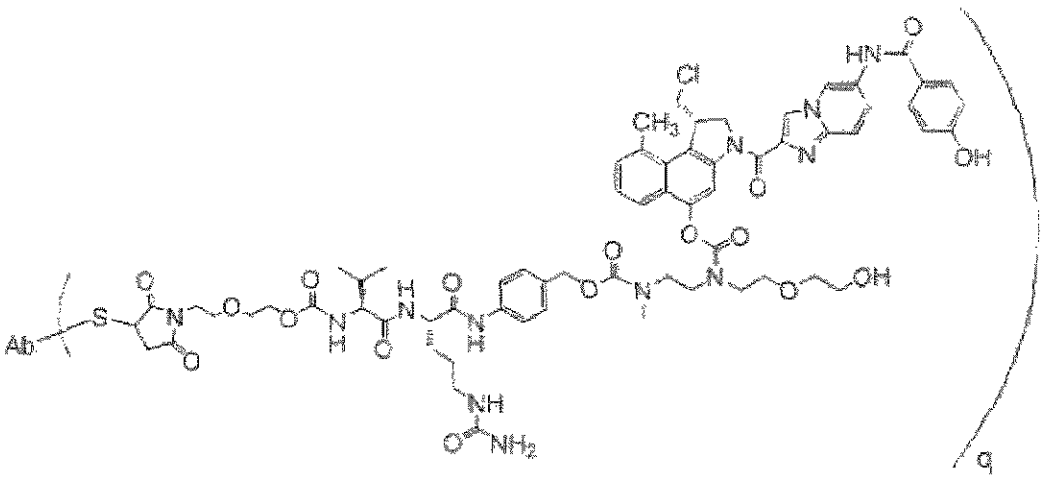
e. eluting said column with a 0-100 mM aqueous salt solution to obtain a purified mixture of cysteine-linked antibody-drug conjugates.

In a particularly preferred embodiment of the present invention, the mixture of cysteine-linked antibody-drug conjugates is of the formula (II)



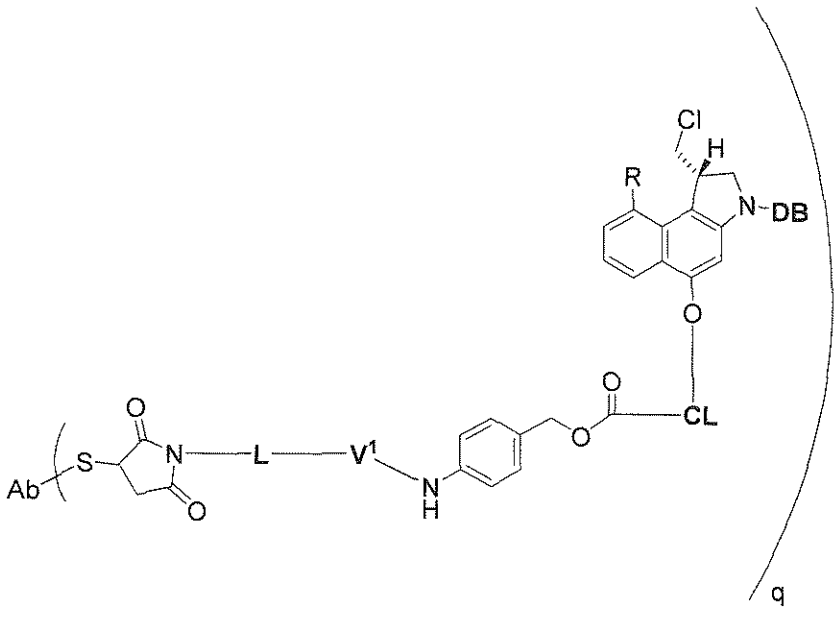
(II)

wherein

Ab is trastuzumab, and

q ranges from 0 to 8.

In another aspect, the present invention provides for a method for purifying a mixture of cysteine-linked antibody-drug conjugates of formula (I)

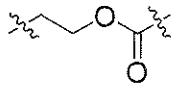
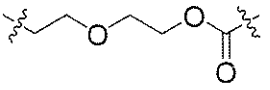
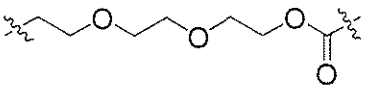


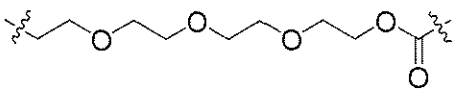
(I),

wherein

Ab is an antibody,

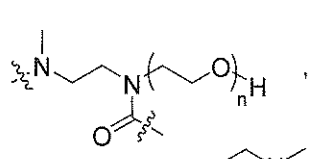
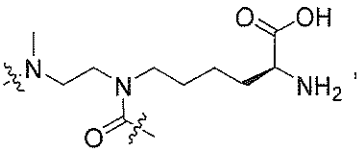
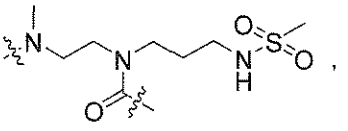
L is a linking group selected from

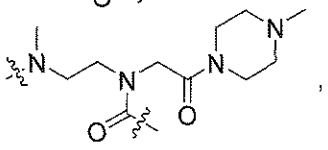
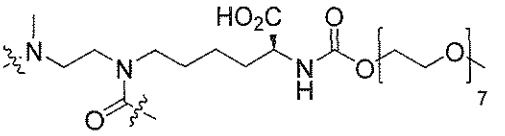
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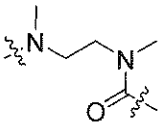
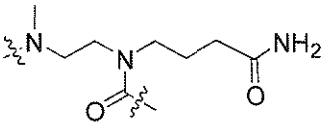
 ,

V^1 is a conditionally-cleavable dipeptide of natural and/or unnatural amino acids,

CL is a cyclization linker selected from

 ,  ,  ,

 ,  ,

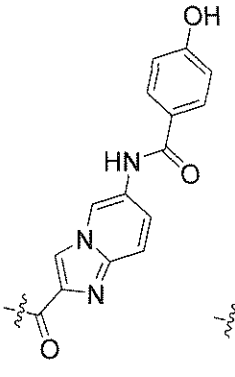
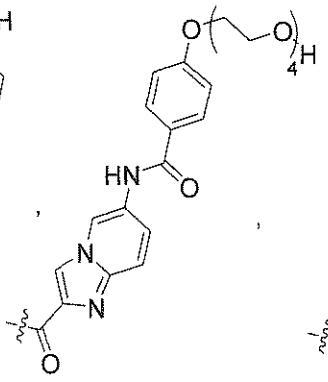
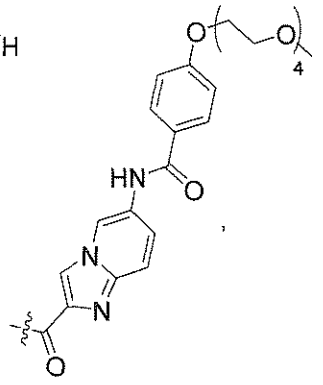
 and  ,

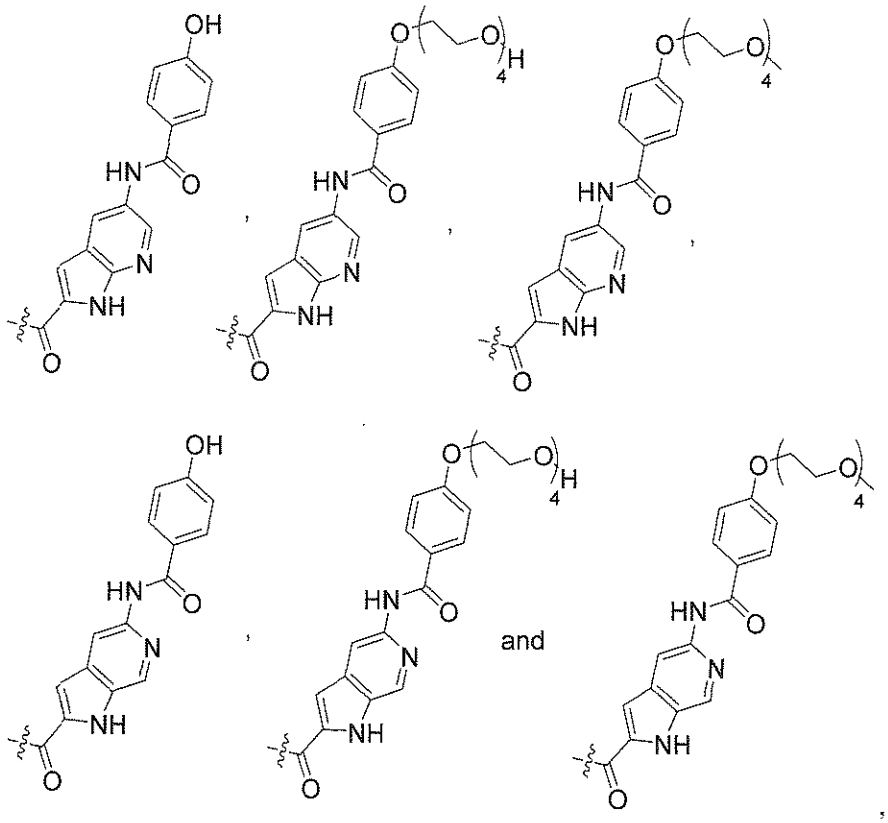
wherein n is an integer of from 1 to 16,

R is selected from H, CH_3, CH_2CH_3, OCH_3, OCH_2CH_3, CF_3, OCF_3, Cl, F,

q ranges from 0 to 8, and

DB is a DNA binding moiety selected from

 ,  ,  ,



wherein the amount of non-conjugated antibody is in the range of 10-40% by weight comprising:

a. providing the mixture in a 0.2-1.5 M aqueous salt solution;

b. loading said solution onto a preparative hydrophobic interaction chromatography column;

c. collecting a flow-through fraction that contains non-conjugated antibody;

d. washing said column with a 0.2-1.5 M aqueous salt solution while collecting the flow-through fraction; and

e. eluting said column with a 0-100 mM aqueous salt solution to obtain a purified mixture of cysteine-linked antibody-drug conjugates;

wherein no added organic solvent is used in either step a, b, d or e.

BRIEF DESCRIPTION OF THE DRAWINGS

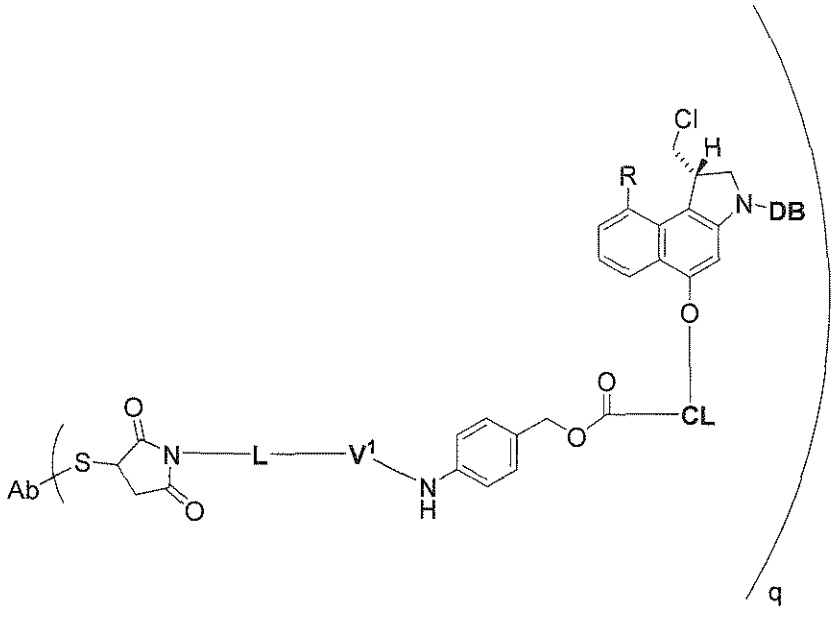
Figure 1 shows an example of the influence of the amount of reductant on the distribution of DAR species. When using 1.0 equivalent of reductant, the percentage of non-conjugated trastuzumab antibody DARO is about 20% by weight.

Figure 2 depicts an analytical HIC chromatogram of a mixture of cysteine-linked antibody-drug conjugates according to formula (II) before and after HIC purification on a preparative scale according to the purification in Example 3.

Figure 3 depicts analytical HIC chromatograms of a mixture of cysteine-linked antibody-drug conjugates according to formula (II) before and after HIC purification on a preparative scale according to the purification in Example 4.

CLAIMS

1. A method for purifying a mixture of cysteine-linked antibody-drug conjugates of formula (**I**)

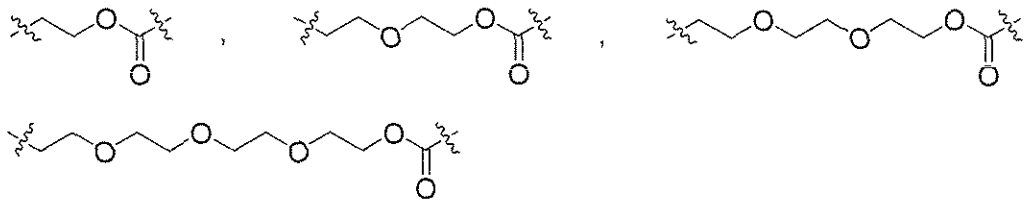


(**I**),

wherein

Ab is an antibody,

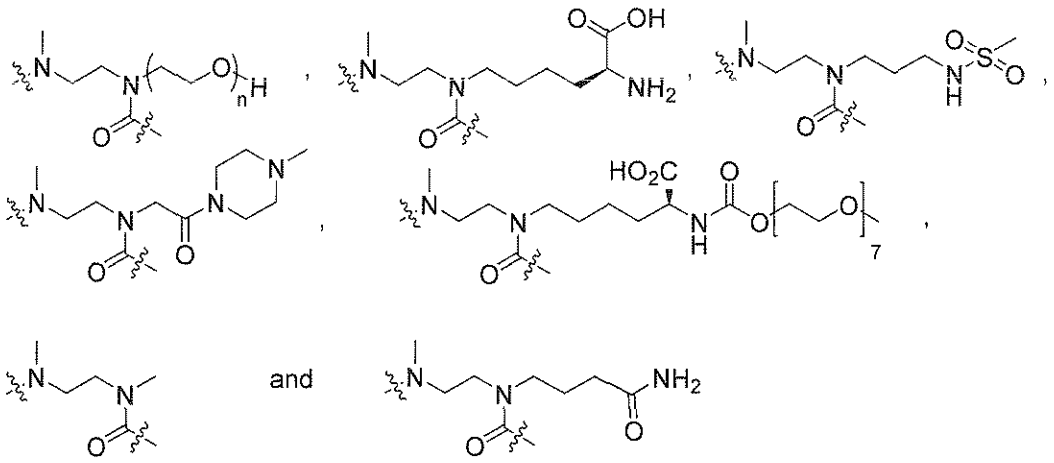
L is a linking group selected from



,

V^1 is a conditionally-cleavable dipeptide of natural and/or unnatural amino acids,

CL is a cyclization linker selected from



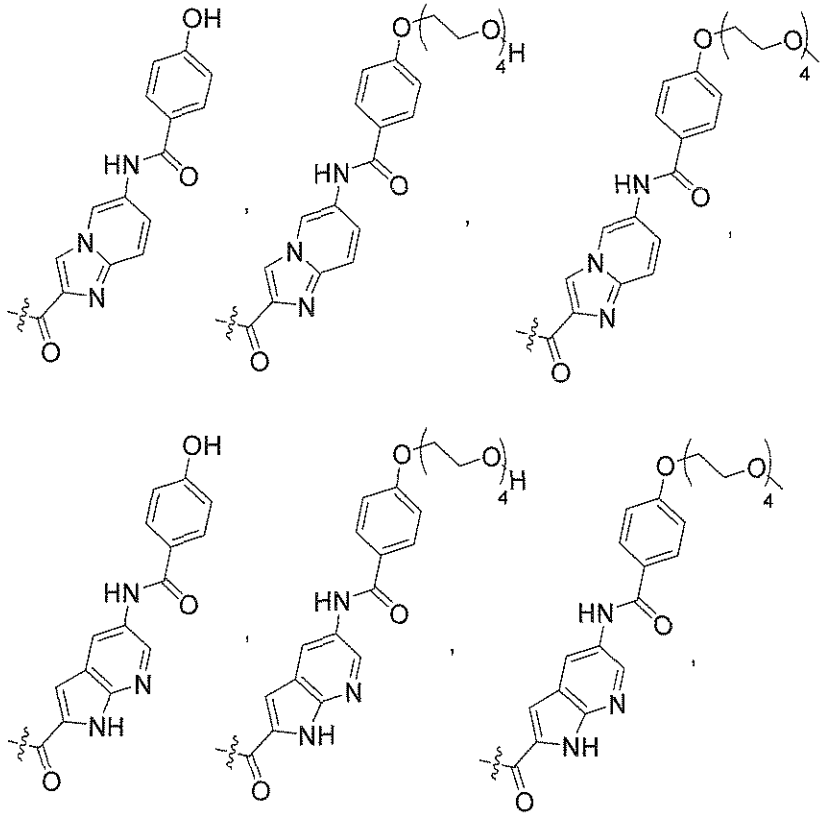
and

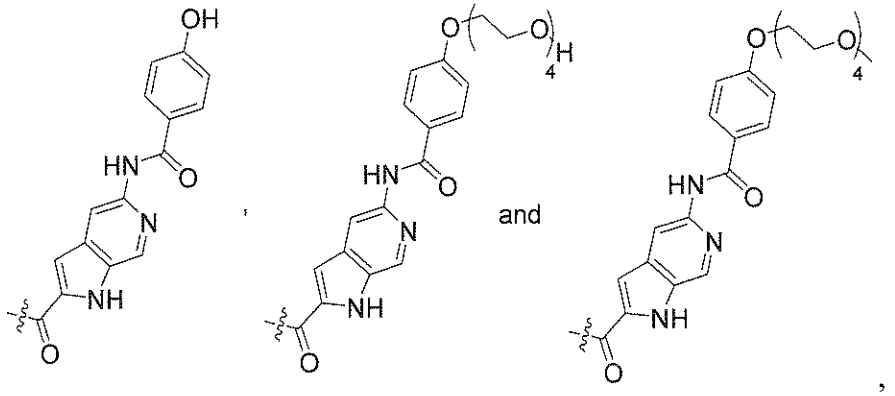
wherein n is an integer of from 1 to 16,

R is selected from H, CH_3, CH_2CH_3, OCH_3, OCH_2CH_3, CF_3, OCF_3, Cl, F,

q ranges from 0 to 8, and

DB is a DNA binding moiety selected from





wherein the amount of non-conjugated antibody is in the range of 10-40% by weight comprising:

a. providing the mixture in a 0.2-1.5 M aqueous salt solution;

b. loading said solution onto a preparative hydrophobic interaction chromatography column;

c. collecting a flow-through fraction that contains non-conjugated antibody;

d. washing said column with a 0.2-1.5 M aqueous salt solution while collecting the flow-through fraction; and

e. eluting said column with a 0-100 mM aqueous salt solution to obtain a purified mixture of cysteine-linked antibody-drug conjugates;

wherein no added organic solvent is used in either step a, b, d or e.

2. The method according to claim 1, wherein said column is packed with Fractogel EMD propyl, Fractrogel EMD phenyl, Butyl-S sepharose, Octyl Sepharose, Capto Octyl, Capto Butyl, Capto Phenyl ImpRes, Capto Butyl ImpRes, Toyopearl PPG-600M, Toyopearl Hexyl-650, Toyopearl Butyl-650, Toyopearl Phenyl-650, Toyopearl Ether-650, Macroprep t-Butyl, Macroprep phenyl, Cellufine Butyl, Cellufine Phenyl or Poros HP2.

3. The method according to claim 1 or 2, wherein said column has a diameter in the range of 4.0-2,000 mm, preferably 15-2,000 mm.

4. The method according to any one of claims 1 to 3, wherein the column loading is in the range of 5-50 g/L, preferably 5-40 g/L of column packing material.

5. The method according to any one of claims 1 to 4, wherein the column packing material has an average particle size in the range of 30-180 μm.

6. The method according to any one of claims 1 to 5, wherein the salt of the aqueous salt solution in step a, d or e is selected from the group consisting of potassium thiocyanate, sodium chloride, potassium chloride, ammonium chloride, sodium sulphate, potassium sulphate and ammonium sulphate, preferably the salt is sodium chloride or ammonium sulphate.

7. The method according to any one of claims 1 to 6, wherein the aqueous salt solution in step a, d or e further contains a buffer.

8. The method according to claim 7, wherein the buffer is selected from the group consisting of sodium phosphate, potassium phosphate, ammonium phosphate, sodium acetate, potassium acetate, sodium citrate, potassium citrate, ammonium citrate and mixtures thereof, preferably the buffer is sodium phosphate or sodium acetate.

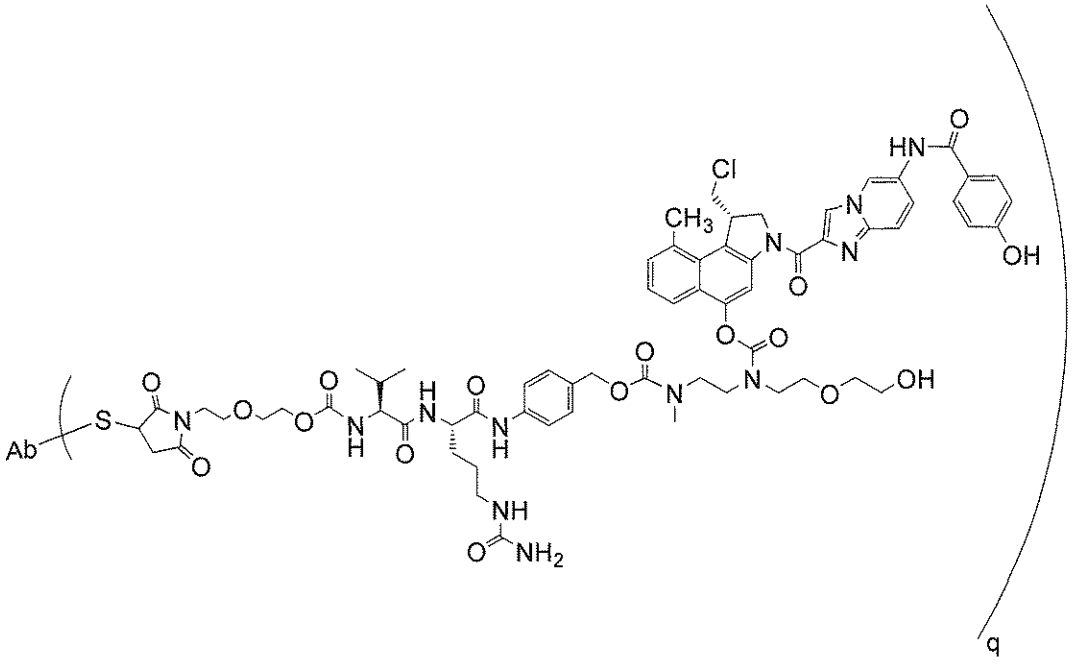
9. The method according to claim 7 or 8, wherein said aqueous salt solution is buffered to a pH of from about 4 to about 8.

10. The method according to any one of claims 1 to 9, wherein the elution in step e is performed in a reverse mode.

11. The method according to any one of claims 1 to 10, wherein the Ab is selected from the group consisting of an anti-CD19 antibody, an anti-CD22 antibody, an anti-CD30 antibody, an anti-CD33 antibody, an anti-CD56 antibody, an anti-CD70 antibody, an anti-CD74 antibody, an anti-CD138 antibody, an anti-CLL-1 antibody, an anti-5T4 antibody, an anti-CD303 antibody, an anti-Tag 72 antibody, an anti-Lewis A like carbohydrate antibody, an anti-EphB3 antibody, an anti-HMW-MAA antibody, an anti-

CD38 antibody, an anti-Cripto antibody, an anti-EphA2 antibody, an anti-GPNMB antibody, an anti-integrin antibody, an anti-MN antibody, an anti-HER2 antibody, an anti-PSMA antibody, an anti-EGFR antibody, an anti-CD203c antibody, an anti-SLC44A4 antibody, an anti-Nectin-4 antibody, an anti-mesothelin antibody, an anti-CD44 antibody, an anti-CD79 antibody, an anti-FcRL5 antibody, an anti-MUC16 antibody, an anti-NaPi2b antibody, an anti-STEAP-1 antibody, an anti-ETBR antibody, an anti-TF antibody, an anti-MUC1 antibody, anti-HGFR antibody, an anti-CD37 antibody, an anti-FOLR1 antibody, an anti-CEACAM antibody, an anti-TROP2 antibody, an anti-GCC antibody, an anti-Lewis Y antibody, an anti-LIV1 antibody, an anti-DLL3 antibody, and an anti-EPCAM antibody.

12. The method according to any one of claims 1 to 11, wherein the mixture of cysteine-linked antibody-drug conjugates is of the formula (II)



(II)

wherein

Ab is trastuzumab and

q ranges from 0 to 8.

13. The method according to claim 12, wherein the purified mixture of said cysteine-linked antibody-drug conjugates of the formula (II) has an average drug-to-antibody ratio (DAR) of from 2.6 to 2.9.

14. The method according to claim 13, wherein the average DAR is 2.80.