. The process of claim 1, wherein the solution in step (b) has a pH of 5.0-9.0, 5.5-9.0, 6.0-9.0 or 6.5-9.0.
14. The process of claim 1, wherein the solution in step (b) comprises a buffering agent selected from the group consisting of a citrate buffer, an acetate buffer, a succinate buffer and a phosphate buffer, or a buffering agent selected from the group consisting of HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid)), POPSO (piperazine-1,4-bis-(2-hydroxy-propane-sulfonic acid) dehydrate), HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), EPPS (4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), MES (2-(N-morpholino)ethanesulfonic acid) and a combination thereof.

15. The process of claim 1, wherein the solution in step (b) further comprises an organic solvent.

16. The process of claim 1, wherein concentration of the cell-binding agent in the solution of step (b) is 5 g/L to 100 g/L.

17. The process of claim 1, wherein the molar ratio of the bifunctional crosslinking reagent to the cell-binding agent is from 2 to 10.

18. The process of claim 1, wherein the process further comprises the step of quenching the reaction in step (b) by adjusting the pH of the solution to 5 or below.

19. The process of claim 1, wherein the antibody is selected from huN901, huMy9-6, huB4, huC242, trastuzumab, bivatuzumab, sibrotuzumab, CNTO95, huDS6, rituximab, anti-Her2 antibody, anti-EGFR antibody, anti-CD27L antibody, anti-EGFRvIII antibody, anti-Cripto antibody, anti-CD138 antibody, anti-CD38 antibody, anti-EphA2 antibody, integrin targeting antibody, anti-CD37 antibody, anti-folate receptor antibody, anti-Her3 antibody, and anti-IGFIR antibody.