

Description

Title of invention: IMPROVED METHOD FOR PRODUCING
ANTIBODY-DRUG CONJUGATE

Technical Field

[0001]

The present invention relates to an improved method for producing a drug-linker intermediate for an antibody-drug conjugate, and an improved method for producing an antibody-drug conjugate wherein the aforementioned method is used.

Background Art

[0002]

An antibody-drug conjugate (ADC) having a drug with cytotoxicity conjugated to an antibody, whose antigen is expressed on the surface of cancer cells and which also binds to an antigen capable of cellular internalization, and therefore can deliver the drug selectively to cancer cells, is thus expected to cause accumulation of the drug within cancer cells and to kill the cancer cells (Non-Patent Literatures 1 to 5).

[0003]

As one such antibody-drug conjugate, an antibody-drug conjugate comprising an antibody and exatecan, which is a topoisomerase I inhibitor, as its components is

known (Patent Literatures 1 to 5 and Non-Patent Literatures 6, 7). Since these antibody-drug conjugates exert a superior antitumor effect and safety, they are currently under clinical studies.

[0004]

As methods for producing drug-linker intermediates for producing the above-described antibody-drug conjugates, methods described in Patent Literatures 1 to 4 are known.

Citation List

Patent Literatures

[0005]

Patent Literature 1: International Publication No. WO 2014/057687

Patent Literature 2: International Publication No. WO 2015/098099

Patent Literature 3: International Publication No. WO 2015/115091

Patent Literature 4: International Publication No. WO 2015/155998

Patent Literature 5: International Publication No. WO 2018/135501

Non-Patent Literatures

[0006]

Non-Patent Literature 1: Ducry, L., et al., Bioconjugate Chem. (2010) 21, 5-13.

Non-Patent Literature 2: Alley, S. C., et al., Current Opinion in Chemical Biology (2010) 14, 529-537.

Non-Patent Literature 3: Damle N. K. Expert Opin. Biol. Ther. (2004) 4, 1445-1452.

Non-Patent Literature 4: Senter P. D., et al., Nature Biotechnology (2012) 30, 631-637.

Non-Patent Literature 5: Howard A. et al., J Clin Oncol 29: 398-405.

Non-Patent Literature 6: Ogitan Y. et al., Clinical Cancer Research (2016) 22 (20), 5097-5108.

Non-Patent Literature 7: Ogitan Y. et al., Cancer Science (2016) 107, 1039-1046.

Summary of Invention

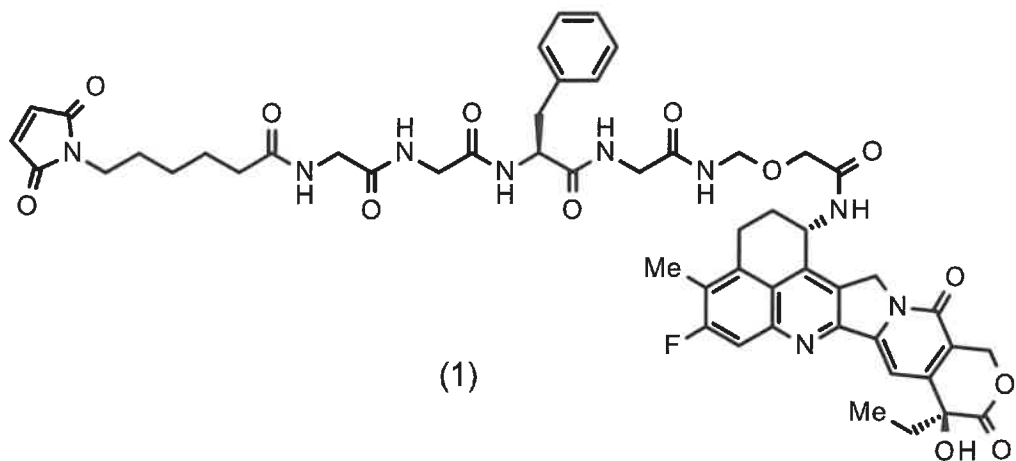
Technical Problem

[0007]

A drug-linker intermediate for producing an antibody-drug conjugate of the present invention is the compound represented by formula (1):

[0008]

[Chem. 1]



[0009]

[0010]

As a method for producing the compound represented by the formula (1), methods described in Patent Literatures 1 to 4 are known. However, it has not been known that the compound represented by the formula (1) can be obtained as crystals, and it is necessary to perform complicated operations such as necessary purification by chromatography. There is therefore a demand for development of an industrially better production method.

[0011]

One object of the present invention is to find an improved industrially excellent method for producing a drug-linker intermediate without the need for purification by chromatography. Another object of the present invention is to construct an improved method for producing an antibody-drug conjugate wherein the

aforementioned improved method for producing a drug-linker intermediate is used.

Solution to Problem

[0012]

The present inventors conducted diligent studies on a method for producing a drug-linker intermediate, and consequently found that, surprisingly, the compound represented by formula (1) can be obtained as crystals. The present inventors also improved a method for producing the compound represented by the formula (1), and consequently found an industrially excellent production method without the need for purification by chromatography. The present inventors further constructed an improved method for producing an antibody-drug conjugate by using the crystals of the compound represented by the formula (1), and completed the present invention.

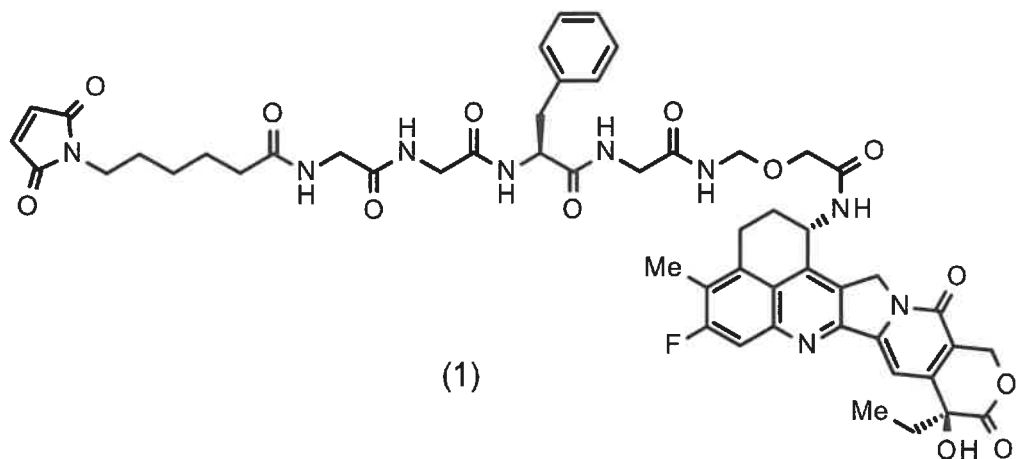
[0013]

Specifically, the present invention relates to the following.

[1] Crystals of the compound represented by formula (1):

[0014]

[Chem. 2]



(1)

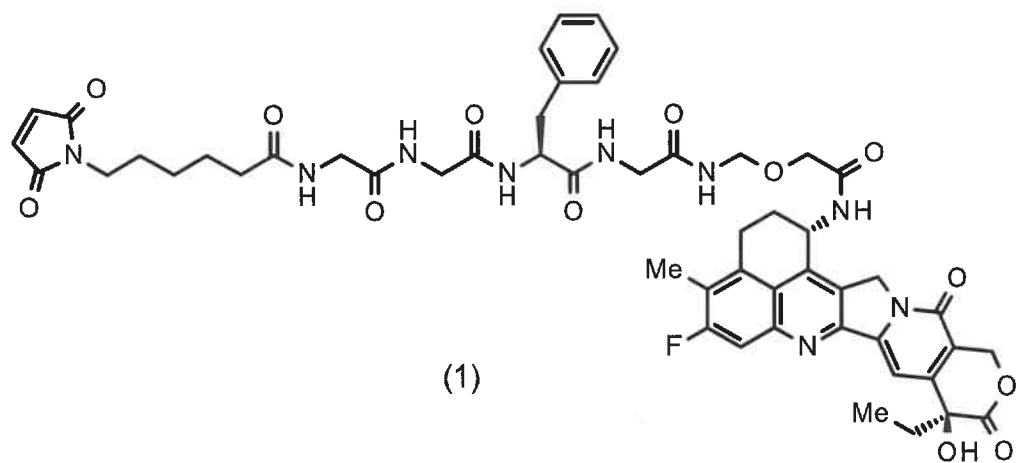
[0015]

[2] The crystals according to [1], wherein the crystals show main peaks at diffraction angles (2θ) of $5.6 \pm 0.2^\circ$, $15.5 \pm 0.2^\circ$ and $22.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper $K\alpha$ radiation.

[3] A method for producing crystals of the compound represented by formula (1):

[0016]

[Chem. 3]



(1)

[0017]

comprising the steps of: preparing a solution in which the compound represented by the formula (1) is dissolved; and then precipitating crystals of the compound represented by the formula (1) from the solution.

[4] The production method according to [3], wherein the crystals of the compound represented by the formula (1) show main peaks at diffraction angles (2θ) of $5.6 \pm 0.2^\circ$, $15.5 \pm 0.2^\circ$ and $22.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper K α radiation.

[5] The production method according to [3] or [4], wherein the solution in which the compound represented by the formula (1) is dissolved comprises a lower ketone and a lower alcohol as solvents.

[6] The production method according to [5], wherein the lower ketone is acetone.

[7] The production method according to [5], wherein the lower ketone is methyl ethyl ketone.

[8] The production method according to any one of [5] to [7], wherein the lower alcohol is 1-propanol.

[9] The production method according to any one of [5] to [7], wherein the lower alcohol is 2-butanol.

[10] The production method according to any one of [3] to [9], comprising a step of adding a seed crystal of the crystals of the compound represented by the formula (1).

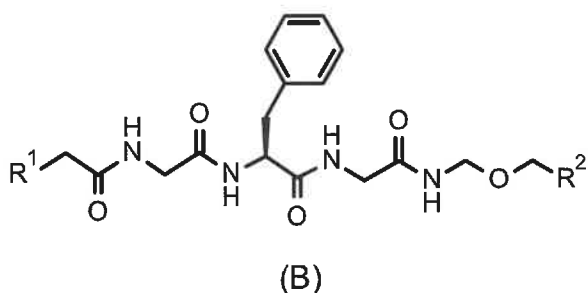
[11] The production method according to any one of [3] to [10], wherein the compound represented by the formula (1) is produced by a production method (I),

wherein the production method (I) is a production method comprising the steps of:

deprotecting protecting groups for an amino group and a carboxy group of a compound represented by formula (B):

[0018]

[Chem. 4]

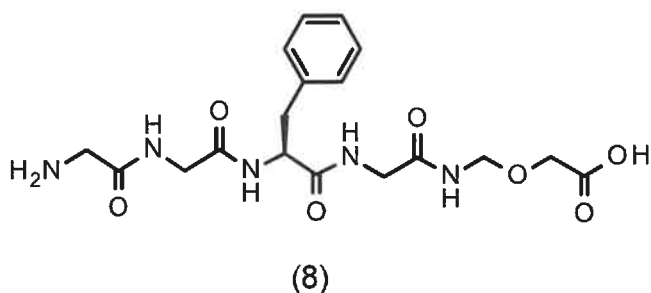


[0019]

wherein R¹ represents an amino group protected with a protecting group, and R² represents a carboxy group protected with a protecting group, to convert it into the compound represented by formula (8):

[0020]

[Chem. 5]



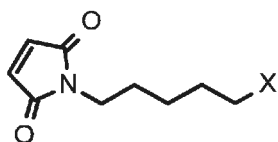
[0021]

; then

condensing the compound represented by the formula (8) with a compound represented by formula (C):

[0022]

[Chem. 6]



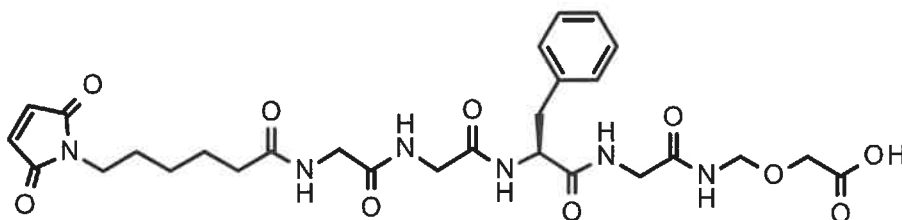
(C)

[0023]

wherein X represents an active ester group or a carboxy group, to convert it into the compound represented by formula (10):

[0024]

[Chem. 7]



(10)

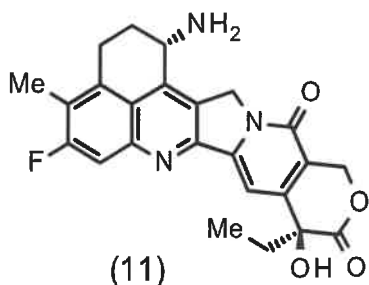
[0025]

; and then

condensing the compound represented by the formula (10) with the compound represented by formula (11):

[0026]

[Chem. 8]

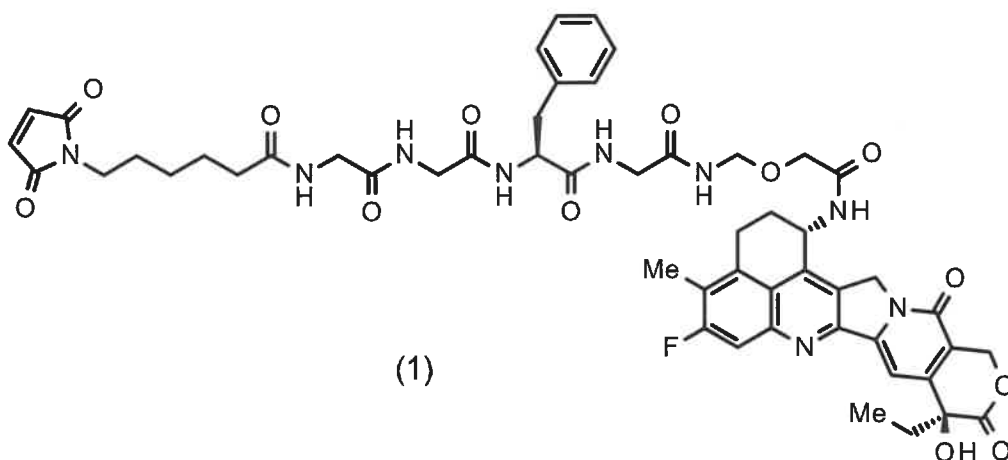


[0027]

to convert it into the compound represented by the formula (1):

[0028]

[Chem. 9]



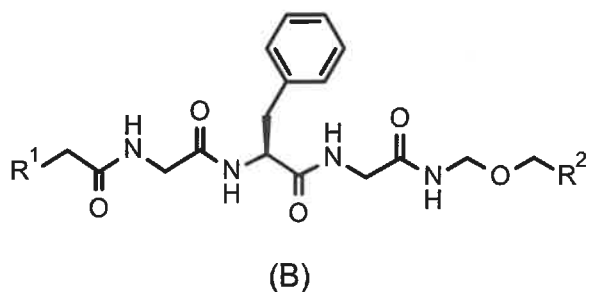
[0029]

[12] The production method according to any one of [3] to [10], wherein the compound represented by the formula (1) is produced by a production method (II), wherein the production method (II) is a production method comprising the steps of:

deprotecting a protecting group for an amino group of a compound represented by formula (B):

[0030]

[Chem. 10]

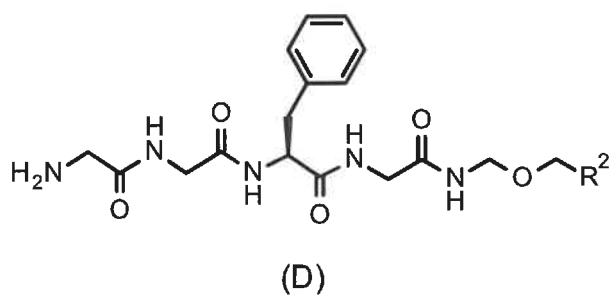


[0031]

wherein R¹ represents an amino group protected with a protecting group, and R² represents a carboxy group protected with a protecting group, to convert it into a compound represented by formula (D):

[0032]

[Chem. 11]

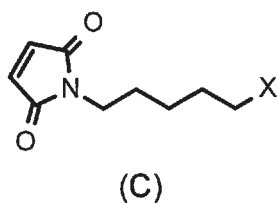


[0033]

wherein R² represents the same meaning as above; then condensing the compound represented by the formula (D) with a compound represented by formula (C):

[0034]

[Chem. 12]

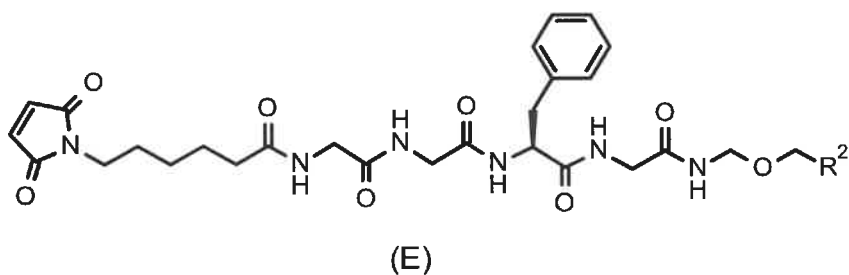


[0035]

wherein X represents an active ester group or a carboxy group, to convert it into a compound represented by formula (E):

[0036]

[Chem. 13]

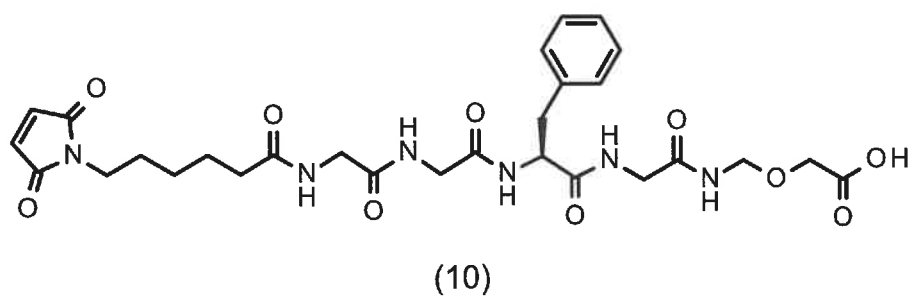


[0037]

wherein R² represents the same meaning as above; then deprotecting the protecting group for the carboxy group of the compound represented by the formula (E) to convert it into the compound represented by formula (10):

[0038]

[Chem. 14]



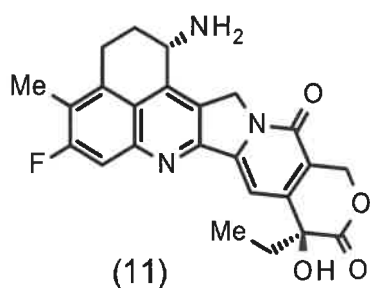
[0039]

; and then

condensing the compound represented by the formula (10) with the compound represented by formula (11):

[0040]

[Chem. 15]

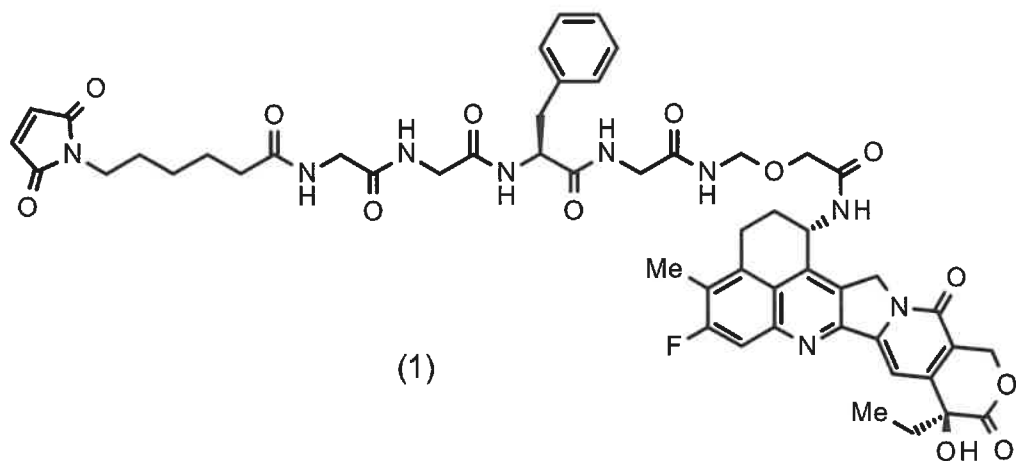


[0041]

to convert it into the compound represented by the formula (1):

[0042]

[Chem. 16]



[0043]

[13] The production method according to [11] or [12], comprising the steps of: dissolving the compound represented by the formula (10) in a solvent containing 1,2-dimethoxyethane; and then precipitating crystals of a 1,2-dimethoxyethane adduct of the compound represented by the formula (10).

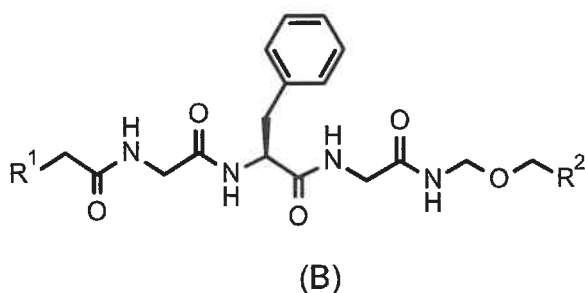
[14] The production method according to [13], wherein the crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper $K\alpha$ radiation.

[15] The production method according to any one of [11] to [14], wherein the step of condensing the compound represented by the formula (10) and the compound represented by the formula (11) to convert it into the compound represented by the formula (1) is performed in a two-phase system of an aqueous sodium sulfate solution and tetrahydrofuran.

[16] The production method according to any one of [3] to [10], wherein the compound represented by the formula (1) is produced by a production method (III), wherein the production method (III) is a production method comprising the steps of:
deprotecting a protecting group for a carboxy group of a compound represented by formula (B):

[0044]

[Chem. 17]

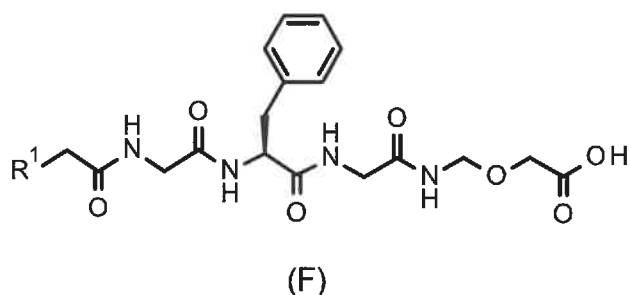


[0045]

wherein R¹ represents an amino group protected with a protecting group, and R² represents a carboxy group protected with a protecting group, to convert it into a compound represented by formula (F):

[0046]

[Chem. 18]

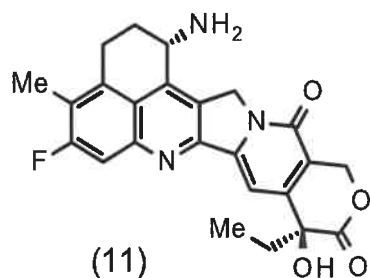


[0047]

wherein R¹ represents the same meaning as above; then condensing the compound represented by the formula (F) with the compound represented by formula (11):

[0048]

[Chem. 19]



[0049]

to convert it into a compound represented by formula (G):

[0050]

[Chem. 20]



[0051]

wherein R¹ represents the same meaning as above; then deprotecting the protecting group for the amino group of the compound represented by the formula (G) to convert it into the compound represented by formula (16):

[0052]

[Chem. 21]



[0053]

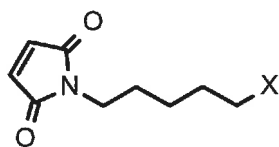
; and then

condensing the compound represented by the formula (16)

with a compound represented by formula (C):

[0054]

[Chem. 22]



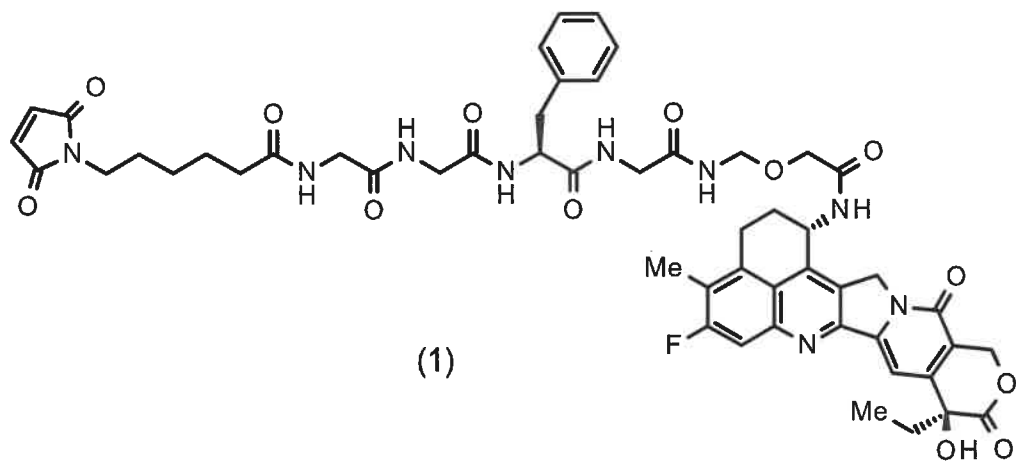
(C)

[0055]

wherein X represents an active ester group or a carboxy group, to convert it into the compound represented by the formula (1):

[0056]

[Chem. 23]



(1)

[0057]

[17] The production method according to any one of [11] to [16], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt.

[18] The production method according to any one of [11] to [16], wherein the compound represented by the formula

(11) is in the form of a methanesulfonic acid salt m-hydrate, wherein m is in the range of 0 to 3.

[19] The production method according to any one of [11] to [16], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt anhydride.

[20] The production method according to any one of [11] to [16], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt monohydrate.

[21] The production method according to any one of [11] to [16], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt dihydrate.

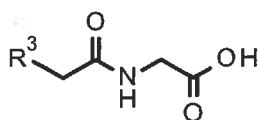
[22] The production method according to any one of [11] to [16], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt trihydrate.

[23] The production method according to any one of [11] to [22], wherein the compound represented by the formula (B) is produced by a production method (IV), wherein the production method (IV) is a production method comprising the steps of:

reacting a compound represented by formula (H):

[0058]

[Chem. 24]



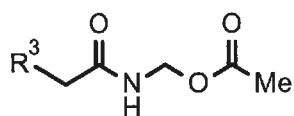
(H)

[0059]

wherein R³ represents an amino group protected with a protecting group, with lead tetraacetate to convert it into a compound represented by formula (J):

[0060]

[Chem. 25]



(J)

[0061]

wherein R³ represents the same meaning as above; then reacting the compound represented by the formula (J) with a compound represented by formula (K):

[0062]

[Chem. 26]



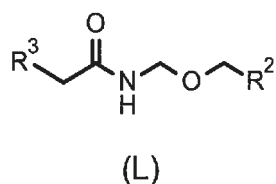
(K)

[0063]

wherein R² represents the same meaning as the R² according to any one of claims 11 to 22, in the presence of an acid or a base to convert it into a compound represented by formula (L):

[0064]

[Chem. 27]



[0065]

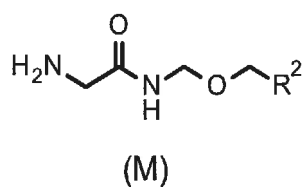
wherein R^2 and R^3 represent the same meanings as above;

then

deprotecting the protecting group for the amino group of the compound represented by the formula (L) to convert it into a compound represented by formula (M):

[0066]

[Chem. 28]

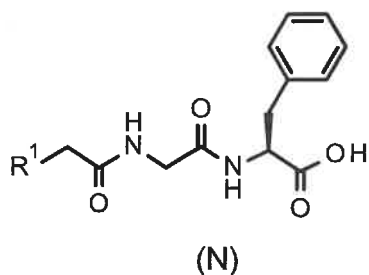


[0067]

wherein R^2 represents the same meaning as above; and then condensing the compound represented by the formula (M) with a compound represented by formula (N):

[0068]

[Chem. 29]

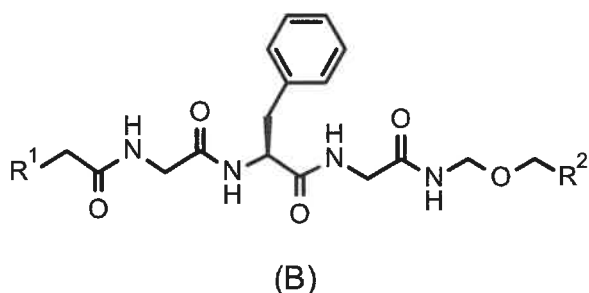


[0069]

wherein R¹ represents the same meaning as the R¹ according to any one of [11] to [22], to convert it into the compound represented by the formula (B):

[0070]

[Chem. 30]



[0071]

wherein R¹ and R² represent the same meanings as above.

[24] The production method according to [23], wherein the step of reacting the compound represented by the formula (H) with lead tetraacetate to convert it into the compound represented by the formula (J) is performed in the presence of acetic acid.

[25] The production method according to [23] or [24], wherein the step of reacting the compound represented by the formula (J) with the compound represented by the formula (K) to convert it into the compound represented

by the formula (L) is performed in the presence of an aqueous sodium hydroxide solution.

[26] The production method according to [23] or [24], wherein the step of reacting the compound represented by the formula (J) with the compound represented by the formula (K) to convert it into the compound represented by the formula (L) is performed in the presence of tris(pentafluorophenyl)borane.

[27] The production method according to any one of [23] to [26], comprising a step of adding an acid to precipitate a salt of the compound represented by the formula (M) and the acid after the step of deprotecting the protecting group for the amino group of the compound represented by the formula (L) to convert it into the compound represented by the formula (M).

[28] The production method according to [27], wherein the acid is 1-hydroxybenzotriazole.

[29] The production method according to any one of [11] to [28], wherein R¹ is an amino group protected with a benzyloxycarbonyl group.

[30] The production method according to any one of [11] to [28], wherein R¹ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[31] The production method according to any one of [11] to [30], wherein R² is a carboxy group protected with a benzyl group.

[32] The production method according to any one of [23] to [31], wherein R³ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

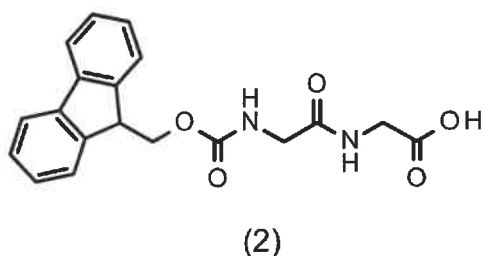
[33] The production method according to any one of [11] to [32], wherein X is a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

[34] The production method according to any one of [3] to [10], wherein the compound represented by the formula (1) is produced by a production method (V), wherein the production method (V) is a production method comprising the steps of:

reacting the compound represented by formula (2):

[0072]

[Chem. 31]

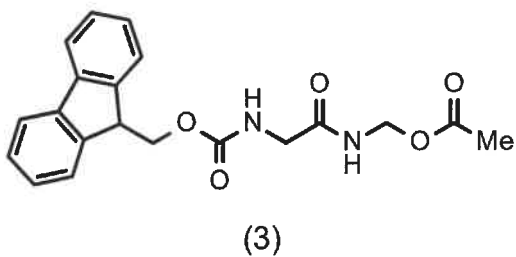


[0073]

with lead tetraacetate to convert it into the compound represented by formula (3):

[0074]

[Chem. 32]



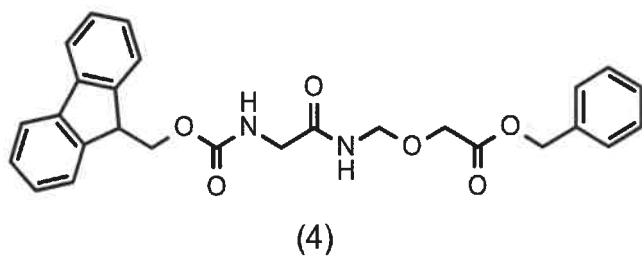
[0075]

; then

reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4):

[0076]

[Chem. 33]



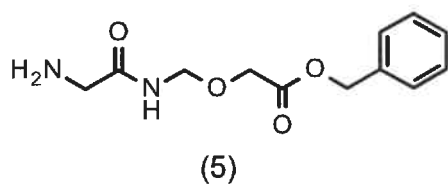
[0077]

; then

deprotecting a protecting group for an amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5):

[0078]

[Chem. 34]



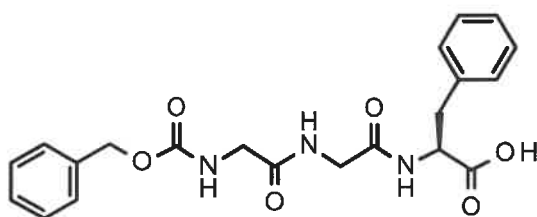
[0079]

; then

condensing the compound represented by the formula (5)
with the compound represented by formula (6):

[0080]

[Chem. 35]



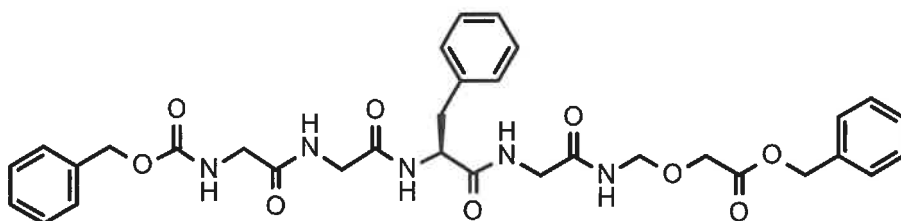
(6)

[0081]

to convert it into the compound represented by formula
(7):

[0082]

[Chem. 36]



(7)

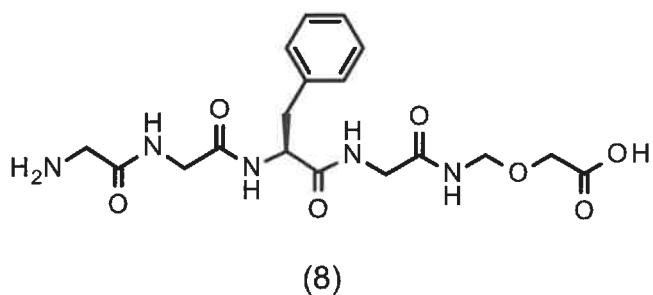
[0083]

; then

deprotecting protecting groups for an amino group and a
carboxy group of the compound represented by the formula
(7) to convert it into the compound represented by
formula (8):

[0084]

[Chem. 37]



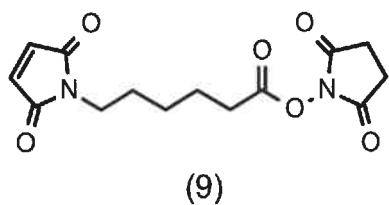
[0085]

; then

condensing the compound represented by the formula (8)
with the compound represented by formula (9):

[0086]

[Chem. 38]

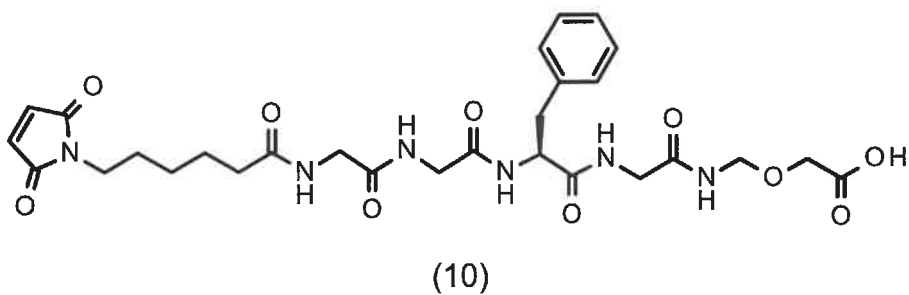


[0087]

to convert it into the compound represented by formula
(10):

[0088]

[Chem. 39]



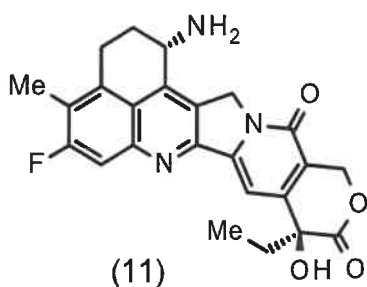
[0089]

; and then

condensing the compound represented by the formula (10)
with the compound represented by formula (11):

[0090]

[Chem. 40]

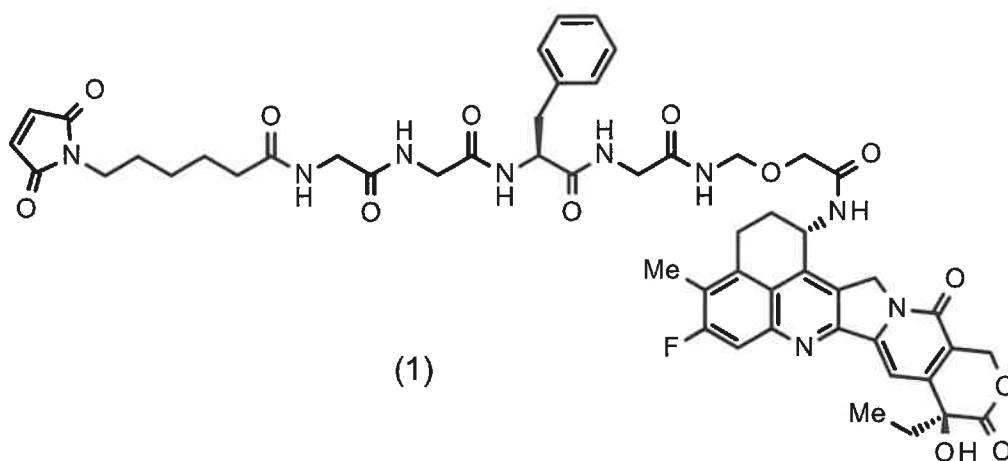


[0091]

to convert it into the compound represented by the
formula (1):

[0092]

[Chem. 41]



[0093]

[35] The production method according to [34], comprising
the steps of: dissolving the compound represented by the

formula (10) in a solvent containing 1,2-dimethoxyethane; and then precipitating crystals of a 1,2-dimethoxyethane adduct of the compound represented by the formula (10).

[36] The production method according to [35], wherein the crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper K α radiation.

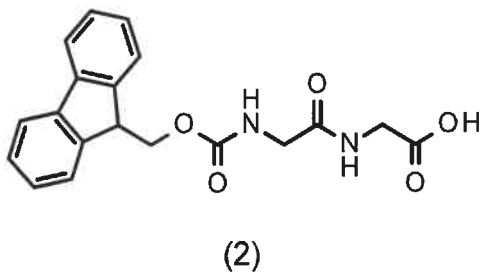
[37] The production method according to any one of [34] to [36], wherein the step of condensing the compound represented by the formula (10) with the compound represented by the formula (11) to convert it into the compound represented by the formula (1) is performed in a two-phase system of an aqueous sodium sulfate solution and tetrahydrofuran.

[38] The production method according to any one of [3] to [10], wherein the compound represented by the formula (1) is produced by a production method (VI), wherein the production method (VI) is a production method comprising the steps of:

reacting the compound represented by formula (2):

[0094]

[Chem. 42]

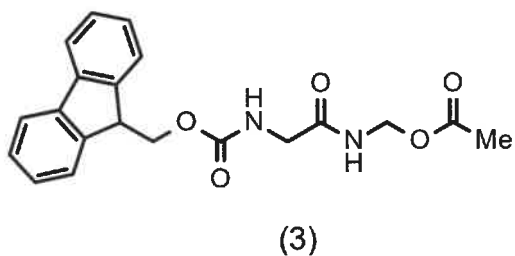


[0095]

with lead tetraacetate to convert it into the compound represented by formula (3):

[0096]

[Chem. 43]



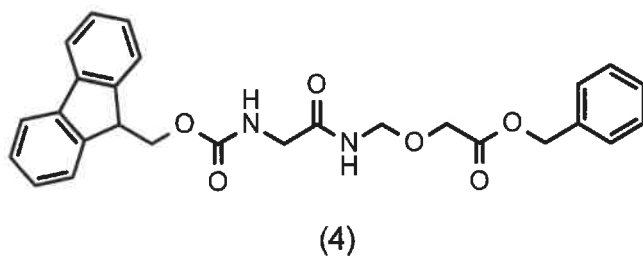
[0097]

; then

reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4):

[0098]

[Chem. 44]



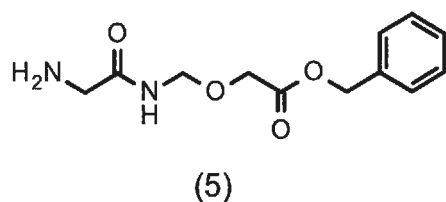
[0099]

; then

deprotecting a protecting group for an amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5):

[0100]

[Chem. 45]



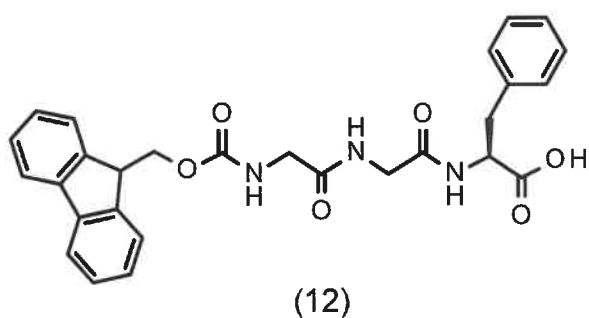
[0101]

; then

condensing the compound represented by the formula (5) with the compound represented by formula (12):

[0102]

[Chem. 46]

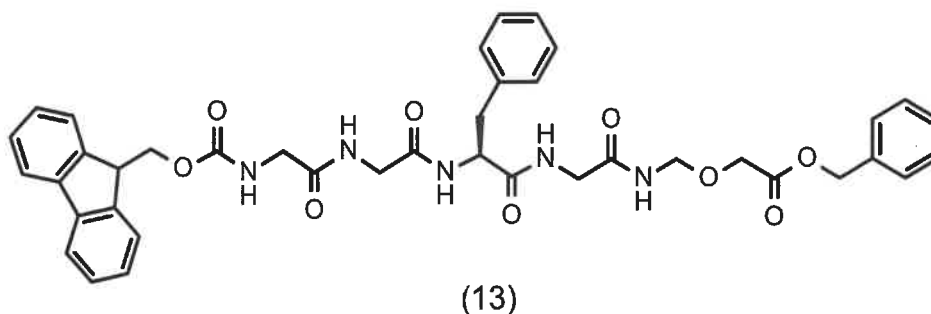


[0103]

to convert it into the compound represented by formula (13):

[0104]

[Chem. 47]



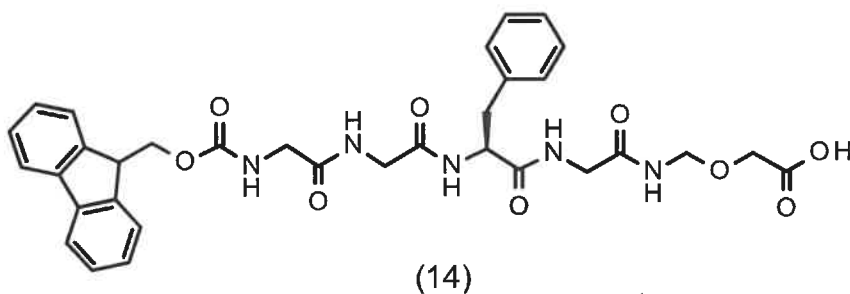
[0105]

; then

deprotecting a protecting group for a carboxy group of the compound represented by the formula (13) to convert it into the compound represented by formula (14):

[0106]

[Chem. 48]



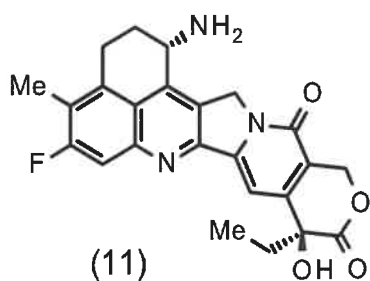
[0107]

; then

condensing the compound represented by the formula (14) with the compound represented by formula (11):

[0108]

[Chem. 49]

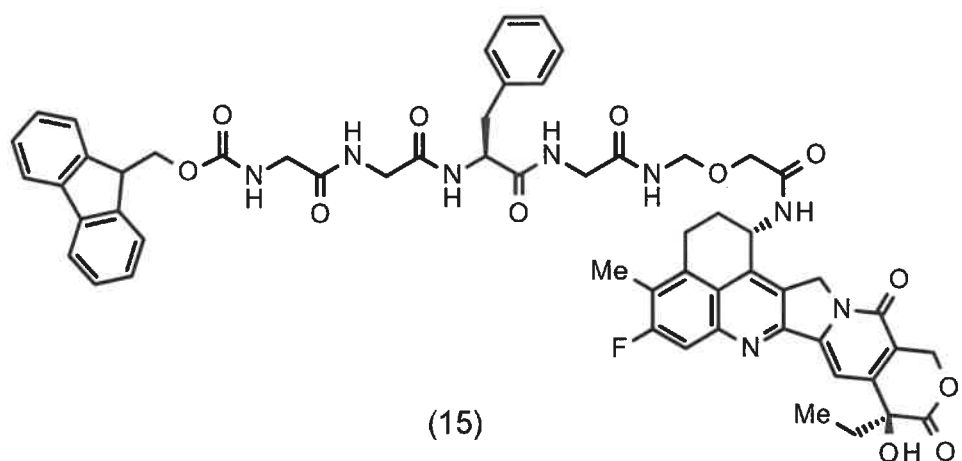


[0109]

to convert it into the compound represented by formula (15):

[0110]

[Chem. 50]



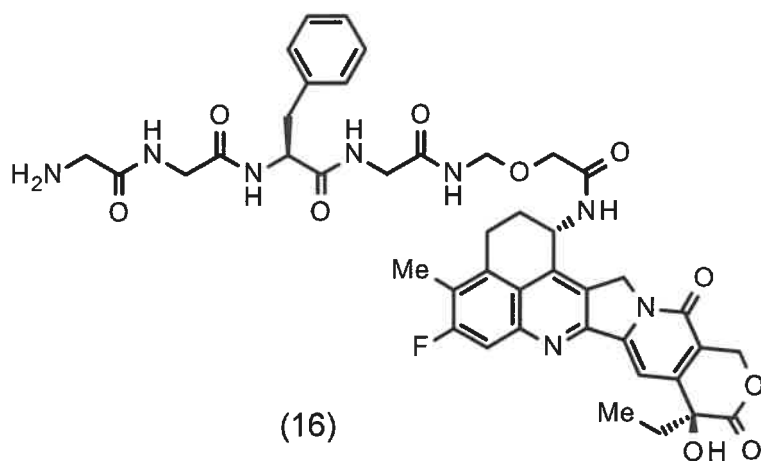
[0111]

; then

deprotecting a protecting group for an amino group of the compound represented by the formula (15) to convert it into the compound represented by formula (16):

[0112]

[Chem. 51]



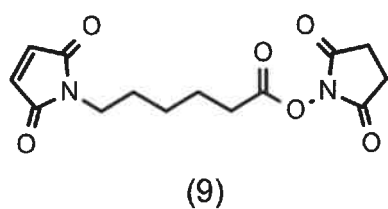
[0113]

; and then

condensing the compound represented by the formula (16)
with the compound represented by formula (9):

[0114]

[Chem. 52]

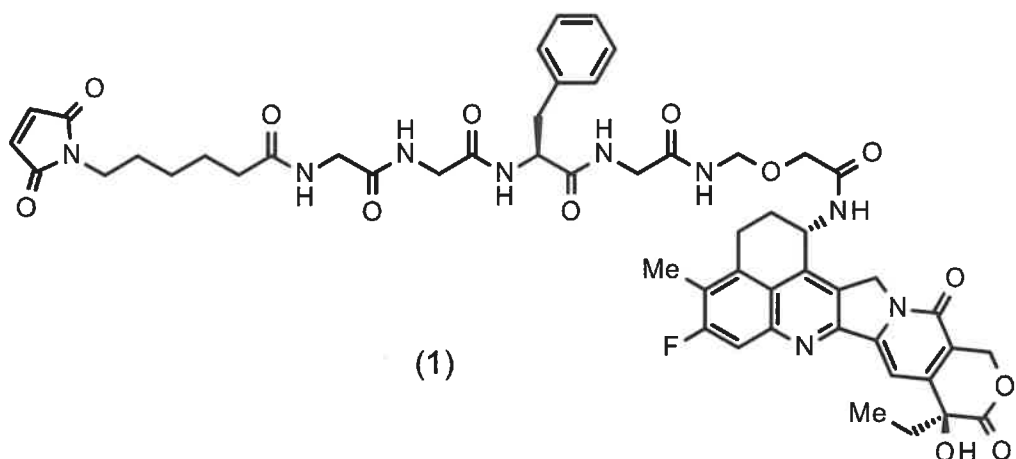


[0115]

to convert it into the compound represented by the
formula (1):

[0116]

[Chem. 53]



[0117]

[39] The production method according to any one of [34] to [38], wherein the step of reacting the compound represented by the formula (2) with lead tetraacetate to convert it into the compound represented by the formula (3) is performed in the presence of acetic acid.

[40] The production method according to any one of [34] to [39], wherein the step of converting the compound represented by the formula (3) into the compound represented by the formula (4) is performed in the presence of an aqueous sodium hydroxide solution.

[41] The production method according to any one of [34] to [39], wherein the step of converting the compound represented by the formula (3) into the compound represented by the formula (4) is performed in the presence of tris(pentafluorophenyl)borane.

[42] The production method according to any one of [34] to [41], comprising a step of adding an acid to precipitate a salt of the compound represented by the

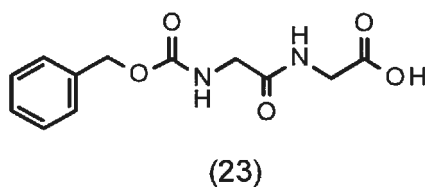
formula (5) and the acid after the step of deprotecting the protecting group for the amino group of the compound represented by the formula (4) to convert it into the compound represented by the formula (5).

[43] The production method according to [42], wherein the acid is 1-hydroxybenzotriazole.

[44] The production method according to any one of [34] to [43], wherein the compound represented by the formula (6) is produced by a method comprising the steps of: condensing the compound represented by formula (23):

[0118]

[Chem. 54]

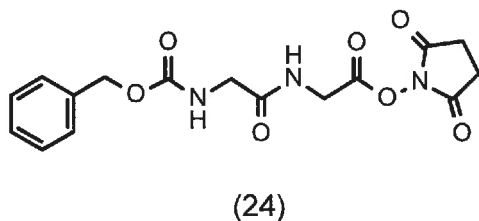


[0119]

with N-hydroxysuccinimide to convert it into the compound represented by formula (24):

[0120]

[Chem. 55]



[0121]

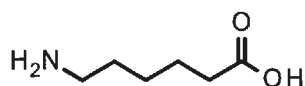
; and then

condensing the compound represented by the formula (24) with L-phenylalanine to convert it into the compound represented by the formula (6).

[45] The production method according to any one of [34] to [44], wherein the compound represented by the formula (9) is produced by a method comprising the steps of: reacting the compound represented by formula (17):

[0122]

[Chem. 56]



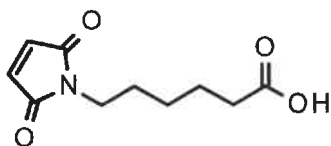
(17)

[0123]

with maleic anhydride to convert it into the compound represented by formula (18):

[0124]

[Chem. 57]



(18)

[0125]

; and then

adding thionyl chloride to the compound represented by the formula (18) and a mixed solution containing N-

hydroxysuccinimide and 2,6-lutidine to convert it into the compound represented by the formula (9).

[46] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt.

[47] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt m-hydrate, wherein m is in the range of 0 to 3.

[48] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt anhydride.

[49] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt monohydrate.

[50] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt dihydrate.

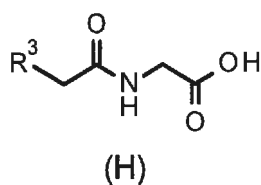
[51] The production method according to any one of [34] to [45], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt trihydrate.

[52] A method for producing a compound represented by formula (J), comprising the step of:

reacting a compound represented by formula (H):

[0126]

[Chem. 58]

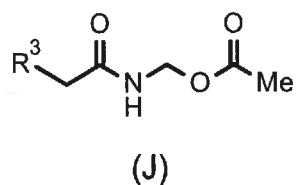


[0127]

wherein R³ represents an amino group protected with a protecting group, with lead tetraacetate in the presence of acetic acid to convert it into the compound represented by the formula (J):

[0128]

[Chem. 59]



[0129]

wherein R³ represents the same meaning as above.

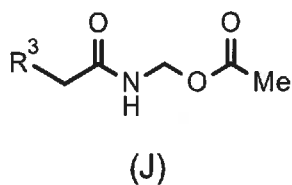
[53] The production method according to [52], wherein R³ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[54] A method for producing a compound represented by formula (L), comprising the step of:

reacting a compound represented by formula (J):

[0130]

[Chem. 60]



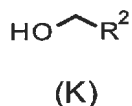
[0131]

wherein R³ represents an amino group protected with a protecting group, with a compound represented by formula

(K):

[0132]

[Chem. 61]



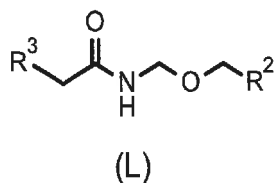
[0133]

wherein R² represents a carboxy group protected with a protecting group, in the presence of an aqueous sodium hydroxide solution or tris(pentafluorophenyl)borane to convert it into the compound represented by the formula

(L):

[0134]

[Chem. 62]



[0135]

wherein R² and R³ represent the same meanings as above.

[55] The production method according to [54], wherein the reaction is performed in the presence of an aqueous sodium hydroxide solution.

[56] The production method according to [54], wherein the reaction is performed in the presence of tris(pentafluorophenyl)borane.

[57] The production method according to any one of [54] to [56], wherein R^2 is a carboxy group protected with a benzyl group.

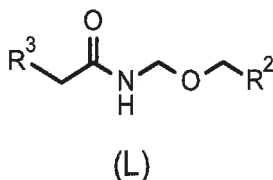
[58] The production method according to any one of [54] to [57], wherein R^3 is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[59] A method for producing a salt of a compound represented by formula (M) and an acid, comprising the steps of:

deprotecting a protecting group for an amino group of a compound represented by formula (L):

[0136]

[Chem. 63]



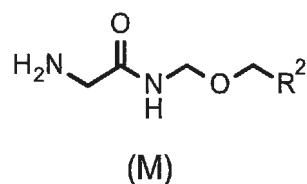
[0137]

wherein R^2 represents a carboxy group protected with a protecting group, and R^3 represents an amino group

protected with a protecting group, to convert it into the compound represented by the formula (M):

[0138]

[Chem. 64]



[0139]

wherein R² represents the same meaning as above; and then adding an acid to precipitate the salt of the compound represented by the formula (M) and the acid.

[60] The production method according to [59], wherein the acid is 1-hydroxybenzotriazole.

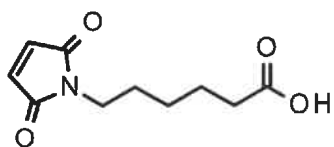
[61] The production method according to [59] or [60], wherein R² is a carboxy group protected with a benzyl group.

[62] The production method according to any one of [59] to [61], wherein R³ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[63] A method for producing the compound represented by formula (9), comprising the step of:
adding thionyl chloride to the compound represented by formula (18):

[0140]

[Chem. 65]



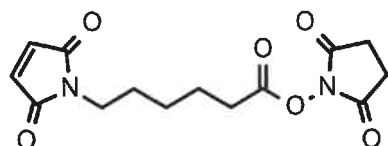
(18)

[0141]

and a mixed solution containing N-hydroxysuccinimide and 2,6-lutidine to convert it into the compound represented by the formula (9):

[0142]

[Chem. 66]



(9)

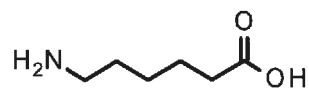
[0143]

[64] The production method according to [63], wherein the compound represented by the formula (18) is produced by a method comprising the step of:

reacting the compound represented by formula (17):

[0144]

[Chem. 67]



(17)

[0145]

with maleic anhydride.

[0146]

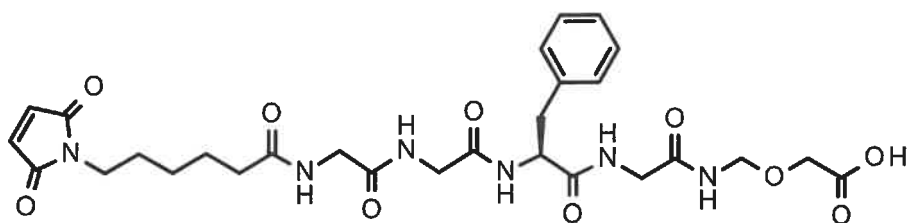
OC(=O)COCCNC(=O)NC(=O)[C@H](Cc1ccccc1)C(=O)NCC(=O)NCC(=O)NCCCCCn2c(=O)c3ccccc3c2=O

(10)

in a solvent containing 1,2-dimethoxyethane; and then precipitating crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10).

[67] A method for producing the compound represented by formula (1), comprising the step of: condensing the compound represented by formula (10):

[Chem. 69]



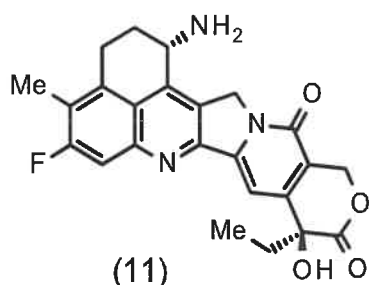
(10)

[0149]

and the compound represented by formula (11):

[0150]

[Chem. 70]



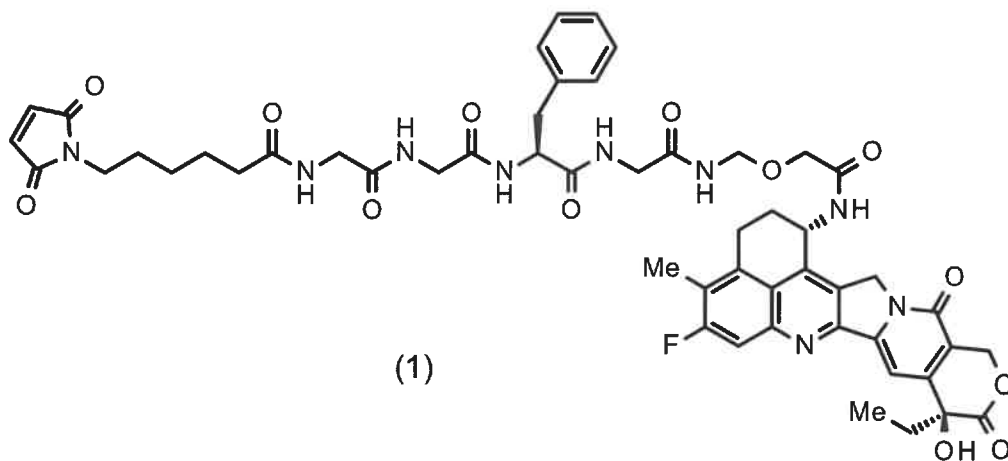
(11)

[0151]

in a two-phase system of an aqueous sodium sulfate solution and tetrahydrofuran to convert it into the compound represented by the formula (1):

[0152]

[Chem. 71]



[0153]

[68] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt.

[69] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt m-hydrate, wherein m is in the range of 0 to 3.

[70] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt anhydride.

[71] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt monohydrate.

[72] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt dihydrate.

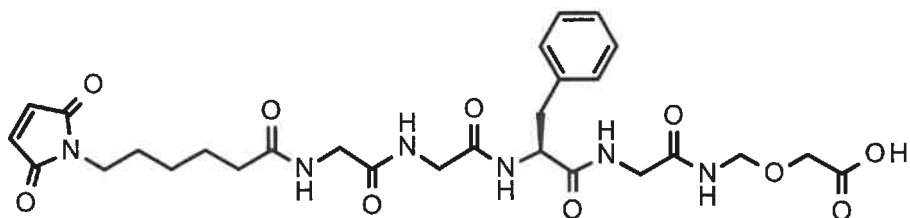
[73] The production method according to [67], wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt trihydrate.

[74] The production method according to any one of [3] to [73], wherein no chromatography is used.

[75] Crystals of a 1,2-dimethoxyethane adduct of the compound represented by formula (10):

[0154]

[Chem. 72]



(10)

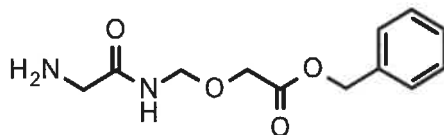
[0155]

[76] The crystals according to [75], wherein the crystals show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper $K\alpha$ radiation.

[77] A salt of the compound represented by formula (5):

[0156]

[Chem. 73]



(5)

[0157]

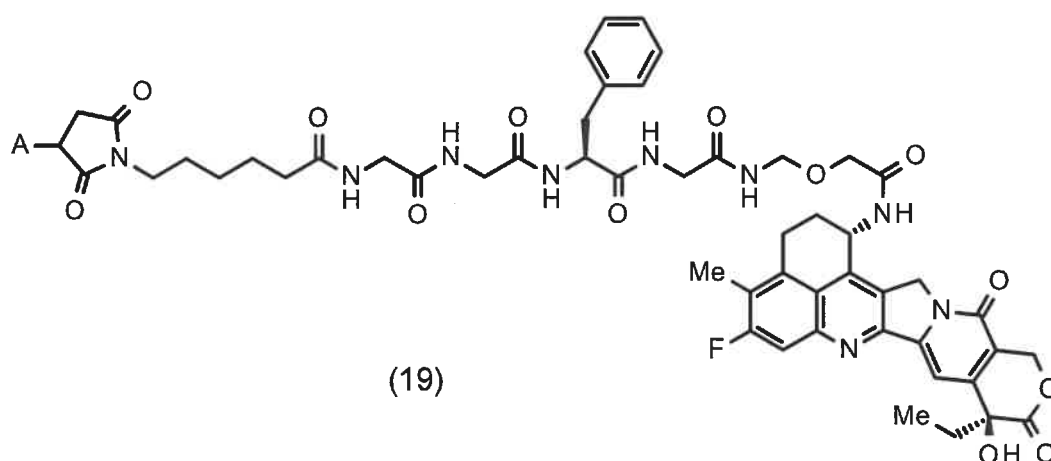
and an acid.

[78] The salt according to [77], wherein the acid is 1-hydroxybenzotriazole.

[79] A method for producing an antibody-drug conjugate, in which a drug-linker represented by formula (19):

[0158]

[Chem. 75]

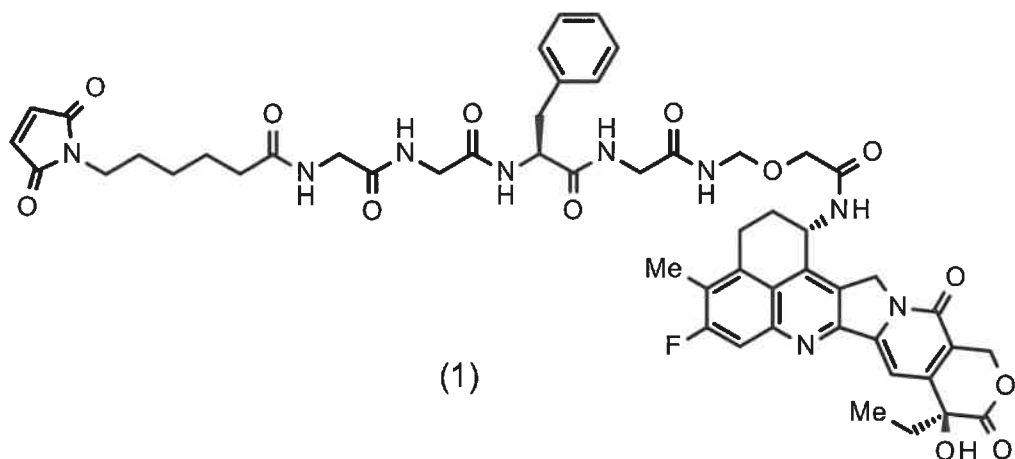


[0159]

wherein A represents the connecting position to an antibody,
is conjugated to the antibody via a thioether bond,
wherein crystals of the compound represented by formula
(1):

[0160]

[Chem. 74]



[0161]

produced by the method according to any one of [3] to [51] are used as a starting material, and the method comprises the steps of:

- i) reducing an antibody; and then
- ii) adding a solution in which the crystals of the compound represented by the formula (1) produced in the above-mentioned method are dissolved, to react the solution with the reduced antibody.

[80] The production method according to [79], wherein the antibody is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, an anti-B7-H3 antibody, or an anti-GPR20 antibody.

[81] The production method according to [80], wherein the antibody is an anti-HER2 antibody.

[82] The production method according to [81], wherein 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 the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[83] The production method according to [81] or [82], 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.

[84] The production method according to [80], wherein the antibody is an anti-HER3 antibody.

[85] The production method according to [84], wherein the anti-HER3 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 3 and a light chain consisting of the 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 of the antibody is deleted.

[86] The production method according to [84] or [85], 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.

[87] The production method according to [80], wherein the antibody is an anti-TROP2 antibody.

[88] The production method according to [87], wherein the anti-TROP2 antibody is an antibody comprising a heavy

chain consisting of an amino acid sequence consisting of amino acid residues 20 to 470 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 of the antibody is deleted.

[89] The production method according to [87] or [88], 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 3 to 5.

[90] The production method according to [80], wherein the antibody is an anti-B7-H3 antibody.

[91] The production method according to [90], wherein the anti-B7-H3 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 of the antibody is deleted.

[92] The production method according to [90] or [91], 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 3 to 5.

[93] The production method according to [80], wherein the antibody is an anti-GPR20 antibody.

[94] The production method according to [93], wherein 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: 9 and a light chain consisting of an amino acid sequence consisting of amino acid residues 21 to 234 of SEQ ID NO: 10, or a variant of the antibody in which a lysine residue at the carboxyl terminus of the heavy chain of the antibody is deleted.

[95] The production method according to [93] or [94], 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.

Advantageous Effects of Invention

[0162]

The present invention enables obtainment of the compound represented by formula (1) as crystals and can provide the compound represented by the formula (1) with given quality. The present invention can also provide an industrially excellent method for producing the compound represented by the formula (1) without the need for purification by chromatography. The present invention can further provide an improved method for producing an

antibody-drug conjugate wherein the aforementioned method is used.

Brief Description of Drawings

[0163]

[Figure 1] Figure 1 shows an amino acid sequence of a heavy chain of an anti-HER2 antibody (SEQ ID NO: 1).

[Figure 2] Figure 2 shows an amino acid sequence of a light chain of an anti-HER2 antibody (SEQ ID NO: 2).

[Figure 3] Figure 3 shows powder X-ray diffraction of a crystals of a 1,2-dimethoxyethane adduct of the compound represented by formula (10).

[Figure 4] Figure 4 shows powder X-ray diffraction of a crystals of the compound represented by formula (1).

[Figure 5] Figure 5 shows an amino acid sequence of a heavy chain of an anti-HER3 antibody (SEQ ID NO: 3).

[Figure 6] Figure 6 shows an amino acid sequence of a light chain of an anti-HER3 antibody (SEQ ID NO: 4).

[Figure 7] Figure 7 shows an amino acid sequence of a heavy chain of an anti-TROP2 antibody (SEQ ID NO: 5).

[Figure 8] Figure 8 shows an amino acid sequence of a light chain of an anti-TROP2 antibody (SEQ ID NO: 6).

[Figure 9] Figure 9 shows an amino acid sequence of a heavy chain of an anti-B7-H3 antibody (SEQ ID NO: 7).

[Figure 10] Figure 10 shows an amino acid sequence of a light chain of an anti-B7-H3 antibody (SEQ ID NO: 8).

[Figure 11] Figure 11 shows an amino acid sequence of a heavy chain of an anti-GPR20 antibody (SEQ ID NO: 9).

[Figure 12] Figure 12 shows an amino acid sequence of a light chain of an anti-GPR20 antibody (SEQ ID NO: 10).

Description of Embodiments

[0164]

Hereinafter, preferred modes for carrying out the present invention are described with reference to the drawings. The embodiments described below are given merely for illustrating one example of a typical embodiment of the present invention and are not intended to limit the scope of the present invention.

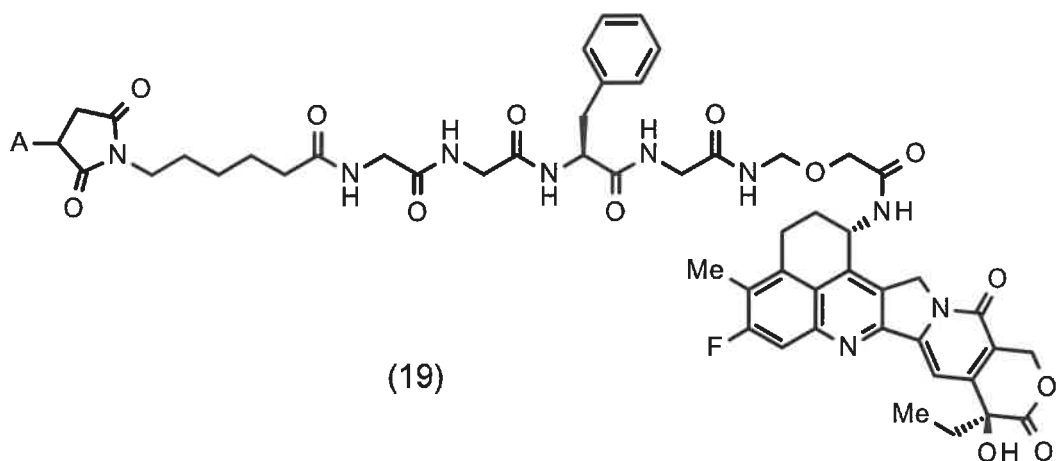
[0165]

[Antibody-drug conjugate]

The antibody-drug conjugate produced by the present invention is an antibody-drug conjugate in which a drug-linker represented by formula (19):

[0166]

[Chem. 76]



[0167]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[0168]

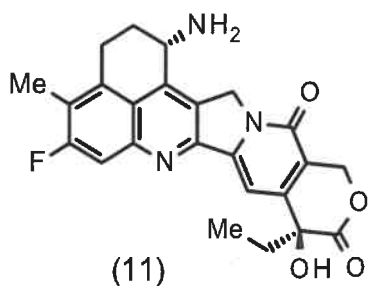
In the present invention, the partial structure consisting of a linker and a drug in the antibody-drug conjugate is referred to as a "drug-linker". The drug-linker is connected to a thiol group (in other words, the sulfur atom of a cysteine residue) formed at an interchain disulfide bond site (two sites between heavy chains, and two sites between a heavy chain and a light chain) in the antibody.

[0169]

The drug-linker of the present invention includes exatecan, which is a topoisomerase I inhibitor, as a component. Exatecan is the compound represented by formula (11):

[0170]

[Chem. 77]



[0171]

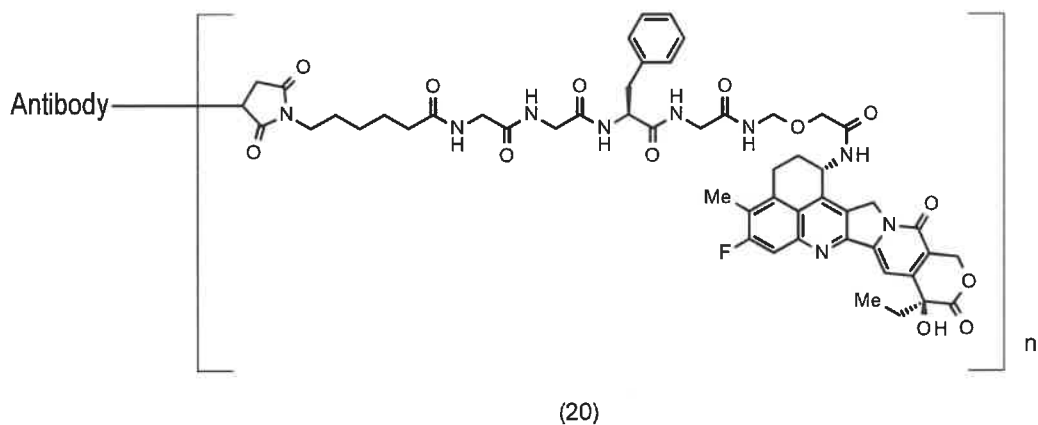
and is a camptothecin derivative having an antitumor effect.

[0172]

The antibody-drug conjugate used in the present invention can also be represented by formula (20):

[0173]

[Chem. 78]



[0174]

[0175]

wherein, the drug-linker is conjugated to an antibody via a thioether bond. The meaning of n is the same as that of what is called the average number of conjugated drug

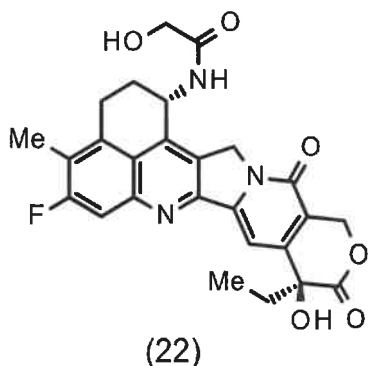
molecules (DAR; Drug-to-Antibody Ratio), and indicates the average number of units of the drug-linker conjugated per antibody molecule.

[0176]

After migrating into cancer cells, the antibody-drug conjugate used in the present invention releases the compound represented by formula (22):

[0177]

[Chem. 79]



[0178]

and thereby exerts an antitumor effect.

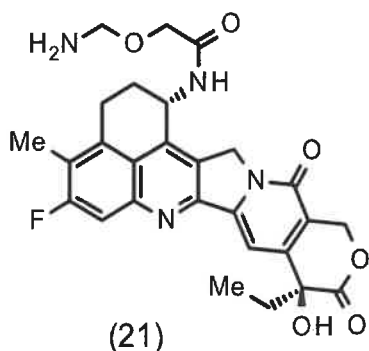
The compound represented by the formula (22) is inferred to be the original source of the antitumor activity of the antibody-drug conjugate produced by the present invention, and has been confirmed to have a topoisomerase I inhibitory effect (Ogitani Y. et al., Clinical Cancer Research, 2016, Oct 15; 22 (20): 5097-5108, Epub 2016 Mar 29).

[0179]

The compound represented by the formula (22) is inferred to be formed by decomposition of an aminor structure of the compound represented by formula (21):

[0180]

[Chem. 80]



[0181]

which is inferred to be formed by cleavage at the linker part of the antibody-drug conjugate produced by the present invention.

[0182]

The antibody-drug conjugate produced by the present invention is known to have a bystander effect (Ogitani Y. et al., Cancer Science (2016) 107, 1039-1046).

[0183]

The bystander effect is exerted through a process in which the antibody-drug conjugate produced according to the present invention is internalized in cancer cells expressing a target and the compound represented by the formula (22) released then exerts an antitumor effect also on cancer cells which are present therearound and not expressing the target.

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it can be evaluated by using, as an index, preservation stability for 3 months, 6 months, 12 months, 24 months, and 36 months in an environment of 25°C/60% RH or 40°C/75% RH, for example.

[0188]

By such quality evaluation, superiority over an amorphous compound represented by the formula (1) can also be confirmed.

[0189]

The production method of the present invention comprises precipitating a crystals of the compound represented by the formula (1) from a solution in which the compound represented by the formula (1) is dissolved, to produce crystals of the compound represented by the formula (1). As a result, highly pure crystals of the compound represented by the formula (1) having given quality can be produced.

[0190]

The crystals of the compound represented by the formula (1) preferably show main peaks at diffraction angles (2θ) of 5.6°, 15.5° and 22.0° in powder X-ray diffraction obtained by irradiation with copper K α radiation. Since diffraction angles (2θ) in powder X-ray diffraction may generally cause an error within the range of $\pm 0.2^\circ$, it should be understood that the above-described values of the diffraction angles include numeric values within the range of $\pm 0.2^\circ$ (for technical

common sense regarding measurement and evaluation by powder X-ray diffraction, see, for example, the Japanese Pharmacopoeia, 16th edition, p. 64-68 (2.58 X-Ray Powder Diffraction Method) or the Japanese Pharmacopoeia, 17th edition, p. 71-74 (2.58 X-Ray Powder Diffraction Method)).

[0191]

Accordingly, crystals having diffraction angles that agree completely with the above-described diffraction angles is identical to crystals having main peaks at diffraction angles (2θ) of $5.6 \pm 0.2^\circ$, $15.5 \pm 0.2^\circ$ and $22.0 \pm 0.2^\circ$, and both of them are included in the present invention. In the present invention, the term " $\pm 0.2^\circ$ " refers to a numeric value in the range of -0.2° to $+0.2^\circ$ with respect to a specific numeric value. For example, the term " $5.6 \pm 0.2^\circ$ " refers to a numeric value in the range of 5.4° to 5.8° .

[0192]

The solution for precipitating the crystals of the compound represented by the formula (1) is preferably a solution containing acetone and a lower alcohol as a solvent. Likewise, a solution containing a lower ketone and a lower alcohol as a solvent can also be preferably used as the solution for precipitating the crystals of the compound represented by the formula (1).

[0193]

In the present invention, the term "lower ketone" refers to a ketone having 3 to 6 carbon atoms. Examples

thereof can include acetone, methyl ethyl ketone, methyl propyl ketone, methyl isopropyl ketone, methyl butyl ketone, methyl isobutyl ketone, methyl tert-butyl ketone, ethyl ethyl ketone, ethyl propyl ketone, and ethyl isopropyl ketone, and acetone and methyl ethyl ketone can be preferably exemplified, and acetone can be more preferably exemplified.

[0194]

In the present invention, the term "lower alcohol" refers to an alcohol having 1 to 4 carbon atoms. Examples thereof can include methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 2-butanol, and tert-butanol, and 1-propanol and 2-butanol can be preferably exemplified, and 1-propanol can be more preferably exemplified.

[0195]

Accordingly, the solution for precipitating the crystals of the compound represented by the formula (1) is preferably a solution containing acetone and 1-propanol or a solution containing acetone and 2-butanol, and more preferably a solution containing acetone and 1-propanol.

[0196]

Precipitation of the crystals of the compound represented by the formula (1) can also be performed by adding a seed crystal of the crystals of the compound

represented by the formula (1) to a solution containing the compound represented by the formula (1).

[0197]

The seed crystal of the crystals of the compound represented by the formula (1) can be obtained by directly performing the above-described method, but can be preferably obtained by purifying a small amount of the compound represented by the formula (1) by chromatography, then dissolving it in a solvent containing acetone and 1-propanol or a solvent containing acetone and 2-butanol, and crystallizing from the solution.

[0198]

The compound represented by the formula (1) can be produced with reference to the descriptions in International Publication No. WO 2014/057687, International Publication No. WO 2015/098099, International Publication No. WO 2015/115091, International Publication No. WO 2015/155998, and so on, but compounds produced by production methods (I), (II), (III), (V), (VI), and (IX) described below can be preferably used. As a result, the crystals of the compound represented by the formula (1) can be produced at a high yield without the use of chromatography in all steps.

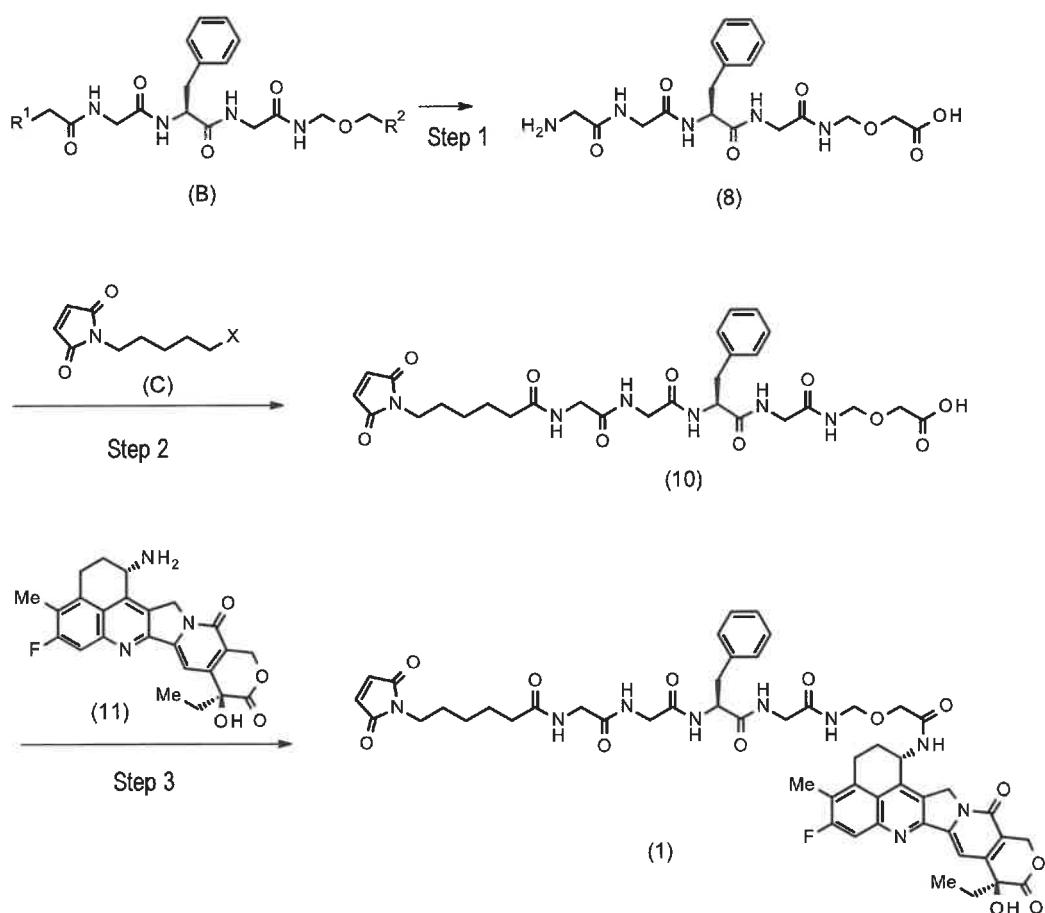
[0199]

[Production method (I)]

The production method (I) is a method of converting a compound represented by formula (B) into the compound represented by the formula (1) through steps 1 to 3. Hereinafter, the steps 1 to 3 will be described in detail.

[0200]

[Chem. 82]



[0201]

In the scheme, R¹ represents an amino group protected with a protecting group, and preferably represents an amino group protected with a benzyloxycarbonyl group, R² represents a carboxy group protected with a protecting

group, and preferably represents a carboxy group protected with a benzyl group, and X represents an active ester group or a carboxy group, and preferably represents a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

[0202]

Step 1:

This step is a step of deprotecting protecting groups for an amino group and a carboxy group of a compound represented by formula (B) to convert it into the compound represented by formula (8).

[0203]

Deprotection of the protecting groups for the amino group and the carboxy group of the compound represented by the formula (B) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0204]

In the case that R^1 is an amino group protected with a benzyloxycarbonyl group, and R^2 is a carboxy group protected with a benzyl group, this step can be preferably performed by the following method.

[0205]

Deprotection of the protecting groups for the amino group and the carboxy group of the compound represented by the formula (B) is not limited by its method as long as the reaction proceeds. It can be preferably performed

using a palladium catalyst, a platinum catalyst, a nickel catalyst, a ruthenium catalyst, or a rhodium catalyst under a hydrogen atmosphere, can be more preferably performed using a palladium catalyst, and can be even more preferably performed using palladium carbon, and 5% palladium carbon can be even more preferably used. The amount of the 5% palladium carbon used in this step is not limited as long as the reaction proceeds. It is preferably 5 to 40% by weight with respect to the compound represented by the formula (B).

[0206]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of tetrahydrofuran and water can be preferably exemplified.

[0207]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as

long as the reaction proceeds. It is preferably 1 to 5 hours.

[0208]

Step 2:

This step is a step of condensing the compound represented by the formula (8) with a compound represented by formula (C) to convert it into the compound represented by formula (10). As the compound represented by the formula (C), a commercially available product or a compound produced by a known method, or a compound produced by a method conforming to the production method (VII) described below can be used. In the case that X is a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group, this step can be preferably performed by the following method.

[0209]

The amount of the compound represented by the formula (C) used in this step is not limited as long as the reaction proceeds. It is preferably 1 to 4 equivalents with respect to the compound represented by the formula (8).

[0210]

This step preferably employs a base. The base used in this step is not particularly limited as long as the reaction proceeds. Examples thereof can include triethylamine, tributylamine, diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine, and N-

methylpiperidine, and N,N-diisopropylethylamine can be preferably exemplified. The amount of the N,N-diisopropylethylamine used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the compound represented by the formula (8).

[0211]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of acetonitrile and water can be preferably exemplified.

[0212]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 7 to 30 hours.

[0213]

The compound represented by the formula (10) can be preferably obtained as a crystals of a 1,2-dimethoxyethane adduct.

[0214]

The quality of the crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) can be evaluated, for example, on the basis of indexes such as an impurity content, an amount of a residual solvent, and appearance. Also, it can be evaluated by using, as an index, preservation stability for 3 months, 6 months, 12 months, 24 months, and 36 months in an environment of 25°C/60% RH or 40°C/75% RH, for example. By such quality evaluation, superiority over an amorphous compound represented by the formula (10) can also be confirmed.

[0215]

The crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) preferably show main peaks at diffraction angles (2θ) of 19.0° and 25.0° in powder X-ray diffraction obtained by irradiation with copper K α radiation. Since diffraction angles (2θ) in powder X-ray diffraction may generally cause an error within the range of $\pm 0.2^\circ$, it should be understood that the above-described values of the diffraction angles include numeric values within the range of $\pm 0.2^\circ$ (for technical common sense regarding measurement and evaluation by powder X-ray diffraction, see, for example,

the Japanese Pharmacopoeia, 16th edition, p. 64-68 (2.58 X-Ray Powder Diffraction Method) or the Japanese Pharmacopoeia, 17th edition, p. 71-74 (2.58 X-Ray Powder Diffraction Method)). Accordingly, crystals having diffraction angles that agree completely with the above-described diffraction angles are identical to crystals having main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$, and both of them are included in the present invention.

[0216]

Step 3:

This step is a step of condensing the compound represented by the formula (10) with the compound represented by formula (11) to convert it into the compound represented by the formula (1). The compound represented by the formula (11) can be preferably used in the form of a methanesulfonic acid salt, can be more preferably used in the form of a methanesulfonic acid salt m -hydrate, wherein m is 0 to 3, can be even more preferably used in the form of a methanesulfonic acid salt anhydride, a methanesulfonic acid salt monohydrate, a methanesulfonic acid salt dihydrate, or a methanesulfonic acid salt trihydrate, and can be even more preferably used in the form of a methanesulfonic acid salt dihydrate, but all of them can be used in the production method of the present invention. The number of water molecules in the above-described hydrate can be

controlled by adjusting the humidity at the time of obtaining or drying of the crystals.

[0217]

The amount of the compound represented by the formula (11) used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the compound represented by the formula (10).

[0218]

The compound represented by the formula (10) can be preferably derivatized into an active ester and condensed with the compound represented by the formula (11). Derivatization into the active ester in this step is not limited by its method as long as the reaction proceeds. It can be performed, for example, by using a condensing agent such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD-HCl) or N,N'-dicyclohexylcarbodiimide (DCC), and reacting with an additive such as 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide, ethyl cyano(hydroxyimino)acetate, or p-nitrophenol, and can be preferably performed using 3-(3-dimethylaminopropyl)carbodiimide hydrochloride and ethyl cyano(hydroxyimino)acetate. The amount of the 3-(3-dimethylaminopropyl)carbodiimide hydrochloride used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the

compound represented by the formula (10). The amount of the ethyl cyano(hydroxyimino)acetate used in this step is not limited as long as the reaction proceeds. It is preferably 0.02 to 0.2 equivalents with respect to the compound represented by the formula (10).

[0219]

This step preferably employs a base. The base used in this step is not particularly limited as long as the reaction proceeds. Examples thereof can include triethylamine, tributylamine, diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine, and N-methylpiperidine, and N-methylmorpholine can be preferably exemplified. The amount of the N-methylmorpholine used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the compound represented by the formula (10).

[0220]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-

pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of tetrahydrofuran and water can be preferably exemplified.

[0221]

The methanesulfonic acid salt of the compound represented by the formula (11) is neutralized with a base to prepare a free form, and then, the reaction proceeds. Here, the methanesulfonic acid salt of the compound represented by the formula (11) is hydrophilic, whereas the free form of the compound represented by the formula (11) is lipophilic. Therefore, in order to allow a series of reactions to proceed efficiently, this step can be preferably performed in a two-phase system of an aqueous layer and an organic layer. In the case that the organic layer contains tetrahydrofuran, an aqueous solution having high ionic strength, for example, an aqueous sodium sulfate solution, can be preferably used as an aqueous layer less miscible therewith.

[0222]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 hours.

[0223]

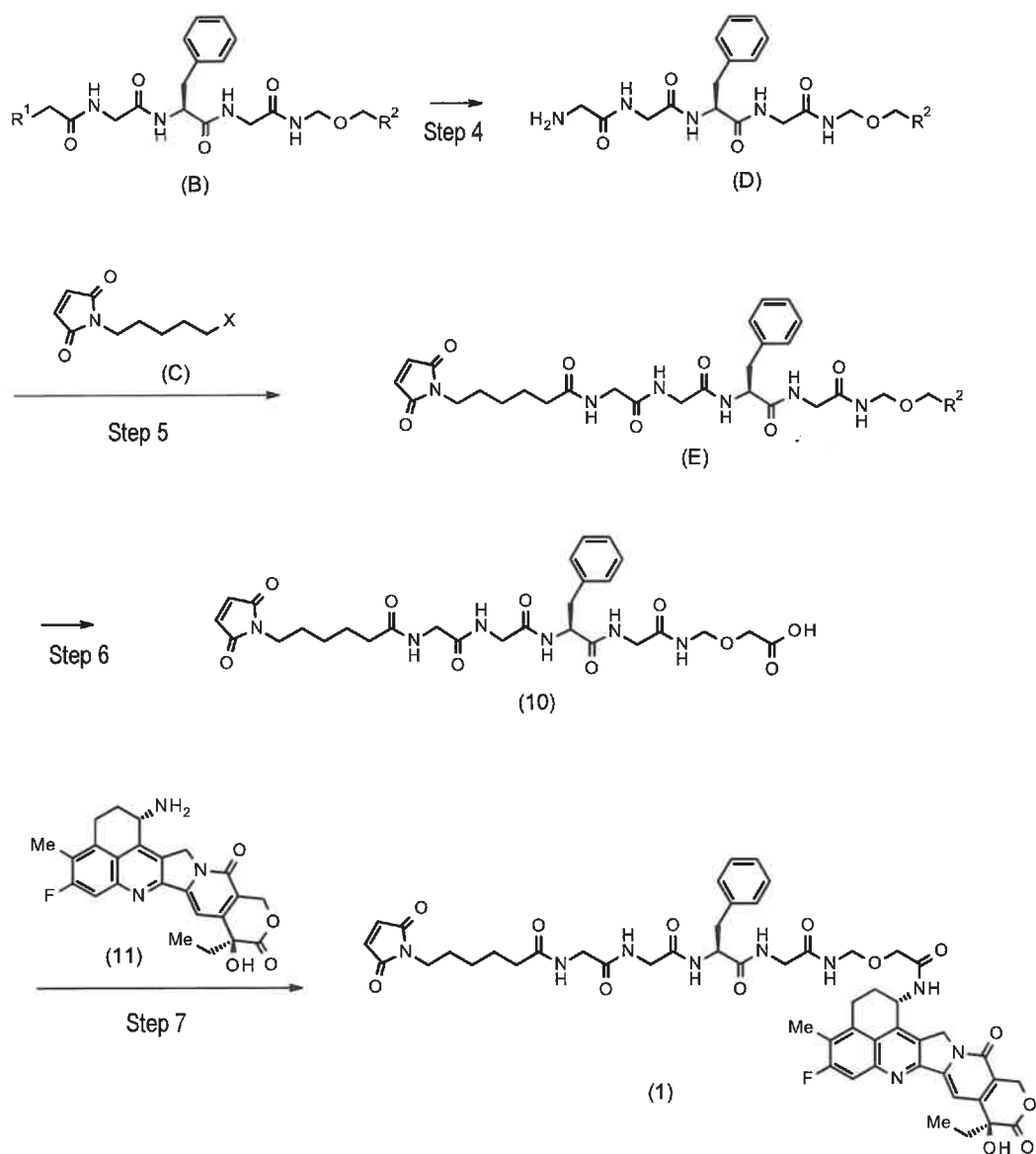
[Production method (II)]

The production method (II) is a method of converting a compound represented by formula (B) into the compound represented by the formula (1) through steps 4 to 7.

Hereinafter the steps 4 to 7 will be described in detail.

[0224]

[Chem. 83]



[0225]

In the scheme, R^1 represents an amino group protected with a protecting group, R^2 represents a carboxy group protected with a protecting group, and X represents an active ester group or a carboxy group, and preferably represents a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

[0226]

Step 4:

This step is a step of deprotecting a protecting group for an amino group of a compound represented by formula (B) to convert it into a compound represented by formula (D).

[0227]

Deprotection of the protecting group for the amino group of the compound represented by the formula (B) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0228]

Step 5:

This step is a step of condensing the compound represented by the formula (D) with a compound represented by formula (C) to convert it into a compound represented by formula (E).

[0229]

This step can be performed in the same manner as in the step 2 of the production method (I).

[0230]

Step 6:

This step is a step of deprotecting the protecting group for the carboxy group of the compound represented by the formula (E) to convert it into the compound represented by formula (10).

[0231]

Deprotection of the protecting group for the carboxy group of the compound represented by the formula (B) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0232]

The compound represented by the formula (10) can be preferably obtained as crystals of a 1,2-dimethoxyethane adduct in the same manner as in the step 2 of the production method (I).

[0233]

Step 7:

This step is a step of condensing the compound represented by the formula (10) with the compound represented by formula (11) to convert it into the compound represented by the formula (1).

[0234]

This step can be performed in the same manner as in the step 3 of the production method (I).

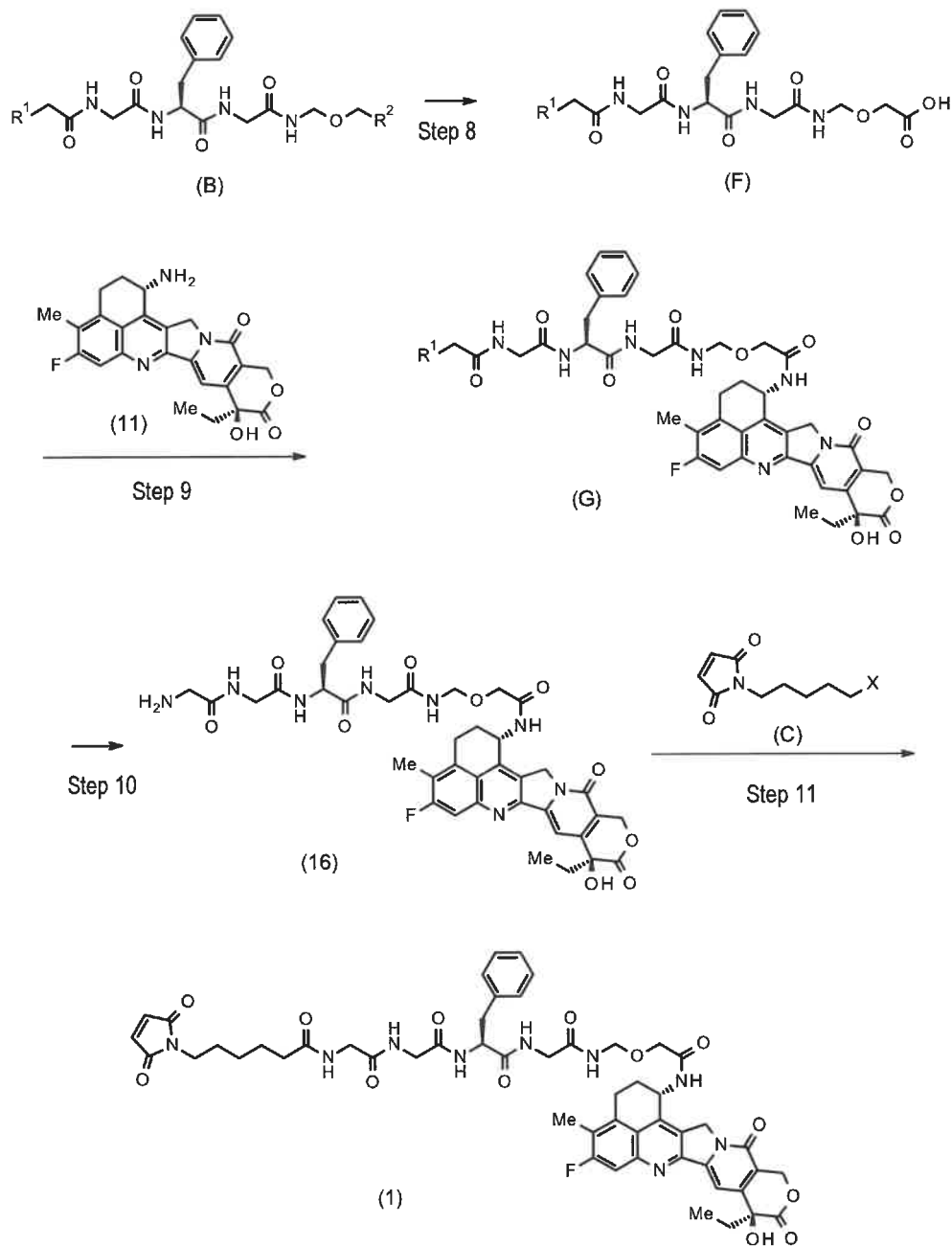
[0235]

[Production method (III)]

The production method (III) is a method of converting a compound represented by formula (B) into the compound represented by the formula (1) through steps 8 to 11. Hereinafter the steps 8 to 11 will be described in detail.

[0236]

[Chem. 84]



[0237]

In the scheme, R¹ represents an amino group protected with a protecting group, and preferably represents an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group, R² represents a carboxy group

protected with a protecting group, and preferably represents a carboxy group protected with a benzyl group, and X represents an active ester group or a carboxy group, and preferably represents a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

[0238]

Step 8:

This step is a step of deprotecting a protecting group for a carboxy group of a compound represented by formula (B) to convert it into a compound represented by formula (F).

[0239]

Deprotection of the protecting group for the carboxy group of the compound represented by the formula (B) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0240]

In the case that R^2 is a carboxy group protected with a benzyl group, this step can be preferably performed by the following method.

[0241]

Deprotection of the protecting group for the carboxy group of the compound represented by the formula (B) is not limited by its method as long as the reaction proceeds. It can be preferably performed using a

palladium catalyst, a platinum catalyst, a nickel catalyst, a ruthenium catalyst, or a rhodium catalyst under a hydrogen atmosphere, can be more preferably performed using a palladium catalyst, and can be even more preferably performed using palladium carbon, and a palladium carbon-ethylenediamine complex can be even more preferably used. The amount of the palladium carbon-ethylenediamine complex used in this step is not limited as long as the reaction proceeds. It is preferably 34 to 136% by weight with respect to the compound represented by the formula (B).

[0242]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of tetrahydrofuran and water can be preferably exemplified.

[0243]

The reaction temperature of this step is preferably 10 to 40°C, but is not limited thereto as long as the

reaction proceeds. The reaction time of this step is preferably 1 to 54 hours, but is not limited thereto as long as the reaction proceeds.

[0244]

Step 9:

This step is a step of condensing the compound represented by the formula (F) with the compound represented by formula (11) to convert it into a compound represented by formula (G).

[0245]

The compound represented by the formula (F) can be preferably derivatized into an active ester and condensed with the compound represented by the formula (11). The amount of the compound represented by the formula (11) used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (F). Derivatization into the active ester in this step is not limited by its method as long as the reaction proceeds. It can be performed, for example, by using a condensing agent such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD-HCl) or N,N'-dicyclohexylcarbodiimide (DCC), and reacting with an additive such as 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide, ethyl cyano(hydroxyimino)acetate, or p-nitrophenol, and can be preferably performed using 3-(3-

dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole. The amount of the 3-(3-dimethylaminopropyl)carbodiimide hydrochloride used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (F). The amount of the 1-hydroxybenzotriazole used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (F).

[0246]

This step preferably employs a base. The base used in this step is not particularly limited as long as the reaction proceeds. Examples thereof can include triethylamine, tributylamine, diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine, and N-methylpiperidine, and triethylamine can be preferably exemplified. The amount of the triethylamine used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (F).

[0247]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-

methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and tetrahydrofuran can be preferably exemplified.

[0248]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 1 to 4 hours.

[0249]

Step 10:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (G) to convert it into the compound represented by formula (16).

[0250]

Deprotection of the protecting group for the amino group of the compound represented by the formula (G) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0251]

In the case that R¹ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group, this step can be preferably performed by the following method.

[0252]

Deprotection of the protecting group for the amino group of the compound represented by the formula (G) is not particularly limited as long as the reaction proceeds. It can be performed using, for example, 1,8-diazabicyclo[5,4,0]-7-undecene, trimethylguanidine, 1,5,7-triazabicyclo[4,4,0]dec-5-ene, or 7-methyl-1,5,7-triazabicyclo[4,4,0]dec-5-ene 1,5-diazabicyclo[4,3,0]-5-nonene, and can be preferably performed using 1,8-diazabicyclo[5,4,0]-7-undecene. The amount of the 1,8-diazabicyclo[5,4,0]-7-undecene used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the compound represented by the formula (15).

[0253]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-

pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and tetrahydrofuran can be preferably exemplified.

[0254]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 1 to 5 hours.

[0255]

Step 11:

This step is a step of condensing the compound represented by the formula (16) with a compound represented by formula (C) to convert it into the compound represented by the formula (1). As the compound represented by the formula (C), a commercially available product, a compound produced by a known method, or a compound produced by a method conforming to the production method (VII) described below can be used. In the case that X is a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group, this step can be preferably performed by the following method.

[0256]

The amount of the compound represented by the formula (C) used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2

equivalents with respect to the compound represented by the formula (16).

[0257]

This step preferably employs a base. The base used in this step is not particularly limited as long as the reaction proceeds. Examples thereof can include triethylamine, tributylamine, diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine, and N-methylpiperidine, and triethylamine can be preferably exemplified. The amount of the triethylamine used in this step is not limited as long as the reaction proceeds. It is preferably 0.75 to 6 equivalents with respect to the compound represented by the formula (16).

[0258]

This step can preferably further employ pyridinium p-toluenesulfonate. The amount of the pyridinium p-toluenesulfonate used in this step is not limited as long as the reaction proceeds. It is preferably 1 to 4 equivalents with respect to the compound represented by the formula (16).

[0259]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane,

pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, pyridine, and water, and mixed solvents thereof, and a mixed solvent of pyridine, acetonitrile, and tetrahydrofuran can be preferably exemplified.

[0260]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 1.5 to 7 hours.

[0261]

As the compound represented by the formula (B) in the production methods (I) to (III), a compound produced by the following production method (IV) can be preferably used.

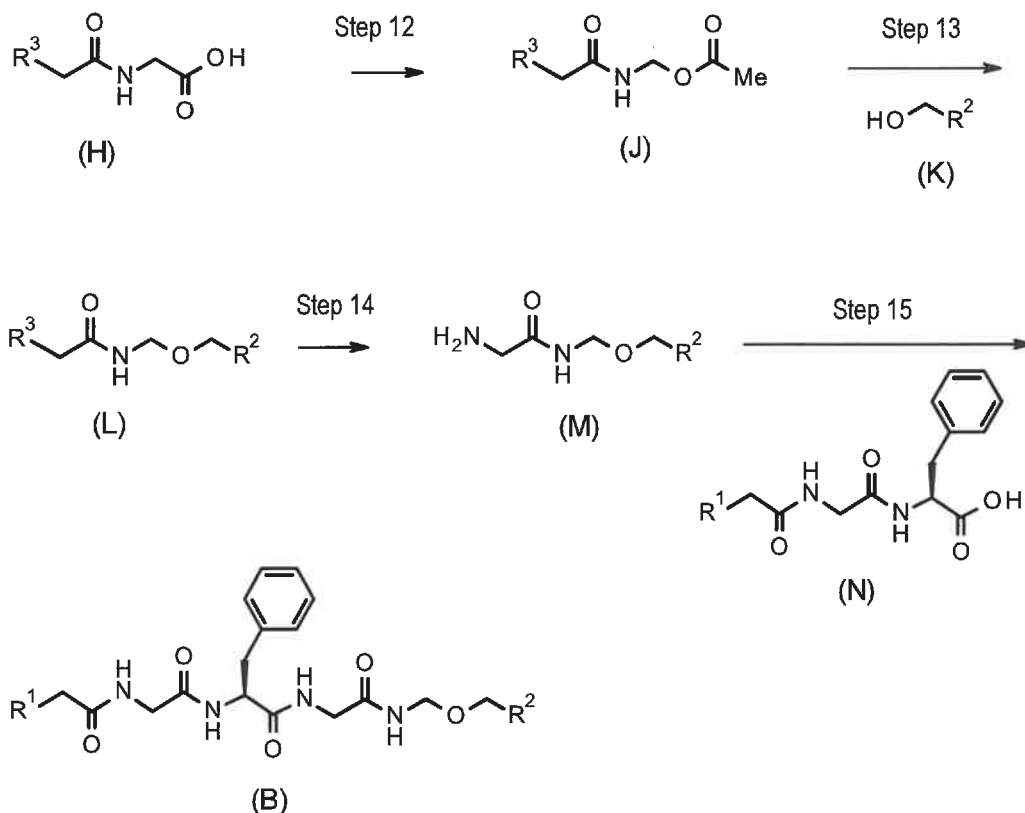
[0262]

[Production method (IV)]

The production method (IV) is a method of converting a compound represented by formula (H) into the compound represented by the formula (B) through steps 12 to 15. Hereinafter the steps 12 to 15 will be described in detail.

[0263]

[Chem. 85]



[0264]

In the scheme, R^1 represents an amino group protected with a protecting group, and preferably represents an amino group protected with a benzyloxycarbonyl group or a (9H-fluoren-9-ylmethoxy)carbonyl group, R^2 represents a carboxy group protected with a protecting group, and preferably represents a carboxy group protected with a benzyl group, R^3 represents an amino group protected with a protecting group, and preferably represents an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group, and X represents an active ester group or a carboxy group, and preferably represents a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

Step 12:

This step is a step of reacting a compound represented by formula (H) with lead tetraacetate to convert it into a compound represented by formula (J). As the compound represented by the formula (H), a commercially available product or a compound produced with reference to a known method can be used. The amount of the lead tetraacetate used in this step is not limited as long as the reaction proceeds. It is preferably 1 to 3 equivalents with respect to the compound represented by the formula (H).

[0265]

This step can be preferably performed in the presence of acetic acid or pyridine, and can be more preferably performed in the presence of acetic acid.

[0266]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, and dimethyl sulfoxide, and mixed solvents thereof, and tetrahydrofuran can be preferably exemplified.

[0267]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 45 to

85°C, and more preferably a temperature that attains heating to reflux of tetrahydrofuran. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 3 hours.

[0268]

Step 13:

This step is a step of reacting the compound represented by the formula (J) with a compound represented by formula (K) in the presence of an acid or a base to convert it into a compound represented by formula (L). The amount of the compound represented by the formula (K) used in this step is not limited as long as the reaction proceeds. It is preferably 1 to 4 equivalents with respect to the compound represented by the formula (J).

[0269]

This step can be performed in the presence of a base or an acid. The base used in this step is preferably an aqueous sodium hydroxide solution. The amount of the aqueous sodium hydroxide solution used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 equivalents with respect to the compound represented by the formula (J). The acid used in this step is preferably tris(pentafluorophenyl)borane. The amount of the tris(pentafluorophenyl)borane used in this step is not limited as long as the reaction proceeds.

It is preferably 0.01 to 0.1 equivalents with respect to the compound represented by the formula (J).

[0270]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, and 1,4-dioxane, and 1,2-dimethoxyethane can be preferably exemplified.

[0271]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably -10 to 15°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 6 hours.

[0272]

Step 14:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (L) to convert it into a compound represented by formula (M).

[0273]

Deprotection of the protecting group for the amino group of the compound represented by the formula (L) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0274]

In the case that R^3 is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group, this step can be preferably performed by the following method.

Deprotection of the protecting group for the amino group of the compound represented by the formula (L) is not particularly limited as long as the reaction proceeds. It can be performed using, for example, 1,8-diazabicyclo[5,4,0]-7-undecene, trimethylguanidine, 1,5,7-triazabicyclo[4,4,0]dec-5-ene, or 7-methyl-1,5,7-triazabicyclo[4,4,0]dec-5-ene 1,5-diazabicyclo[4,3,0]-5-nonene, and can be preferably performed using 1,8-diazabicyclo[5,4,0]-7-undecene. The amount of the 1,8-diazabicyclo[5,4,0]-7-undecene used in this step is not limited as long as the reaction proceeds. It is preferably 0.25 to 1 equivalents with respect to the compound represented by the formula (L).

[0275]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-

pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and acetonitrile and N,N-dimethylacetamide can be preferably exemplified.

[0276]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 2 to 8 hours.

[0277]

The compound represented by the formula (M) can be precipitated from the reaction solution by forming a salt with an acid, and preferably isolated and purified. As a result, by-products, which can be a factor inhibiting reactions in the subsequent steps, can be removed.

The above-described acid is preferably 1-hydroxybenzotriazole. The 1-hydroxybenzotriazole used in this step can also function as one of the condensing agents in the next step 15. Likewise, an acid other than 1-hydroxybenzotriazole can be preferably used in this step as long as it functions as one of the condensing agents.

[0278]

Step 15:

This step is a step of condensing the compound represented by the formula (M) with a compound represented by formula (N) to convert it into the

compound represented by the formula (B). As the compound represented by the formula (N), a commercially available product, a compound produced by a known method, or a compound produced by a method conforming to the production method (VIII) described below can be used. The amount of the compound represented by the formula (N) used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (M).
[0279]

The compound represented by the formula (M) can be preferably derivatized into an active ester and condensed with the compound represented by the formula (N). Derivatization into the active ester can be performed, for example, by using a condensing agent such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD-HCl) or N,N'-dicyclohexylcarbodiimide (DCC), and reacting with an additive such as 1-hydroxybenzotriazole (HOBT), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide, ethyl cyano(hydroxyimino)acetate, or p-nitrophenol, and can be preferably performed using 3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole. The amount of the 3-(3-dimethylaminopropyl)carbodiimide hydrochloride used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (5). The amount

of the 1-hydroxybenzotriazole used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (M).

[0280]

In the case that the compound represented by the formula (M) is in the form of a 1-hydroxybenzotriazole salt, this step can be preferably performed without the addition of fresh 1-hydroxybenzotriazole.

[0281]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of acetonitrile and water can be preferably exemplified.

[0282]

In the case that the compound represented by the formula (M) is not isolated in the step 14, and this step is performed continuously therefrom, the solvent used in

the step 14 can be used as it is as the solvent of this step.

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably -10 to 15°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 1.5 to 7 hours.

[0283]

In a more specific aspect, the compound represented by the formula (1) can be preferably produced by the following production method (V) or (VI), and used.

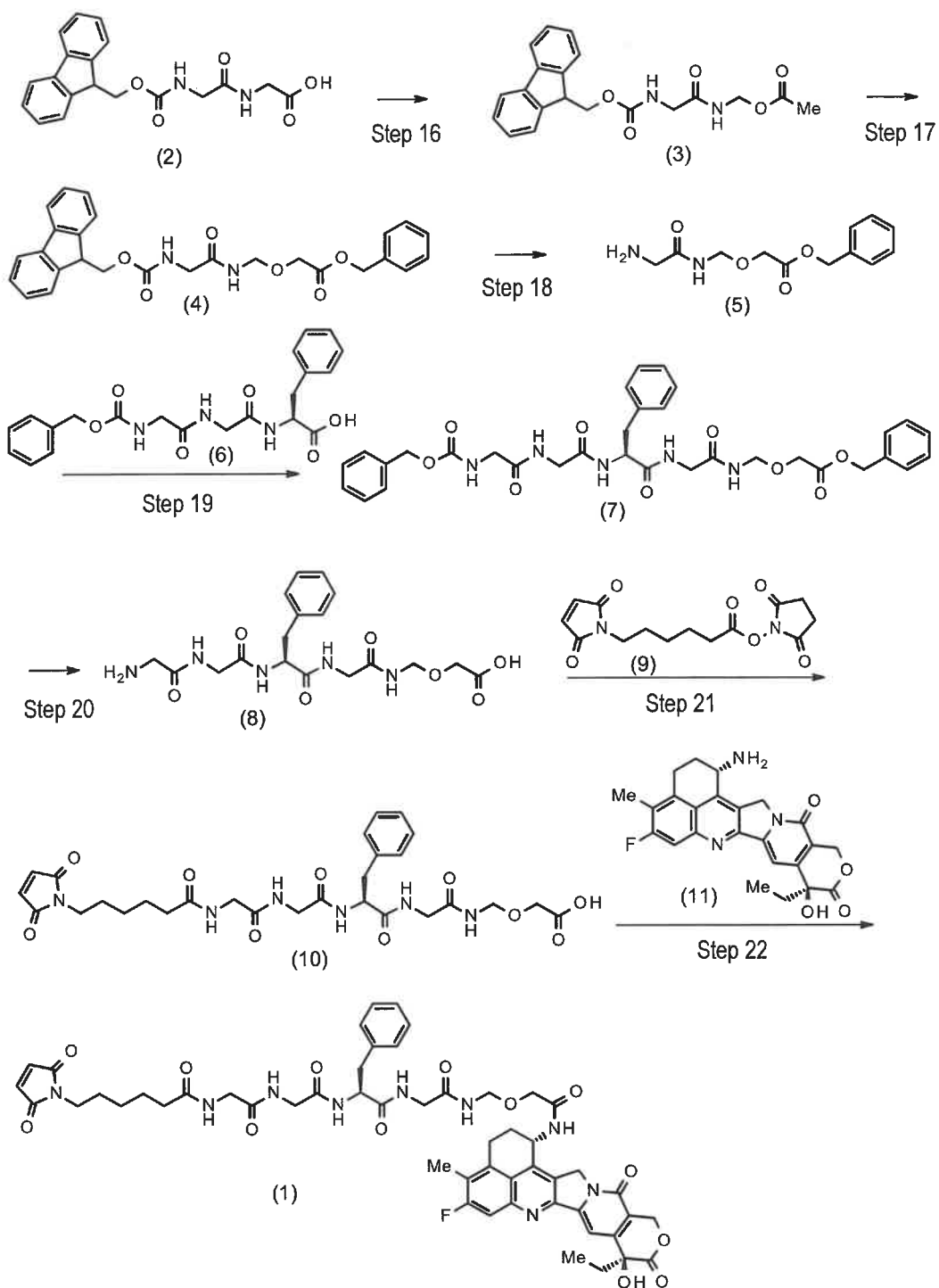
[0284]

[Production method (V)]

The production method (V) is a method of converting the compound represented by formula (2) into the compound represented by the formula (1) through steps 16 to 22. Hereinafter the steps 16 to 22 will be described in detail.

[0285]

[Chem. 86]



[0286]

Step 16:

This step is a step of reacting the compound represented by formula (2) with lead tetraacetate to convert it into the compound represented by formula (3). As the compound represented by the formula (2), a commercially available product or a compound produced with reference to a known method can be used. This step can be performed in the same manner as in the step 12 of the production method (IV).

[0287]

Step 17:

This step is a step of reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4). This step can be performed in the same manner as in the step 13 of the production method (IV).

[0288]

Step 18:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5). This step can be performed in the same manner as in the step 14 of the production method (IV).

[0289]

Step 19:

This step is a step of condensing the compound represented by the formula (5) with the compound represented by formula (6) to convert it into the compound represented by formula (7). As the compound represented by the formula (6), a commercially available product, a compound produced by a known method, or a compound produced by the production method (VIII) described below can be used. This step can be performed in the same manner as in the step 15 of the production method (IV).

[0290]

Step 20:

This step is a step of deprotecting the protecting groups for the amino group and the carboxy group of the compound represented by the formula (7) to convert it into the compound represented by formula (8). This step can be performed in the same manner as in the step 1 of the production method (I).

[0291]

Step 21:

This step is a step of condensing the compound represented by the formula (8) with the compound represented by formula (9) to convert it into the compound represented by formula (10). This step can be performed in the same manner as in the step 2 of the production method (I).

[0292]

Step 22:

This step is a step of condensing the compound represented by the formula (10) with the compound represented by formula (11) to convert it into the compound represented by the formula (1). This step can be performed in the same manner as in the step 3 of the production method (I).

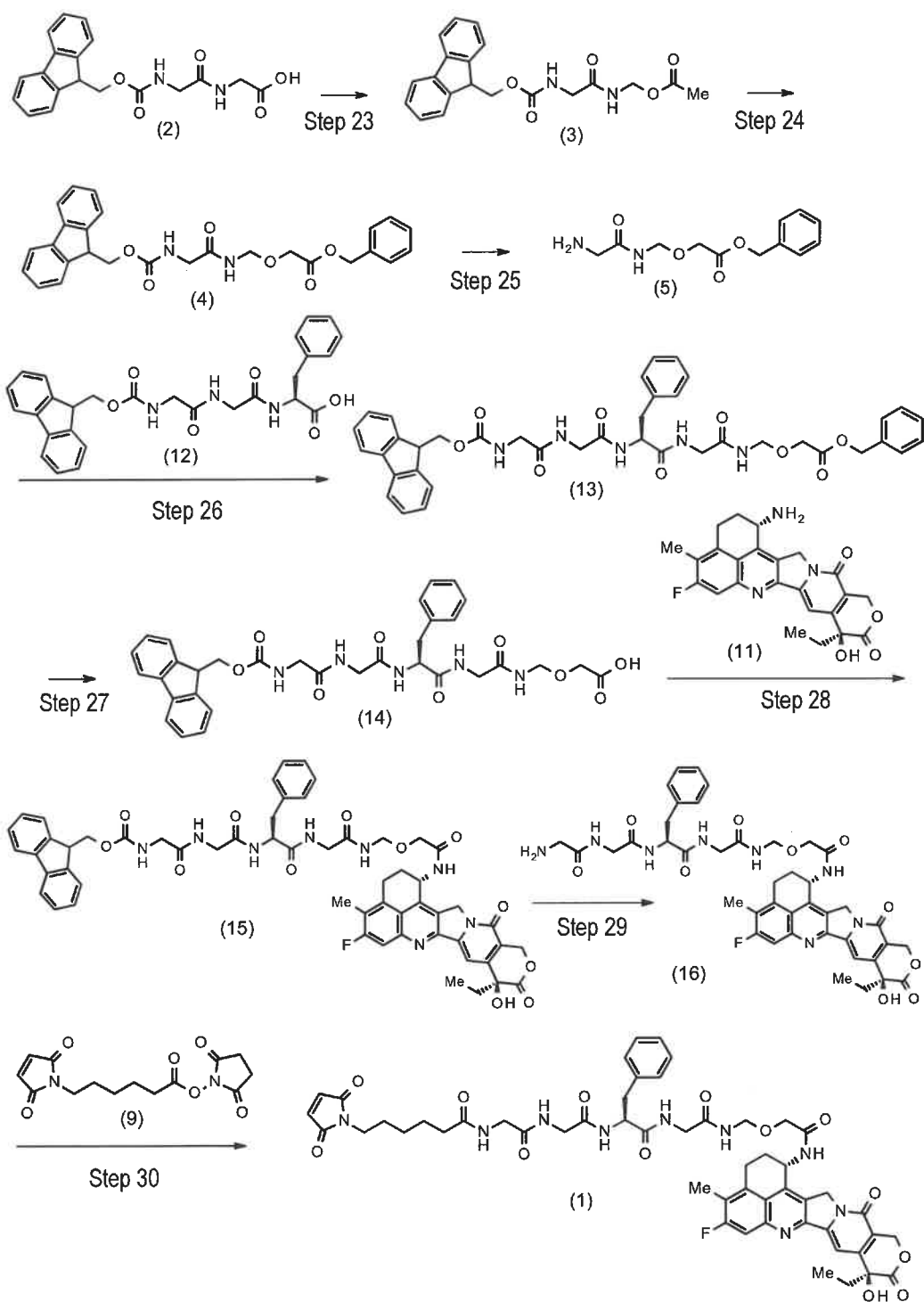
[0293]

[Production method (VI)]

The production method (VI) is a method of converting the compound represented by formula (2) into the compound represented by the formula (1) through steps 23 to 30. Hereinafter the steps 23 to 30 will be described in detail.

[0294]

[Chem. 87]



[0295]

Step 23:

This step is a step of reacting the compound represented by formula (2) with lead tetraacetate to convert it into the compound represented by formula (3). As the compound represented by the formula (2), a commercially available product or a compound produced with reference to a known method can be used. This step can be performed in the same manner as in the step 12 of the production method (IV).

[0296]

Step 24:

This step is a step of reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4). This step can be performed in the same manner as in the step 13 of the production method (IV).

[0297]

Step 25:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5). This step can be performed in the same manner as in the step 14 of the production method (IV).

[0298]

Step 26:

This step is a step of condensing the compound represented by the formula (5) with the compound represented by formula (12) to convert it into the compound represented by formula (13). As the compound represented by the formula (13), a commercially available product or a compound produced by a known method can be used. This step can be performed in the same manner as in the step 15 of the production method (IV).

[0299]

Step 27:

This step is a step of deprotecting the protecting group for the carboxy group of the compound represented by the formula (13) to convert it into the compound represented by formula (14). This step can be performed in the same manner as in the step 8 of the production method (III).

[0300]

Step 28:

This step is a step of condensing the compound represented by the formula (14) with the compound represented by formula (11) to convert it into the compound represented by formula (15). This step can be performed in the same manner as in the step 9 of the production method (III).

[0301]

Step 29:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (15) to convert it into the compound represented by formula (16). This step can be performed in the same manner as in the step 10 of the production method (III).

[0302]

Step 30:

This step is a step of condensing the compound represented by the formula (16) with the compound represented by formula (9) to convert it into the compound represented by the formula (1). This step can be performed in the same manner as in the step 11 of the production method (III).

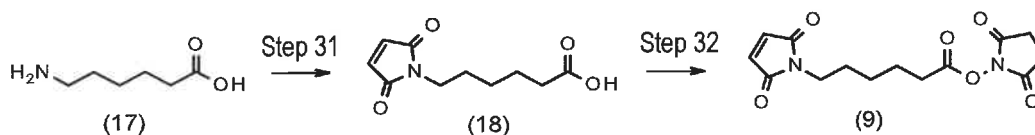
[0303]

[Production method (VII)]

The compound represented by the formula (9) can be preferably produced by the production method (VII) described below, and used. As a result, impurities that might influence the quality of compounds produced in the subsequent steps can be suppressed, and this can contribute to obtainment of the compound represented by the formula (1) with high quality.

[0304]

[Chem. 88]



[0305]

Step 31:

This step is a step of condensing the compound represented by formula (17) with maleic anhydride to convert it into the compound represented by formula (18). The amount of the maleic anhydride used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (17).

[0306]

This step is preferably performed in acetic acid.

[0307]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 80 to 120°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 8 to 32 hours.

[0308]

Step 32:

This step is a step of condensing the compound represented by the formula (18) with N-hydroxysuccinimide to convert it into the compound represented by the formula (9). The amount of the N-hydroxysuccinimide used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (17).

[0309]

The compound represented by the formula (18) can be derivatized into an active ester, a mixed acid anhydride, or an acid halide, etc. and condensed with the N-hydroxysuccinimide, or can be preferably derivatized into an acid halide and condensed with the N-hydroxysuccinimide.

[0310]

Derivatization into the acid halide can be preferably performed by using thionyl chloride. The amount of the thionyl chloride used in this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 1.5 equivalents with respect to the compound represented by the formula (18). In this step, a base is preferably used. The base used in this step is preferably 2,6-lutidine. The amount of the 2,6-lutidine used in this step is not limited as long as the reaction proceeds. It is preferably 1 to 3 equivalents with respect to the compound represented by the formula (18).

[0311]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, and chlorobenzene,

and mixed solvents thereof, and acetonitrile can be preferably exemplified.

[0312]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably -25°C to 0°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 0.5 to 2 hours.

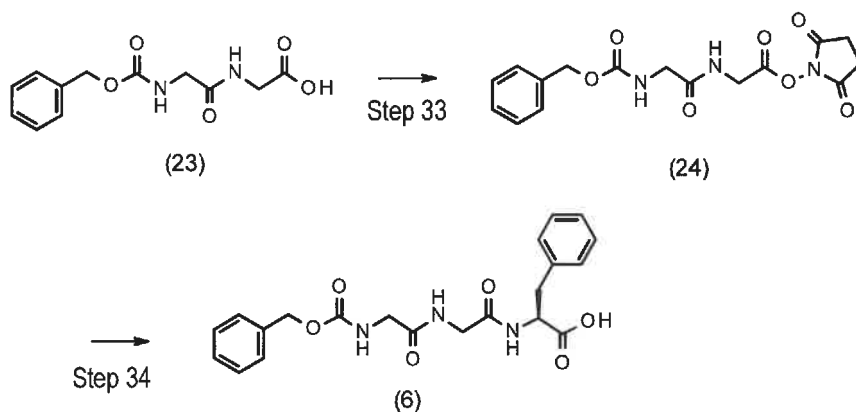
[0313]

[Production method (VIII)]

The compound represented by the formula (6) can be produced by the following production method (VIII), and used.

[0314]

[Chem. 89]



[0315]

Step 33:

This step is a step of condensing the compound represented by formula (23) with N-hydroxysuccinimide to convert it into the compound represented by formula (24).

The amount of the compound represented by the formula (23) used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.5 equivalents with respect to the compound represented by the formula (23).

[0316]

The compound represented by the formula (23) can be derivatized into an active ester, a mixed acid anhydride, or an acid halide, etc. and condensed with the N-hydroxysuccinimide, and can be preferably derivatized into an active esterified form and condensed with the N-hydroxysuccinimide.

[0317]

Active esterification can be preferably performed by using 3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The amount of the 3-(3-dimethylaminopropyl)carbodiimide hydrochloride used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.5 equivalents with respect to the compound represented by the formula (23).

[0318]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane,

pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and acetonitrile can be preferably exemplified.

[0319]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably 10 to 40°C. The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 2 to 8 hours.

[0320]

Step 34:

This step is a step of condensing the compound represented by the formula (24) with L-phenylalanine to convert it into the compound represented by the formula (6). The amount of the L-phenylalanine used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (24).

[0321]

This step preferably employs a base. The base used in this step is not particularly limited as long as the reaction proceeds. Examples thereof can include triethylamine, tributylamine, diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine, and N-

methylpiperidine, and triethylamine can be preferably exemplified. The amount of the triethylamine used in this step is not limited as long as the reaction proceeds. It is preferably 0.7 to 1.3 equivalents with respect to the compound represented by the formula (24).

[0322]

The solvent used in this step is not particularly limited as long as the reaction is not inhibited. Examples thereof can include acetonitrile, dichloromethane, chloroform, methanol, ethanol, diethyl ether, 1,2-dimethoxyethane, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, ethyl acetate, hexane, pentane, heptane, cyclohexane, ethylcyclohexane, benzene, toluene, chlorobenzene, acetone, 2-butanone, N,N-dimethylformamide, N,N-dimethylacetamide, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, and water, and mixed solvents thereof, and a mixed solvent of acetonitrile and water can be preferably exemplified.

[0323]

The reaction temperature of this step is not limited as long as the reaction proceeds. It is preferably the compound represented by formula (23). The reaction time of this step is not limited as long as the reaction proceeds. It is preferably 1 to 4 hours.

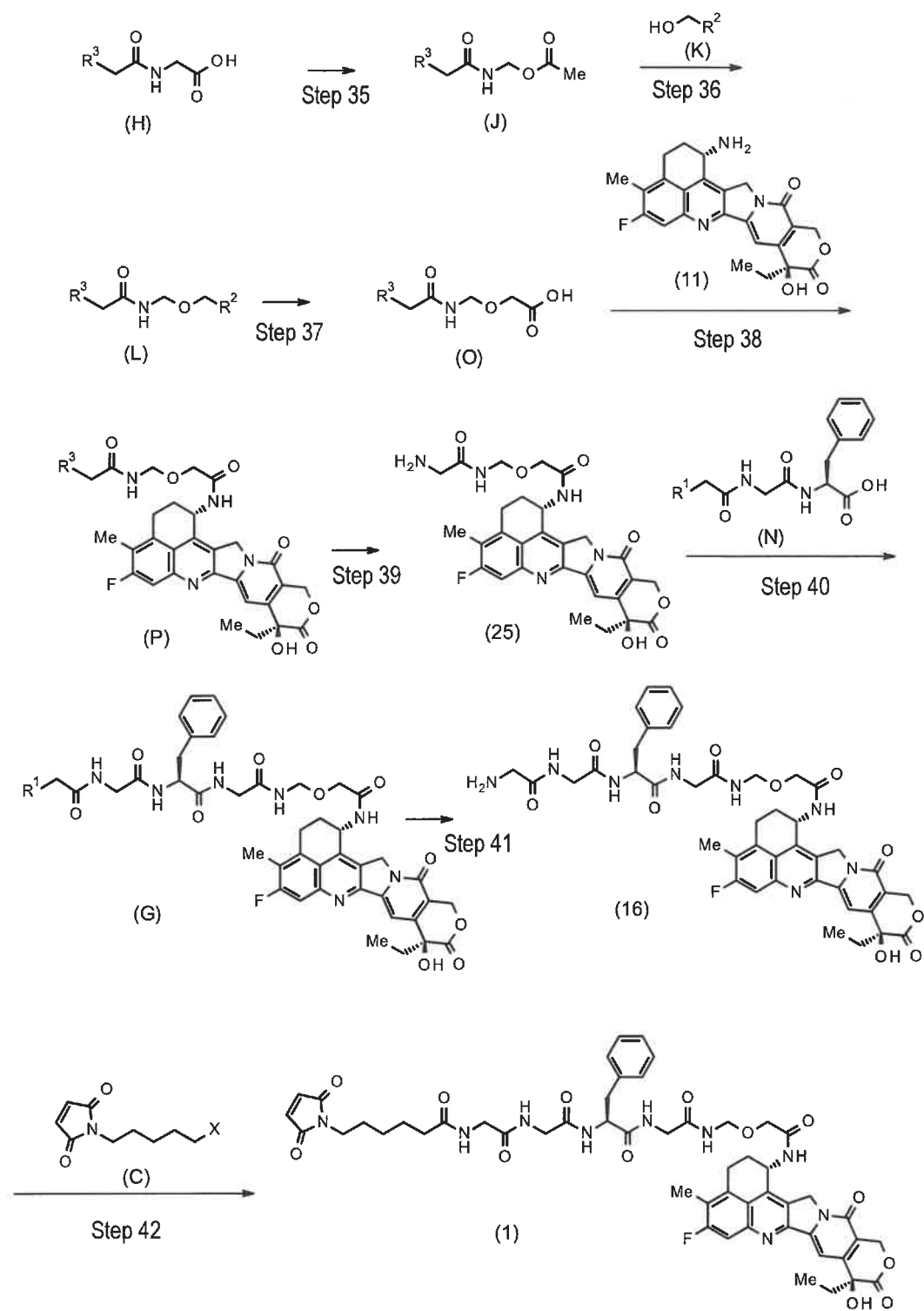
[0324]

[Production method (IX)]

The compound represented by the formula (1) can also be produced by the following production method (IX), and used.

[0325]

[Chem. 90]



[0326]

In the scheme, R^1 represents an amino group protected with a protecting group, R^2 represents a carboxy group protected with a protecting group, R^3 represents an amino group protected with a protecting group, and X represents an active ester group or a carboxy group, and preferably represents a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.
[0327]

Step 35:

This step is a step of reacting a compound represented by formula (H) with lead tetraacetate to convert it into a compound represented by formula (J). As the compound represented by the formula (H), a commercially available product or a compound produced with reference to a known method can be used. This step can be performed in the same manner as in the step 12 of the production method (IV).
[0328]

Step 36:

This step is a step of reacting the compound represented by the formula (J) with a compound represented by formula (K) in the presence of an acid or a base to convert it into a compound represented by formula (L). This step can be performed in the same manner as in the step 13 of the production method (IV).
[0329]

Step 37:

This step is a step of deprotecting the protecting group for the carboxy group of the compound represented by the formula (L) to convert it into a compound represented by formula (O).

[0330]

Deprotection of the protecting group for the carboxy group of the compound represented by the formula (L) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0331]

Step 38:

This step is a step of condensing the compound represented by the formula (O) with the compound represented by formula (11) to convert it into a compound represented by formula (P).

[0332]

The compound represented by the formula (O) can be preferably derivatized into an active ester and condensed with the compound represented by the formula (11).

[0333]

Step 39:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (P) to convert it into the compound represented by formula (25).

[0334]

Deprotection of the protecting group for the carboxy group of the compound represented by the formula (P) can be performed by a method well known in the art (see, for example, Peter G. M. Wuts, Theodora W. Greene, Greene's Protective Groups in Organic Synthesis 4th Edition (2007), Wiley-Interscience).

[0335]

Step 40:

This step is a step of condensing the compound represented by the formula (25) with a compound represented by formula (N) to convert it into a compound represented by formula (G).

[0336]

The compound represented by the formula (N) can be preferably derivatized into an active ester and condensed with the compound represented by the formula (25).

[0337]

Step 41:

This step is a step of deprotecting the protecting group for the amino group of the compound represented by the formula (G) to convert it into the compound represented by formula (16). This step can be performed in the same manner as in the step 10 of the production method (III).

[0338]

Step 42:

This step is a step of condensing the compound represented by the formula (16) with a compound represented by formula (C) to convert it into the compound represented by the formula (1). This step can be performed in the same manner as in the step 11 of the production method (III).

[0339]

[Antibody for use in the production of an antibody-drug conjugate]

The antibody for use in the production of the antibody-drug conjugate of the present invention may be derived from any species, and is preferably an antibody derived from a human, a rat, a mouse, or a rabbit. In cases where the antibody is derived from species other than human species, it is preferably chimerized or humanized using a well-known technique. The antibody of the present invention may be a polyclonal antibody or a monoclonal antibody and is preferably a monoclonal antibody.

[0340]

The antibody for use in the production of the antibody-drug conjugate of the present invention is an antibody preferably having a characteristic of being capable of targeting cancer cells, and is preferably an antibody possessing, for example, the property of recognizing a cancer cell, the property of binding to a

cancer cell, the property of internalizing in a cancer cell, and/or cytocidal activity against cancer cells.

[0341]

The binding activity of the antibody against cancer cells can be confirmed using flow cytometry. The internalization of the antibody into tumor cells can be confirmed using (1) an assay of visualizing an antibody incorporated in cells under a fluorescence microscope using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Cell Death and Differentiation (2008) 15, 751-761), (2) an assay of measuring a fluorescence intensity incorporated in cells using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Molecular Biology of the Cell, Vol. 15, 5268-5282, December 2004), or (3) a Mab-ZAP assay using an immunotoxin binding to the therapeutic antibody wherein the toxin is released upon incorporation into cells to inhibit cell growth (Bio Techniques 28: 162-165, January 2000). As the immunotoxin, a recombinant complex protein of a diphtheria toxin catalytic domain and protein G may be used.

[0342]

The antitumor activity of the antibody can be confirmed in vitro by determining inhibitory activity against cell growth. For example, a cancer cell line overexpressing a target protein for the antibody is

cultured, and the antibody is added into the culture system at varying concentrations to determine inhibitory activity against focus formation, colony formation, and spheroid growth. The antitumor activity can be confirmed *in vivo*, for example, by administering the antibody to a nude mouse with a transplanted cancer cell line highly expressing the target protein, and determining change in the cancer cell.

[0343]

Since the compound conjugated in the antibody-drug conjugate exerts an antitumor effect, it is preferred but not essential that the antibody itself should have an antitumor effect. For the purpose of specifically and selectively exerting the cytotoxic activity of the antitumor compound against cancer cells, it is important and also preferred that the antibody should have the property of internalizing to migrate into cancer cells.

[0344]

The antibody for use in the production of the antibody-drug conjugate of the present invention can be obtained by a procedure known in the art. For example, the antibody of the present invention can be obtained using a method usually carried out in the art, which involves immunizing animals with an antigenic polypeptide and collecting and purifying antibodies produced *in vivo*. The origin of the antigen is not limited to humans, and the animals may be immunized with an antigen derived from

a non-human animal such as a mouse, a rat and the like. In this case, the cross-reactivity of antibodies binding to the obtained heterologous antigen with human antigens can be tested to screen for an antibody applicable to a human disease.

[0345]

Alternatively, antibody-producing cells which produce antibodies against the antigen are fused with myeloma cells according to a method known in the art (e.g., Kohler and Milstein, *Nature* (1975) 256, p. 495-497; and Kennet, R. ed., *Monoclonal Antibodies*, p. 365-367, Plenum Press, N.Y. (1980)) to establish hybridomas, from which monoclonal antibodies can in turn be obtained.

[0346]

The antigen can be obtained by genetically engineering host cells to produce a gene encoding the antigenic protein. Specifically, vectors that permit expression of the antigen gene are prepared and transferred to host cells so that the gene is expressed. The antigen thus expressed can be purified. The antibody can also be obtained by a method of immunizing animals with the above-described genetically engineered antigen-expressing cells or a cell line expressing the antigen.

[0347]

The antibody for use in the production of the antibody-drug conjugate of the present invention is preferably a recombinant antibody obtained by artificial

modification for the purpose of decreasing heterologous antigenicity to humans such as a chimeric antibody or a humanized antibody, or is preferably an antibody having only the gene sequence of an antibody derived from a human, that is, a human antibody. These antibodies can be produced using a known method.

[0348]

As the chimeric antibody, an antibody in which antibody variable and constant regions are derived from different species, for example, a chimeric antibody in which a mouse- or rat-derived antibody variable region is connected to a human-derived antibody constant region can be exemplified (Proc. Natl. Acad. Sci. USA, 81, 6851-6855, (1984)).

[0349]

As the humanized antibody, an antibody obtained by integrating only the complementarity determining region (CDR) of a heterologous antibody into a human-derived antibody (Nature (1986) 321, pp. 522-525), and an antibody obtained by grafting a part of the amino acid residues of the framework of a heterologous antibody as well as the CDR sequence of the heterologous antibody to a human antibody by a CDR-grafting method (WO 90/07861), and an antibody humanized using a gene conversion mutagenesis strategy (U.S. Patent No. 5821337) can be exemplified.

[0350]

As the human antibody, an antibody generated by using a human antibody-producing mouse having a human chromosome fragment including genes of a heavy chain and light chain of a human antibody (see Tomizuka, K. et al., Nature Genetics (1997) 16, p. 133-143; Kuroiwa, Y. et. al., Nucl. Acids Res. (1998) 26, p. 3447-3448; Yoshida, H. et. al., Animal Cell Technology: Basic and Applied Aspects vol. 10, p. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et. al., Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727, etc.) can be exemplified. As an alternative, an antibody obtained by phage display, the antibody being selected from a human antibody library (see Wormstone, I. M. et. al, Investigative Ophthalmology & Visual Science. (2002)43 (7), p. 2301-2308; Carmen, S. et. al., Briefings in Functional Genomics and Proteomics (2002), 1 (2), p. 189-203; Siriwardena, D. et. al., Ophthalmology (2002) 109 (3), p. 427-431, etc.) can be exemplified.

[0351]

In the present invention, modified variants of the antibody for use in the production of the antibody-drug conjugate of the present invention are also included. The modified variant refers to a variant obtained by subjecting the antibody according to the present invention to chemical or biological modification. Examples of the chemically modified variant include

variants including a linkage of a chemical moiety to an amino acid skeleton, variants including a linkage of a chemical moiety to an N-linked or O-linked carbohydrate chain, etc. Examples of the biologically modified variant include variants obtained by post-translational modification (such as N-linked or O-linked glycosylation, N- or C-terminal processing, deamidation, isomerization of aspartic acid, or oxidation of methionine), and variants in which a methionine residue has been added to the N terminus by being expressed in a prokaryotic host cell. Further, an antibody labeled so as to enable the detection or isolation of the antibody or an antigen according to the present invention, for example, an enzyme-labeled antibody, a fluorescence-labeled antibody, and an affinity-labeled antibody are also included in the meaning of the modified variant. Such a modified variant of the antibody according to the present invention is useful for improving the stability and blood retention of the antibody, reducing the antigenicity thereof, detecting or isolating an antibody or an antigen, and so on.

[0352]

Further, by regulating the modification of a glycan which is linked to the antibody according to the present invention (glycosylation, defucosylation, etc.), it is possible to enhance antibody-dependent cellular cytotoxic activity. As the technique for regulating the

modification of a glycan of antibodies, WO 99/54342, WO 00/61739, WO 02/31140, etc. are known. However, the technique is not limited thereto. In the antibody according to the present invention, antibodies in which the modification of a glycan is regulated are also included.

[0353]

It is known that a lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell is deleted (Journal of Chromatography A, 705: 129-134 (1995)), and it is also known that two amino acid residues (glycine and lysine) at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell are deleted and a proline residue newly located at the carboxyl terminus is amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion and modification of the heavy chain sequence do not affect the antigen-binding affinity and the effector function (the activation of complement, antibody-dependent cellular cytotoxicity, etc.) of the antibody. Therefore, in the antibody according to the present invention, antibodies subjected to such modification and functional fragments of the antibody are also included, and deletion variants in which one or two amino acids have been deleted at the carboxyl terminus of the heavy chain, variants obtained by amidation of deletion variants (for

example, a heavy chain in which the carboxyl terminal proline residue has been amidated), and the like are also included. The type of deletion variant having a deletion at the carboxyl terminus of the heavy chain of the antibody according to the present invention is not limited to the above variants as long as the antigen-binding affinity and the effector function are conserved. The two heavy chains constituting the antibody according to the present invention may be of one type selected from the group consisting of a full-length heavy chain and the above-described deletion variant, or may be of two types in combination selected therefrom. The ratio of the amount of each deletion variant can be affected by the type of cultured mammalian cells which produce the antibody according to the present invention and the culture conditions; however, an antibody in which one amino acid residue at the carboxyl terminus has been deleted in both of the two heavy chains in the antibody according to the present invention can be preferably exemplified.

[0354]

As isotypes of the antibody according to the present invention, for example, IgG (IgG1, IgG2, IgG3, IgG4) can be exemplified, and IgG1 or IgG2 can be exemplified preferably.

[0355]

Examples of antibodies applicable to the production of the antibody-drug conjugate of the present invention can include, but are not particularly limited to, an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, an anti-B7-H3 antibody, an anti-CD3 antibody, an anti-CD30 antibody, an anti-CD33 antibody, an anti-CD37 antibody, an anti-CD56 antibody, an anti-CD98 antibody, an anti-DR5 antibody, an anti-EGFR antibody, an anti-EPHA2 antibody, an anti-FGFR2 antibody, an anti-FGFR4 antibody, an anti-FOLR1 antibody, an anti-VEGF antibody, and an anti-GPR20 antibody, and preferably an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, an anti-B7-H3 antibody, and an anti-GPR20 antibody can be exemplified.

[0356]

In the present invention, the term "anti-HER2 antibody" refers to an antibody which specifically binds to HER2 (Human Epidermal Growth Factor Receptor Type 2; ErbB-2), and preferably has an activity of internalizing in HER2-expressing cells by binding to HER2.

[0357]

Examples of the anti-HER2 antibody can include trastuzumab (U.S. Patent No. 5821337) and pertuzumab (International Publication No. WO 01/00245), and trastuzumab can be preferably exemplified.

[0358]

In the present invention, the term "trastuzumab" is a humanized anti-HER2 monoclonal antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 (Figure 1) and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2 (Figure 2).

[0359]

In the present invention, the term "anti-HER3 antibody" refers to an antibody which specifically binds to HER3 (Human Epidermal Growth Factor Receptor Type 3; ErbB-3), and preferably has an activity of internalizing in HER3-expressing cells by binding to HER3 on HER3-expressing cell surface.

[0360]

Examples of the anti-HER3 antibody can include patritumab (U3-1287), U1-59 (International Publication No. WO 2007/077028), MM-121 (seribantumab), an anti-ERBB3 antibody described in International Publication No. WO 2008/100624, RG-7116 (lumretuzumab), and LJM-716 (elgemtumab), and patritumab and U1-59 can be preferably exemplified.

[0361]

In the present invention, the term "anti-TROP2 antibody" refers to an antibody which specifically binds to TROP2 (TACSTD2: Tumor-associated calcium signal transducer 2; EGP-1), and preferably has an activity of

internalizing in TROP2-expressing cells by binding to TROP2.

[0362]

Examples of the anti-TROP2 antibody can include hTINA1-H1L1 (International Publication No. WO 2015/098099).

[0363]

In the present invention, the term "anti-B7-H3 antibody" refers to an antibody which specifically binds to B7-H3 (B cell antigen #7 homolog 3; PD-L3; CD276), and preferably has an activity of internalizing in B7-H3-expressing cells by binding to B7-H3.

[0364]

Examples of the anti-B7-H3 antibody can include M30-H1-L4 (International Publication No. WO 2014/057687).

[0365]

In the present invention, the term "anti-GPR20 antibody" refers to an antibody which specifically binds to GPR20 (G protein-coupled receptor 20), and preferably has an activity of internalizing in GPR20-expressing cells by binding to GPR20.

Examples of the anti-GPR20 antibody can include h046-H4e/L7 (International Publication No. WO 2018/135501).

[0366]

[Conjugation between the antibody and the drug-linker intermediate]

The antibody-drug conjugate of the present invention can be produced by reacting the compound represented by the formula (1) and an antibody having a thiol group (alternatively referred to as a sulfhydryl group).

[0367]

The crystals of the compound represented by the formula (1) of the present invention is preferably dissolved in a solvent to prepare a solution containing the compound represented by the formula (1), which can then be used in reaction. The solvent for use in this step is not particularly limited as long as the reaction is not inhibited. Preferably, a solvent containing dimethyl sulfoxide, dimethylformamide, dimethylacetamide, or N-methylpyrrolidone can be used, and a solvent containing dimethyl sulfoxide can be more preferably used.

[0368]

The antibody having a sulfhydryl group can be obtained by a method well known in the art (Hermanson, G. T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996)). For example, by using 0.3 to 3 molar equivalents of a reducing agent such as tris(2-carboxyethyl)phosphine hydrochloride (TCEP) per interchain disulfide within the antibody and reacting with the antibody in a buffer solution containing a chelating agent such as ethylenediamine tetraacetic acid (EDTA), an antibody having a sulfhydryl group with

partially or completely reduced interchain disulfides within the antibody can be obtained.

[0369]

Further, by using 2 to 20 molar equivalents of the compound represented by the formula (1) per antibody having a sulfhydryl group, an antibody-drug conjugate in which 2 to 8 drug molecules are conjugated per antibody molecule can be produced.

[0370]

The average number of conjugated drug molecules per antibody molecule of the antibody-drug conjugate produced can be determined, for example, by a method of calculation based on measurement of UV absorbance for the antibody-drug conjugate and the conjugation precursor thereof at two wavelengths of 280 nm and 370 nm (UV method), or a method of calculation based on quantification through HPLC measurement for fragments obtained by treating the antibody-drug conjugate with a reducing agent (HPLC method).

[0371]

Conjugation between the antibody and the drug-linker intermediate (compound represented by the formula (1)) and calculation of the average number of conjugated drug molecules per antibody molecule of the antibody-drug conjugate can be performed with reference to descriptions in International Publication No. WO 2014/057687, International Publication No. WO 2015/098099,

International Publication No. WO 2015/115091,
International Publication No. WO 2015/155998, and
International Publication No. WO 2018/135501, and so on.
[0372]

In the present invention, the term "anti-HER2
antibody-drug conjugate" refers to an antibody-drug
conjugate in which the antibody in the antibody-drug
conjugate is an anti-HER2 antibody.

[0373]

The anti-HER2 antibody is preferably 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 the amino acid sequence represented by SEQ
ID NO: 1 and a light chain consisting of the amino acid
sequence represented by SEQ ID NO: 2.

[0374]

The average number of units of the drug-linker
conjugated per antibody molecule in the anti-HER2
antibody-drug conjugate produced according to the present
invention is preferably 2 to 8, more preferably 3 to 8,
even more preferably 7 to 8, even more preferably 7.5 to
8, and even more preferably about 8.

[0375]

The anti-HER2 antibody-drug conjugate can be produced with reference to descriptions in International Publication No. WO 2015/115091 and so on by using the crystals of the compound represented by the formula (1) produced by the production method of the present invention.

[0376]

In the present invention, the term "anti-HER3 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in the antibody-drug conjugate is an anti-HER3 antibody.

[0377]

The anti-HER3 antibody is preferably an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 3 and a light chain consisting of the 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 of the antibody is deleted.

[0378]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-HER3 antibody-drug conjugate produced according to the present invention is preferably 2 to 8, more preferably 3 to 8, even more preferably 7 to 8, even more preferably 7.5 to 8, and even more preferably about 8.

[0379]

The anti-HER3 antibody-drug conjugate can be produced with reference to descriptions in International Publication No. WO 2015/155998 and so on by using the crystals of the compound represented by the formula (1) produced by the production method of the present invention.

[0380]

In the present invention, the term "anti-TROP2 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in the antibody-drug conjugate is an anti-TROP2 antibody.

[0381]

The anti-TROP2 antibody is preferably an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 20 to 470 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 of the antibody is deleted.

[0382]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-TROP2 antibody-drug conjugate produced according to the present invention is preferably 2 to 8, more preferably 3 to 5, even more preferably 3.5 to 4.5, and even more preferably about 4.

[0383]

The anti-TROP2 antibody-drug conjugate can be produced with reference to descriptions in International Publication No. WO 2015/098099 and so on by using the crystals of the compound represented by the formula (1) produced by the production method of the present invention.

[0384]

In the present invention, the term "anti-B7-H3 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in the antibody-drug conjugate is an anti-B7-H3 antibody.

[0385]

The anti-B7-H3 antibody is preferably 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 of the antibody is deleted.

[0386]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-B7-H3 antibody-drug conjugate produced according to the present invention is preferably 2 to 8, more preferably 3 to 5,

even more preferably 3.5 to 4.5, and even more preferably about 4.

[0387]

The anti-B7-H3 antibody-drug conjugate can be produced with reference to descriptions in International Publication No. WO 2014/057687 and so on by using the crystals of the compound represented by the formula (1) produced by the production method of the present invention.

[0388]

In the present invention, the term "anti-GPR20 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in the antibody-drug conjugate is an anti-GPR20 antibody.

[0389]

The anti-GPR20 antibody is preferably an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 20 to 472 of SEQ ID NO: 9 and a light chain consisting of an amino acid sequence consisting of amino acid residues 21 to 234 of SEQ ID NO: 10, or a variant of the antibody in which a lysine residue at the carboxyl terminus of the heavy chain of the antibody is deleted.

[0390]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-GPR20 antibody-drug conjugate produced according to the present

invention is preferably 2 to 8, more preferably 3 to 8, even more preferably 7 to 8, even more preferably 7.5 to 8, and even more preferably about 8.

[0391]

The anti-GPR20 antibody-drug conjugate can be produced with reference to descriptions in International Publication No. WO 2018/135501 and so on by using the crystals of the compound represented by the formula (1) produced by the production method of the present invention.

[0392]

[Pharmaceutical compositions]

The antibody-drug conjugate produced by the present invention can contain at least one pharmaceutically suitable ingredient and be administered. The pharmaceutically suitable ingredient can be suitably selected and applied from formulation additives or the like that are generally used in the art, according to the dosage, administration concentration, and so on of the antibody-drug conjugate produced by the present invention. For example, the antibody-drug conjugate produced by the present invention can be administered as a pharmaceutical composition containing a buffer such as a histidine buffer, an excipient such as sucrose or trehalose, and a surfactant such as polysorbate 80 or polysorbate 20.

[0393]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be expected to exert a therapeutic effect by application as a systemic therapy to patients, and additionally, by local application to cancer tissues.

[0394]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be preferably used for a mammal, and can be more preferably used for a human.

[0395]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be preferably used as an injection, can be more preferably used as an aqueous injection or a lyophilized injection, and can be even more preferably used as a lyophilized injection.

[0396]

In the case that the pharmaceutical composition containing the antibody-drug conjugate produced by the present invention is an aqueous injection, preferably, it can be diluted with a suitable diluent and then intravenously administered by drip infusion. Examples of the diluent can include glucose solution (preferably a 5% glucose solution) and physiological saline.

[0397]

In the case that the pharmaceutical composition containing the antibody-drug conjugate produced by the present invention is a lyophilized injection, preferably, it can be dissolved in water for injection, and then, a necessary amount can be diluted with a suitable diluent and then intravenously administered by drip infusion. Examples of the diluent can include a glucose solution (preferably a 5% glucose solution) and physiological saline.

[0398]

Examples of administration routes that can be used for administering the pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can include intravenous, intradermal, subcutaneous, intramuscular, and intraperitoneal routes, and an intravenous route can be preferably exemplified.

[0399]

The antibody-drug conjugate produced by the present invention can be administered to a human at intervals of once a day to every 180 days, preferably can be administered at intervals of once a week, every 2 weeks, every 3 weeks, or every 4 weeks, and even more preferably can be administered at intervals of once every 3 weeks. Also, the antibody-drug conjugate produced by the present invention can be administered at a dosage of about 0.001 to 100 mg/kg per dose, and preferably can be administered at a dosage of 0.8 to 12.4 mg/kg per dose. In the case

that the antibody-drug conjugate produced by the present invention is an anti-HER2 antibody-drug conjugate, it can be preferably administered at a dosage of 5.4, 6.4, or 7.4 mg/kg per dose, and more preferably can be administered at a dosage of 5.4 mg/kg or 6.4 mg/kg per dose.

[0400]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be used for treating cancer, and can be preferably used for treating at least one type of cancer selected from the group consisting of breast cancer, gastric cancer (also called gastric adenocarcinoma), colorectal cancer (also called colon and rectal cancer, and including colon cancer and rectal cancer), lung cancer (including small cell lung cancer and non-small cell lung cancer), esophageal cancer, salivary gland cancer, esophagogastric junction adenocarcinoma, bile duct cancer, Paget's disease, pancreatic cancer, ovarian cancer, uterine carcinosarcoma, urothelial cancer, prostate cancer, bladder cancer, gastrointestinal stromal tumor, digestive stromal tumor, uterine cervix cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, colon cancer, rectal cancer, endometrial cancer, uterine cancer, kidney cancer, vulval cancer, thyroid cancer, penile cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma,

neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, and sarcoma, and in the case that the antibody-drug conjugate produced by the present invention is an anti-HER2 antibody-drug conjugate, for example, it can be more preferably used for treating at least one type of cancer selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, esophageal cancer, salivary gland cancer, esophagogastric junction adenocarcinoma, bile duct cancer, Paget's disease, pancreatic cancer, ovarian cancer, and uterine carcinosarcoma, and can be even more preferably used for treating at least one type of cancer selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, esophageal cancer, salivary gland cancer, esophagogastric junction adenocarcinoma, bile duct cancer, and Paget's disease, and can be even more preferably used for treating breast cancer, gastric cancer, colorectal cancer, or non-small cell lung cancer.

[0401]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be selectively used as an agent for drug therapy, which is a main method for treating cancer, and as a result, can delay development of cancer cells, inhibit

growth thereof, and further kill cancer cells. These effects can allow cancer patients to be free from symptoms caused by cancer or achieve improvement in QOL of cancer patients and attain a therapeutic effect by sustaining the lives of the cancer patients. Even if the pharmaceutical composition and therapeutic method of the present invention do not accomplish killing cancer cells, it can achieve higher QOL of cancer patients while achieving longer-term survival, by inhibiting or controlling the growth of cancer cells.

[0402]

In such drug therapy, the pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be used as an agent alone and in addition, it can be used in combination with an additional therapy in adjuvant therapy and can be combined with surgery, radiotherapy, hormone therapy, or the like. Furthermore, it can also be used as an agent for drug therapy in neoadjuvant therapy.

[0403]

In addition to the therapeutic use as described above, for example, a prophylactic effect such as suppressing the growth of small metastatic cancer cells and further killing them can also be expected for the pharmaceutical composition containing the antibody-drug conjugate produced by the present invention. For example, an effect of inhibiting and killing cancer cells in a

body fluid in the course of metastasis or an effect of, for example, inhibiting and killing small cancer cells immediately after implantation in any tissue can be expected. Accordingly, inhibition of cancer metastasis or a prophylactic effect can be expected, particularly, after surgical removal of cancer.

[0404]

The pharmaceutical composition containing the antibody-drug conjugate produced by the present invention can be administered in combination with other cancer treating agents. The anti-cancer effect may be enhanced accordingly. Examples of other cancer treating agents used for such purpose can include 5-fluorouracil (5-FU), pertuzumab, trastuzumab, paclitaxel, carboplatin, cisplatin, gemcitabine, capecitabine, irinotecan (CPT-11), docetaxel, pemetrexed, sorafenib, vinblastin, vinorelbine, everolims, tanespimycin, bevacizumab, oxaliplatin, lapatinib, trastuzumab emtansine (T-DM1) or agents described in International Publication No. WO 2003/038043, LH-RH analogues (leuprorelin, goserelin, or the like), estramustine phosphate, estrogen antagonists (tamoxifen, raloxifene, or the like), and aromatase inhibitors (anastrozole, letrozole, exemestane, or the like), but are not limited as long as they are agents having an antitumor activity.

Examples

[0405]

The present invention is described in more detail below by way of examples. However, the present invention is not limited to these.

[0406]

In the Examples, the terms "¹H-NMR" and "¹³C-NMR" mean "nuclear magnetic resonance spectrum". Within parentheses, CDCl₃ means deuterated chloroform which is a measuring solvent, DMSO-d₆ means deuterated dimethyl sulfoxide which is a measuring solvent, D₂O means deuterium oxide which is a measuring solvent, and MeOH-d₄ means deuterated methanol which is a measuring solvent. TMS (tetramethylsilane) was used as an internal standard. The meanings of multiplicity in ¹H-NMR are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and brs = broad singlet.

[0407]

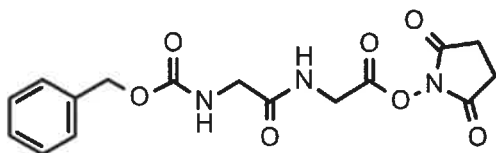
(Example 1)

2,5-Dioxopyrrolidin-1-yl N-

[(benzyloxy)carbonyl]glycylglycinate

[0408]

[Chem. 91]



(24)

[0409]

To a mixture of N-[(benzyloxy)carbonyl]glycylglycine (200.00 g, 0.751 mol) and acetonitrile (2.0 L), N-hydroxysuccinimide (95.10 g, 0.826 mol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (172.80 g, 0.901 mol) were added, and the resulting mixture was stirred at room temperature for about 4 hours. The reaction solution was cooled to 1°C and stirred for about 3 hours. Precipitates were filtered, and a powder separated by the filtration was washed with acetonitrile (400 mL). The obtained powder was dried under reduced pressure at 40°C to obtain 2,5-dioxopyrrolidin-1-yl N-[(benzyloxy)carbonyl]glycylglycinate (221.6 g, 0.610 mol, yield: 81.2%).

[0410]

¹H-NMR (400 MHz, DMSO-d₆) δ 2.81 (4H, s), δ 3.69 (2H, d, 6.7 Hz), δ 4.28 (2H, d, 6.1 Hz), δ 5.04 (2H, s), δ 7.29-7.39 (5H, m), δ 7.56 (1H, t, 6.4 Hz), δ 8.55 (1H, t, 5.8 Hz).

¹³C-NMR (100 MHz, DMSO-d₆) δ 25.4, 38.2, 43.3, 65.6, 127.7, 127.8, 128.3, 137.0, 156.5, 166.3, 170.0, 170.0.

MS (ESI) (m/z): 364 ([M+H]⁺).

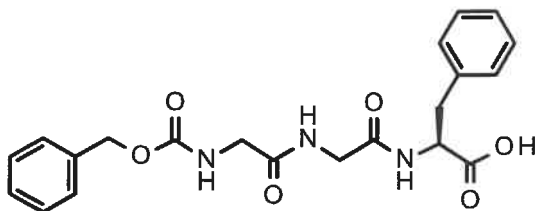
[0411]

(Example 2)

N-[(Benzyloxy)carbonyl]glycylglycyl-L-phenylalanine

[0412]

[Chem. 92]



(6)

[0413]

To a mixture of L-phenylalanine (80.0 g, 0.487 mol), acetonitrile (400 mL), and water (400 mL), triethylamine (74.7 mL, 0.536 mol) and 2,5-dioxopyrrolidin-1-yl N-[(benzyloxy)carbonyl]glycylglycinate (212.4 g, 0.585 mol) were added, and the resulting mixture was stirred at room temperature for about 2 hours. Water (800 mL) and concentrated hydrochloric acid (40.6 mL) were added to the reaction solution, then N-[(benzyloxy)carbonyl]glycylglycyl-L-phenylalanine (80 mg) was added, and the resulting mixture was stirred at room temperature for about 6 hours. Precipitates were filtered, and a powder separated by the filtration was washed with water (160 mL). The obtained powder was dried under reduced pressure at 40°C to obtain N-[(benzyloxy)carbonyl]glycylglycyl-L-phenylalanine (157.2 g, 0.380 mol, yield: 78.0%).

[0414]

^1H -NMR (400 MHz, DMSO- d_6) δ 2.86-2.91 (1H, m), δ 3.03-3.08 (1H, m), δ 3.64-3.78 (4H, m), δ 4.41-4.47 (1H, m), δ 5.04 (2H, s), δ 7.18-7.40 (10H, m), δ 7.50 (1H, t, 6.1 Hz), δ

8.05 (1H, t, 5.8 Hz), δ 8.17 (1H, d, 7.9 Hz), δ 12.77 (1H, s).

^{13}C -NMR (100 MHz, DMSO- d_6) δ 36.8, 41.6, 43.5, 53.5, 65.5, 126.5, 127.7, 127.8, 128.2, 128.3, 129.1, 137.0, 137.4, 156.5, 168.6, 169.3, 172.7.

MS (ESI) (m/z): 412 ($[\text{M}-\text{H}]^-$).

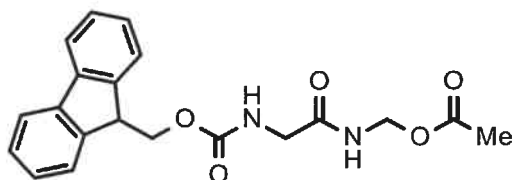
[0415]

(Example 3)

({N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate

[0416]

[Chem. 93]



(3)

[0417]

To N-[(9H-fluoren-ylmethoxy)carbonyl]glycylglycine (650.0 g, 1.834 mol), tetrahydrofuran (9.75 L) and acetic acid (1.95 L) were added, and the resulting mixture was dissolved by warming to 40°C. Lead tetraacetate (1301.3 g, 2.935 mol) was added thereto, and the resulting mixture was refluxed for about 1.5 hours. After cooling to room temperature, insoluble matter was filtered off, then the insoluble matter separated by the filtration was washed with ethyl acetate (3.25 L), and the washes were

combined with the filtrate. A 20 (w/v)% aqueous trisodium citrate dihydrate solution (3.25 L) was added to the obtained solution, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. The obtained organic layer was washed twice with a 20 (w/v)% aqueous trisodium citrate dihydrate solution (3.25 L), and then, the organic layer was concentrated to 6.5 L under reduced pressure. Water (1.95 L) was added to the residue, then ({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate (0.65 g) was added, and the resulting mixture was stirred at room temperature for about 1 hour. Water (6.5 L) was added dropwise thereto, and the resulting mixture was cooled to 0 to 5°C and stirred for about 3 hours. Precipitates were filtered, and a powder separated by the filtration was washed with a cold 30 (v/v)% aqueous tetrahydrofuran solution (2.6 L). The obtained powder was dried under reduced pressure at 40°C to obtain ({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate (617.1 g, 1.675 mol, yield: 91.3%).

[0418]

¹H-NMR (400 MHz, CDCl₃) δ 2.06 (3H, s), δ 3.90 (2H, d, 4.9 Hz), δ 4.23 (1H, t, 6.7 Hz), δ 4.45 (2H, d, 6.7 Hz), δ 5.25 (2H, d, 7.3 Hz), δ 5.39 (1H, brs), δ 7.05 (1H, brs), δ 7.30-7.34 (2H, m), δ 7.41 (2H, t, 7.3 Hz), δ 7.59 (2H, d, 7.3 Hz), δ 7.77 (2H, d, 7.3 Hz).

^{13}C -NMR (100 MHz, CDCl_3) δ 20.8, 44.4, 47.0, 63.9, 67.2, 120.0, 125.0, 127.1, 127.7, 141.3, 143.6, 156.6, 169.8, 171.7.

MS (ESI) (m/z): 369 ($[\text{M}+\text{H}]^+$).

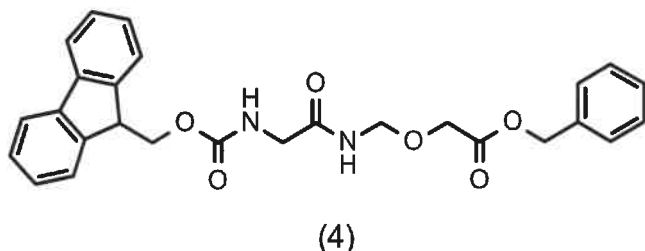
[0419]

(Example 4)

Benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate

[0420]

[Chem. 94]



[0421]

To a mixture of ({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate (610.0 g, 1.656 mol) and 1,2-dimethoxyethane (9.15 L), benzyl glycolate (470 mL, 3.312 mol) was added, and the resulting mixture was cooled to 0 to 5°C. A 10 mol/L sodium hydroxide solution (162.6 mL, 1.626 mol) was added thereto, and the resulting mixture was stirred for about 1 hour. Acetic acid (47.4 mL) was added thereto, and the resulting mixture was stirred at 1°C for about 1 hour. Then, water (2.0 L) was added dropwise thereto, then benzyl [({N-[(9H-fluoren-9-

ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (0.61 g) was added, and the resulting mixture was stirred at 0 to 5°C for about 1 hour. Water (4.7 L) was added dropwise thereto, and the resulting mixture was stirred at 0 to 5°C for about 2.5 hours. Precipitates were filtered, and a powder separated by the filtration was washed with a cold 50 (v/v)% aqueous 1,2-dimethoxyethane solution (2.44 L). To the obtained wet powder, 1,2-dimethoxyethane (9.15 L) was added, and the resulting mixture was dissolved by stirring at room temperature for about 30 minutes. Water (3.66 L) was added thereto, then benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (0.61 g) was added, and the resulting mixture was stirred at room temperature for about 1 hour. Water (3.05 L) was added dropwise thereto, and the resulting mixture was stirred at room temperature for about 1 hour. After cooling to 0 to 5°C and stirring for about 1 hour, precipitates were filtered, and a powder separated by the filtration was washed with a cold 50 (v/v)% aqueous 1,2-dimethoxyethane solution (2.44 L). To the obtained wet powder, 1,2-dimethoxyethane (9.0 L) was added, and the resulting mixture was dissolved by stirring at room temperature for about 30 minutes. Water (3.6 L) was added thereto, then benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (0.01 g) was added, and the resulting mixture was stirred at room

temperature for about 1 hour. Water (3.0 L) was added dropwise thereto, and the resulting mixture was stirred at room temperature for about 1 hour. After cooling to 0 to 5°C and stirring for about 1 hour, precipitates were filtered, and a powder separated by the filtration was washed with a cold 50% (v/v) aqueous 1,2-dimethoxyethane solution (2.4 L). To the obtained wet powder, 1,2-dimethoxyethane (9.0 L) was added, and the resulting mixture was dissolved by stirring at room temperature for about 20 minutes. Water (3.6 L) was added thereto, then benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (0.15 g) was added, and the resulting mixture was stirred at room temperature for about 2 hours. Water (3.0 L) was added dropwise thereto, and the resulting mixture was stirred at room temperature for about 1 hour. After cooling to 0 to 5°C and stirring for about 2 hours, precipitates were filtered, and a powder separated by the filtration was washed with a cold 50 (v/v)% aqueous 1,2-dimethoxyethane solution (2.4 L). The obtained powder was dried under reduced pressure at 40°C to obtain crude benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate. To the obtained crude benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate, toluene (12 L) was added, and the resulting mixture was dissolved by heating to 70°C. After cooling to 0 to 5°C and

stirring for about 2 hours, precipitates were filtered, and a powder separated by the filtration was washed with cold toluene (2.4 L). The obtained powder was dried under reduced pressure at 40°C to obtain benzyl [(N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl)amino)methoxy]acetate (575.5 g, 1.213 mol, yield: 73.2%).

[0422]

¹H-NMR (400 MHz, CDCl₃) δ 3.82 (2H, d, 4.9 Hz), δ 4.19-4.22 (3H, m), δ 4.45 (2H, d, 6.7 Hz), δ 4.83 (2H, d, 6.7 Hz), δ 5.15 (2H, s), δ 5.34 (1H, brs), δ 6.95 (1H, brs), δ 7.29-7.37 (7H, m), δ 7.40 (2H, t, 7.3 Hz), δ 7.58 (2H, d, 7.3 Hz), δ 7.76 (2H, d, 7.9 Hz).

¹³C-NMR (100 MHz, CDCl₃) δ 44.5, 47.1, 66.6, 66.8, 67.1, 70.6, 120.0, 124.9, 127.1, 127.8, 128.4, 128.5, 128.6, 135.2, 141.3, 143.6, 170.2, 170.2, 170.4.

MS (ESI) (m/z): 475 ([M+H]⁺).

[0423]

(Example 5)

Glycylglycyl-L-phenylalanyl-N-

[(carboxymethoxy)methyl]glycineamide

[0424]

[Chem. 95]

[0425]

To a mixture of benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (340.0 g, 0.717 mol) and acetonitrile (10.2 L), 1,8-diazabicyclo[5,4,0]-7-undecene (53.6 mL, 0.358 mol) was added, and the resulting mixture was stirred at room temperature for about 2 hours. After cooling to 0 to 5°C, 1-hydroxybenzotriazole monohydrate (132.0 g, 0.862 mol) and N-[(benzyloxy)carbonyl]glycylglycyl-L-phenylalanine (311.0 g, 0.752 mol) were added thereto, then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (158.0 g, 0.824 mol) was added in divided portions, and the resulting mixture was stirred at 0 to 5°C for about 1 hour. A 10 (w/v)% phosphate buffer solution (pH 3, 3.4 L) was added thereto, and the resulting mixture was warmed to room temperature. After separation into organic and aqueous layers and removal of the aqueous layer, the organic layer was concentrated to 3.7 L under reduced pressure. Ethyl acetate (3.4 L) and water (1.7 L) were added to the residue, and the resulting mixture was separated into organic and aqueous layers. The aqueous layer was removed. A 10 (w/v)% aqueous potassium

bicarbonate solution (3.4 L) was added thereto, and the resulting mixture was separated into organic and aqueous layers. The aqueous layer was removed. A 10 (w/v)% aqueous potassium bicarbonate solution (3.4 L) was added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. Water (3.4 L) was added thereto, and the resulting mixture was stirred. After separation into organic and aqueous layers and removal of the aqueous layer, the organic layer was concentrated to 1.5 L under reduced pressure. 2-Methoxyethanol (3.74 L) was added to the residue, and the resulting mixture was concentrated to 3.06 L under reduced pressure. The residue was transferred to a 20 L autoclave, then tetrahydrofuran (1.36 L), water (3.4 L), and 5% palladium carbon (72.6 g, water content: 53.2%) were added thereto, and the atmosphere was exchanged to hydrogen. After stirring at room temperature for about 19 hours, the atmosphere was exchanged to nitrogen, then water (360 mL) was added thereto, and the resulting mixture was stirred at room temperature for about 30 minutes. The palladium carbon was separated by filtration, then the palladium carbon was washed with water (1.36 L), and the washes were combined with the filtrate. Ethyl acetate (0.85 L) and n-heptane (2.55 L) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The organic layer was removed and

concentrated to 1.6 L under reduced pressure. Water (221 mL) and 2-methoxyethanol (126 mL) were added to the residue, then glycyglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (0.34 g) was added, and the resulting mixture was heated to 40°C and stirred for about 19 hours. Ethanol (3.4 L) was added dropwise thereto, and the resulting mixture was stirred at room temperature for about 18 hours. Precipitates were filtered, and a powder separated by the filtration was washed with ethanol (1.02 L). The obtained powder was dried under reduced pressure at 40°C to obtain glycyglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (243.7 g, 0.576 mol, yield: 80.3%).

[0426]

¹H-NMR (400 MHz, D₂O) δ 3.03-3.20 (2H, m), δ 3.79-3.96 (6H, m), δ 3.97 (2H, s), δ 4.64 (1H, t, 7.9 Hz), δ 4.67-4.75 (2H, m), δ 7.29-7.42 (5H, m).

¹³C-NMR (100 MHz, D₂O) δ 37.3, 41.0, 42.7, 43.2, 56.0, 67.3, 70.0, 127.8, 129.4, 129.8, 136.9, 168.4, 171.7, 172.9, 174.4, 178.1.

MS (ESI) (m/z): 422 ([M-H]⁻).

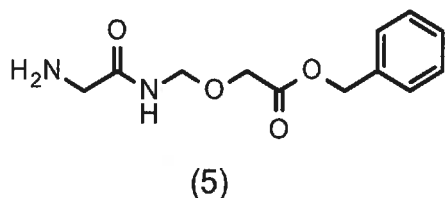
[0427]

(Example 6)

Benzyl [(glycylamino)methoxy]acetate 1H-benzotriazol-1-ol

[0428]

[Chem. 96]



[0429]

To a mixture of benzyl [(N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl)amino)methoxy]acetate (50.00 g, 105.4 mmol) and acetonitrile (1.5 L), 1,8-diazabicyclo[5,4,0]-7-undecene (8.02 g, 52.7 mmol) was added, and the resulting mixture was stirred at room temperature for about 4 hours. 1-Hydroxybenzotriazole monohydrate (35.51 g, 231.9 mmol) was added thereto in divided portions at room temperature, and the resulting mixture was stirred for about 30 minutes. The reaction mixture was cooled to 1°C and stirred for about 11 hours. Precipitates were filtered, and a powder separated by the filtration was washed with acetonitrile (250 mL). The obtained powder was dried under reduced pressure at 40°C to obtain benzyl [(glycylamino)methoxy]acetate 1H-benzotriazol-1-ol (38.98 g, 100.6 mol, yield: 95.4%).

[0430]

¹H-NMR (500 MHz, MeOH-d₄) δ 3.63-3.68 (2H, brs), δ 4.19-4.23 (2H, brs), δ 4.79 (2H, s), δ 5.16-5.20 (2H, brs), δ 7.25-7.38 (7H, m), δ 7.64-7.72 (2H, dd, 17.3 Hz, 7.8 Hz).
¹³C-NMR (125 MHz, MeOH-d₄) δ 41.9, 66.3, 67.6, 71.0, 112.3, 118.7, 125.24, 125.27, 128.8, 129.25, 129.30, 129.5, 136.9, 144.4, 169.5, 171.8

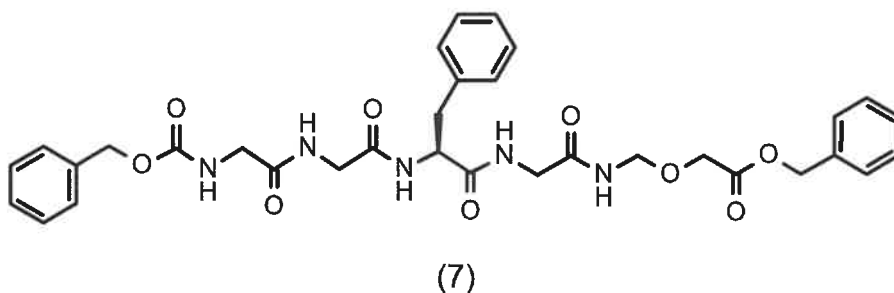
[0431]

(Example 7)

N-[(Benzyloxy)carboxyl]glycylglycyl-L-phenylalanyl-N-{[2-(benzyloxy)-2-oxoethoxy]methyl}glycinamide

[0432]

[Chem. 97]



[0433]

To a mixture of N-[(benzyloxy)carbonyl]glycylglycyl-L-phenylalanine (10.99 g, 26.58 mmol), acetonitrile (120 mL), and water (20 mL), benzyl [(glycylamino)methoxy]acetate 1H-benzotriazol-1-ol (10.00 g, 25.81 mmol) was added, and the resulting mixture was cooled to 2°C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.70 g, 29.73 mmol) was added thereto, and the resulting mixture was stirred at 0 to 5°C for about 3.5 hours. Ethanol (100 mL) and water (150 mL) were added to the reaction solution, and the resulting mixture was stirred at room temperature for 14 hours. Water (130 mL) was added thereto in divided portions, and the resulting mixture was stirred for 2 hours, then cooled to 1°C, and stirred for about 1 hour. Precipitates were filtered, and a powder separated by the

filtration was washed with acetonitrile:water = 1:2 (60 mL). The obtained powder was dried under reduced pressure at 40°C to obtain N-[(benzyloxy)carboxyl]glycylglycyl-L-phenylalanyl-N-{[2-(benzyloxy)-2-oxoethoxy)methyl]glycinamide (15.34 g, 23.68 mmol, yield: 91.7%).

[0434]

¹H-NMR (500 MHz, DMSO-d₆) δ 2.79 (1H, dd, 14 Hz, 9.2 Hz), δ 3.06 (1H, dd, 14 Hz, 4.5 Hz), δ 3.55-3.80 (6H, m), δ 4.15 (2H, s), δ 4.51 (1H, ddd, Hz, 9.2 Hz, 8.6 Hz, 4.5 Hz), δ 4.63 (2H, d, 6.5 Hz), δ 5.03 (2H, s), δ 5.15 (2H, s), δ 7.15-7.40 (15H, m), δ 7.15-7.40 (15H, m), δ 7.50 (1H, t, 6 Hz), δ 8.02 (1H, t, 5.8 Hz), δ 8.15 (1H, d, 8.6 Hz), δ 8.33 (1H, t, 5.8 Hz), δ 8.60 (1H, t, 7 Hz)

¹³C-NMR (125 MHz, DMSO-d₆) δ 37.4, 41.9, 42.2, 43.6, 54.2, 64.5, 65.6, 65.7, 69.1, 126.3, 127.77, 127.85, 128.10, 128.14, 128.2, 128.4, 128.5, 129.2, 135.8, 137.0, 137.9, 156.6, 168.9, 169.5, 169.9, 170.2, 171.5.

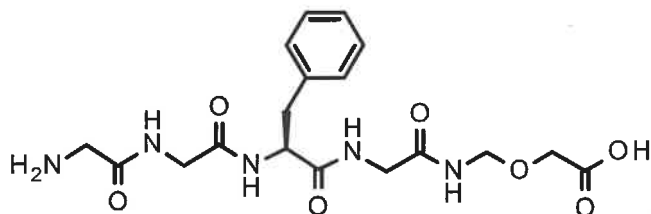
[0435]

(Example 8)

Glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide

[0436]

[Chem. 98]



[0437]

To a mixture of N-[(benzyloxy)carboxyl]glycylglycyl-L-phenylalanyl-N-{[2-(benzyloxy)-2-oxoethoxy]methyl}glycinamide (15.0 g, 23.16 mmol), tetrahydrofuran (315 mL), and water (210 mL), 5% palladium carbon (3.31 g, water content: 54.7%) was added, and the atmosphere was exchanged to hydrogen. After stirring at room temperature for about 2.5 hours, the atmosphere was exchanged to nitrogen. The palladium carbon was separated by filtration, then the palladium carbon was washed with water (60 mL), and the washes were combined with the filtrate. The resulting filtrate was concentrated to 240 mL under reduced pressure. Ethanol (150 mL) was added to the residue, and the resulting mixture was concentrated to 180 mL under reduced pressure. Ethanol (150 mL) was added to the residue, and the resulting mixture was concentrated to 135 mL under reduced pressure. Ethanol (150 mL) was added to the residue, and the resulting mixture was concentrated to 90 mL under reduced pressure. Ethanol (300 mL) was added to the residue, and the resulting mixture was stirred for 17 hours. Precipitates were filtered, and a powder

separated by the filtration was washed with ethanol (75 mL). The obtained powder was dried under reduced pressure at 40°C to obtain glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (8.95 g, 21.14 mmol, yield: 91.3%).

[0438]

$^1\text{H-NMR}$ (400 MHz, D_2O) δ 3.03–3.20 (2H, m), δ 3.79–3.96 (6H, m), δ 3.97 (2H, s), δ 4.64 (1H, t, 7.9 Hz), δ 4.67–4.75 (2H, m), δ 7.29–7.42 (5H, m).

$^{13}\text{C-NMR}$ (100 MHz, D_2O) δ 37.3, 41.0, 42.7, 43.2, 56.0, 67.3, 70.0, 127.8, 129.4, 129.8, 136.9, 168.4, 171.7, 172.9, 174.4, 178.1.

MS (ESI) (m/z): 422 ($[\text{M-H}]^-$).

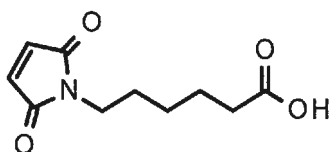
[0439]

(Example 9)

6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid

[0440]

[Chem. 99]



(18)

[0441]

To a solution of 6-aminohexanoic acid (2.5 kg, 19.1 mol) in acetic acid (10 L), a solution of maleic anhydride (1.87 kg, 19.1 mol) in acetic acid (10 L) was added dropwise at 25 to 30°C over 1 hour, and the

resulting mixture was stirred at the same temperature as the above for 2 hours. To the obtained slurry, sulfuric acid (0.93 kg, 9.55 mol) was added dropwise, and the resulting mixture was heated to 100°C and then stirred for 16 hours. The reaction solution was cooled to 30°C and then concentrated to 7.0 L under reduced pressure. The obtained concentrate (about 7.0 L) was added dropwise to cold water (20 L) of 0 to 5°C over 1 hour under stirring conditions, and the resulting mixture was stirred at the same temperature as the above for 1 hour. Precipitates were filtered, and a powder separated by the filtration was washed with cold water (5.0 L). The obtained powder was dried under reduced pressure at 40°C to obtain 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (1.46 kg, 6.95 mol, yield: 36.4%).

[0442]

The obtained 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (1.40 kg, 6.66 mol) was dissolved in a mixed solution of acetic acid (2.1 L) and purified water (1.4 L) at 25 to 30°C. To the solution, purified water (0.7 L) was added, and then, the resulting mixture was cooled to 20 to 25°C and then stirred for 2 hours. To the obtained suspension, purified water (7.0 L) was added dropwise over 1 hour, and the resulting mixture was cooled to 0 to 5°C and then stirred for 1 hour. Precipitates were filtered, and a powder separated by the filtration was washed with cold water (2.1 L). The

obtained powder was dried under reduced pressure at 40°C to obtain 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (1.27 kg, 6.02 mol, yield: 90.4%).

[0443]

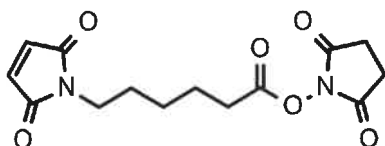
¹H-NMR (400 MHz, DMSO-d₆) δ 1.18-1.24 (2H, m), δ 1.45-1.52 (4H, m), δ 2.18 (2H, t, 7.5 Hz), δ 3.38 (2H, t, 7.5 Hz), δ 7.01 (2H, s), δ 11.98 (1H, s).

(Example 10)

1-{6-[(2,5-Dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione

[0444]

[Chem. 100]



(9)

[0445]

To a mixture of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (5.0 g, 23.6 mmol), N-hydroxysuccinimide (3.0 g, 26.0 mmol), and acetonitrile (50 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.45 g, 28.4 mmol) was added, and the resulting mixture was stirred at room temperature for about 3.5 hours. Water (100 mL) and toluene (100 mL) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The aqueous layer was removed. The organic layer was washed

twice with water (50 mL), and the organic layer was concentrated to 25 mL under reduced pressure. A silica gel cartridge (KP-sil 10 g) was charged with the residue, then toluene:acetone = 9:1 (100 mL) was passed therethrough, and an eluate was recovered and concentrated to 25 mL under reduced pressure. 1-Butanol (50 mL) was added to the residue, then 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione (10 mg) was added, and the resulting mixture was stirred at room temperature for 1 hour. 1-Butanol (50 mL) was added dropwise thereto, and the resulting mixture was cooled to -10°C and stirred. Precipitates were filtered, and a powder separated by the filtration was washed with cold 1-butanol (20 mL). The obtained powder was dried under reduced pressure at 40°C to obtain 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione (6.52 g, 21.1 mmol, yield: 89.4%).

[0446]

¹H-NMR (400 MHz, DMSO-d₆) δ 1.27-1.35 (2H, m), δ 1.48-1.56 (2H, m), δ 1.59-1.67 (2H, m), δ 2.65 (2H, t, 7.3 Hz), δ 2.81 (4H, s), δ 3.39 (2H, t, 7.0 Hz), δ 7.00 (2H, s).

¹³C-NMR (100 MHz, DMSO-d₆) δ 23.7, 25.1, 25.4, 27.4, 30.0, 36.8, 134.4, 168.9, 170.2, 171.1.

MS (ESI) (m/z): 309 ([M+H]⁺).

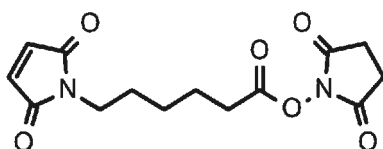
[0447]

(Example 11)

1-{6-[(2,5-Dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione

[0448]

[Chem. 101]



(9)

[0449]

A mixed solution of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (1.1 kg, 5.21 mol), N-hydroxysuccinimide (0.72 kg, 6.25 mol), and acetonitrile (11 L) was cooled to -15°C. 2,6-Lutidine (1.34 kg, 12.50 mol) was added thereto, and then, thionyl chloride (0.74 kg, 6.25 mol) was added dropwise at -15°C to -10°C over 1 hour. Water (11 L) and toluene (11 L) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The aqueous layer was removed. The organic layer was washed twice with cold water (11 L) of 0 to 5°C and washed with 20% saline (11 L) of 0 to 5°C, and the organic layer was concentrated to 5.5 L under reduced pressure. Then, toluene (5.5 L) was added to the residue, and the resulting mixture was concentrated again to 5.5 L under reduced pressure. A funnel was packed with neutral silica gel (Silica gel 60N, 3.3 kg) wetted with toluene, then the concentrate was passed therethrough, and the funnel was washed with

toluene:acetone = 9:1 (29 L) to obtain a filtrate. The obtained filtrate was concentrated to 5.5 L under reduced pressure, then 1-butanol (8.8 L) was added to the residue, and then, the resulting mixture was stirred at 20 to 25°C for 16 hours. 1-Butanol (13.2 L) was added dropwise thereto, and the resulting mixture was cooled to -15°C and stirred for 1 hour. Precipitates were filtered, and a powder separated by the filtration was washed with cold 1-butanol (4.4 L). The obtained powder was dried under reduced pressure at 40°C to obtain 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione (1.45 kg, 4.72 mol, yield: 90.5%).

[0450]

¹H-NMR (400 MHz, DMSO-d₆) δ 1.27-1.35 (2H, m), δ 1.48-1.56 (2H, m), δ 1.59-1.67 (2H, m), δ 2.65 (2H, t, 7.3 Hz), δ 2.81 (4H, s), δ 3.39 (2H, t, 7.0 Hz), δ 7.00 (2H, s).

¹³C-NMR (100 MHz, DMSO-d₆) δ 23.7, 25.1, 25.4, 27.4, 30.0, 36.8, 134.4, 168.9, 170.2, 171.1.

MS (ESI) (m/z): 309 ([M+H]⁺).

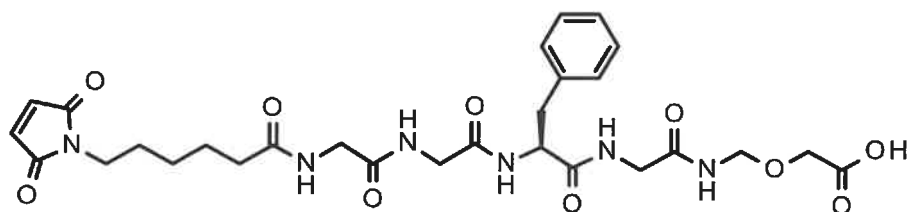
[0451]

(Example 12)

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide

[0452]

[Chem. 102]



(10)

[0453]

To a solution of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione (291.3 g, 0.945 mol) in acetonitrile (1.8 L), glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (200.0 g, 0.472 mol), water (4.2 L), and N,N-diisopropylethylamine (48.8 g, 0.378 mol) were added, and the resulting mixture was stirred at room temperature for about 9 hours. Isopropyl acetate (2.0 L), anhydrous sodium dihydrogen phosphate (400.0 g), and anhydrous disodium hydrogen phosphate (26.0 g) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The organic layer was removed. Tetrahydrofuran (1.0 L), ethyl acetate (1.0 L), and anhydrous sodium dihydrogen phosphate (160.0 g) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The aqueous layer was removed. A 10 (w/v)% phosphate buffer solution (pH 3.4, 0.6 L) was added thereto, and the resulting mixture was stirred. After separation into organic and aqueous layers and removal of the aqueous layer, the organic layer was concentrated to 1.0 L under

reduced pressure. 1,2-Dimethoxyethane (4.0 L) was added to the residue, and the resulting mixture was concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (4.0 L) and acetonitrile (0.4 L) were added to the residue, and the resulting mixture was concentrated to 1.0 L under reduced pressure. Acetonitrile (20 mL) was added to the residue, and the water content of the solution was measured and was consequently 6.1% (corresponding to 18.8 mL of water). Water (19 mL) was added thereto, then N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (0.2 g) was added, then 1,2-dimethoxyethane (0.8 L) was added dropwise, and the resulting mixture was stirred at room temperature for about 16 hours. 1,2-Dimethoxyethane (3.2 L) was added dropwise thereto, and the resulting mixture was concentrated to 4.0 L under reduced pressure. 1,2-Dimethoxyethane (1.0 L) was added to the residue, and the resulting mixture was cooled to 0 to 5°C and stirred for about 19.5 hours. Precipitates were filtered, and a powder separated by the filtration was washed with 1,2-dimethoxyethane (0.8 L). The obtained powder was dried under reduced pressure at 40°C to obtain N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (268.3 g, 0.435 mol, yield: 92.1%).

[0454]

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ 1.15-1.23 (2H, m), δ 1.43-1.52 (4H, m), δ 2.11 (2H, t, 7.3 Hz), δ 2.78-2.84 (1H, m), δ 3.04-3.09 (1H, m), δ 3.37 (2H, t, 7.0 Hz) δ 3.61-3.79 (6H, m), δ 3.94 (2H, s), δ 4.47-4.52 (1H, m), δ 4.61 (2H, d, 6.7 Hz), δ 6.99 (2H, s), δ 7.15-7.27 (5H, m), δ 8.11-8.15 (2H, m), δ 8.22 (1H, d, 8.5 Hz), δ 8.31 (1H, t, 5.8 Hz), δ 8.63 (1H, t, 6.4 Hz).

$^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6) δ 24.6, 25.8, 27.8, 34.9, 37.0, 37.2, 41.9, 42.1, 42.1, 54.2, 65.1, 69.2, 126.2, 128.1, 129.1, 134.4, 137.9, 168.9, 169.5, 169.8, 171.1, 171.4, 171.9, 172.6.

MS (ESI) (m/z): 615 ($[\text{M-H}]^-$).

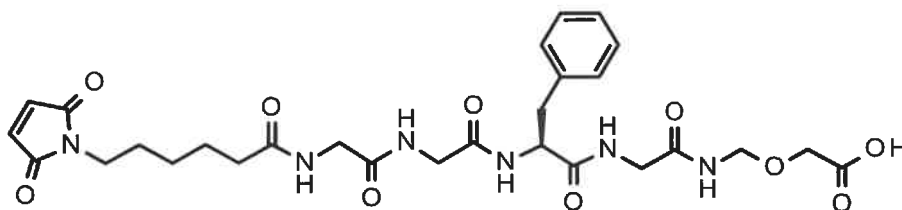
[0455]

(Example 13)

1,2-Dimethoxyethane adduct of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide

[0456]

[Chem. 103]



(10)

[0457]

To a solution of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione (72.8 g, 0.236

mol) in acetonitrile (450.0 mL), glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (50.0 g, 0.118 mol), water (1050.0 mL), and N,N-diisopropylethylamine (16.5 mL, 0.095 mol) were added, and the resulting mixture was stirred at room temperature for about 15 hours. Isopropyl acetate (500.0 mL), anhydrous sodium dihydrogen phosphate (100.0 g), and anhydrous disodium hydrogen phosphate (6.5 g) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The organic layer was removed. Isopropyl acetate (500.0 mL) was added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The organic layer was removed. 1,2-Dimethoxyethane (250.0 mL), ethyl acetate (250.0 mL), acetonitrile (25.0 mL), and anhydrous sodium dihydrogen phosphate (400.0 g) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. The aqueous layer was removed. Acetonitrile (750.0 mL), water (113.0 mL), sodium chloride (30.0 g), anhydrous sodium dihydrogen phosphate (7.5 g), and phosphoric acid (85%, 1.5 g, 0.012 mol) were added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. Water (113.0 mL), sodium chloride (30.0 g), and anhydrous sodium dihydrogen phosphate (7.5 g) were added thereto, and the resulting mixture was stirred and separated into

organic and aqueous layers. The aqueous layer was removed. Water (113 mL), sodium chloride (30.0 g), and anhydrous sodium dihydrogen phosphate (7.5 g) were added thereto, and the resulting mixture was stirred. After separation into organic and aqueous layers and removal of the aqueous layer, the organic layer was concentrated to 500.0 mL under reduced pressure. 1,2-Dimethoxyethane (750.0 mL) was added to the residue, and then, the resulting mixture was concentrated to 500.0 mL under reduced pressure. The water content of the solution was measured and was consequently 6.9% (corresponding to 31.3 g of water). Water (9.5 mL) and 1,2-dimethoxyethane (1.0 L) were added thereto, and then, the resulting mixture was stirred at room temperature for about 13 hours. 1,2-Dimethoxyethane (250.0 mL) was added dropwise thereto, and the resulting mixture was stirred at room temperature for about 5 hours and then concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (1.0 L) was added dropwise to the residue, and the resulting mixture was stirred at room temperature for about 1 hour and then concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (250.0 mL) was added dropwise to the residue, and the resulting mixture was stirred at room temperature for about 16 hours. Then, precipitates were filtered, and a powder separated by the filtration was washed with 1,2-dimethoxyethane (250.0 L). To the obtained wet powder, 1,2-dimethoxyethane (2.0 L) and

water (65.0 mL) were added, and the resulting mixture was heated to 45°C. After stirring for 30 minutes, the sodium chloride was separated by filtration, then the sodium chloride was washed with 1,2-dimethoxyethane/water (97/3, 150 mL), and the washes were combined with the filtrate. The resulting mixture was concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (1.0 L) was added to the residue, and the resulting mixture was stirred at room temperature for about 3 hours and then concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (1.0 L) was added dropwise to the residue, and the resulting mixture was concentrated to 1.0 L under reduced pressure. 1,2-Dimethoxyethane (250.0 mL) was added dropwise to the residue, and the resulting mixture was stirred at room temperature for about 16 hours. Then, precipitates were filtered, and a powder separated by the filtration was washed with 1,2-dimethoxyethane (250 mL). The obtained powder was dried under reduced pressure (4 kPa) at 25°C to obtain a 1,2-dimethoxyethane adduct of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (65.7 g, 0.107 mol, yield: 90.3%) in the form of crystals.

[0458]

¹H-NMR (500 MHz, DMSO-d₆) δ 1.16-1.23 (2H, m), δ 1.44-1.52 (4H, m), δ 2.11 (2H, t, 7.5 Hz), δ 2.79-2.84 (1H, m), δ 3.05-3.09 (1H, m), δ 3.24 (6H, s), δ 3.37 (2H, t, 7.3 Hz),

δ 3.43 (4H, s), δ 3.56-3.78 (6H, m), δ 3.99 (2H, s), δ 4.48-4.52 (1H, m), δ 4.61 (2H, d, 6.5 Hz), δ 7.00 (2H, s), δ 7.16-7.27 (5H, m), δ 8.02-8.10 (2H, m), δ 8.15 (1H, d, 8.0 Hz), δ 8.32 (1H, t, 6.0 Hz), δ 8.58 (1H, t, 6.8 Hz). δ 12.61 (1H, brs)

^{13}C -NMR (100 MHz, DMSO- d_6) δ 25.5, 26.8, 28.7, 35.9, 37.9, 38.2, 42.8, 43.0, 43.1, 55.1, 59.0, 65.2, 69.8, 72.0, 127.2, 129.0, 130.1, 135.4, 138.8, 169.8, 170.4, 170.9, 172.0, 172.3, 172.4, 173.6.

MS (ESI) (m/z): 615 ([M-H]⁻).

[0459]

Powder X-ray diffraction:

The crystals of the title compound were subjected to powder X-ray diffraction obtained by irradiation with copper K α radiation. The results are shown in Table 1 and Figure 3. Main peaks were observed at diffraction angles (2 θ) of 19.0° and 25.0°.

[0460]

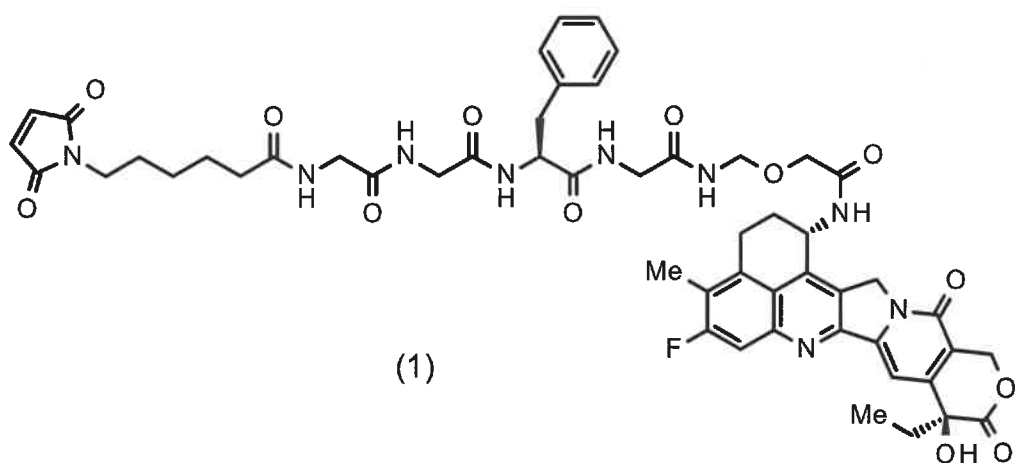
[Table 1]

Diffraction angle 2 θ (°)	Interplanar spacing d (Å)	Relative intensity (%)
7.0	12.6	32.0
12.4	7.1	40.9
19.0	4.7	82.9
25.0	3.6	100.0
25.2	3.5	59.5

[0461]

(Example 14)

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide
 [0462]
 [Chem. 104]



[0463]

To a suspension of (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-aminium methanesulfonate dihydrate (gross amount: 154.6 g, internal content after correction with a water content value of 2.95%: 150.0 g, 0.282 mol) in tetrahydrofuran (1.8 L), a 5 (w/v)% aqueous sodium sulfate solution (1.5 L) and N-methylmorpholine (28.5 g, 0.282 mol) were added, and the resulting mixture was stirred at 32°C for about 1 hour. Ethyl

cyano(hydroxyimino)acetate (8.0 g, 56.3 mmol), N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (gross amount: 232.0 g, internal content after conversion into 2.50% 1,2-dimethoxyethane: 226.2 g, 0.367 mol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (108.2 g, 0.564 mol) were added thereto, and the resulting mixture was stirred at 29 to 32°C for about 1 hour and then separated into organic and aqueous layers. The aqueous layer was removed. Ethyl acetate (1.8 L) and a 5 (v/v)% aqueous acetic acid solution (0.45 L) were added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. Activated carbon (15.0 g, Kyoryoku Shirasagi (manufactured by Osaka Gas Chemicals Co., Ltd.)) was added thereto, and the resulting mixture was stirred at room temperature for about 30 minutes. Then, the activated carbon was separated by filtration, then the activated carbon was washed with tetrahydrofuran (0.45 L), and the washes were combined with the filtrate. The resulting mixture was concentrated to 0.75 L under reduced pressure. 1-Propanol (1.5 L) was added to the residue, and the resulting mixture was concentrated to 0.75 L under reduced pressure. Acetone:1-propanol = 1:1 (3.0 L) was added to the residue. N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-

4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide (0.15 g) was added thereto, and the resulting mixture was stirred at room temperature for about 45 hours. Precipitates were filtered, and a powder separated by the filtration was washed with acetone:1-propanol = 1:1 (0.6 L). The obtained wet powder was dissolved by the addition of tetrahydrofuran (1.5 L) and water (0.3 L), and the solution was concentrated to 0.75 L under reduced pressure. 1-Propanol (1.5 L) was added to the residue, and the resulting mixture was concentrated to 0.75 L under reduced pressure. Acetone:1-propanol = 1:1 (3.0 L) was added to the residue. N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide (0.15 g) was added thereto, and the resulting mixture was stirred at room temperature for about 24 hours. Precipitates were filtered, and crystals separated by the filtration were washed with acetone:1-propanol = 1:1 (0.6 L). The obtained crystals were dried under reduced pressure at 40°C to obtain N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-

2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (254.6 g, yield: 87.3%) in the form of crystals.

[0464]

¹H-NMR (400 MHz, DMSO-d₆) δ 0.87 (3H, t, 7.3 Hz), δ 1.14-1.21 (2H, m), δ 1.41-1.50 (4H, m), δ 1.78-1.93 (2H, m), δ 2.09 (2H, t, 7.3 Hz), δ 2.13-2.23 (2H, m), δ 2.36 (3H, s), δ 2.74-2.80 (1H, m), δ 3.00-3.04 (1H, m), δ 3.08-3.25 (2H, m), δ 3.32-3.37 (2H, m), δ 3.56-3.77 (6H, m), δ 4.02 (2H, s), δ 4.44-4.50 (1H, m), δ 4.64 (2H, d, 6.7 Hz), δ 5.17 (2H, d, 5.5 Hz), δ 5.41 (2H, s), δ 5.57-5.62 (1H, m), δ 6.51 (1H, s), δ 6.99 (2H, s), δ 7.14-7.26 (5H, m), δ 7.30 (1H, s), δ 7.75 (1H, d, 11.0 Hz), δ 8.00 (1H, t, 5.8 Hz), δ 8.06 (1H, t, 5.3 Hz), δ 8.12 (1H, d, 7.9 Hz), δ 8.29 (1H, t, 5.8 Hz), δ 8.49 (1H, d, 8.5 Hz), δ 8.62 (1H, t, 6.7 Hz).

¹³C-NMR (100 MHz, DMSO-d₆) δ 7.6, 10.8, 10.9, 23.5, 24.6, 25.7, 27.7, 30.2, 30.6, 34.8, 36.9, 37.1, 41.7, 42.0, 44.4, 49.5, 54.1, 65.1, 66.9, 69.7, 72.2, 96.6, 109.6, 109.8, 119.0, 121.5, 123.4, 123.6, 125.3, 126.2, 128.0, 129.0, 134.3, 136.2, 136.3, 137.7, 140.4, 145.0, 147.7, 147.8, 149.9, 152.2, 156.6, 160.2, 162.7, 168.8, 169.1, 169.3, 170.0, 171.0, 171.3, 172.3, 172.5.

MS (ESI) (m/z): 1034 ([M+H]⁺).

[0465]

Powder X-ray diffraction:

The crystals of the title compound were subjected to powder X-ray diffraction obtained by irradiation with copper K α radiation. The results are shown in Table 2 and Figure 4. Main peaks were observed at diffraction angles (2θ) of 5.6°, 15.5°, and 22.0°.

[0466]

[Table 2]

Diffraction angle 2θ (°)	Interplanar spacing d (Å)	Relative intensity (%)
5.6	15.9	100.0
5.8	15.3	41.6
15.5	5.7	73.6
17.9	5.0	35.0
20.5	4.3	35.1
21.4	4.2	31.4
22.0	4.0	74.9

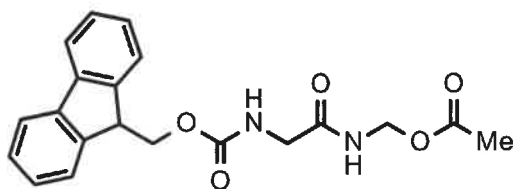
[0467]

(Example 15)

((N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycyl)amino)methyl acetate

[0468]

[Chem. 105]



(3)

[0469]

To a suspension of N-9-fluorenylmethoxycarbonylglycylglycine (2.85 kg, 8.04 mol) in anhydrous tetrahydrofuran (38.0 kg), acetic acid (2.41 kg, 40.1 mol) and lead(IV) tetraacetate (5.35 kg, 12.0 mol) were added under a nitrogen atmosphere, and the resulting mixture was refluxed for 1.5 hours. After cooling to room temperature, the precipitated solid was separated by filtration, and the solid thus separated by filtration was washed with tetrahydrofuran (10.1 kg). The obtained filtrate and washes were concentrated under reduced pressure until the amount of the liquid became about 16 L. To the obtained concentrate, ethyl acetate (26 kg), a 10% aqueous citric acid solution (17.1 L), and 20% saline (5.7 L) were added, and the resulting mixture was stirred and then separated into organic and aqueous layers. The obtained organic layer was separated into organic and aqueous layers and washed with a 10% aqueous citric acid solution (17.1 L), a 9% aqueous sodium bicarbonate solution (28.5 L), and 20% saline (14.3 L) in this order. To the obtained organic layer, silica gel 60 (5.7 kg) and ethyl acetate (10.3 kg) were added, and the resulting mixture was stirred for 1 hour. Then, a solid was separated by filtration, and the solid thus separated by filtration was washed with ethyl acetate (7.7 kg). The obtained filtrate and washes were concentrated under reduced pressure until the amount of the liquid became about 5 L. Cyclopentyl methyl ether (24.5 kg) was added

to the residue. The resulting mixture was concentrated again under reduced pressure until the amount of the liquid became about 5 L. To the obtained concentrate, cyclopentyl methyl ether (14.7 kg) was added, and the resulting mixture was stirred at about 5°C for 1 hour. The precipitated solid was filtered, and the obtained crystals were washed with cyclopentyl methyl ether (4.9 kg) cooled to about 5°C. The obtained crystals were dried under reduced pressure at 40°C to obtain the title compound (2.01 kg, yield: 68%) as a colorless solid.

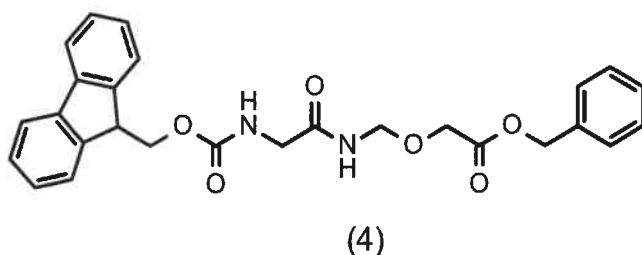
[0470]

(Example 16)

Benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate

[0471]

[Chem. 106]



[0472]

To a suspension of ({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methyl acetate (2.01 kg, 5.46 mol) in anhydrous 1,2-dimethoxyethane (21 kg), benzyl glycolate (1.81 kg, 10.9 mol) was added under a nitrogen atmosphere, and the resulting mixture was cooled

to about 0°C. Tris(pentafluorophenyl)borane (142 g, 0.27 mol) was added thereto, and the resulting mixture was stirred at the same temperature as the above for 3 hours. Then, ethyl acetate (27.1 kg) and a 10% aqueous potassium bicarbonate solution were added thereto, and the resulting mixture was heated to room temperature and separated into organic and aqueous layers. The obtained organic layer was separated into organic and aqueous layers and washed by the addition of 10% saline (20.1 L). The obtained organic layer was concentrated under reduced pressure until the amount of the liquid became about 4 L. Methanol (15.7 kg) was added to the residue. The resulting mixture was concentrated under reduced pressure until the amount of the liquid became about 4 L. To the obtained concentrate, methanol (7.8 kg) was added. The resulting mixture was concentrated under reduced pressure until the amount of the liquid became about 4 L. To the obtained concentrate, methanol (12.5 kg) was added, and the resulting mixture was cooled to about 5°C and stirred for 1 hour. The precipitated crystals were filtered, and the obtained crystals were washed with methanol (4.7 kg) cooled to about 5°C. The obtained solid was dried under reduced pressure at 40°C to obtain the title compound (2.28 kg, yield: 88%).

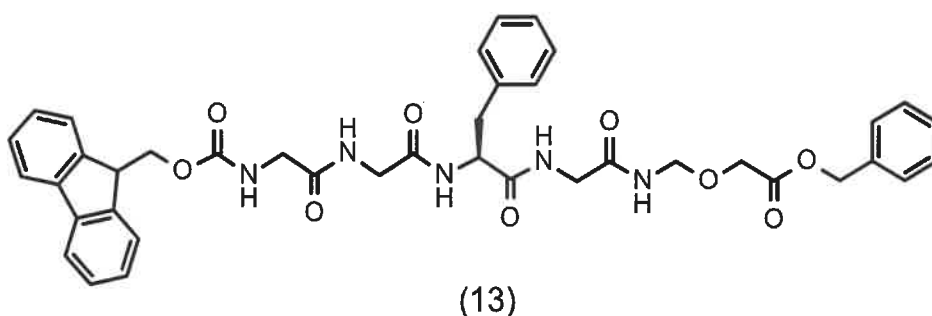
[0473]

(Example 17)

Benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl}amino)methoxy]acetate

[0474]

[Chem. 107]



[0475]

To a solution of benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycyl}amino)methoxy]acetate (2.28 kg, 4.81 mol) in N,N-dimethylacetamide (15.0 kg), 1,8-diazabicyclo[5.4.0]undec-7-ene (0.37 kg, 2.4 mol) was added under a nitrogen atmosphere, and the resulting mixture was stirred at room temperature for 30 minutes. Pyridinium p-toluenesulfonate (0.60 kg, 2.4 mol), 1-hydroxybenzotriazole monohydrate (0.74 kg, 4.8 mol), N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanine (2.19 kg, 4.37 mol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.84 kg, 4.37 mol) were added thereto, and the resulting mixture was stirred at room temperature for 3 hours. Ethyl acetate (21.0 kg) and 10% saline (34 L) were added thereto, and the resulting mixture was stirred and then

separated into organic and aqueous layers. The obtained organic layer was separated into organic and aqueous layers and washed with a 10% aqueous citric acid solution (11.4 L). To the obtained organic layer, tetrahydrofuran (20 kg) and a 15% aqueous potassium bicarbonate solution (22.8 L) were added, and the resulting mixture was stirred and separated into organic and aqueous layers. The obtained organic layer was separated into organic and aqueous layers and washed with 10% saline (22.8 L). The obtained organic layer was concentrated under reduced pressure until the amount of the liquid became about 6.8 L. 2-Propanol (12.4 kg) was added to the residue. The resulting mixture was concentrated again under reduced pressure until the amount of the liquid became about 6.8 L. To the obtained concentrate, 2-propanol (30.2 kg) was added under warming at about 50°C. The resulting mixture was stirred at the same temperature as the above for 1 hour, then cooled to about 5°C, and further stirred for 2 hours. The precipitated solid was filtered, and the solid separated by the filtration was washed with 2-propanol (14.2 kg) cooled to about 5°C. The obtained crystals 2 were suspended in 2-propanol (36 kg), and the suspension was stirred at about 5°C for 1 hour. Then, the precipitated solid was filtered, and the solid separated by the filtration was washed with 2-propanol (28.5 kg) cooled to about 5°C. The obtained crystals

were dried under reduced pressure at 50°C to obtain the title compound (3.34 kg, yield: 94%).

[0476]

¹H-NMR (400 MHz, DMSO-d₆) δ 2.79 (1H, dd, J=14.0, 9.8 Hz), 3.05 (1H, dd, J=14.0, 4.3 Hz), 3.58-3.79 (6H, m), 4.15 (2H, s), 4.20-4.24 (1H, m), 4.28-4.30 (2H, m), 4.48-4.53 (1H, m), 4.63 (2H, d, J=6.7 Hz), 5.14 (2H, s), 7.15-7.43 (13H, m), 7.58 (1H, t, J=6.1 Hz), 7.71 (2H, d, J=7.3 Hz), 7.89 (2H, d, J=7.9 Hz), 8.01 (1H, t, J=5.5 Hz), 8.15 (1H, d, J=7.9 Hz), 8.33 (1H, t, J=5.8 Hz), 8.59 (1H, t, J=6.4 Hz).

¹³C-NMR (100 MHz, DMSO-d₆) δ 37.3, 41.8, 42.1, 43.5, 46.6, 54.1, 64.4, 65.6, 65.7, 69.0, 120.1, 125.2, 126.3, 127.1, 127.6, 128.0, 128.1, 128.1, 128.4, 129.1, 135.8, 137.8, 140.7, 143.8, 156.5, 168.8, 169.4, 169.9, 170.1, 171.4.

MS (ESI) (m/z): 736 ([M+H]⁺).

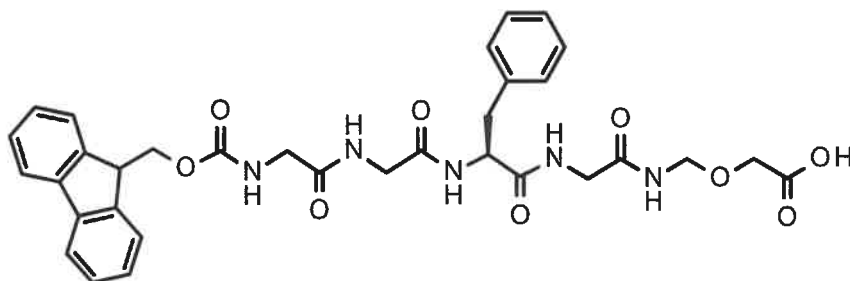
[0477]

(Example 18)

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide

[0478]

[Chem. 108]



(14)

[0479]

To a suspension of benzyl [({N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl}amino)methoxy]acetate (367 g, 0.499 mol) in tetrahydrofuran (5.88 kg) and water (1.61 L), a palladium carbon-ethylenediamine complex (28 g) was added, and the resulting mixture was stirred at room temperature for 1 hour to 3 hours under a hydrogen gas atmosphere at ordinary pressure. The catalyst was separated by filtration, and the catalyst thus separated by filtration was washed with tetrahydrofuran (1.63 kg) to obtain a filtrate and washes. The above-described operation of reaction and separation of a catalyst by filtration was repetitively performed 9 times, and the obtained 9 portions of filtrates and washes were combined. The resulting mixture was concentrated under reduced pressure until the amount of the liquid became about 17 L. To the obtained concentrate, 2-propanol (39 kg) was added, and the operation of concentration under reduced pressure until the amount of the liquid became about 17 L was repetitively performed three times. To the obtained concentrate, ethyl acetate (45 kg) was added, and the resulting mixture was stirred at room temperature for 6 hours. This suspension was further stirred at about 5°C for 1 hour. The precipitated solid was filtered, and the solid separated by the filtration was washed with a 1:3 mixed solution of 2-propanol and ethyl acetate (23.1 L)

cooled to about 5°C. The obtained crystals were dried under reduced pressure at 40°C to obtain a crude form of the title compound (2.18 kg, yield: 75%). The obtained crude form (400 g, 0.62 mol) was suspended in tetrahydrofuran (2.4 L) and ethyl acetate (5.6 L). To the suspension, a 1% aqueous potassium bisulfate solution (4 L) was added, and the resulting mixture was dissolved by warming to about 32°C and stirring. After separation into organic and aqueous layers, the obtained organic layer was separated into organic and aqueous layers and washed with water (2 L). The obtained organic layer was concentrated under reduced pressure until the amount of the liquid became about 2 L. To the obtained concentrate, acetonitrile (6 L) was added, and the resulting mixture was concentrated under reduced pressure until the amount of the liquid became about 2.8 L. As a result, a solid was precipitated. Ethyl acetate (6 L) was added thereto, and the resulting mixture was stirred at room temperature for 18 hours, then cooled to about 5°C, and stirred for 3 hours. The precipitated solid was filtered, and the crystals separated by the filtration were washed with a 1:2 mixed solution of acetonitrile and ethyl acetate (7 L). The obtained solid was dried under reduced pressure at 40°C to obtain the title compound (356 g, yield: 89%).

[0480]

¹H-NMR (400 MHz, DMSO-d₆) δ 2.79 (1H, dd, J=14.0, 9.8 Hz), 3.06 (1H, dd, J=13.7, 4.6 Hz), 3.58-3.79 (6H, m), 3.98

(2H, s), 4.21-4.25 (1H, m), 4.28-4.30 (2H, m), 4.48-4.54 (1H, m), 4.61 (2H, d, J=6.7 Hz), 7.16-7.20 (1H, m), 7.22-7.27 (4H, m), 7.33 (2H, t, J=7.3 Hz), 7.42 (2H, t, J=7.3 Hz), 7.59 (1H, t, J=6.1 Hz), 7.71 (2H, d, J=7.3 Hz), 7.89 (2H, d, J=7.3 Hz), 8.03 (1H, t, J=5.5 Hz), 8.16 (1H, d, J=7.9 Hz), 8.33 (1H, t, J=5.8 Hz), 8.57 (1H, t, J=6.7 Hz).

¹³C-NMR (100 MHz, CDCl₃) δ 37.4, 41.8, 42.1, 43.5, 46.6, 54.1, 64.2, 65.7, 68.8, 120.1, 125.2, 126.3, 127.1, 127.6, 128.1, 129.1, 137.8, 140.7, 143.8, 156.5, 168.8, 169.4, 170.0, 171.4, 171.4.

MS (ESI) (m/z): 646 ([M+H]⁺).

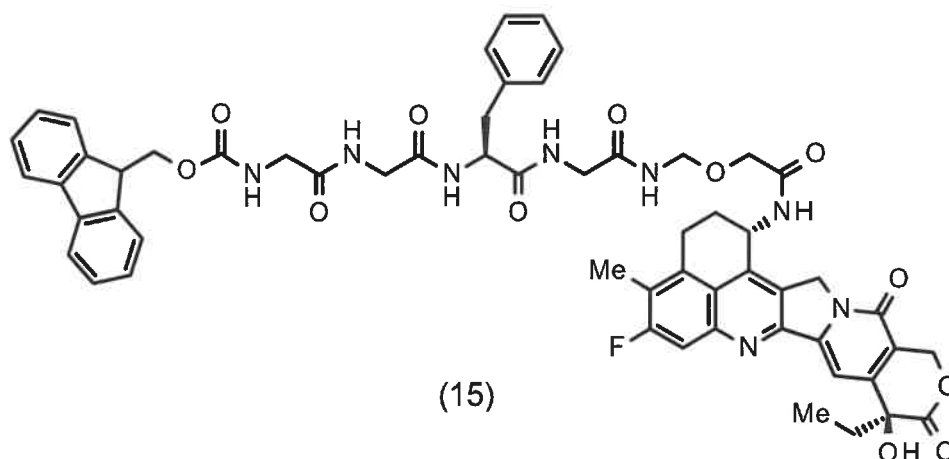
[0481]

(Example 19)

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide

[0482]

[Chem. 109]



[0483]

To a suspension of (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-aminium methanesulfonate dihydrate (260 g, 0.458 mol) in dimethyl sulfoxide (1.8 L) and tetrahydrofuran (1.3 L), triethylamine (55.6 g, 0.549 mol), 1-hydroxybenzotriazole monohydrate (84.2 g, 0.549 mol), N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (325 g, 0.503 mol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (114 g, 0.595 mol) were added under a nitrogen atmosphere, and the resulting mixture was stirred at room temperature for 2 hours. Tetrahydrofuran (3.9 L), ethyl acetate (2.6 L), and a 11% aqueous potassium bicarbonate solution (5.2 L) were added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The obtained organic layer was washed with a 19% aqueous citric acid

solution (3.9 L), a 22% aqueous potassium bicarbonate solution (2.6 L), and 18% saline (0.78 L) in this order. To the obtained organic layer, activated carbon (52 g) was added, and the resulting mixture was stirred for 30 minutes. Then, tetrahydrofuran (0.78 L) and anhydrous magnesium sulfate (0.78 g) were added thereto, and the resulting mixture was stirred for 30 minutes. A solid was separated by filtration, and the solid thus separated by filtration was washed with tetrahydrofuran (0.78 L). The obtained filtrate and washes were concentrated under reduced pressure until the amount of the liquid became about 200 mL. To the obtained concentrate, ethyl acetate (1.3 L) was added, and the resulting mixture was concentrated under reduced pressure until the amount of the liquid became about 200 mL. To the obtained concentrate, tetrahydrofuran (1.8 L) was added. The obtained solution was added dropwise over 12 minutes to a mixed solution of ethyl acetate (1.3 L) and cyclopentyl methyl ether (1.3 L) prepared in another container. To this suspension, cyclopentyl methyl ether (2.6 L) was added, and the resulting mixture was stirred for 18 hours, then cooled to about 5°C, and further stirred for 1 hour. The precipitated solid was filtered, and the solid separated by the filtration was washed with a 1:3 mixed solution of tetrahydrofuran and cyclopentyl methyl ether (1.3 L). The obtained solid was dried under reduced

pressure at 40°C to obtain the title compound (408 g, yield: 84%).

[0484]

¹H-NMR (400 MHz, DMSO-d₆) δ 0.86 (3H, t, J=7.3), 1.79-1.90 (2H, m), 2.11-2.22 (2H, m), 2.37 (3H, s), 2.77 (1H, dd, J=14.0, 9.8 Hz), 3.02 (1H, dd, J=13.7, 4.6 Hz), 3.07-3.25 (2H, m), 3.58-3.79 (6H, m), 4.02 (2H, s), 4.18-4.23 (1H, m), 4.26-4.30 (2H, m), 4.45-4.54 (1H, m), 4.64 (2H, d, J=6.7 Hz), 5.17 (2H, dd, J=23.5, J=19.2 Hz), 5.40 (2H, s), 5.56-5.61 (1H, m), 6.52 (1H, s), 7.14-7.43 (10H, m), 7.58 (1H, t, J=6.1 Hz), 7.68 (2H, d, J=7.3 Hz), 7.76 (1H, d, J=11.0 Hz), 7.86 (2H, d, J=7.3 Hz), 8.02 (1H, t, J=5.5 Hz), 8.15 (1H, d, J=7.9 Hz), 8.32 (1H, t, J=5.8 Hz), 8.50 (1H, d, J=8.5 Hz), 8.63 (1H, t, J=6.4 Hz).

¹³C-NMR (100 MHz, DMSO-d₆) δ 7.7, 10.9, 11.0, 23.1, 23.7, 27.8, 30.3, 31.4, 37.3, 41.8, 42.1, 43.5, 44.6, 46.6, 49.6, 54.2, 55.6, 65.2, 65.8, 67.0, 69.8, 72.3, 82.0, 96.7, 109.7, 109.9, 119.1, 120.0, 121.6, 123.5, 123.7, 125.2, 125.3, 126.3, 127.0, 127.6, 128.1, 129.1, 136.3, 136.4, 137.8, 140.5, 140.7, 143.8, 143.8, 145.1, 147.8, 147.9, 150.0, 152.3, 156.5, 156.7, 160.3, 162.8, 168.9, 169.2, 169.4, 170.2, 171.4, 172.4.

MS (ESI) 1063: (M+H)⁺

[0485]

(Example 20)

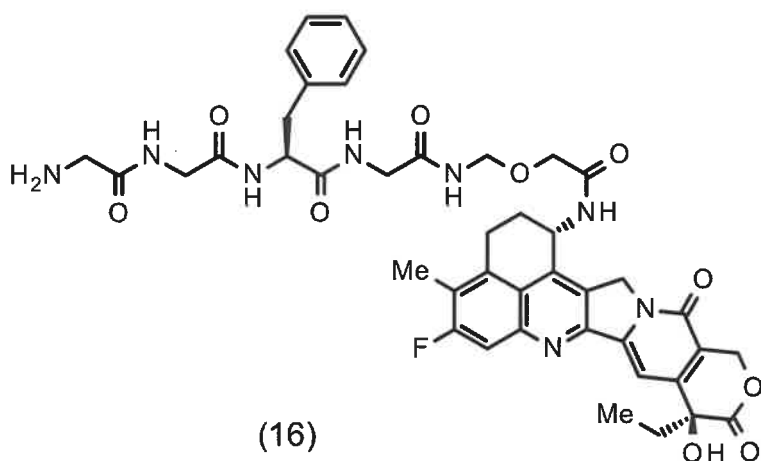
Glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-

hexahydro-1H,12H-

benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide

[0486]

[Chem. 110]



[0487]

To a suspension of N-[(9H-fluoren-9-ylmethoxy)carbonyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (400 g, 0.376 mol) in dehydrated tetrahydrofuran (8 L), 1,8-diazabicyclo[5.4.0]undec-7-ene (51.6 g, 0.339 mol) was added every 5 minutes in 8 divided portions under a stream of nitrogen gas, and the resulting mixture was stirred for 2.5 hours. The precipitated solid was filtered under the stream of nitrogen gas, and the solid separated by the filtration was washed with

tetrahydrofuran (2.4 L). The obtained solid was dried under reduced pressure at 40°C to obtain a mixture containing the title compound (363 g, yield: 115%).

[0488]

¹H-NMR (400 MHz, DMSO-d₆) δ 0.87 (3H, t, J=7.3), 1.57-1.67 (6H, m), 1.80-1.92 (2H, m), 2.06-2.25 (2H, m), 2.35-2.38 (3H, m), 2.61-2.63 (2H, m), 2.73-2.89 (1H, m), 3.00-3.79 (29H, m), 3.80 (1H, dd, J=16.2, 7.0 Hz), 3.99-4.10 (2H, m), 4.30-4.51 (1H, m), 4.58 (1H, dd, J=9.8, 6.1 Hz), 4.63-4.69 (1H, m), 5.01 (0.5H, br), 5.15 (1H, t, J=18.3 Hz), 5.24 (1H, t, J=18.3 Hz), 5.41 (2H, s), 5.54-5.62 (1H, m), 6.52 (0.6H, br), 7.11-7.31 (6H, m), 7.75-7.79 (1H, m), 8.12-8.15 (0.6H, m), 8.22 (0.2H, d, J=8.5 Hz), 8.36 (0.2H, t, J=5.8 Hz), 8.52 (0.2H, t, J=5.5 Hz), 8.66 (0.2H, t, J=6.4 Hz), 8.93 (0.6H, t, J=5.5 Hz), 9.10 (1H, dd, J=20.1, 9.2 Hz), 9.82 (0.6H, br).

MS (ESI) 841: (M+H)⁺

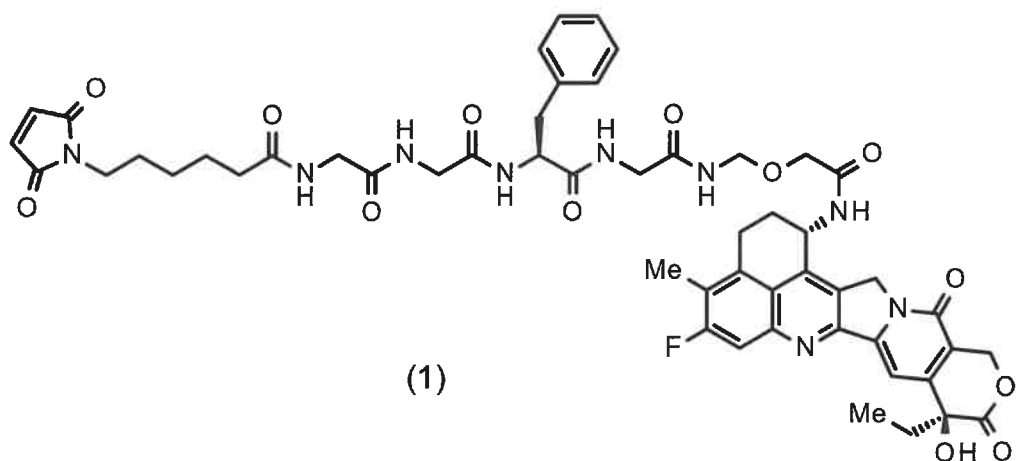
[0489]

(Example 21)

Preparation of seed crystals of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide

[0490]

[Chem. 111]



[0491]

To a suspension of glycylglycyl-L-phenylalanyl-N-[(2-{{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide (200 mg, 0.24 mmol) in pyridine (0.2 mL), tetrahydrofuran (2.0 mL) and acetonitrile (0.6 mL), pyridinium p-toluenesulfonate (120 mg, 0.48 mmol), triethylamine (100 μ L, 0.72 mmol), and N-succinimidyl 6-maleimidohexanoate (73 mg, 0.24 mmol) were added, and the resulting mixture was stirred at room temperature for 3 hours. The reaction solution was purified by silica gel flash column chromatography (Biotage AB) [tetrahydrofuran:acetone = 3:7 to 7:3 (v/v)] to obtain the title compound as an oil. To 19.5 mg of the obtained oil, acetone (0.4 mL) and 2-butanol (0.2 mL) were added, and the resulting mixture was warmed to about 60°C. The precipitated solid was filtered at room temperature, and the solid separated by the filtration

was washed with 2-butanol (about 0.2 mL) to obtain the title compound (14.3 mg) as a colorless powder. The obtained powder was used as a seed crystal in the next reaction.

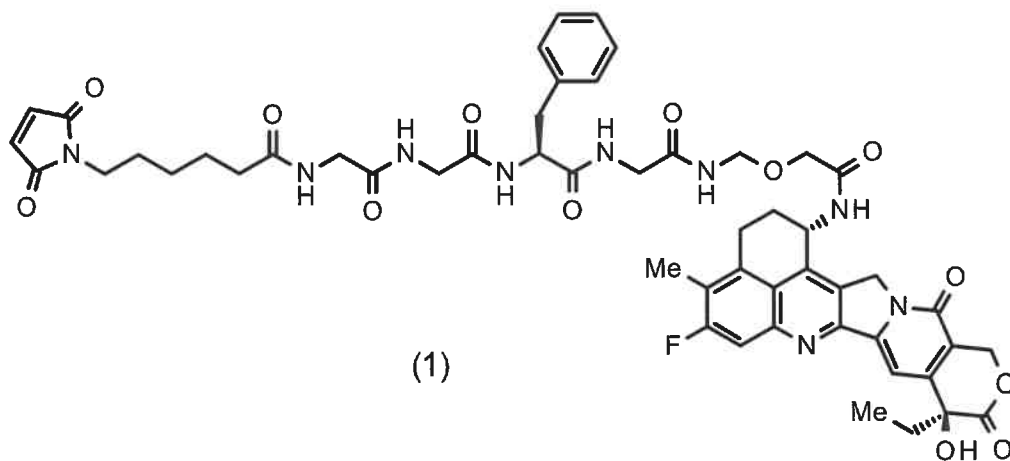
[0492]

(Example 22)

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide

[0493]

[Chem. 112]



[0494]

In pyridine (0.35 L), acetonitrile (1.1 L), and tetrahydrofuran (3.5 L), pyridinium p-toluenesulfonate (209 g, 0.832 mol), N-succinimidyl 6-maleimidohexanoate (128 g, 0.415 mol), and glycylglycyl-L-phenylalanyl-N-

[(2-([(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (350 g, 0.416 mol) were dissolved under a nitrogen atmosphere. To the solution, triethylamine (63.2 g, 0.625 mol) was then added, and the resulting mixture was stirred at room temperature for 3.5 hours. Tetrahydrofuran (3.5 L), a 19% aqueous citric acid solution (3.5 L), ethyl acetate (2.5 L), and 18% saline (2.5 L) were added thereto, and the resulting mixture was stirred and then separated into organic and aqueous layers. To the obtained organic layer, a 19% aqueous citric acid solution (2.5 L) and 18% saline (2.5 L) were added, and the resulting mixture was stirred and then separated into organic and aqueous layers. The obtained organic layer was separated into organic and aqueous layers and washed with a 22% aqueous potassium bicarbonate solution (2.1 L) and subsequently with 18% saline (1.8 L). The obtained organic layer was added dropwise to a suspension of activated carbon (35 g) in acetonitrile (35 L) prepared in another container, and the resulting mixture was stirred for 30 minutes. Then, the activated carbon was separated by filtration, and the activated carbon thus separated by filtration was washed with acetonitrile (1.8 L). The obtained filtrate and washes were concentrated under reduced pressure at an external temperature of about 40°C until the solvent was

no longer distilled off. To the obtained concentrated residue, acetone (1.8 L) and 1-propanol (3.5 L) were added in this order, and the resulting mixture was dissolved by warming to 55°C. Then, the solution was cooled to room temperature. The powder (0.2 g) obtained in Example 21 was added thereto as a seed crystal, and the resulting mixture was stirred for 86 hours. Then, the precipitated solid was filtered, and the solid separated by the filtration was washed with acetone (1.1 L). The obtained solid was dried under reduced pressure at 40°C to obtain the title compound (191 g, yield: 44%) in the form of crystals.

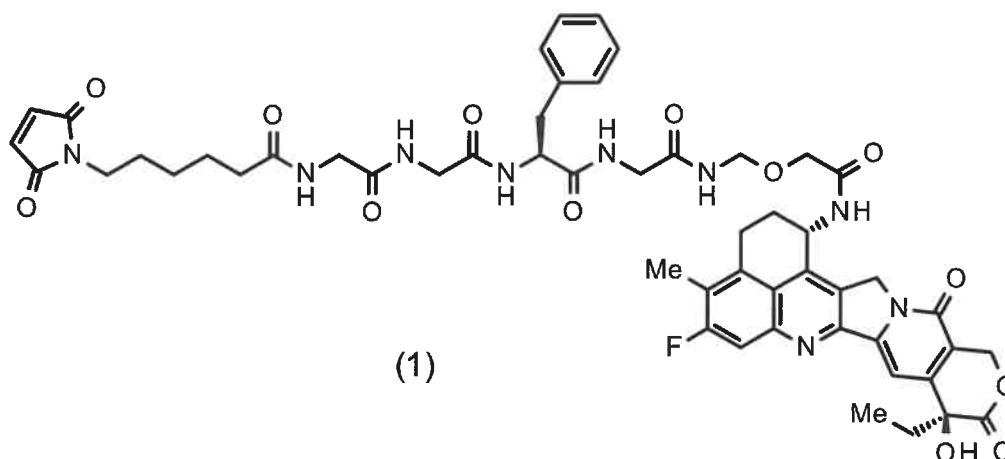
[0495]

(Example 23)

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide

[0496]

[Chem. 113]



[0497]

To anhydrous sodium sulfate (1.8 g), ethyl cyano(hydroxyimino)acetate (0.16 g, 1.13 mmol), and a mixed solution of purified water and tetrahydrofuran (24 mL and 18 mL) containing N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (gross amount: 5.76 g, internal content after conversion into 12.40% 1,2-dimethoxyethane: 5.05 g, 8.18 mmol), a mixed suspension of purified water and tetrahydrofuran (9 mL and 15 mL) containing (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-aminium methanesulfonate (3.0 g, 5.64 mmol) was added at 20 to 30°C. To the mixed solution, tetrahydrofuran (9 mL) and a solution of tetrahydrofuran (7.5 mL) containing N-methylmorpholine (0.63 g, 6.23 mmol) were added, and the resulting mixture was stirred at the same temperature as the above for 15 minutes. Then, 1-(3-

dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.16 g, 11.27 mmol) and a mixed solution of purified water and tetrahydrofuran (1.5 mL and 1.5 mL) were added thereto. The mixed solution was stirred at 20 to 30°C for 30 minutes or longer. After confirmation of termination of the reaction, the reaction mixture was separated into organic and aqueous layers, and the aqueous layer was removed. The temperature of the organic layer was adjusted to 15 to 25°C, then ethyl acetate (36 mL), anhydrous sodium sulfate (1.26 g), and purified water (24 mL) containing N-methylmorpholine (0.14 g, 1.38 mmol) were added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. Purified water (9 mL) was added to the organic layer, and the resulting mixture was stirred and separated into organic and aqueous layers. Purified water (9 mL) containing acetic acid (0.45 mL) was further added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers to obtain an organic layer. Activated carbon (0.30 g, Kyoryoku Shirasagi (manufactured by Osaka Gas Chemicals Co., Ltd.)) was added thereto, and the resulting mixture was stirred at room temperature for about 15 minutes. Then, the activated carbon was separated by filtration, then the activated carbon was washed with tetrahydrofuran (9 mL), and the washes were combined with the filtrate. The resulting mixture was

concentrated to 30 mL under reduced pressure. Tetrahydrofuran (75 mL) was added to the concentrate, and the resulting mixture was concentrated to 30 mL under reduced pressure. Tetrahydrofuran (45 mL) was further added to the residue, and the resulting mixture was concentrated to 30 mL under reduced pressure. After confirmation that the water content of the concentrate was 8.0% (v/v) or less, a mixed solution of acetone and 1-propanol (30 mL and 71 mL) was added thereto. N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (30 mg) was added thereto, and the resulting mixture was stirred at 20 to 30°C for 12 hours or longer. The suspension was cooled to 0 to 5°C and then further stirred for 24 hours or longer. Precipitates were filtered, and a powder separated by the filtration was washed with a 1:1 mixed solution of acetone and 1-propanol (30 mL) at 0 to 5°C. The obtained crystals were dried under reduced pressure at 35°C to obtain crude crystals of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-

yl]amino}-2-oxoethoxy)methyl]glycinamide (5.23 g, yield: 89.6%).

[0498]

To the crude crystals of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (4.50 g, 4.35 mmol), a mixed solution of acetone and purified water (10.6 mL and 2.9 mL) containing acetic acid (15 μ L) was added, and the resulting mixture was stirred at 34 to 38°C for 1 hour or longer. After confirmation of dissolution, the solution was cooled to 20 to 25°C. A mixed solution of acetone and 1-propanol (31.5 mL and 64.8 mL) was added thereto, then N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (27 mg) was added, and the resulting mixture was stirred at 20 to 25°C for 24 hours or longer. The suspension was cooled to 0 to 5°C and then further stirred for 12 hours or longer. Precipitates were filtered, and a powder separated by the filtration was washed with a 1:1 mixed solution of acetone and 1-propanol (27 mL) at 0 to 5°C.

The obtained crystals were dried under reduced pressure at 35°C to obtain purified crystals of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-([(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (4.37 g, yield: 93.0%).

The instrumental data was similar to that of the compound described in Example 14.

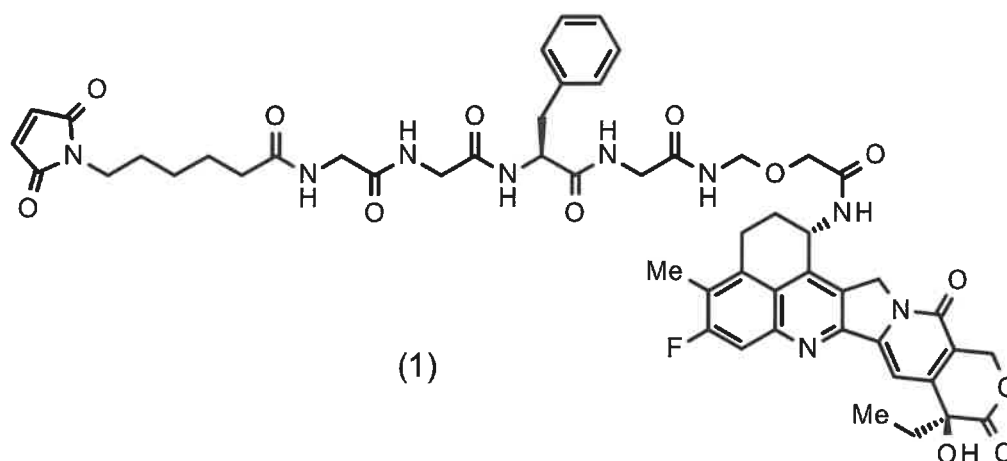
[0499]

(Example 24)

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-([(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide

[0500]

[Chem. 114]



[0501]

To anhydrous sodium sulfate (1.8 g), ethyl cyano(hydroxyimino)acetate (0.16 g, 1.13 mmol), and a mixed solution of purified water and tetrahydrofuran (24 mL and 18 mL) containing N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(carboxymethoxy)methyl]glycinamide (gross amount: 5.76 g, internal content after conversion into 12.40% 1,2-dimethoxyethane: 5.05 g, 8.18 mmol), a mixed suspension of purified water and tetrahydrofuran (9 mL and 15 mL) containing (1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-aminium methanesulfonate (3.0 g, 5.64 mmol) was added at 20 to 30°C. To the mixed solution, tetrahydrofuran (9 mL) and a solution of tetrahydrofuran (7.5 mL) containing N-methylmorpholine (0.63 g, 6.23 mmol) were added, and the resulting mixture was stirred at the same temperature as the above for 15 minutes. Then, 1-(3-

dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.16 g, 11.27 mmol) and a mixed solution of purified water and tetrahydrofuran (1.5 mL and 1.5 mL) were added thereto. The mixed solution was stirred at 20 to 30°C for 30 minutes or longer. After confirmation of termination of the reaction, the reaction mixture was separated into organic and aqueous layers, and the aqueous layer was removed. The temperature of the organic layer was adjusted to 15 to 25°C, then ethyl acetate (36 mL), anhydrous sodium sulfate (1.26 g), and purified water (24 mL) containing N-methylmorpholine (0.14 g, 1.38 mmol) were added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers. The aqueous layer was removed. Purified water (9 mL) was added to the organic layer, and the resulting mixture was stirred and separated into organic and aqueous layers. Purified water (9 mL) containing acetic acid (0.45 mL) was further added thereto, and the resulting mixture was stirred and separated into organic and aqueous layers to obtain an organic layer. Activated carbon (0.30 g, Kyoryoku Shirasagi (manufactured by Osaka Gas Chemicals Co., Ltd.)) was added thereto, and the resulting mixture was stirred at room temperature for about 15 minutes or longer. Then, the activated carbon was separated by filtration, then the activated carbon was washed with tetrahydrofuran (9 mL), and the washes were combined with the filtrate. The resulting mixture

was concentrated to 30 mL under reduced pressure. Tetrahydrofuran (75 mL) was added to the concentrate, and the resulting mixture was concentrated to 30 mL under reduced pressure. Tetrahydrofuran (45 mL) was further added to the residue, and the resulting mixture was concentrated to 30 mL under reduced pressure. After confirmation that the water content of the concentrate was 8.0% (v/v) or less, a mixed solution of acetone and 1-propanol (30 mL and 71 mL) was added thereto, and the resulting mixture was stirred at 20 to 30°C for 22 hours. The suspension was cooled to 0 to 5°C and then further stirred for 24 hours or longer. Precipitates were filtered, and a powder separated by the filtration was washed with a 1:1 mixed solution of acetone and 1-propanol (30 mL) at 0 to 5°C. The obtained crystals were dried under reduced pressure at 35°C to obtain crystals of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide (5.08 g, yield: 87.0%).

[0502]

The instrumental data was similar to that of the compound described in Example 14.

Free Text of Sequence Listing

[0503]

SEQ ID NO: 1 - Amino acid sequence of a heavy chain of the anti-HER2 antibody

SEQ ID NO: 2 - Amino acid sequence of a light chain of the anti-HER2 antibody

SEQ ID NO: 3 - Amino acid sequence of a heavy chain of the anti-HER3 antibody

SEQ ID NO: 4 - Amino acid sequence of a light chain of the anti-HER3 antibody

SEQ ID NO: 5 - Amino acid sequence of a heavy chain of the anti-TROP2 antibody

SEQ ID NO: 6 - Amino acid sequence of a light chain of the anti-TROP2 antibody

SEQ ID NO: 7 - Amino acid sequence of a heavy chain of the anti-B7-H3 antibody

SEQ ID NO: 8 - Amino acid sequence of a light chain of the anti-B7-H3 antibody

SEQ ID NO: 9 - Amino acid sequence of a heavy chain of the anti-GPR20 antibody

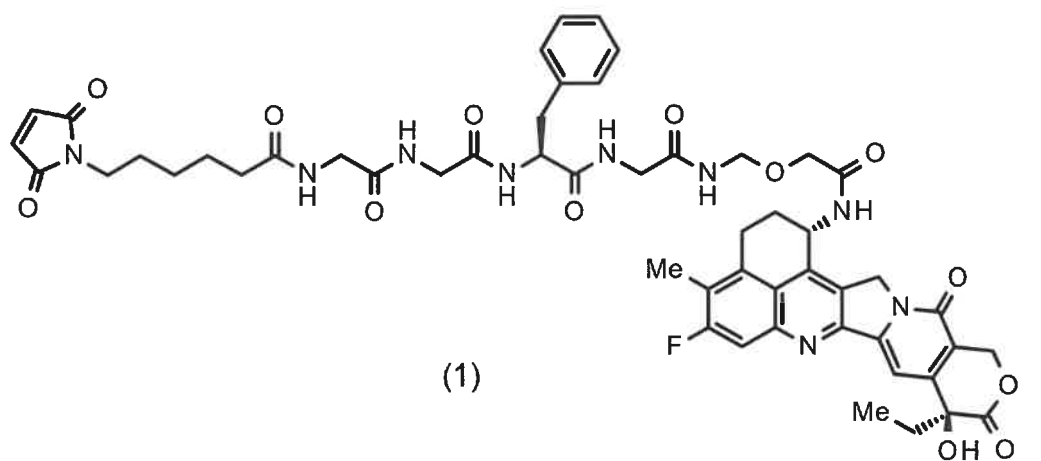
SEQ ID NO: 10 - Amino acid sequence of a light chain of the anti-GPR20 antibody

Claims

[Claim 1]

Crystals of the compound represented by formula (1):

[Chem. 1]



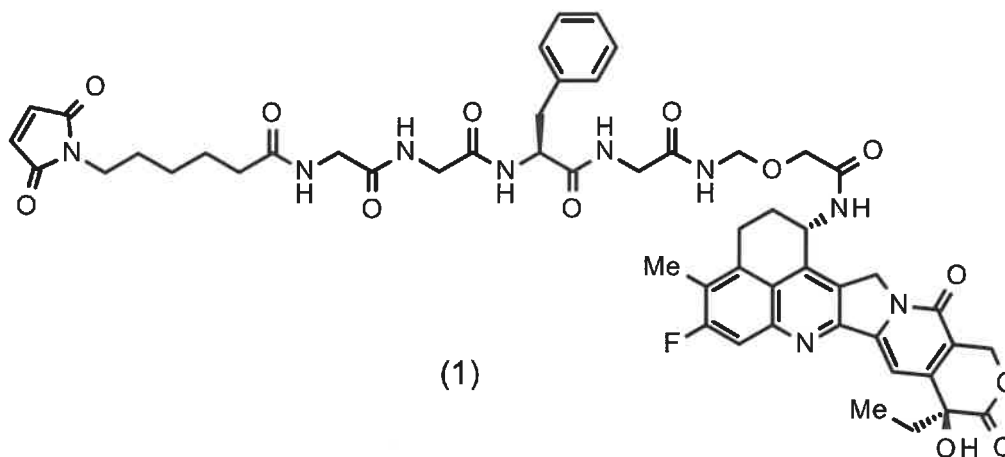
[Claim 2]

The crystals according to claim 1, wherein the crystals show main peaks at diffraction angles (2θ) of $5.6 \pm 0.2^\circ$, $15.5 \pm 0.2^\circ$ and $22.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with copper $K\alpha$ radiation.

[Claim 3]

A method for producing crystals of the compound represented by formula (1):

[Chem. 2]



comprising the steps of: preparing a solution in which the compound represented by the formula (1) is dissolved; and then precipitating crystals of the compound represented by the formula (1) from the solution.

[Claim 4]

The production method according to claim 3, wherein the crystals of the compound represented by the formula (1) show main peaks at diffraction angles (2θ) of $5.6 \pm 0.2^\circ$, $15.5 \pm 0.2^\circ$ and $22.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with a copper $K\alpha$ radiation.

[Claim 5]

The production method according to claim 3 or 4, wherein the solution in which the compound represented by the formula (1) is dissolved comprises a lower ketone and a lower alcohol as solvents.

[Claim 6]

The production method according to claim 5, wherein the lower ketone is acetone.

[Claim 7]

The production method according to claim 5, wherein the lower ketone is methyl ethyl ketone.

[Claim 8]

The production method according to any one of claims 5 to 7, wherein the lower alcohol is 1-propanol.

[Claim 9]

The production method according to any one of claims 5 to 7, wherein the lower alcohol is 2-butanol.

[Claim 10]

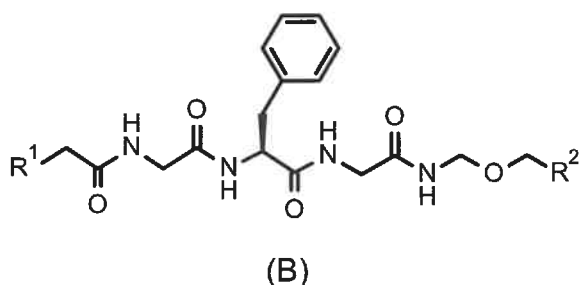
The production method according to any one of claims 3 to 9, comprising a step of adding a seed crystal of the crystals of the compound represented by the formula (1).

[Claim 11]

The production method according to any one of claims 3 to 10, wherein the compound represented by the formula (1) is produced by a production method (I), wherein the production method (I) is a production method comprising the steps of:

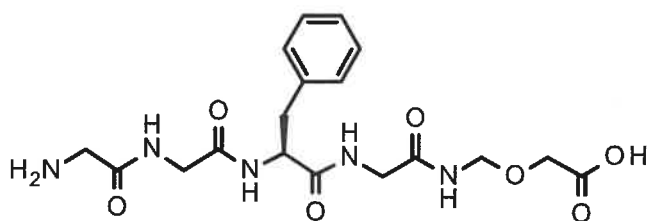
deprotecting protecting groups for an amino group and a carboxy group of a compound represented by formula (B):

[Chem. 3]



wherein R^1 represents an amino group protected with a protecting group, and R^2 represents a carboxy group protected with a protecting group, to convert it into the compound represented by formula (8):

[Chem. 4]

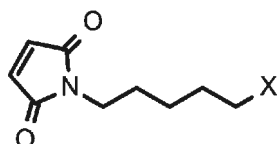


(8)

; then

condensing the compound represented by the formula (8) with a compound represented by formula (C):

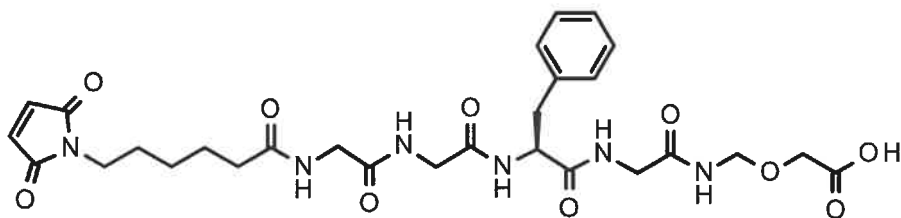
[Chem. 5]



(C)

wherein X represents an active ester group or a carboxy group, to convert it into the compound represented by formula (10):

[Chem. 6]



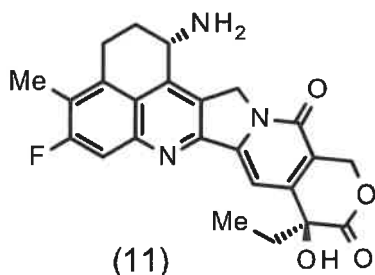
(10)

; and

then

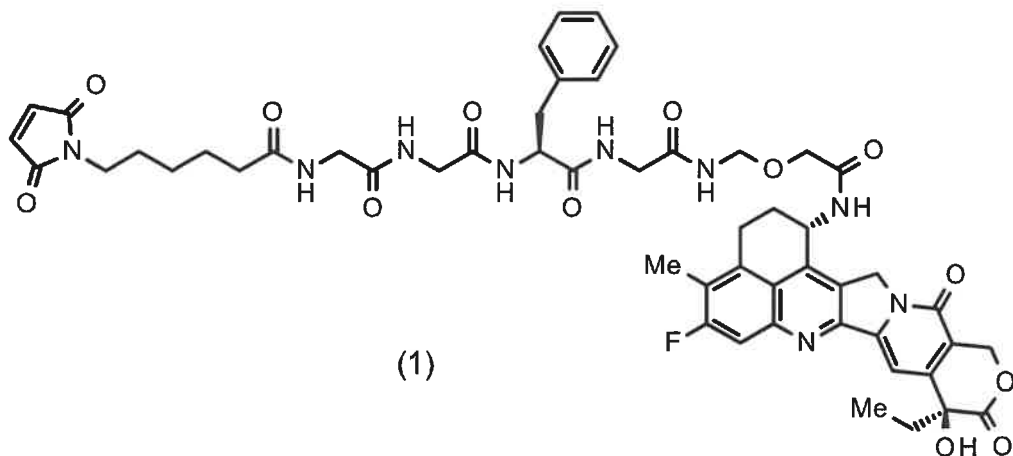
condensing the compound represented by the formula (10)
with the compound represented by formula (11):

[Chem. 7]



to convert it into the compound represented by the
formula (1):

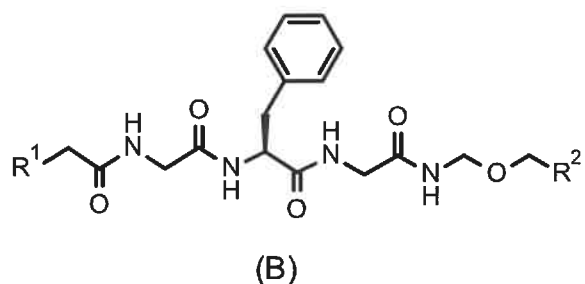
[Chem. 8]



[Claim 12]

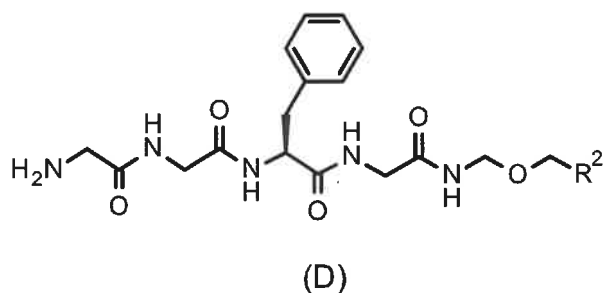
The production method according to any one of claims
3 to 10, wherein the compound represented by the formula
(1) is produced by a production method (II),
wherein the production method (II) is a production method
comprising the steps of:
deprotecting a protecting group for an amino group of a
compound represented by formula (B):

[Chem. 9]



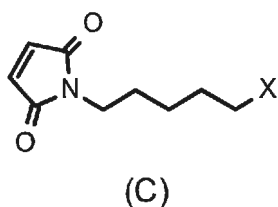
wherein R¹ represents an amino group protected with a protecting group, and R² represents a carboxy group protected with a protecting group, to convert it into a compound represented by formula (D):

[Chem. 10]



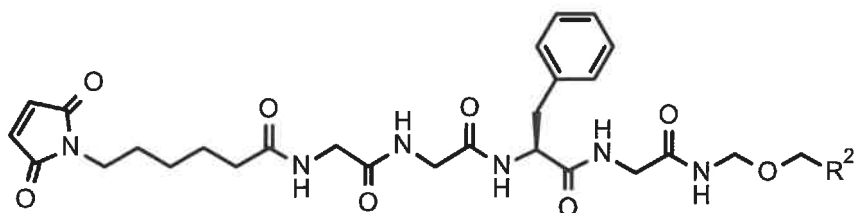
wherein R² represents the same meaning as above; then condensing the compound represented by the formula (D) with a compound represented by formula (C):

[Chem. 11]



wherein X represents an active ester group or a carboxy group, to convert it into a compound represented by formula (E):

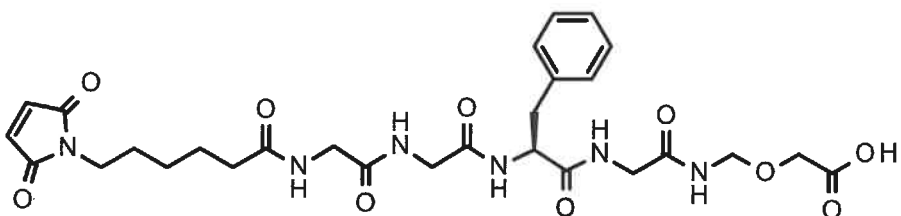
[Chem. 12]



(E)

wherein R² represents the same meaning as above; then deprotecting the protecting group for the carboxy group of the compound represented by the formula (E) to convert it into the compound represented by formula (10):

[Chem. 13]



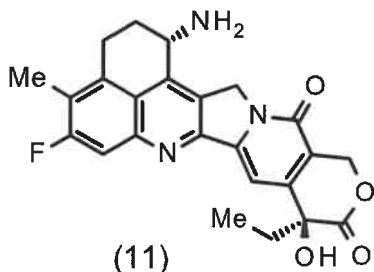
(10)

; and

then

condensing the compound represented by the formula (10) with the compound represented by formula (11):

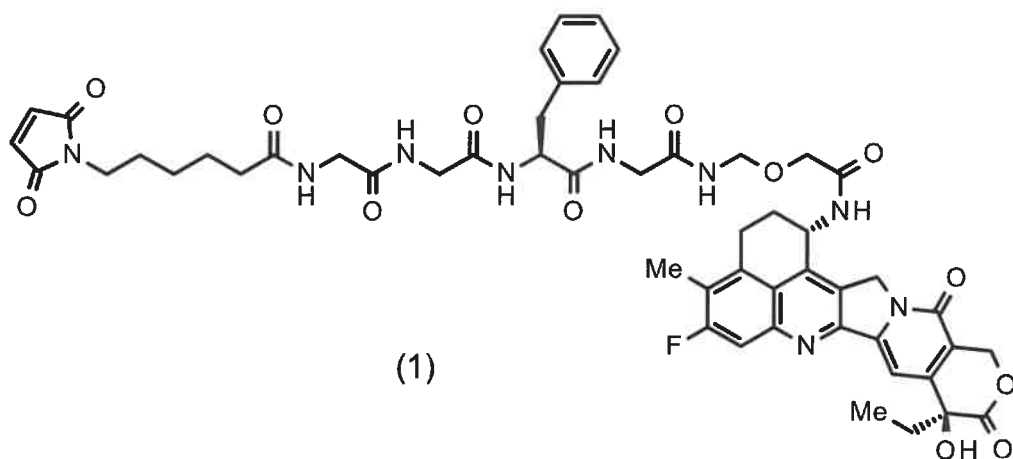
[Chem. 14]



(11)

to convert it into the compound represented by the formula (1):

[Chem. 15]



[Claim 13]

The production method according to claim 11 or 12, comprising the steps of: dissolving the compound represented by the formula (10) in a solvent containing 1,2-dimethoxyethane; and then precipitating crystals of a 1,2-dimethoxyethane adduct of the compound represented by the formula (10).

[Claim 14]

The production method according to claim 13, wherein the crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with a copper $K\alpha$ radiation.

[Claim 15]

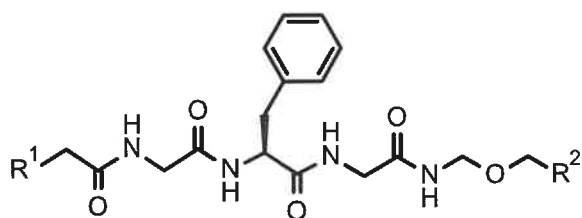
The production method according to any one of claims 11 to 14, wherein the step of condensing the compound represented by the formula (10) and the compound

represented by the formula (11) to convert it into the compound represented by the formula (1) is performed in a two-phase system of an aqueous sodium sulfate solution and tetrahydrofuran.

[Claim 16]

The production method according to any one of claims 3 to 10, wherein the compound represented by the formula (1) is produced by a production method (III), wherein the production method (III) is a production method comprising the steps of:
deprotecting a protecting group for a carboxy group of a compound represented by formula (B):

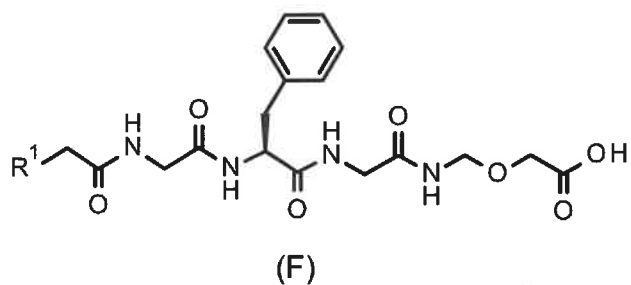
[Chem. 16]



(B)

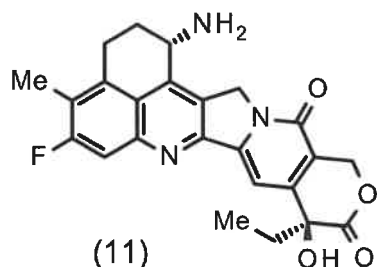
wherein R¹ represents an amino group protected with a protecting group, and R² represents a carboxy group protected with a protecting group, to convert it into a compound represented by formula (F):

[Chem. 17]



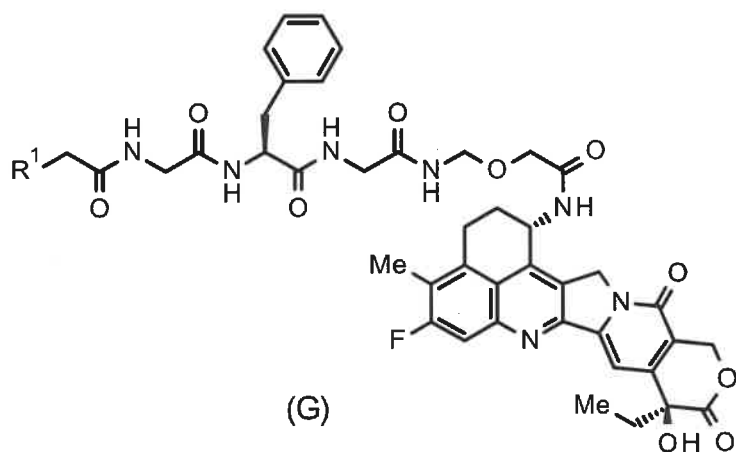
wherein R¹ represents the same meaning as above; then
condensing the compound represented by the formula (F)
with the compound represented by formula (11):

[Chem. 18]



to convert it into a compound represented by formula (G):

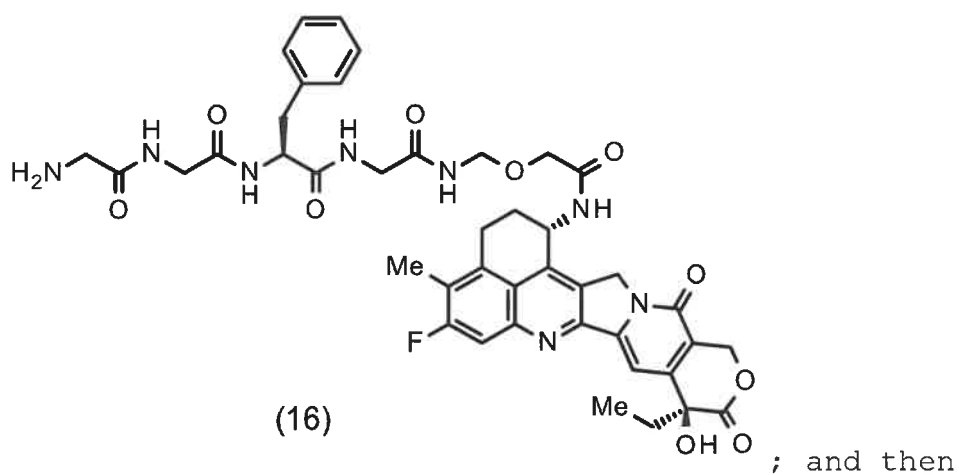
[Chem. 19]



wherein R¹ represents the same meaning as above; then

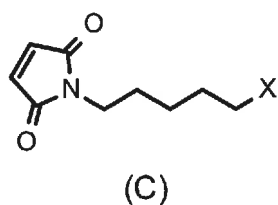
deprotecting the protecting group for the amino group of the compound represented by the formula (G) to convert it into the compound represented by formula (16):

[Chem. 20]



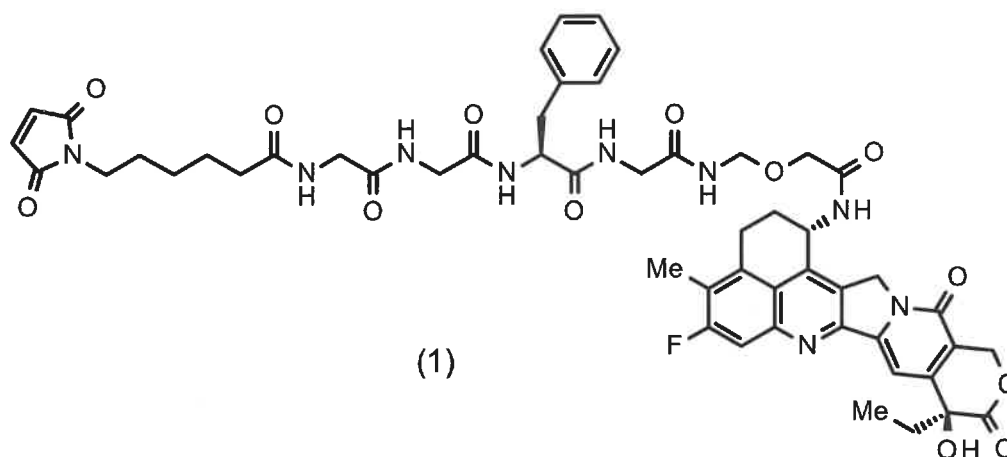
condensing the compound represented by the formula (16) with a compound represented by formula (C):

[Chem. 21]



wherein X represents an active ester group or a carboxy group, to convert it into the compound represented by the formula (1):

[Chem. 22]



[Claim 17]

The production method according to any one of claims 11 to 16, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt.

[Claim 18]

The production method according to any one of claims 11 to 16, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt m-hydrate, wherein m is in the range of 0 to 3.

[Claim 19]

The production method according to any one of claims 11 to 16, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt dihydrate.

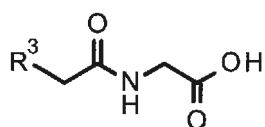
[Claim 20]

The production method according to any one of claims 11 to 19, wherein the compound represented by the formula (B) is produced by a production method (IV),

wherein the production method (IV) is a production method comprising the steps of:

reacting a compound represented by formula (H):

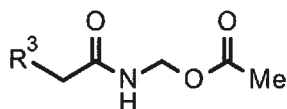
[Chem. 23]



(H)

wherein R³ represents an amino group protected with a protecting group, with lead tetraacetate to convert it into a compound represented by formula (J):

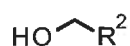
[Chem. 24]



(J)

wherein R³ represents the same meaning as above; then reacting the compound represented by the formula (J) with a compound represented by formula (K):

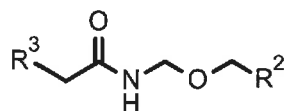
[Chem. 25]



(K)

wherein R² represents the same meaning as the R² according to any one of claims 11 to 19, in the presence of an acid or a base to convert it into a compound represented by formula (L):

[Chem. 26]



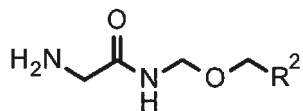
(L)

wherein R^2 and R^3 represent the same meanings as above;

then

deprotecting the protecting group for the amino group of the compound represented by the formula (L) to convert it into a compound represented by formula (M):

[Chem. 27]



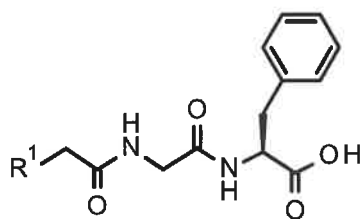
(M)

wherein R^2 represents the same meaning as above; and then

condensing the compound represented by the formula (M)

with a compound represented by formula (N):

[Chem. 28]



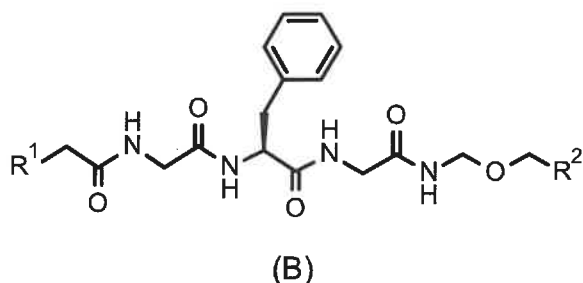
(N)

wherein R^1 represents the same meaning as the R^1

according to any one of claims 11 to 19, to convert it

into the compound represented by the formula (B):

[Chem. 29]



wherein R¹ and R² represent the same meanings as above.

[Claim 21]

The production method according to claim 20, wherein the step of reacting the compound represented by the formula (H) with lead tetraacetate to convert it into the compound represented by the formula (J) is performed in the presence of acetic acid.

[Claim 22]

The production method according to claim 20 or 21, wherein the step of reacting the compound represented by the formula (J) with the compound represented by the formula (K) to convert it into the compound represented by the formula (L) is performed in the presence of an aqueous sodium hydroxide solution.

[Claim 23]

The production method according to claim 20 or 21, wherein the step of reacting the compound represented by the formula (J) with the compound represented by the formula (K) to convert it into the compound represented by the formula (L) is performed in the presence of tris(pentafluorophenyl)borane.

[Claim 24]

The production method according to any one of claims 20 to 23, comprising a step of adding an acid to precipitate a salt of the compound represented by the formula (M) and the acid after the step of deprotecting the protecting group for the amino group of the compound represented by the formula (L) to convert it into the compound represented by the formula (M).

[Claim 25]

The production method according to claim 24, wherein the acid is 1-hydroxybenzotriazole.

[Claim 26]

The production method according to any one of claims 11 to 25, wherein R¹ is an amino group protected with a benzyloxycarbonyl group.

[Claim 27]

The production method according to any one of claims 11 to 25, wherein R¹ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[Claim 28]

The production method according to any one of claims 11 to 27, wherein R² is a carboxy group protected with a benzyl group.

[Claim 29]

The production method according to any one of claims 20 to 28, wherein R³ is an amino group protected with a (9H-fluoren-9-ylmethoxy)carbonyl group.

[Claim 30]

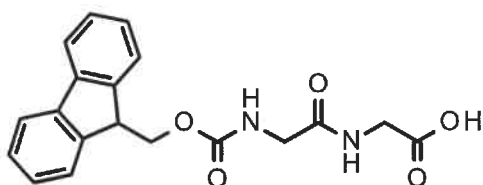
The production method according to any one of claims 11 to 29, wherein X is a (2,5-dioxopyrrolidin-1-yl)oxycarbonyl group.

[Claim 31]

The production method according to any one of claims 3 to 10, wherein the compound represented by the formula (1) is produced by a production method (V), wherein the production method (V) is a production method comprising the steps of:

reacting the compound represented by formula (2):

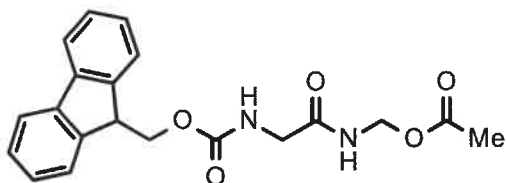
[Chem. 30]



(2)

with lead tetraacetate to convert it into the compound represented by formula (3):

[Chem. 31]

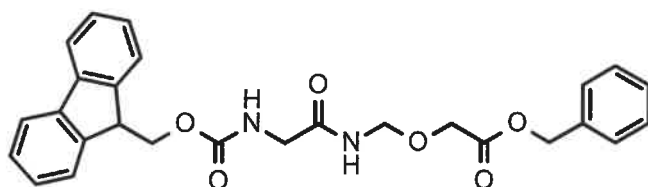


(3)

; then

reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4):

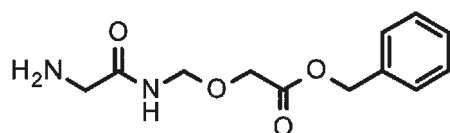
[Chem. 32]



(4) ; then

deprotecting a protecting group for an amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5):

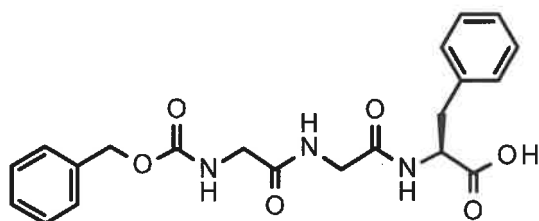
[Chem. 33]



(5) ; then

condensing the compound represented by the formula (5) with the compound represented by formula (6):

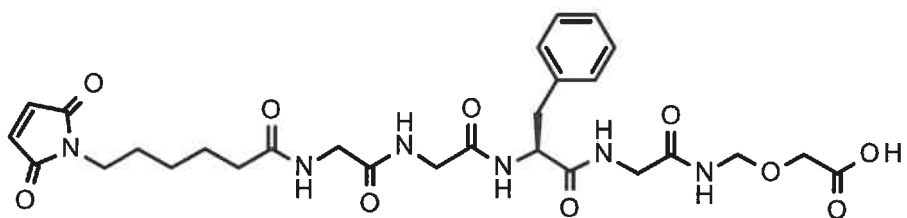
[Chem. 34]



(6)

to convert it into the compound represented by formula (7):

[Chem. 35]



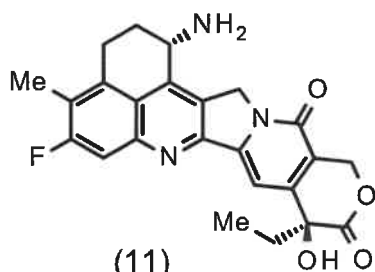
(10)

; and

then

condensing the compound represented by the formula (10) with the compound represented by formula (11):

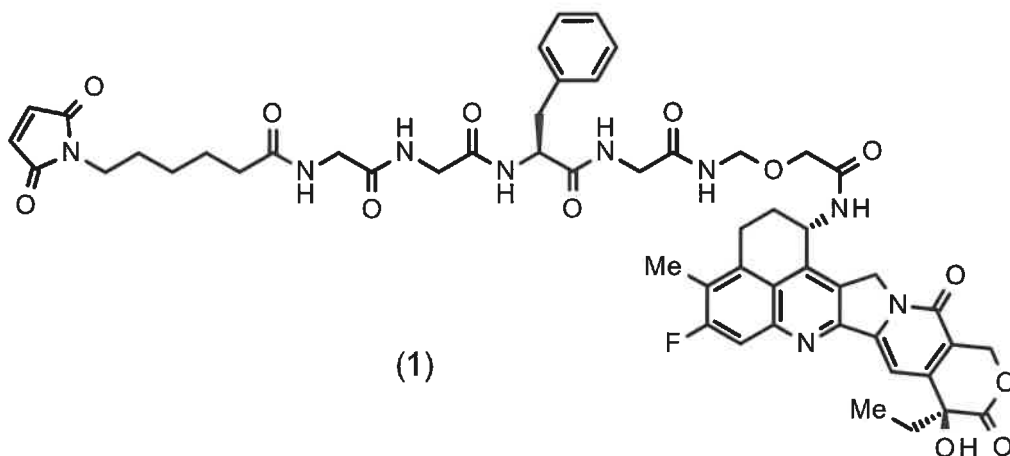
[Chem. 39]



(11)

to convert it into the compound represented by formula (1):

[Chem. 40]



(1)

[Claim 32]

The production method according to claim 31, comprising the steps of: dissolving the compound represented by the formula (10) in a solvent containing 1,2-dimethoxyethane; and then precipitating crystals of a 1,2-dimethoxyethane adduct of the compound represented by the formula (10).

[Claim 33]

The production method according to claim 32, wherein the crystals of the 1,2-dimethoxyethane adduct of the compound represented by the formula (10) show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with a copper K α radiation.

[Claim 34]

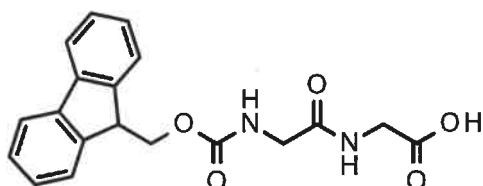
The production method according to any one of claims 31 to 33, wherein the step of condensing the compound represented by the formula (10) with the compound represented by the formula (11) to convert it into the compound represented by the formula (1) is performed in a two-phase system of an aqueous sodium sulfate solution and tetrahydrofuran.

[Claim 35]

The production method according to any one of claims 3 to 10, wherein the compound represented by the formula (1) is produced by a production method (VI), wherein the production method (VI) is a production method comprising the steps of:

reacting the compound represented by formula (2):

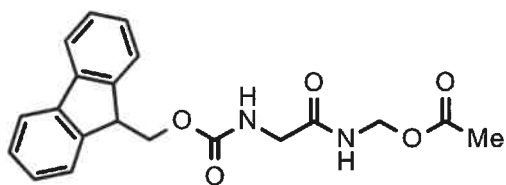
[Chem. 41]



(2)

with lead tetraacetate to convert it into the compound represented by formula (3):

[Chem. 42]

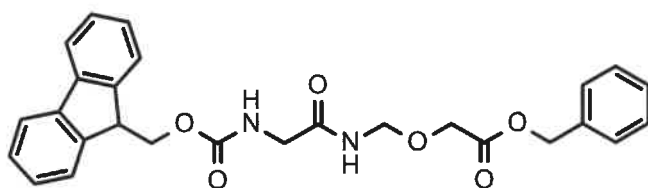


(3)

; then

reacting the compound represented by the formula (3) with benzyl glycolate in the presence of an acid or a base to convert it into the compound represented by formula (4):

[Chem. 43]

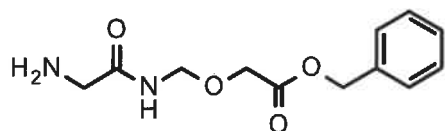


(4)

; then

deprotecting a protecting group for an amino group of the compound represented by the formula (4) to convert it into the compound represented by formula (5):

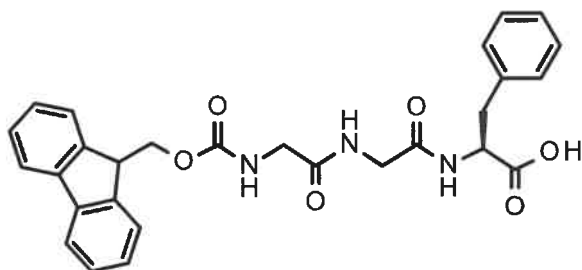
[Chem. 44]



(5) ; then

condensing the compound represented by the formula (5)
with the compound represented by formula (12):

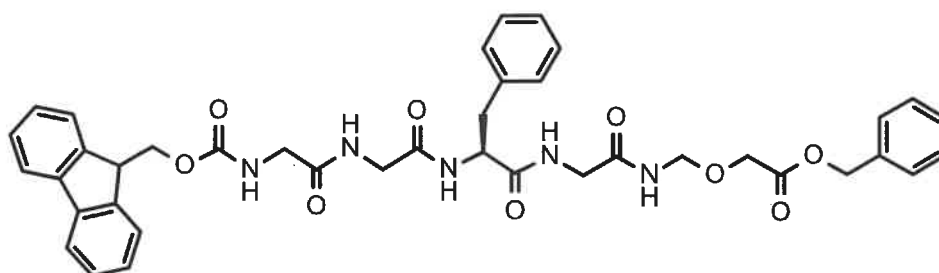
[Chem. 45]



(12)

to convert it into the compound represented by formula
(13):

[Chem. 46]



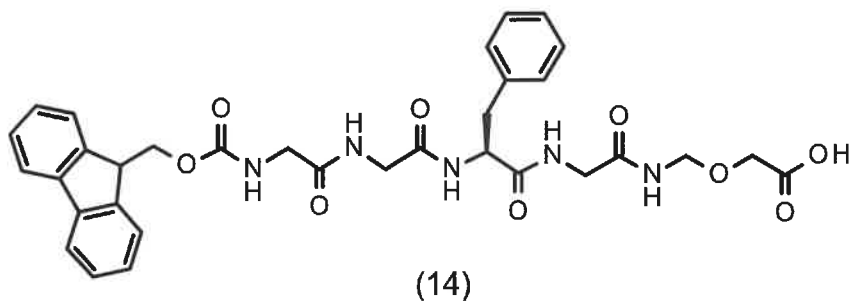
(13)

;

then

deprotecting a protecting group for a carboxy group of
the compound represented by the formula (13) to convert
it into the compound represented by formula (14):

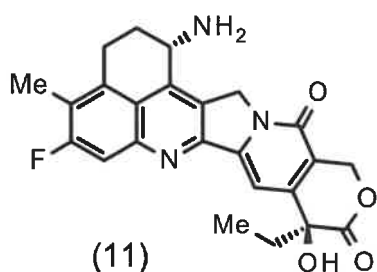
[Chem. 47]



; then

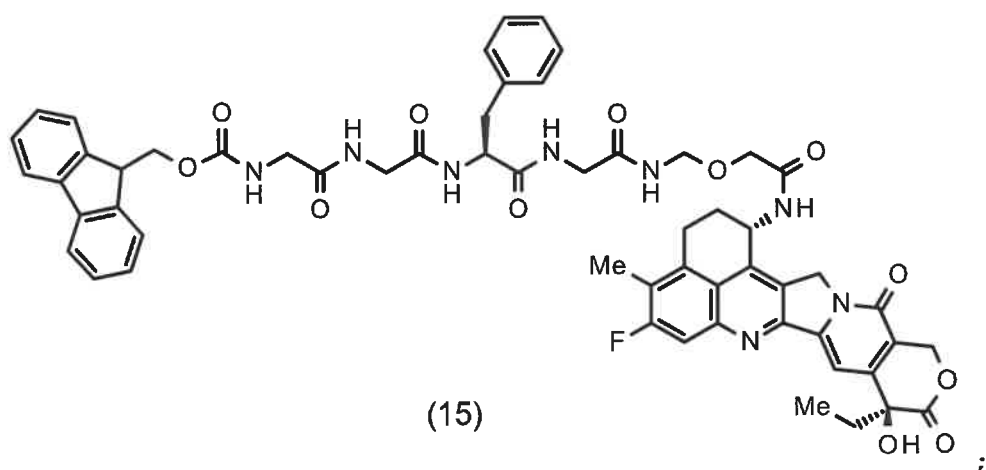
condensing the compound represented by the formula (14) with the compound represented by formula (11):

[Chem. 48]



to convert it into the compound represented by formula (15):

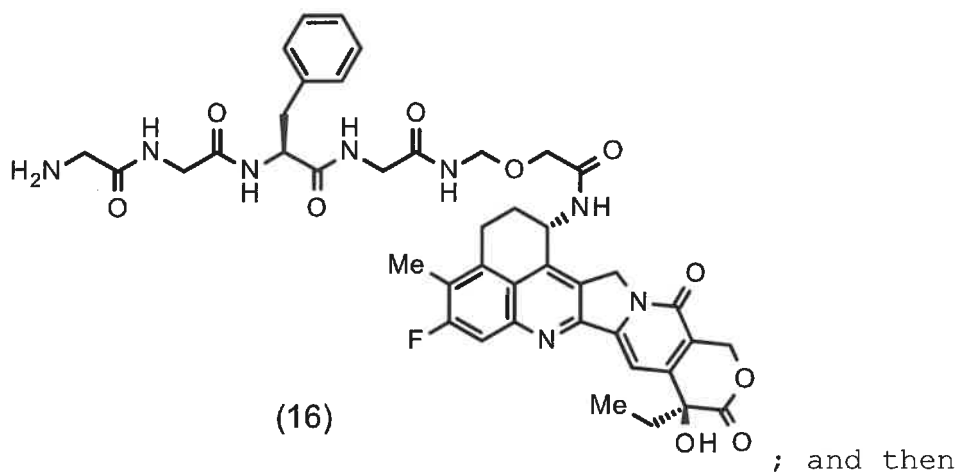
[Chem. 49]



then

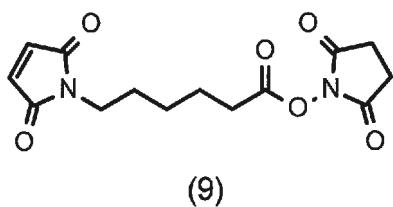
deprotecting a protecting group for an amino group of the compound represented by the formula (15) to convert it into the compound represented by formula (16):

[Chem. 50]



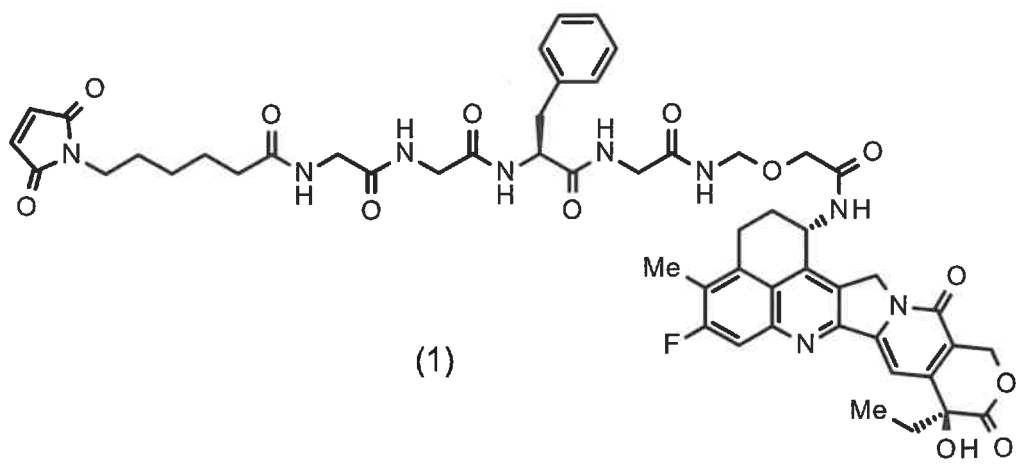
condensing the compound represented by the formula (16) with the compound represented by formula (9):

[Chem. 51]



to convert it into the compound represented by the formula (1):

[Chem. 52]



[Claim 36]

The production method according to any one of claims 31 to 35, wherein the step of reacting the compound represented by the formula (2) with lead tetraacetate to convert it into the compound represented by the formula (3) is performed in the presence of acetic acid.

[Claim 37]

The production method according to any one of claims 31 to 36, wherein the step of converting the compound represented by the formula (3) into the compound represented by the formula (4) is performed in the presence of an aqueous sodium hydroxide solution.

[Claim 38]

The production method according to any one of claims 31 to 36, wherein the step of converting the compound represented by the formula (3) into the compound represented by the formula (4) is performed in the presence of tris(pentafluorophenyl)borane.

[Claim 39]

The production method according to any one of claims 31 to 38, comprising a step of adding an acid to precipitate a salt of the compound represented by the formula (5) and the acid after the step of deprotecting the protecting group for the amino group of the compound represented by the formula (4) to convert it into the compound represented by the formula (5).

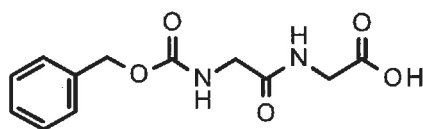
[Claim 40]

The production method according to claim 39, wherein the acid is 1-hydroxybenzotriazole.

[Claim 41]

The production method according to any one of claims 31 to 40, wherein the compound represented by the formula (6) is produced by a method comprising the steps of: condensing the compound represented by formula (23):

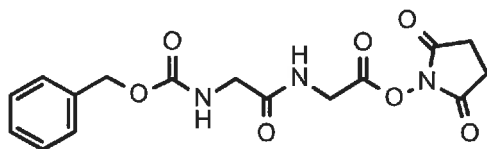
[Chem. 53]



(23)

with N-hydroxysuccinimide to convert it into the compound represented by formula (24):

[Chem. 54]



(24)

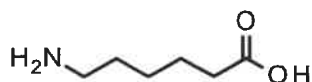
; and then

condensing the compound represented by the formula (24) with L-phenylalanine to convert it into the compound represented by the formula (6).

[Claim 42]

The production method according to any one of claims 31 to 41, wherein the compound represented by the formula (9) is produced by a method comprising the steps of: reacting the compound represented by formula (17):

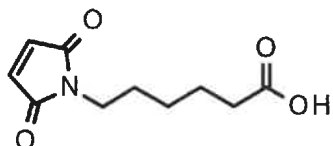
[Chem. 55]



(17)

with maleic anhydride to convert it into the compound represented by formula (18):

[Chem. 56]



(18)

; and then

adding thionyl chloride to the compound represented by the formula (18) and a mixed solution containing N-hydroxysuccinimide and 2,6-lutidine to convert it into the compound represented by the formula (9).

[Claim 43]

The production method according to any one of claims 31 to 42, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt.

[Claim 44]

The production method according to any one of claims 31 to 42, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt m-hydrate, wherein m is in the range of 0 to 3.

[Claim 45]

The production method according to any one of claims 31 to 42, wherein the compound represented by the formula (11) is in the form of a methanesulfonic acid salt dihydrate.

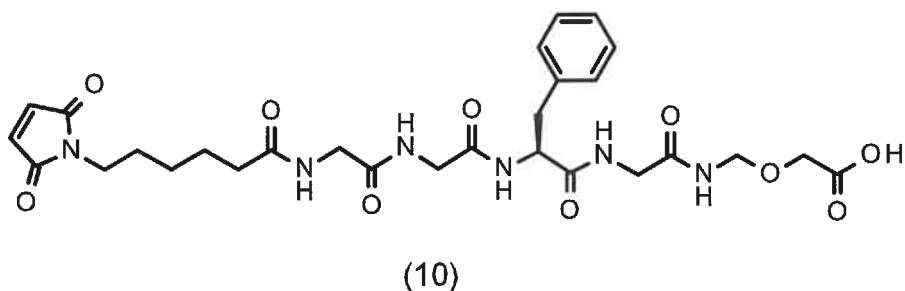
[Claim 46]

The production method according to any one of claims 3 to 45, wherein no chromatography is used.

[Claim 47]

Crystals of a 1,2-dimethoxyethane adduct of the compound represented by formula (10):

[Chem. 57]



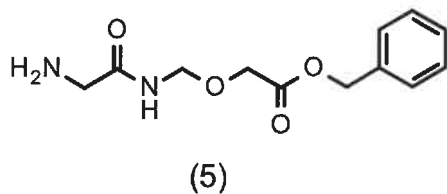
[Claim 48]

The crystals according to claim 47, wherein the crystals show main peaks at diffraction angles (2θ) of $19.0 \pm 0.2^\circ$ and $25.0 \pm 0.2^\circ$ in powder X-ray diffraction obtained by irradiation with a copper $K\alpha$ radiation.

[Claim 49]

A salt of the compound represented by formula (5):

[Chem. 58]



and an acid.

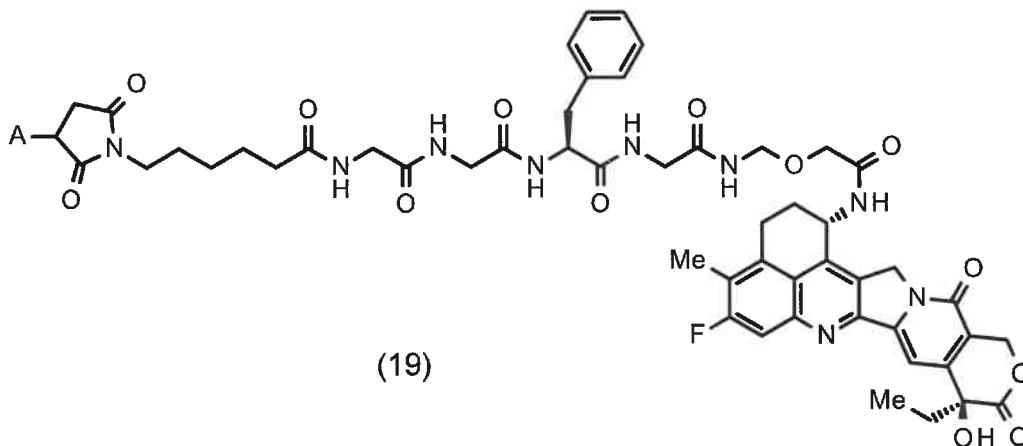
[Claim 50]

The salt according to claim 49, wherein the acid is 1-hydroxybenzotriazole.

[Claim 51]

A method for producing an antibody-drug conjugate, in which a drug-linker represented by formula (19):

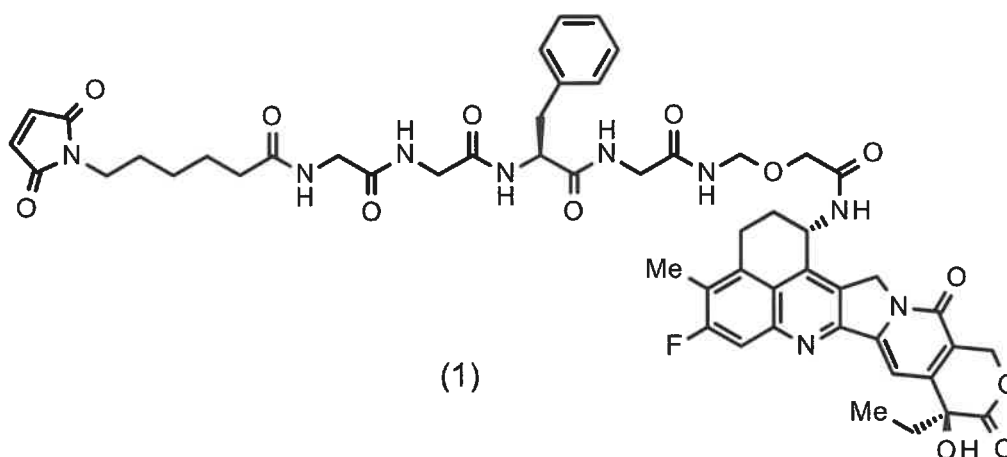
[Chem. 60]



wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond,
wherein crystals of the compound represented by formula
(1):

[Chem. 59]



produced by the method according to any one of claims 3
to 46 are used as a starting material, and the method
comprises the steps of:

- i) reducing an antibody; and then
- ii) adding a solution in which the crystals of the
compound represented by the formula (1) produced in the
above-mentioned method is dissolved, to react the
solution with the reduced antibody.

[Claim 52]

The production method according to claim 51, wherein
the antibody is an anti-HER2 antibody, an anti-HER3
antibody, an anti-TROP2 antibody, an anti-B7-H3 antibody,
or an anti-GPR20 antibody.