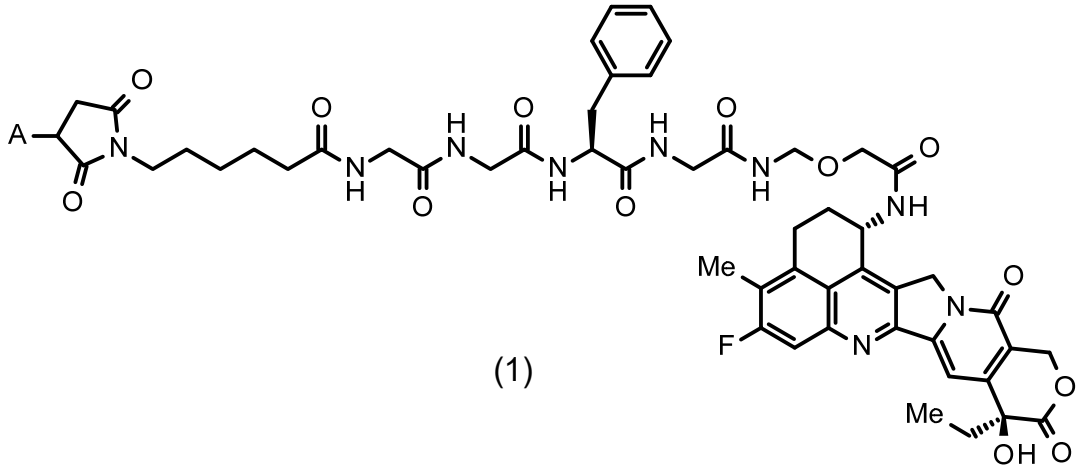
Claims

[Claim 1]

A method for producing an antibody-drug conjugate, in which

a drug-linker represented by formula (1)

[Chem. 1]



(1)

wherein A represents the connecting position to an antibody,

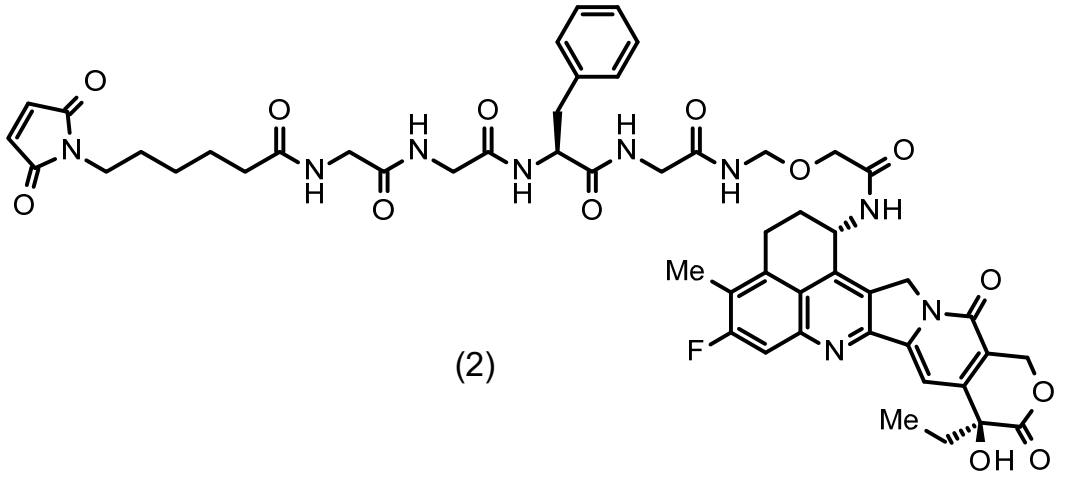
is conjugated to the antibody via a thioether bond,

wherein the method comprises the steps of:

(i) reducing the antibody with a reducing agent;

(ii) reacting a compound represented by formula (2)

[Chem. 2]



(2)

with the antibody reduced in step (i);

(iii) adding a reagent having a thiol group to react with the residual compound represented by formula (2) in step (ii); and then

(iv) removing a compound in which the reducing agent used in step (i) is added to the maleimidyl group of the compound represented by formula (2), and a compound in which the reagent having a thiol group used in step (iii) is added to the maleimidyl group of the compound represented by formula (2), through ultrafiltration using a buffer solution containing a salt of a strong acid and a strong base,

wherein the buffer solution used in step (iv) is a buffer solution selected from the group consisting of a histidine buffer solution, an acetate buffer solution and a phosphate buffer solution,

the salt of a strong acid and strong base in step (iv) is at least one salt selected from the group consisting of sodium chloride, potassium chloride, sodium sulfate, and

potassium sulfate, or a salt comprising a combination of two or more of them,
the pH of the buffer solution used in step (iv) is 4.7 to 5.3, and
the concentration of the salt of a strong acid and a strong base used in step (iv) is 0.2 to 1 wt% with respect to the buffer solution used in step (iv).

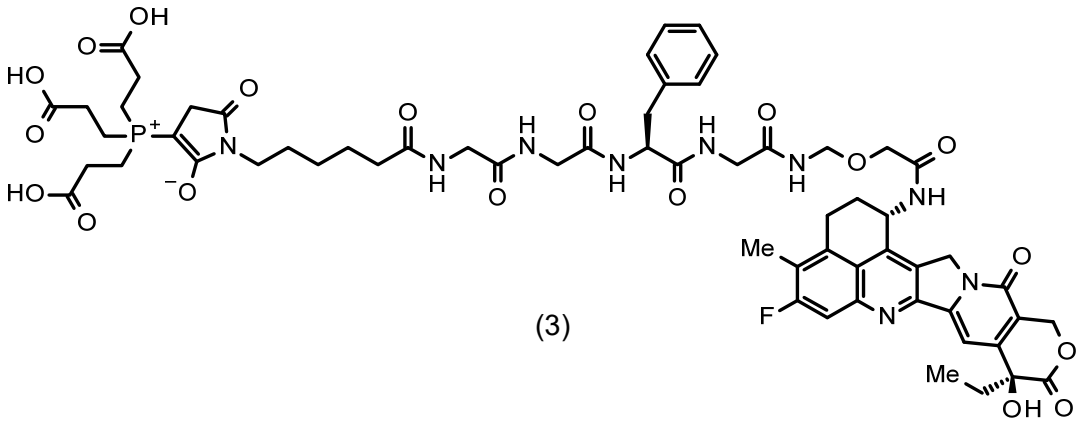
[Claim 2]

The production method according to claim 1, wherein the reducing agent used in step (i) is tris(2-carboxyethyl)phosphine or a salt thereof.

[Claim 3]

The production method according to claim 1 or 2, wherein the reducing agent used in step (i) is tris(2-carboxyethyl)phosphine hydrochloride, and
the compound in which the reducing agent used in step (i) is added to the maleimidyl group of the compound represented by formula (2) is a compound represented by formula (3)

[Chem. 3]



(3)

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[Claim 4]

The production method according to any one of claims 1 to 3, wherein step (i) is performed in a buffer solution.

[Claim 5]

The production method according to claim 4, wherein the pH of the buffer solution is adjusted to 6 to 8 by using an aqueous solution of disodium hydrogen phosphate.

[Claim 6]

The production method according to claim 4 or 5, wherein the buffer solution is an acetate buffer solution.

[Claim 7]

The production method according to any one of claims 1 to 6, wherein step (i) is performed in the presence of a chelating agent.

[Claim 8]

The production method according to claim 7, wherein the chelating agent is ethylenediaminetetraacetic acid.

[Claim 9]

The production method according to any one of claims 4 to 8, wherein the buffer solution used in step (i) contains a surfactant.

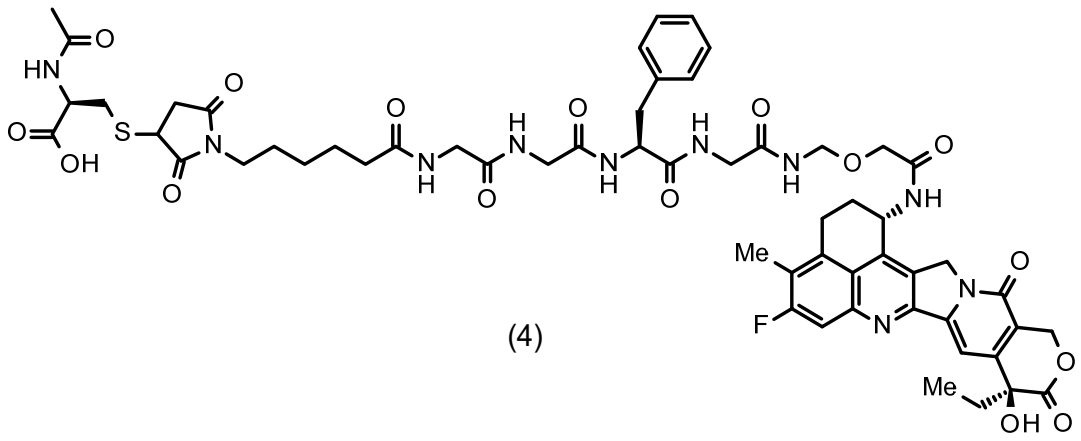
[Claim 10]

The production method according to claim 9, wherein the surfactant is polysorbate 20 or polysorbate 80.

[Claim 11]

The production method according to any one of claims 1 to 10, wherein the reagent having a thiol group used in step (iii) is N-acetylcysteine, and
the compound in which the reagent having a thiol group used in step (iii) is added to the maleimidyl group of the compound represented by formula (2) is a compound represented by formula (4)

[Chem. 4]



(4)

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[Claim 12]

The production method according to any one of claims 1 to 11, wherein the buffer solution used in step (iv) is a histidine buffer solution.

[Claim 13]

The production method according to any one of claims 1 to 12, wherein the concentration of the salt of a strong acid and a strong base used in step (iv) is about 0.5 wt% with respect to the buffer solution used in step (iv).

[Claim 14]

The production method according to any one of claims 1 to 13, wherein the salt of a strong acid and a strong base used in step (iv) is sodium chloride.

[Claim 15]

The production method according to any one of claims 1 to 14, comprising a step subsequent to step (iv) of

(v) removing the salt of a strong acid and a strong base through ultrafiltration using a buffer solution.

[Claim 16]

The production method according to claim 15, wherein the pH of the buffer solution used in step (v) is in the range of from 4 to 6.

[Claim 17]

The production method according to claim 15, wherein the pH of the buffer solution used in step (v) is about 5.

[Claim 18]

The production method according to any one of claims 15 to 17, wherein the buffer solution used in step (v) is a histidine buffer solution.

[Claim 19]

The production method according to any one of claims 1 to 18, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[Claim 20]

The production method according to any one of claims 1 to 18, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[Claim 21]

The production method according to any one of claims 1 to 20, wherein the antibody is an anti-HER2 antibody, an anti-HER3 antibody, an anti-GPR20 antibody or an anti-CDH6 antibody.

[Claim 22]

The production method according to claim 21, wherein the antibody is an anti-HER2 antibody, and the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2, or an antibody comprising a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of an amino acid sequence represented by SEQ ID NO: 2.

[Claim 23]

The production method according to claim 21, wherein the antibody is an anti-HER3 antibody, and the anti-HER3 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 3 and a light chain consisting of an amino acid

sequence represented by SEQ ID NO: 4, or a variant of the antibody in which a lysine residue at the carboxyl terminus of the heavy chain is deleted.

[Claim 24]

The production method according to claim 21, wherein the antibody is an anti-GPR20 antibody, and the anti-GPR20 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 20 to 472 of SEQ ID NO: 5 and a light chain consisting of an amino acid sequence consisting of amino acid residues 21 to 234 of SEQ ID NO: 6, or a variant of the antibody in which a lysine residue at the carboxyl terminus of the heavy chain is deleted.

[Claim 25]

The production method according to claim 21, wherein the antibody is an anti-CDH6 antibody, and the anti-CDH6 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 20 to 471 of SEQ ID NO: 7 and a light chain consisting of an amino acid sequence consisting of amino acid residues 21 to 233 of SEQ ID NO: 8, or a variant of the antibody in which a lysine residue at the carboxyl terminus of the heavy chain is deleted.

[Claim 26]

The production method according to any one of claims 1 to 25, comprising no purification step involving chromatography.

[Claim 27]

The production method according to claim 26, wherein the chromatography is at least one selected from the group consisting of gel filtration chromatography, ion exchange chromatography, hydrophobic chromatography, and affinity chromatography.

[Claim 28]

A method for producing a pharmaceutical composition containing an antibody-drug conjugate, a buffer solution, and an excipient, by producing the antibody-drug conjugate by the production method according to any one of claims 1 to 27, and then

performing the steps of at least one selected from the group consisting of:

(vi) adding a buffer solution to a solution containing the antibody-drug conjugate;

(vii) concentrating the solution containing the antibody-drug conjugate; and

(viii) adjusting the pH of the solution containing the antibody-drug conjugate to a predetermined pH; and also

performing the step of

(ix) adding the excipient to the solution containing the antibody-drug conjugate.

[Claim 29]

The production method according to claim 28, wherein the buffer solution is a histidine buffer solution.

[Claim 30]

The production method according to claim 28 or 29, wherein the excipient is sucrose or trehalose.

[Claim 31]

A method for producing a pharmaceutical composition containing an antibody-drug conjugate, a buffer solution, an excipient, and a surfactant, by producing the pharmaceutical composition containing an antibody-drug conjugate, a buffer solution, and an excipient by the production method according to any one of claims 28 to 30, and then

performing the step of

(x) adding the surfactant to the pharmaceutical composition.

[Claim 32]

The production method according to claim 31, wherein the surfactant is polysorbate 80 or polysorbate 20.