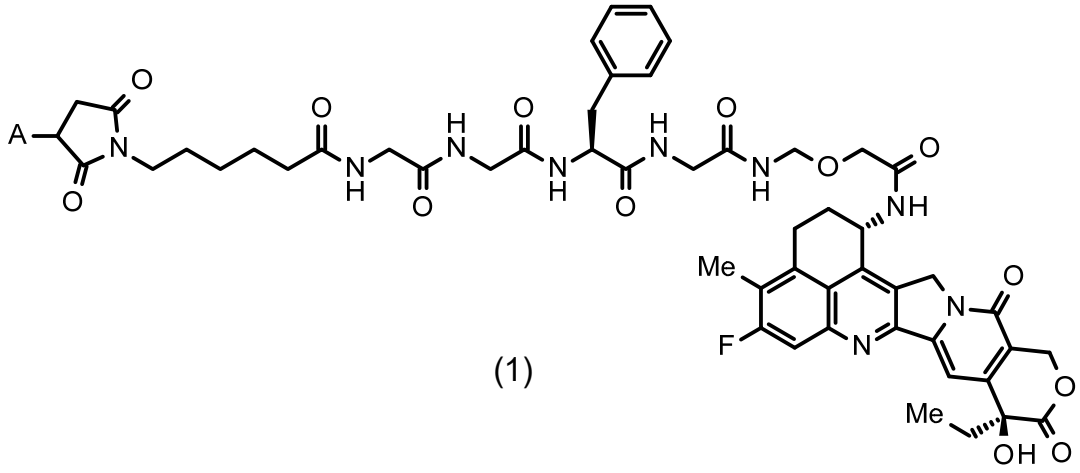
Claims

[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,

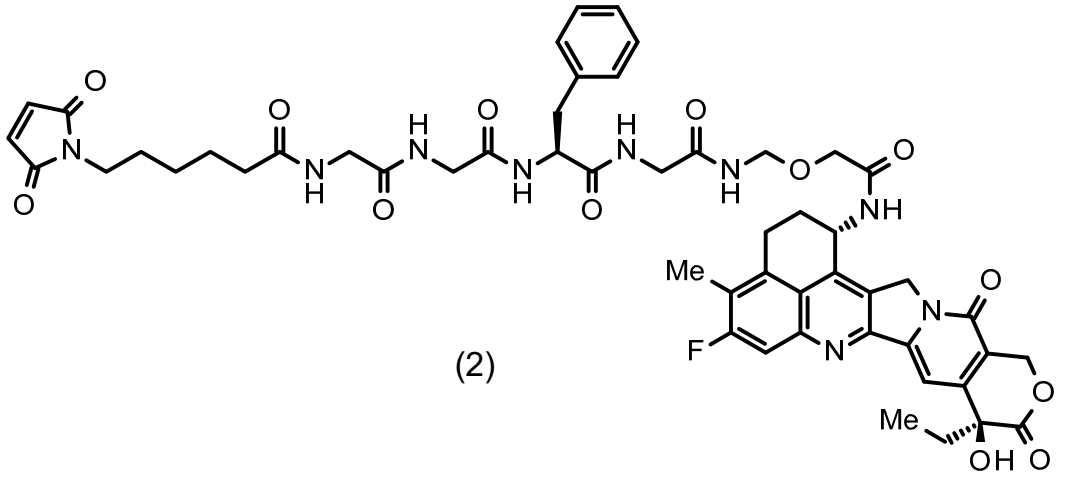
is conjugated to the antibody via a thioether bond,

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 ~~consisting~~ 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).

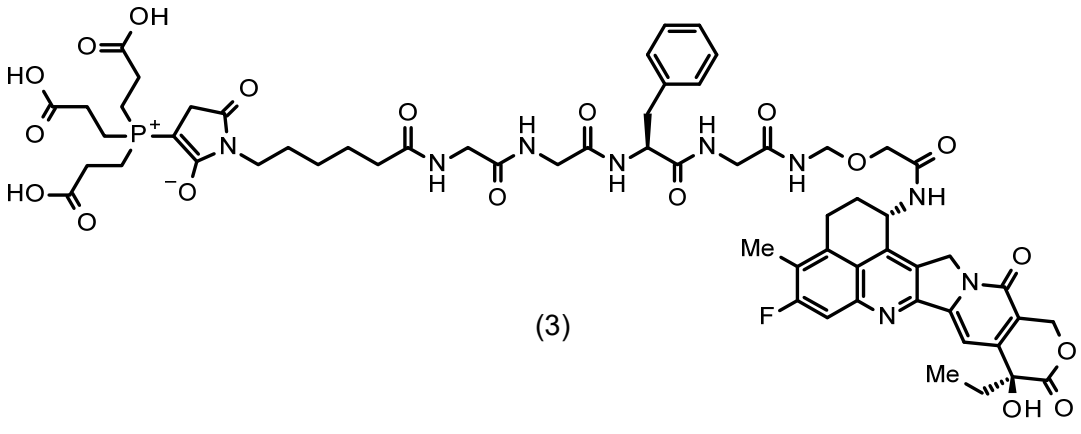
[Claim 2]

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)

.

[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]

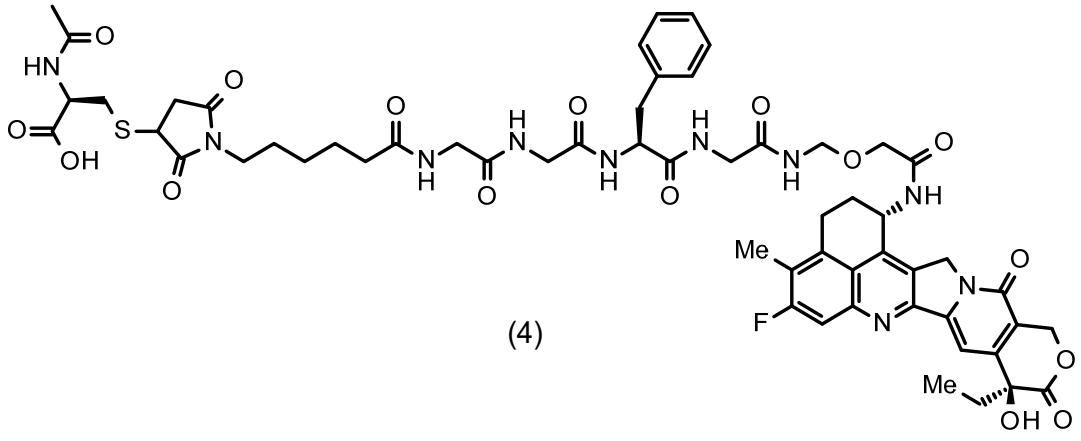
The production method according to any one of claims 4 to 8, wherein the buffer solution used in step (i) contains a surfactant.

[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)

.

~~[Claim 12]~~

~~The production method according to any one of claims 1 to 11, wherein the pH of the buffer solution used in step (iv) is about 5.~~

[Claim ~~13~~12]

The production method according to any one of claims 1 to ~~12~~11, wherein the buffer solution used in step (iv) is a histidine buffer solution.

~~[Claim 14]~~

~~The production method according to any one of claims 1 to 13, wherein the concentration of the salt consisting~~

~~of a strong acid and a strong base used in step (iv) is in the range of from 0.2 wt% to 1 wt% with respect to the buffer solution used in step (iv).~~

[Claim ~~15~~13]

The production method according to any one of claims 1 to ~~13~~12, wherein the concentration of the salt ~~consisting~~ 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 ~~16~~14]

The production method according to any one of claims 1 to ~~15~~13, wherein the salt ~~consisting~~ of a strong acid and a strong base used in step (iv) is sodium chloride.

[Claim ~~17~~15]

The production method according to any one of claims 1 to ~~16~~14, comprising a step subsequent to step (iv) of

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

[Claim ~~18~~16]

The production method according to claim ~~17~~15, wherein the pH of the buffer solution used in step (v) is in the range of from 4 to 6.

[Claim ~~19~~17]

The production method according to claim ~~17~~15, wherein the pH of the buffer solution used in step (v) is about 5.

[Claim ~~20~~18]

The production method according to any one of claims ~~17~~ 15 to ~~19~~17, wherein the buffer solution used in step (v) is a histidine buffer solution.

[Claim ~~21~~19]

The production method according to any one of claims 1 to ~~20~~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 ~~22~~20]

The production method according to any one of claims 1 to ~~20~~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 ~~23~~21]

The production method according to any one of claims 1 to ~~22~~20, wherein the antibody is an anti-HER2 antibody, an anti-HER3 antibody, an anti-GPR20 antibody or an anti-CDH6 antibody.

[Claim ~~24~~22]

The production method according to claim ~~23~~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 ~~25~~23]

The production method according to claim ~~23~~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 ~~26~~24]

The production method according to claim ~~23~~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 ~~27~~25]

The production method according to claim ~~23~~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 ~~28~~26]

The production method according to any one of claims 1 to ~~27~~25, comprising no purification step involving chromatography.

[Claim ~~29~~27]

The production method according to claim ~~28~~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 ~~30~~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 ~~29~~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 ~~31~~29]

The production method according to claim ~~30~~28, wherein the buffer solution is a histidine buffer solution.

[Claim ~~32~~30]

The production method according to claim ~~30 —~~28 or ~~31~~29, wherein the excipient is sucrose or trehalose.

[Claim ~~33~~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 ~~30 —~~28 to ~~32~~30, and then

performing the step of

(x) adding the surfactant to the pharmaceutical composition.

[Claim ~~34~~32]

The production method according to claim ~~33~~31, wherein the surfactant is polysorbate 80 or polysorbate 20.