

TRANSLATOR'S DECLARATION

I, Carl Sullivan, certify that I am familiar with both the Japanese and the English languages, that I have prepared the attached English translation (Exhibit 1039) of Japanese Patent Application No. 2018-113962 (Exhibit 1038), and that the English translation is a true, faithful and accurate translation of Japanese Patent Application No. 2018-113962 (Exhibit 1038).

I further declare that all statements made in this declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of legal decisions of any nature based on them.



December 23, 2024
Date

Name: Carl Sullivan

[Title of Invention]

[Technical Field]

[0001] The present invention relates to compounds having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof, and the like.

[Prior Art]

[0002] In recent years, research and development of an antibody-drug conjugate (ADC) have been actively conducted. An ADC, as implied by the name, is a medicine in which a drug (e.g., an anti-cancer agent) is conjugated with an antibody and has a direct cytotoxic activity on cancer cells and the like. A typical ADC is T-DM1 (trade name: Kadcyla (registered trademark) (Non-Patent Documents 1 to 3).

[0003] ADCs including T-DM1 have had the problem of their nonuniformity from the beginning of their development. That is, a small compound drug is randomly reacted with about 70 to 80 Lys residues in an antibody, and thus a drug / antibody ratio (DAR) and a conjugation position are not constant. It is known that such a random conjugation method normally provides a DAR within a range of 0 to 8, producing a plurality of medicines having different numbers of bonds of a drug. In recent years, it has been reported that when the number of bonds and the bond positions of a drug of an ADC are changed, pharmacokinetics, and a releasing rate and effects of the drug change. Given these circumstances, next-generation ADCs are required to control the number and positions of a drug to be conjugated. It is believed that when the number and positions are fixed, the problems of expected efficacy, variations in conjugation medicines, will be solved (Non-Patent Document 4).

[0004] Although methods for regioselectively modifying antibodies are being investigated worldwide, most of them are methods of modification using genetic engineering techniques or enzymes. For the genetic engineering methods of modification, problems have been pointed out such as reductions in the expression efficiency of antibodies themselves (reductions in total yield when ADCs are prepared), although regioselectivity and number selectivity can be controlled. In addition, there is a problem in that it takes long years to construct an antibody expression system and the like (Non-Patent Documents 5 to 7).

[0005] In recent years, methods that chemically modify proteins under complicated environments such as intracellular ones using a small molecule probe have been reported. The methods are used for imaging or identification of receptors in repositioning small compound drugs. In the field of chemical biology, organic chemical methods of protein modification using a synthesized small molecule probe are attracting attention (Non-Patent Documents 8 to 11).

[0006] A chemical conjugation by affinity peptide (CCAP) method has recently been developed. This method has succeeded in regioselective modification of antibodies by a method that reacts a peptide reagent in which an NHS activated ester and a drug are coupled with an affinity peptide with an antibody (that is, a method for producing an ADC through a linker comprising a peptide portion). This method has succeeded in regioselectively modifying an antibody Fc region with a drug by a chemical synthetic technique first in the world, and besides, practically favorable results [reaction time: 30 minutes, yield: 70 % (for DAR 1), and regioselectivity: 100 %] have been determined. It has been demonstrated that control with a DAR of 2 can be achieved by adding about five equivalents of the peptide reagent, which is epoch-making in that a modified position can also be controlled (Patent Document 1).

[Prior Art Literature]

[Patent Literature]

[0007]

[Patent Document 1] International Publication No. 2016/186206

[Non Patent Literature]

[0008]

[Non-patent document 1] Reichert JM et al., Nat Biotechnol 2005;23:1073-8

[Non-patent document 2] Kubota T et al., Cancer Sci 2009;100:1566-72

[Non-patent document 3] Wu AM et al., Nat Biotechnol 2005;23:1137-46

[Non-patent document 4] Junutula JR et al., Nat Biotechnol 2008;26:925-32

[Non-patent document 5] Shen BQ et al., Nat Biotechnol 2012;30:184-9

[Non-patent document 6] Hofer T et al., Biochemistry 2009;48:12047-57

[Non-patent document 7] Liu W et al., Nat Methods 2007;4:239-44

[Non-patent document 8] S. T. Laughlin et al., Science 2008; 320, 664

[Non-patent document 9] A. E. Speers et al., ChemBioChem 2004; 5, 41

[Non-patent document 10] Y. Takaoka et al., Angew. Chem. Int. Ed. 2013; 52, 4088

[Non-patent literature 11] S. Fujishima et al., J. Am. Chem. Soc, 2012; 134: 3961-64

[Summary of the invention]

[Problems to be Solved by the Invention]

[0009] The object of the present invention is to provide novel techniques enabling modification of an antibody, particularly regioselective modification of an anti-body.

[Means for Solving the Problem]

[0010] As a result of intensive research, the present inventors have discovered that a compound having a structural unit of A-L-E (where A is an affinity substance to an antibody, L is a divalent group comprising a certain leaving group, and E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody) or a salt thereof is useful for regiospecific modification of an antibody. For example, it has been found out that a certain compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by Formula (I), is useful for regiospecific modification of an antibody (e.g., FIG. 1 and various Examples). In addition, it has been found out that a compound having an affinity substance to an antibody and a functional substance, represented by formula (IV), or a salt thereof is useful for regiospecific modification of an antibody (e.g., Examples 13 and 14). The inventors of the present invention have also found out that use of such a compound can prepare an antibody comprising no peptide portion as a linker and regioselectively having a functional substance or functional substances (e.g., a drug) (antibody drug conjugate (ADC)), for example. Avoidance of use of a linker comprising a peptide portion, which has potential immunogenicity and is easily hydrolyzed in the blood, is desirable in the clinical application of ADC. That is, the method developed by the inventors of the present invention has succeeded in regioselectively modifying an antibody Fc region with a drug by a chemical synthetic technique, and besides, without using any linker comprising a peptide portion.

[0011]

[0010] Specifically, the present invention is as follows.

[0011] (1) A compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I): A-L-E-B (I)

wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof.

(2) The compound or salt thereof according to (1), wherein the affinity substance is a peptide.

(3) The compound or salt thereof according to (2), wherein the peptide is a peptide having ability to bind to a constant region of a monoclonal antibody.

(4) The compound or salt thereof according to (2) or (3), wherein the peptide is a peptide having ability to bind to an Fc region of a monoclonal antibody.

(5) The compound or salt thereof according to (4), wherein the peptide is a peptide having ability to bind to an Fc region of IgG.

(6) The compound or salt thereof according to any of (2) to (5), wherein the peptide has 10 to 40 amino acid residues.

(7) The compound or salt thereof according to any of (2) to (6), wherein the peptide comprises

(a) (a-1-1) the amino acid sequence of FNMQQRRFYALHDPNLNNEQRNARIRSIRDD (SEQ ID NO: 11) or

(a-1-2) the amino acid sequence of FNMQCRRFYALHDPNLNNEQRNARIRSIRDDC (SEQ ID NO: 12), wherein any one to three amino acid residues in the sequence, which may be the same or different, are each substituted with one amino acid residue selected from the group consisting of a lysine residue, an aspartic acid residue, and a glutamic acid residue, or (a-2-1) the amino acid sequence of O-Ala NMQQRRFYALHDPNLNNEQRNARIRSIRDD (SEQ ID NO: 13) or

(a-2-2) the amino acid sequence of O-Ala NMOCRRFYALHDPNLNNEQRNARIRSIRDDC (SEQ ID NO: 14), wherein any one to three amino acid residues in the sequence, which may be the same or different, are each substituted with one amino acid residue selected from the group consisting of a lysine residue, an aspartic acid residue, and a glutamic acid residue, and

(b) an amino acid sequence having 85 % or more identity to each of the amino acid sequences of SEQ ID NOs: 11 to 14

(8) The compound or salt thereof according to any of (2) to (6), wherein the peptide comprises any of amino acid sequences of

Formula	1-1 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-I-I-W-C-(X_{0-3})_b$	(SEQ ID NO: 15)
Formula	1-2 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-I-V-W-C-(X_{0-3})_b$	(SEQ ID NO: 16)
Formula	1-3 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-V-V-W-C-(X_{0-3})_b$	(SEQ ID NO: 17)
Formula	1-4 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-A-V-W-C-(X_{0-3})_b$	(SEQ ID NO: 18)
Formula	1-5 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-L-L-W-C-(X_{0-3})_b$	(SEQ ID NO: 19)
Formula	1-6 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-L-I-W-C-(X_{0-3})_b$	(SEQ ID NO: 20)
Formula	1-7 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-L-V-F-C-(X_{0-3})_b$	(SEQ ID NO: 21)
Formula	1-8 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-X_{aa6}-Q-V-W-C-(X_{0-3})_b$	(SEQ ID NO: 22)
Formula	1-9 : $(X_{0-3})_a-C-X_{aa1}-X_{aa2}-X_{aa3}-X_{aa4}-X_{aa5}-$	

wherein

(X₀₋₃), is absence, or an arginine residue-glycine residue-asparagine residue, an aspartic acid residue, a glycine residue-asparagine residue, or an asparagine residue, (X₀₋₃), is absence, or a threonine residue-tyrosine residue-histidine residue, or a threonine residue,

Xaa1 is an alanine residue,

Xaa2 is a tyrosine residue, a tryptophan residue, or a histidine residue,

Xaa3 is a histidine residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, an arginine residue, or a glycine residue,

Xaa4 is a lysine residue, an aspartic acid residue, or a glutamic acid residue,

Xaa5 is a glycine residue, a serine residue, an asparagine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, a histidine residue, a threonine residue, a leucine residue, an alanine residue, a valine residue, an isoleucine residue, or an arginine residue,

Xaa6 is a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue, and

Formula 2-1: (X₀₋₃')a-C-(Xaa1')-(Xaa2')-(Xaa3')-(Xaa4')-(Xaa5')-(Xaa6')-L-V-W-C-(X₀₋₃')₆
(SEQ ID NO: 24)

wherein

(X₀₋₃')a and (X₀₋₃')₆ are the same as the above (X₀₋₃)a and (X₀₋₃)₆, respectively, and

Xaa1', Xaa2', Xaa3', Xaa4', Xaa5', and Xaa6' are the same as the above Xaa1, Xaa2, Xaa3, Xaa4, Xaa5, and Xaa6, respectively.

(9) The compound or salt thereof according to any of (1) to (8), wherein the leaving group is (1) a group selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- where R is a hydrogen atom or C₁₋₆ alkyl, or (2) heteroaryl.

(10) The compound or salt thereof according to any one of (1) to (9), wherein the nucleophilic group is selected from the group consisting of NH₂ in a side chain of a lysine residue, OH in a side chain of a tyrosine residue, OH in a side chain of a serine residue, OH in a side chain of a threonine residue, and SH in a side chain of a cysteine residue.

(11) The compound or salt thereof according to any one of (1) to (10), wherein the electrophilic group is selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-.

(12) The compound or salt thereof according to any of (1) to (11), wherein the bioorthogonal functional group is selected from the group consisting of an azide residue, an aldehyde residue, a thiol residue, an alkyne residue, an alkene residue, a halogen residue, a tetrazine residue, a nitron residue, a hydroxylamine residue, a nitrile residue, a hydrazine residue, a ketone residue, a boronic acid residue, a cyanobenzothiazole residue, an allyl residue, a phosphine residue, a maleimide residue, a disulfide residue, a thioester residue, an α-halocarbonyl residue, an isonitrile residue, a sydnone residue, and a selenium residue.

(13) The compound or salt thereof according to any one of (1) to (12), wherein the bioorthogonal functional group is selected from the group consisting of an azide residue, a thiol residue, an alkyne residue, a maleimide residue, and a disulfide residue.

(14) The compound or salt thereof according to any one of (1) to (13), wherein the compound represented by the above Formula (I) is a compound represented by Formula (I-1) below:

A-L1-L2-E1-E2-E3-B (I-1)

wherein

A and B are the same as those in the above Formula (I),

L₁ is a bond or a divalent group,

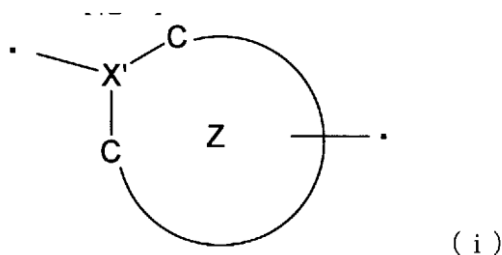
L₂ is a leaving group,

E₁, is an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

E₂ is a group represented by (a) —X—Y— wherein X which binds to E₁, is C (R₁) (R₂) (where R₁, and R₂ are each independently a hydrogen atom or C1-6 alkyl, N (R₃) where R₃ is a hydrogen atom or C1-6 alkyl), O, S, or Se, and Y which binds to E₃ is C (R₄) (R₅) (where R₄ and R₅, are each independently a hydrogen atom or C1-6 alkyl, or

E₂ is a group represented by (b) the following formula (i):

[Formula 1]



where ring Z is a divalent cyclic group in which all of a ring-constituting atom X ' which binds to E₁, and ring constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X ' which binds to E₁, is a nitrogen atom, and ring constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond,

when E₂ is —X—Y—, E₃ is a divalent group, and when E₂, is a group represented by Formula (i), E₃ is a bond or a divalent group, and

the leaving group has ability to be cleaved and eliminated from E₁, by a reaction between the nucleophilic group and the electrophilic group.

(15) The compound or salt thereof according to (14), wherein the L₂ is (a) ring P-Q- wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)—

where R is a hydrogen atom or C₁₋₆ alkyl,

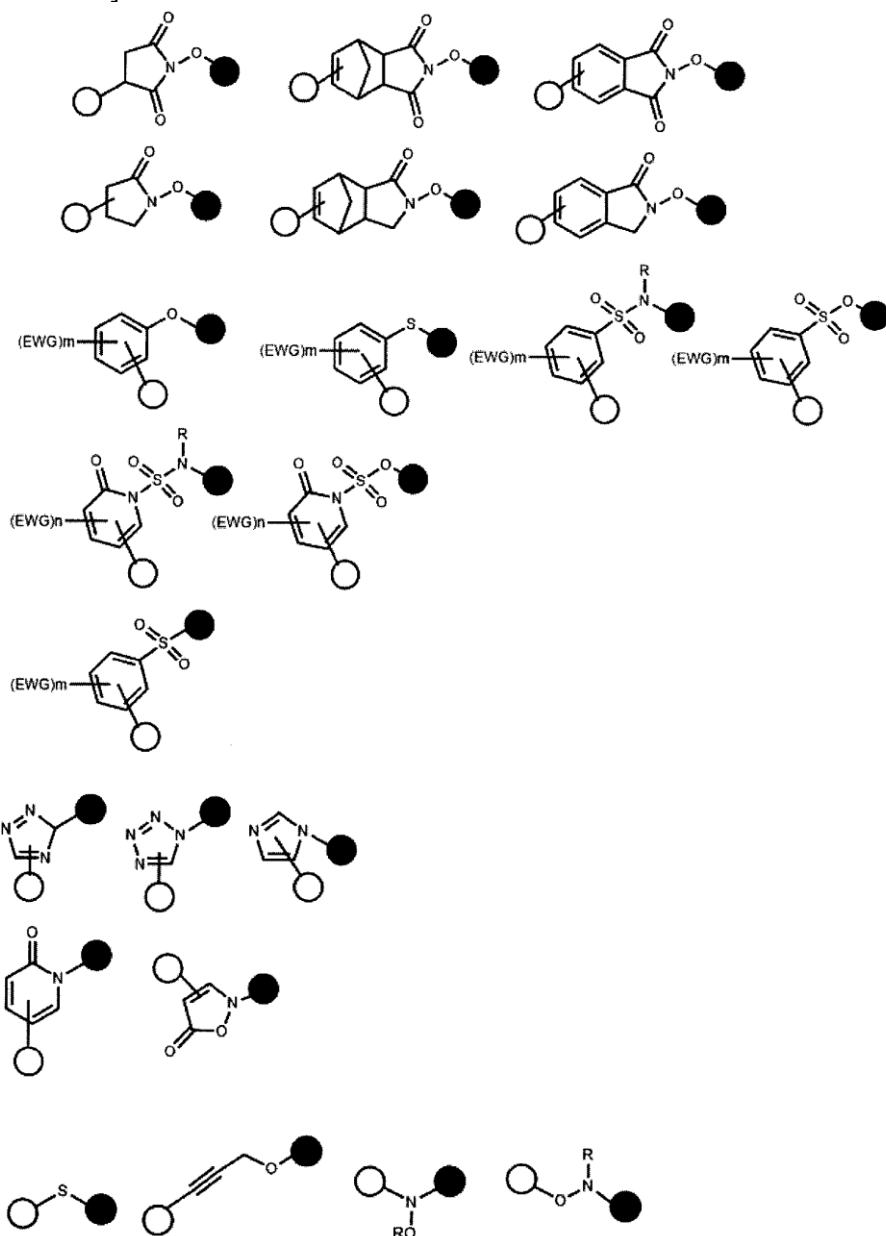
(b) heteroarylene, or

—Q—

wherein Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)— where R is a hydrogen atom or C₁₋₆alkyl.

(16) The compound or salt thereof according to (14) or (15), wherein the L₂ is a group selected from the group consisting of the following structural formulae:

[Formula 2]



where EWG is an electron-withdrawing group,

m is an integer of 0 to 4,

n is an integer of 0 to 3,

R is a hydrogen atom or C1-6 alkyl,

$\circ$ is a bond to L_1 , and

$\bullet$ is a bond to E_1 .

(17) The compound or salt thereof according to any of (1) to (16), wherein a main chain of L_1 or L_1 - L_2 coupling A with E consists of 20 or less atoms.

(18) The compound or salt thereof according to any of (14) to (17), wherein the compound represented by the above Formula (I-1) is a compound represented by the following Formula (I-2):

A-L₁-L₂-E₁-X-Y-E₃-B (I-2)

(wherein

A, L₁, X, Y, and B are the same as those in the above Formula (I-1),

L₂ is

(a) ring P—Q— wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂-N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)— (R)— where R is a hydrogen atom or C₁₋₆ alkyl,

(b) heteroarylene, or

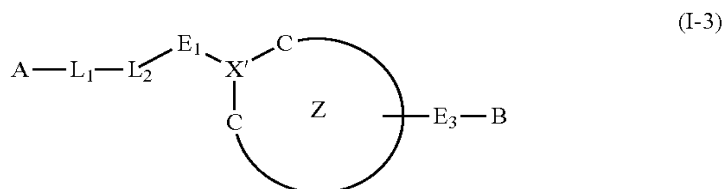
(c) —Q— wherein Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂-N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)— (R)—,

where R is a hydrogen atom or C₁₋₆ alkyl,

E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and —CH₂—, and E₃ is a divalent group.

(19) The compound or salt thereof according to any of (14) to (17), wherein the compound represented by the above Formula (I-1) is a compound represented by the following Formula (I-3):

[Formula 3]



wherein

A, L₁, ring Z, and B are the same as those in the above Formula (I-1),

L₂ is

(a) ring P-Q- wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂-N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)—, where R is a hydrogen atom or C₁₋₆ alkyl,

(b) heteroarylene, or

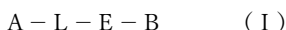
(c) -Q- wherein is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂-N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)—,

where R is a hydrogen atom or C₁₋₆ alkyl,

E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and —CH₂—, and

E₃ is a bond or a divalent group.

(20) A reagent of regioselectively modifying an antibody by a bioorthogonal functional group, the reagent comprising a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

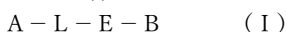
E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof.

(21) A method for producing an antibody having a bioorthogonal functional group or bioorthogonal functional groups or a salt thereof, the method comprising:

reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

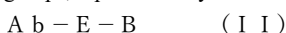
A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):



wherein

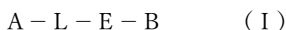
E and B are the same as those in the above Formula (I), and

Ab is an antibody, or

a salt thereof.

(22) A method for producing an antibody having a functional substance or functional substances or a salt thereof, the method comprising:

(1) reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

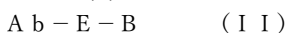
L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody

to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):



wherein

E and B are the same as those in the above Formula (I), and

Ab is an antibody, or

a salt thereof; and

(2) reacting the antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the above

to form an antibody having a functional substance or functional substances, represented by the following Formula (III):



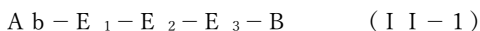
wherein Ab is the same as that in Formula (II),

E is the same as that in Formula (I),

B' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and

F is a functional substance, or a salt thereof.

(23) An antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-1):



wherein

Ab is an antibody,

E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a) -X-Y

wherein X which binds to E₁, is C (R₁) (R₂)

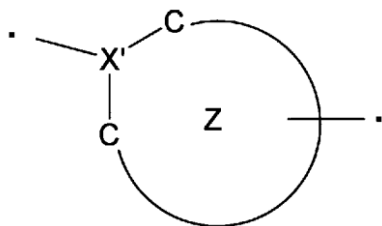
where R₁ and R₂ are each independently a hydrogen atom or C1-6 alkyl, N (R₃) where R₃ is a hydrogen atom or C1-6 alkyl, O, S, or Se, and

Y which binds to E₃ is C (R₄) (R₅)

where R₄ and R₅ are each independently a hydrogen atom or C1-6 alkyl, or

E₂ is a group represented by (b) the following formula (i):

[Formula 4]



wherein ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring constituting atom X' which binds to E, is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond,

when E₂, is —X—Y—, E₃ is a divalent group, and when E₂ is a group represented by Formula (i), E₃ is a bond or a divalent group, and

B is a bioorthogonal functional group, or

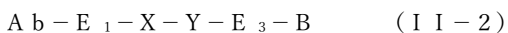
a salt thereof.

(24) The antibody or salt thereof according to (23), wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in a constant region of a monoclonal antibody.

(25) The antibody or salt thereof according to (23) or (24), wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in an Fc region of a monoclonal antibody.

(26) The antibody or salt thereof according to any of (23) to (25), wherein the antibody is a human IgG regioselectively having a bioorthogonal functional group or bioorthogonal functional groups in a region consisting of amino acid residues at positions 246 to 248 or 288 to 290 in a human IgG Fc region.

(27) The antibody or salt thereof according to any of (23) to (26), wherein the antibody represented by the above Formula (II-1) is an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following formula (II-2):



wherein

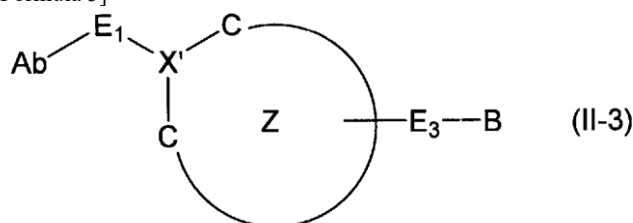
Ab, X, Y, and B are the same as those in the above Formula (II-1),

E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and CH₂, and

E₃ is a divalent group.

(28) The antibody or salt thereof according to any of (23) to (26), wherein the antibody represented by the above Formula (II-1) is an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following formula (II-3):

[Formula 5]



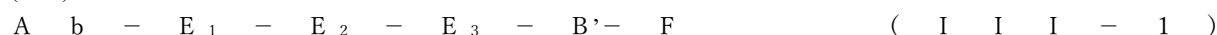
wherein

Ab, ring-constituting atom X', ring Z, and B are the same as those in the above Formula (II-1),

E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and CH₂, and

E₃ is a bond or a divalent group.

(29) An antibody regioselectively having a functional substance or functional substances, represented by the following Formula (III-1):



wherein

Ab is an antibody,

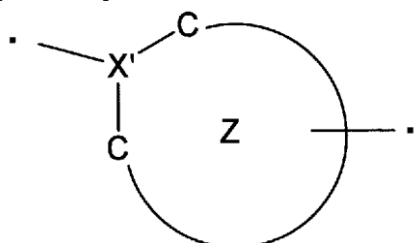
E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a) —X—Y—

wherein X which binds to E₁, is C (R₁) (R₂) where R₁ and R₂ are each independently a hydrogen atom or C₁₋₆ alkyl, N (R₃)

where R₃ is a hydrogen atom or C₁₋₆ alkyl, O, S, or Se, and Y which binds to E₃ is C (R₄) (R₅) where R₄ and R₅ are each independently a hydrogen atom or C₁₋₆ alkyl, or E₂ is a group represented by (b) the following formula (i):

[Formula 6]



wherein ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring constituting atom X' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond, E₂ is a divalent group when E₃ is —X—Y—, and is a bond or a divalent group when E₂ is a group represented by Formula (i), B' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and

F is a functional substance, or a salt thereof.

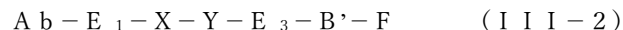
(30) The antibody or salt thereof according to (29), wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in a constant region of a monoclonal antibody.

(31) The antibody or salt thereof according to (29) or (30), wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in an Fc region of a monoclonal antibody.

(32) The antibody or salt thereof according to any of (29) to (31), wherein the antibody is a human IgG regioselectively

having a functional substance or functional substances in a region consisting of amino acid residues at positions 246 to 248 or 288 to 290 in a human IgG Fc region.

(33) The antibody or salt thereof according to any of (29) to (32), wherein the antibody represented by the above Formula (III-1) is an antibody regioselectively having a functional substance or functional substances, represented by the following formula (III-2):



wherein

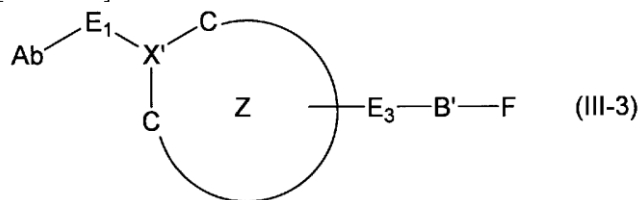
Ab, X, Y, B', and F are the same as those in the above Formula (III-1),

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and CH_2 , and

E₃ is a divalent group.

(34) The antibody or salt thereof according to any of (29) to (32), wherein the antibody represented by the above Formula (III-1) is an antibody regioselectively having a functional substance or functional substances, represented by the following formula (III-3):

[Formula 7]



wherein

Ab, ring-constituting atom X', ring Z, B', and F are the same as those in the above Formula (III-1),

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and CH_2 , and

E₃ is a divalent group.

[Effects of the Invention]

[0012]

The compound of the present invention having an affinity substance to an antibody and a bioorthogonal functional group or a functional substance, or a salt thereof is useful for regioselective modification of an antibody, for example.

The antibody of the present invention regioselectively having a bioorthogonal functional group or a salt thereof is useful as an intermediate in preparation of an antibody regioselectively having a functional substance or functional substances or a salt thereof, for example.

The antibody of the present invention regioselectively having a functional substance or functional substances or a salt thereof is useful as pharmaceuticals or reagents (e.g., diagnostic reagents and reagents for research), for example.

[Brief Description of the Drawings]

[0013]

[FIG. 1] FIG. 1 is a schematic diagram illustrating an outline of the present invention.

[FIG. 2] FIG. 2 is a diagram illustrating analysis results by SDS-PAGE in synthesis of an IgG antibody trastuzumab-peptide conjugate. Lanes 1, 3, 6, and 8: molecular weight markers; Lane 2: unreacted IgG antibody trastuzumab (control, a band near a molecular weight of 50,000 indicates a heavy chain, and a band near a molecular weight of 25,000 indicates a light chain); Lane 4: a conjugate of LgG antibody trastuzumab and compound 10 (An upper band near a molecular weight of 50,000 indicates that compound 10 has been conjugated with a heavy chain of trastuzumab. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain); Lane 5: a conjugate of IgG antibody trastuzumab and compound 11 (An upper band near a molecular weight of 50,000 indicates that compound 11 has been conjugated with a heavy chain of trastuzumab. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain); Lane 7: a reaction mixture after conjugation of IgG antibody trastuzumab with compound 12 (A band near a molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. A band indicating conjugation of compound 12 is not observed).

[FIG. 3] FIG. 3 is a diagram illustrating analysis results by SDS-PAGE of a trastuzumab-peptide conjugate synthesized by regiospecifically introducing maleimide to IgG antibody trastuzumab and then conjugating the resulting product with a peptide reagent having a thiol. Lanes 1 and 9: molecular weight markers. Lane 2: a conjugate obtained by treating IgG antibody trastuzumab with compound 22 (10 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 3: a conjugate obtained by treating IgG antibody trastuzumab with compound 22 (20 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 4: a conjugate obtained by treating IgG antibody trastuzumab with compound 22 (40 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 5: a conjugate obtained by treating IgG antibody trastuzumab with compound 23 (10 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 6: a conjugate obtained by treating IgG antibody trastuzumab with compound 23 (20 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 7: a conjugate obtained by treating IgG antibody trastuzumab with compound 23 (40 molar equivalents to the antibody) to introduce maleimide thereto regioselectively, and then conjugating the resulting product with compound 25. An upper band near a molecular weight of 50,000 indicates that maleimide has been introduced to a heavy chain of trastuzumab and the trastuzumab has been conjugated with compound 25. A lower band near the molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lanes 8 and 10: unreacted IgG antibody trastuzumab (control, A band near a molecular weight of 50,000 indicates a heavy chain, and a band near a molecular weight of 25,000 indicates a light chain) Lane 11: a reaction mixture obtained by treating IgG antibody trastuzumab with compound 24 (10 molar equivalents to the antibody) and then adding compound 25 thereto. A band near a molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 12: a reaction mixture obtained by treating IgG antibody trastuzumab with compound 24 (20 molar equivalents to the antibody) and then adding compound 25 thereto. A band near a molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain. Lane 13: a reaction mixture obtained by treating IgG antibody trastuzumab with compound 24 (40 molar equivalents to the antibody) and then adding compound 25 thereto. A band near a molecular weight of 50,000 indicates an unreacted heavy chain, and a band near a molecular weight of 25,000 indicates an unreacted light chain.

[FIG. 4] FIG. 4 is a diagram illustrating ESI-TOFMS of maleimide-modified trastuzumab synthesized in (4-5-1) under post-reduction conditions. An upper part indicates measurement results of unreacted trastuzumab, and a lower part indicates modified trastuzumab.

[FIG. 5] FIG. 5 is a diagram illustrating ESI-TOFMS of azide-modified trastuzumab (azide-modified antibody 1) synthesized in (6-1-1) under post-reduction conditions. A lower part indicates measurement results of unreacted trastuzumab, and an upper part indicates modified trastuzumab.

[FIG. 6] FIG. 6 is a diagram illustrating ESI-TOFMS of a trastuzumab-Cy3 conjugate synthesized in (7-1-1). A lower part indicates measurement results of unreacted trastuzumab, a middle part indicates measurement results of azide-modified trastuzumab synthesized in (6-1-1), and an upper part indicates the trastuzumab-Cy3 conjugate.

[FIG. 7] FIG. 7 is a diagram illustrating ESI-TOFMS of a trastuzumab-Cy3 conjugate treated in (7-1-2) under reduction conditions. A lower part indicates measurement results of unreacted trastuzumab, a middle part indicates measurement results of azide-modified trastuzumab synthesized in (6-1-1), and an upper part indicates the trastuzumab-Cy3 conjugate.

[FIG. 8] FIG. 8 is a diagram illustrating ESI-TOFMS of a trastuzumab-peptide conjugate synthesized in (7-2-2). A lower part indicates measurement results of unreacted trastuzumab, a middle part indicates measurement results of azide-modified trastuzumab synthesized in (6-1-1), and an upper part indicates the trastuzumab-Cy3 conjugate.

[FIG. 9] FIG. 9 is a diagram illustrating ESI-TOFMS of a trastuzumab-Cy3 conjugate treated in (7-2-3) under reduction conditions. A lower part indicates measurement results of unreacted trastuzumab, a middle part indicates measurement results of azide-modified trastuzumab synthesized in (6-1-1), and an upper part indicates the trastuzumab – peptide conjugate.

[FIG. 10] FIG. 10 shows (1) the amino acid sequence of the heavy chain of trastuzumab whose sugar chain has been cleaved with PNGase (SEQ ID NO: 2), and (2) the amino acid sequence of the light chain of trastuzumab (SEQ ID NO: 4).

[FIG. 11] FIG. 11 shows the CID spectrum (m/x 905.73328, 4-valent) of a peptide fragment of THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR (sequence number 11), which contains a modification site (amine benzoic acid introduced (+119.037 Da)) on a lysine residue by trypsin digestion of trastuzumab modified with azidobenzoic acid.

[FIG. 12] FIG. 12 shows a CID spectrum of a peptide fragment of THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR (sequence number 11), which contains a modification site (amine benzoic acid introduced (+119.037 Da)) on a lysine residue by trypsin digestion of trastuzumab modified with azidobenzoic acid.

[FIG. 13] FIG. 13 shows the MS spectrum (m/s 760.38287, trivalent) of a peptide fragment of THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR (SEQ ID NO: 12) containing a modification site (amine benzoic acid introducer (+119.037 Da)) on a lysine residue by trypsin digestion of trastuzumab modified with an azidobenzoic acid introducer.

[FIG. 14] FIG. 14 shows the CID spectrum of a peptide fragment of FNWYVDGVEVHNAKTKPR (SEQ ID NO: 12) containing a modification site (amine benzoic acid introducer (+119.037 Da)) on a lysine residue by trypsin digestion of trastuzumab modified with an azidobenzoic acid introducer.

[FIG. 15] FIG. 15 shows (1) the consensus amino acid sequence of the Fc region in the heavy chain of trastuzumab and the IgG1 Fc region (SEQ ID NO: 1), and (2) the amino acid sequence of the IgG1 Fc region (SEQ ID NO: 3).

[Form for Carrying Out the Invention]

[0014] 1. Compound Having Affinity Substance to Anti- body and Bioorthogonal Functional Group, or Salt Thereof

1-1. Outline

The present invention provides a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by Formula (I), or a salt thereof

A-L-E-B (I)

wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic

group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group.

[0015] In expressions of Formula (I) and other formulae presented in relation to the present invention, - (a hyphen) indicates that two units present on both sides thereof covalently bind to each other. Consequently, in Formula (I), A covalently binds to L, L covalently binds to A and E, E covalently binds to L and B, and B covalently binds to E.

[0016] 1-2. Affinity Substance to Antibody (A)

In Formula (I), A is an affinity substance to an antibody. The affinity substance to an antibody is a substance having binding ability through a noncovalent bond to an antibody.

[0017] The affinity substance used in the present invention targets an antibody. The antibody may be an antibody modified with a biomolecule (e.g., sugar) or an antibody unmodified with a biomolecule. As the antibody, any anti-body to any component such as a bio-derived component, a virus-derived component, or a component found in an environment can be used, but an antibody to a bio-derived component or a virus-derived component is preferable. Examples of the bio-derived component include components derived from animals such as mammals and birds (e.g., chickens), insects, microorganisms, plants, fungi, and fishes (e.g., protein). The bio-derived component is preferably a component derived from mammals. Examples of the mammals include primates (e.g., humans, monkeys, and chimpanzees), rodents (e.g., mice, rats, guinea pigs, hamsters, and rabbits), pets (e.g., dogs and cats), domestic animals (e.g., cows, pigs, and goats), and work animals (e.g., horses and sheep). The bio-derived component is more preferably a component derived from primates or rodents (e.g., protein), and even more preferably a human-derived component (e.g., protein) in view of the clinical application of the present invention. Examples of the virus-derived component include components derived from influenza viruses (e.g., avian influenza viruses and swine influenza viruses), AIDS virus, Ebola virus, and phage viruses (e.g., protein).

[0018] The antibody is a polyclonal antibody or a monoclonal antibody, and is preferably a monoclonal antibody. Examples of the monoclonal antibody include chimeric antibodies, humanized antibodies, human antibodies, anti-bodies with a certain sugar chain added (e.g., an antibody modified so as to have a sugar chain-binding consensus sequence such as an N-type sugar chain-binding consensus sequence), bi-specific antibodies, scFv antibodies, Fab anti-bodies, F(ab')₂ antibodies, VHH antibodies, Fe region proteins, and Fe-fusion proteins. The antibody may be a divalent antibody (e.g., IgG, IgD, or IgE) or a tetravalent or higher antibody (e.g., IgA antibody or IgM antibody).

[0019] The antibody as a target of the affinity substance may comprise any amino acid residues and preferably comprises 20 natural L-α-amino acid residues normally contained in proteins. Examples of such amino acid residues include L-alanine (A), L-asparagine (N), L-cysteine (C), L-glutamine (Q), L-isoleucine (I), L-leucine (L), L-methionine (M), L-phenylalanine (F), L-proline (P), L-serine (S), L-threonine (T), L-tryptophan (W), L-tyrosine (Y), L-valine (V), L-aspartic acid (D), L-glutamic acid (E), L-arginine (R), L-histidine (H), L-lysine (K), and glycine (G) (hereinafter, the expression of L is omitted). The antibody may

comprise e.g., 100 or more, preferably 120 or more, more preferably 150 or more, even more preferably 180 or more, and particularly preferably 200 or more amino acid residues. The antibody may comprise e.g., 1,000 or less, preferably 900 or less, more preferably 800 or less, even more preferably 700 or less, and particularly preferably 600 or less amino acid residues. More specifically, the antibody may comprise e.g., 100 to 1,000, preferably 120 to 900, more preferably 150 to 800, even more preferably 180 to 700, and particularly preferably 200 to 600 amino acid residues. When the antibody is an antibody (e.g., the monoclonal antibody described above), the above number of amino acid residues may correspond to amino acid residues of a heavy chain of the antibody.

[0020] The antibody as the target of the affinity substance is further a protein comprising specific amino acid residues having a side chain or a terminal (an N-terminal and/or a C-terminal), preferably a side chain, with which a bioorthogonal functional group described below is capable of reacting at one position or a plurality of positions (preferably a plurality of positions). Examples of such specific amino acid residues include amino acid residues described below; preferred are amino acid residues selected from the group consisting of a lysine residue, a tyrosine residue, a serine residue, a threonine residue, and a cysteine residue. Considering that the compound of the present invention can regioselectively modify an antibody, preferred is an antibody comprising such specific amino acid residues at a plurality of positions. The positions are not limited to particular positions so long as they are two or more positions and may be e.g., three or more positions, preferably five or more positions, more preferably ten or more positions, even more preferably 20 or more positions, and particularly preferably 30 or more positions. The positions may be e.g., 200 or less positions, preferably 180 or less positions, more preferably 150 or less positions, even more preferably 120 or less positions, and particularly preferably 100 or less positions. More specifically, the positions may be e.g., 3 to 200 positions, preferably 5 to 180 positions, more preferably 10 to 150 positions, even more preferably 20 to 120 positions, and particularly preferably 30 to 100 positions. Even for such an antibody comprising the specific amino acid residues at a plurality of positions, the compound of the present invention can regioselectively modify one or two specific amino acid residues present in a specific region. It is said that the number of lysine residues of human IgG1 is generally about 70 to 90, for example, although it depends on an amino acid composition in a variable region. The present invention has succeeded in regioselectively modifying such one or two lysine residues present in a specific region of human IgG1.

[0021] More specifically, in the present invention, in view of, while maintaining the function of an antibody (that is, while maintaining native folding without denaturing the antibody), modifying amino acid residues present at specific target sites in the antibody, preferred is regioselective modification of amino acid residues exposed to the surface of the antibody. In human IgG such as human IgG1, for example, exposed lysine residues and exposed tyrosine residues are present at the following positions (refer to http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnb er.html by EU numbering).

(1) Exposed lysine residues

CH2 domain (position 246, position 248, position 274, position 288, position 290, position 317, position 320, position 322, and position 338)

CH3 domain (position 360, position 414, and position 439)

(2) Exposed tyrosine residues

CH2 domain (position 278, position 296, and position 300)

CH3 domain (position 436)

(3) Exposed serine residues

CH2 domain (position 254, position 267, position 298, and position 324)

CH3 domain (position 375, position 400, position 415, position 440, and position 442)

(4) Exposed threonine residue

CH2 domain (position 256, position 289, and position 307)

CH3 domain (position 335, position 359, position 393, and position 437)

Consequently, when human IgG such as human IgG1 is modified with a lysine residue or a tyrosine residue, modification at the above positions is preferred.

[0022] When human IgG such as human IgG1 is modified with a lysine residue, a tyrosine residue, a serine residue, or a threonine residue, among the positions of (1) to (4), lysine residues, tyrosine residues, serine residues, or tyrosine residues present at the following positions, which are high in the degree of exposure to the surface, may be preferably modified

(1') Exposed Lysine Residues

CH2 domain (position 246, position 248, position 274, position 288, position 290, position 317, position 320, and position 322)

CH3 domain (position 360, position 414, and position 439)

(2') Exposed Tyrosine Residues

CH2 domain (position 278, position 296, and position 300)

CH3 domain (position 436)

(3') Exposed Serine Residues

CH2 domain (position 254, position 267, and position 298)

CH3 domain (position 400, position 415, and position 440)

(4') Exposed threonine residues

CH2 domain (position 256 and position 289)

CH3 domain (position 335 and position 359) Consequently, when human IgG such as human IgG1 is modified with a lysine residue, a tyrosine residue, a serine residue, or a threonine residue, modification at the above positions is more preferred.

[0023] When human IgG such as human IgG1 is modified with a lysine residue, among the positions of (1), lysine residues present at certain positions (e.g., position 246, position 248, position 288, position 290, and position 317) in the CH2 domain, which can be efficiently modified in the present invention, may be more preferably modified.

[0024] In a specific embodiment, the antibody as the target of the affinity substance, when comprising the specific amino acid residues at a plurality of positions as described above, may comprise one or more specific amino acid residues in a target region consisting of 1 to 50 continuous amino acid residues and comprise five or more of the specific amino acid residues in a non-target region other than the target region. The target region may consist of preferably 1 to 30, more preferably 1 to 20, and even more preferably one to ten, one to five, or one to three (that is, one, two, or three) amino acid residues. The target region may be particularly preferably a region consisting of a specific amino acid residue present at a specific position. Such a specific position, which varies depending on the types of the target protein and the affinity substance and the like, may be e.g., a specific position in a specific region of a constant region of an antibody (e.g., CH1, CH2, and CH3) and preferably a position in CH2 of an antibody. The target region may be more specifically the following residues following Eu numbering in human IgG Fe:

(1) a Lys248 residue (hereinafter, also referred to simply as "Lys248" in the present specification and corresponding to the 18th residue in a human IgG CH2 region (SEQ ID NO: 1)) or a Lys246 residue (hereinafter, also referred to simply as "Lys246" in the present specification and corresponding to the 16th residue in the human IgG CH2 region (SEQ ID NO: 1));

(2) a Lys288 residue (hereinafter, also referred to simply as "Lys288" in the present specification and corresponding to the 58th residue in the human IgG CH2 region (SEQ ID NO: 1)) or a Lys290 residue (hereinafter, also referred to simply as "Lys290" in the present specification and corresponding to the 60th residue in the human IgG CH2 region (SEQ ID NO: 1)); and

(3) a Lys317 residue (hereinafter, also referred to simply as "Lys317" in the present specification and corresponding to the 87th residue in the human IgG CH2 region (SEQ ID NO: 1)).

[0025] The present invention can modify the specific amino acid residue in the target region highly regioselectively. Such regioselectivity may be e.g., 30% or more, preferably 40% or more, more preferably 50% or more, even more preferably 60% or more, and particularly preferably 70% or more, 80% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 100%.

[0026] The target region does not necessarily comprise the same kind of amino acid residue as the specific amino acid residue other than the specific amino acid residue present at the specific position in a region up to a remote position of "a" (where "a" is any integer of 1 to 10) amino acid residues to an N-terminal side and a C-terminal side each with respect to the specific amino acid present at the specific position. The symbol "a" is preferably an integer of 1 to 5, more preferably an integer of 1 to 3, even more preferably 1 or 2, and particularly preferably 1.

[0027] In a preferred embodiment, the antibody is a monoclonal antibody. Examples of the isotype of the monoclonal antibody and the like include IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA, IgD, IgE, and IgY. The monoclonal antibody is a full-length antibody or an antibody fragment (e.g., F(ab')₂, Fab', Fab, Fv, and a single-chain antibody); the full-length antibody is preferred. The antibody is particularly preferably a human antibody, a humanized antibody, or a chimeric antibody having human IgG (e.g., IgG1, IgG2, IgG3, and IgG4) in a constant region.

[0028] The antibody is an antibody to any antigen. Such an antigen may be a component found in organisms and viruses described above, for example. Examples of such an antigen include a protein [comprising an oligopeptide and a poly-

peptide, which may be a protein modified with a biomolecule such as sugar (e.g., glycoprotein)], a sugar chain, a nucleic acid, and a small compound.

[0029] The antibody may be preferably an antibody with a protein as an antigen. Examples of the protein include cell membrane receptors, cell membrane proteins other than cell membrane receptors (e.g., extracellular matrix proteins), ligands, and soluble receptors.

[0030] More specifically, the protein as the antigen of the antibody may be a disease target protein. Examples of the disease target protein include the following.

[0031] (1) Cancerous Region

PD-L1, GD2, PDGFR α (a platelet-derived growth factor receptor), CD22, HER2, phosphatidyl serine (PS), EpCAM, fibronectin, PD-1, VEGFR-2, CD33, HGF, gpNMB, CD27, DEC-205, folic acid receptors, CD37, CD19, Trop2, CEACAM5, SiP, HER3, IGF-IR, DLL4, TNT-1/B, CPAAAs, PSMA, CD20, CD105 (Endoglin), ICAM-1, CD30, CD16A, CD38, MUC1, EGFR, KIR2DL1, KIR2DL2, NKG2A, tenascin-C, IGF (insulin-like growth factor), CTLA-4, mesothelin, CD138, c-Met, Ang2, VEGF-A, CD79b, ENPD3, folic acid receptor α , TEM-1, GM2, Glypican 3, macrophage inhibitory factor, CD74, Notch1, Notch2, Notch3, CD37, TLR-2, CD3, CSF-IR, FGFR2b, HLA-DR, GM-CSF, EphA3, B7-H3, CD123, gpA33, Frizzled 7 receptor, DLL4, VEGF, RSPO, LIV-1, SLITRK6, Nectin-4, CD70, CD40, CD19, SEMA4D (CD100), CD25, MET, Tissue Factor, IL-8, EGFR, cMet, KIR3DL2, Bst1 (CD157), P-Cadherin, CEA, GITR, TAM (tumor associated macrophage), CEA, DLL4, Ang2, CD73, FGFR2, CXCR4,

LAG-3, GITR, Fucosyl GM1, IGF-1, Angiopoietin 2, CSF-1R, FGFR3, OX40, BCMA, ErbB3, CD137 (4-1BB), PTK7, EFNA4, FAP, DRS, CEA, Ly6E, CA6, CEACAM5, LAMPI, tissue factor, EPHA2, DRS, B7-H3, FGFR4, FGFR2, α 2-PI, A33, GDF15, CAIX, CD166, ROR1, GITR, BCMA, TBA, LAG-3, EphA2, TIM-3, CD-200, EGFRvIII, CD16A, CD32B, PIGF, Ax1, MICA/B, Thomsen-Friedenreich, CD39, CD37, CD73, CLEC2A, Lgr3, transferrin receptors, TGF, IL-17, 5T4, RTK, Immune Suppressor Protein, NaPi2b, Lewis blood group B antigen, A34, Lysil-Oxidase, DLK-1, TROP-2, α 9 Integrin, TAG-72 (CA72-4), and CD70.

[0032] (2) Autoimmune Diseases and Inflammatory Diseases

IL-17, IL-6R, IL-17R, INF- α , IL-5R, IL-13, IL-23, IL-6, ActRIIB, 7-Integrin, IL-4aR, HAS, Eotaxin-1, CD3, CD19, TNF- α , IL-15, CD3, Fibronectin, IL-1, IL-1a, IL-17, TSLP (Thymic Stromal Lymphopoietin), LAMP (Al-pha4 Beta 7 Integrin), IL-23, GM-CSFR, TSLP, CD28, CD40, TLR-3, BAFF-R, MAdCAM, IL-31R, IL-33, CD74, CD32B, CD79B, IgE (immunoglobulin E), IL-17A, IL-17F, CS, FcRn, CD28, TLR4, MCAM, B7RP1, CXCR1/2 Ligands, IL-21, Cadherin-11, CX3CL1, CCL20, IL-36R, IL-10R, CD86, TNF- α , IL-7R, Kv1.3, α 9 Integrin, and LIFHT.

[0033] (3) Brain or Nerve Diseases

CGRP, CD20, amyloid, amyloid protofibril, Calcitonin Gene-Related Peptide Receptor, LINGO (Ig Domain Containing 1), α Synuclein, extracellular tau, CD52, insulin receptors, tau protein, TDP-43, SOD1, TauC3, and JC virus.

[0034] (4) Infectious Diseases

Clostridium Difficile toxin B, cytomegalovirus, RS viruses, LPS, *S. Aureus* Alpha-toxin, M2e protein, Psi, PcrV, *S. Aureus* toxin, influenza A, Alginate, *Staphylococcus aureus*, PD-L1, influenza B, *Acinetobacter*, F-protein, Env, CD3, enteropathogenic *Escherichia coli*, *Klebsiella*, and *Streptococcus pneumoniae*.

[0035] (5) Hereditary Rare Diseases

amyloidAL, SEMA4D (CD100), insulin receptors, ANGPTL3, IL4, IL13, FGF23, adrenocorticotrophic hormone, transthyretin, and huntingtin.

[0036]

(6) Eye Diseases

Factor D, IGF-IR, PGDFR, Ang2, VEGF-A, CD-105 (Endoglin), IGF-IR, and β amyloid.

[0037] (7) Bone and Orthopedic Region

Sclerostin, Myostatin, Dickkopf-1, GDF8, RANKL, HAS, Siglec-15

[0038] (8) Blood Diseases

vWF, Factor IXa, Factor X, IFN γ , CS, BMP-6, Ferroportin, TFPI

[0039] (9) Other Diseases

BAFF (B cell activating factor), IL-1 β , PCSK9, NGF, CD45, TLR-2, GLP-1, TNFR1, CS, CD40, LPA, prolactin receptors, VEGFR-1, CBI, Endoglin, PTH1R, CXCL1, CXCL8, IL-1, AT2-R, and IAPP.

[0040] In a more preferred embodiment, the affinity substance to an antibody is an affinity substance to a monoclonal antibody. The isotype of the monoclonal antibody is similar to those described above for the antibody; IgG (e.g., IgG1, IgG2, IgG3, and IgG4) is preferred. The monoclonal antibody is preferably a full-length monoclonal antibody.

[0041] In an even more preferred embodiment, the affinity substance to an antibody is an affinity substance to a chimeric antibody, a humanized antibody, or a human antibody (e.g., IgG including IgG1, IgG2, IgG3, and IgG4) as a full-length monoclonal antibody.

[0042] In a particularly preferred embodiment, the affinity substance to an antibody is an affinity substance to an antibody, comprising any one Fe region protein selected from the group consisting of the following (A) to (C) and having antigen-binding ability:

(A) an Fe region protein comprising the amino acid sequence of SEQ ID NO: 1;

(B) an Fe region protein comprising an amino acid sequence with one or several amino acid residues inserted, added, deleted, or substituted in the amino acid sequence of SEQ ID NO: 1; and

(C) an Fe region protein comprising an amino acid sequence having 90% or more identity to the amino acid sequence of SEQ ID NO: 1.

[0043] The amino acid sequence of SEQ ID NO: 1 is an Fe region protein. It is known that such an Fe region protein has secretion ability. Consequently, the Fe region proteins of (A) to (C) can have secretion ability. An antibody comprising such an Fe region protein can have antigen-binding ability. The amino acid residue at position 18 in SEQ ID NO: 1 is any amino acid residue, preferably a neutral amino acid residue, more preferably an amino acid residue having a nonpolar side chain described below, and even more preferably leucine, isoleucine, or alanine, and particularly preferably leucine or alanine. The amino acid residue at position 19 in SEQ ID NO: 1 is any amino acid residue, preferably a neutral amino acid residue or an acidic amino acid residue, more preferably an amino acid residue having a nonpolar side chain or an acidic amino acid residue, and even more preferably leucine or glutamic acid. The amino acid residue at position 21 in SEQ ID NO: 1 is any amino acid residue,

preferably a neutral amino acid residue, more preferably an amino acid residue having a nonpolar side chain, and even more preferably glycine or alanine. The amino acid residue at position 140 in SEQ ID NO: 1 is any amino acid residue, preferably an acidic amino acid residue, and more preferably glutamic acid or aspartic acid. The amino acid residue at position 142 in SEQ ID NO: 1 is any amino acid residue, preferably a neutral amino acid residue, more preferably an amino acid residue having a nonpolar side chain, even more preferably methionine, leucine, or isoleucine, and particularly preferably methionine or leucine. The amino acid residue at position 177 in SEQ ID NO: 1 is any amino acid residue, preferably a neutral amino acid residue, more preferably an amino acid residue having an uncharged polar side chain or an amino acid residue having a nonpolar side chain described below, even more preferably threonine, alanine, or glycine, and particularly preferably threonine or alanine.

[0044] In a preferred embodiment, the amino acid sequence of SEQ ID NO: 1 may be an amino acid sequence consisting of the amino acid residues at positions 220 to 449 in the amino acid sequence of SEQ ID NO: 2.

[0045] In another preferred embodiment, the amino acid sequence of SEQ ID NO: 1 may be an amino acid sequence consisting of the amino acid residues at positions 7 to 236 in the amino acid sequence of SEQ ID NO: 3.

[0046] In a specific embodiment, the antibody comprising the Fe region protein comprising the amino acid sequence described above may be an antibody comprising the Fe region protein comprising the amino acid sequence described above and a constant region of an antibody. Such a constant region of an antibody may be a constant region of a chimeric antibody, a humanized antibody, or a human antibody (e.g., IgG including IgG1, IgG2, IgG3, and IgG4).

[0047] In (B) the Fe region protein, one or several amino acid residues can be modified by one, two, three, or four variations selected from the group consisting of deletion, substitution, addition, and insertion of amino acid residues. The variations of amino acid residues may be introduced to one region in the amino acid sequence or intruded to a plurality of different regions. The term "one or several" indicates a number that does not significantly impair protein activity. The number indicated by the term "one or several" is e.g., 1 to 100, preferably 1 to 80, more preferably 1 to 50,

1 to 30, 1 to 20, 1 to 10, or 1 to 5 (e.g., one, two, three, four, or five).

[0048] In (C) the Fe region protein, the percent identity to the amino acid sequence of SEQ ID NO: 1 may be 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more. In the present invention, calculation of the percent identity of peptides and polypeptides (proteins) can be performed by Algorithm blast. More specifically, calculation of the percent identity of polypeptides can be performed using Scoring Parameters (Matrix: BLO- SUM62; Gap Costs: Existence=11 Extension=11; Compositional Adjustments: Conditional compositional score matrix adjustment) as default settings in Algorithm blastp provided in National Center for Biotechnology Information (NCBI). Calculation of the percent identity of polynucleotides (genes) can be performed by Algorithm blastn. More specifically, calculation of the percent identity of polynucleotides can be performed using Scoring Parameters (Match/Mismatch Scores=1, -2; Gap Costs=Linear) as default settings in Algorithm blastn provided in NCBI.

[0049] Secretion in secretion ability has the same meaning as the secretion (what is called solubility) of the secretory protein. Consequently, "having secretion ability" means functioning as an antibody in a manner similar to normal antibodies.

[0050] In the antibody comprising the Fe region protein, a variation may be introduced to a specific site so long as target characteristics (e.g., secretion ability and antigen-binding ability) are maintained. The position of an amino acid residue to which a variation may be introduced that can maintain the target characteristics is obvious to a person skilled in the art. Specifically, a person skilled in the art can

1) compare amino acid sequences of a plurality of proteins having homogeneous characteristics with each other, 2) clarify a relatively preserved region and a relatively non-preserved region, and then 3) predict a region capable of playing an important role for a function and a region incapable of playing an important role for a function from the relatively preserved region and the relatively non-preserved region each and can thus recognize structure-function correlation. Consequently, a person skilled in the art can identify the position of an amino acid residue to which a variation may be introduced in the amino acid sequence of the antibody comprising the Fe region protein.

[0051] When an amino acid residue is varied by substitution, the substitution of the amino acid residue may be preservative substitution. The term "preservative substitution" when used in the present specification refers to substituting a certain amino acid residue with an amino acid residue having a similar side chain. Families of amino acid residues having a similar side chain are known in the field concerned. Examples of such families include amino acids having a basic side chain (e.g., lysine, arginine, and histidine), amino acids having an acidic side chain (e.g., aspartic acid, and glutamic acid), amino acids having an uncharged polar side chain (e.g., asparagine, glutamine, serine, threonine, tyrosine, and cysteine), amino acids having a nonpolar side chain (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan), amino acids having a β -position-branched side chain (e.g., threonine, valine, and isoleucine), amino acids having an aromatic side chain (e.g., tyrosine, phenylalanine, tryptophan, and histidine), amino acids having a hydroxy group (e.g., alcoholic and phenolic)-containing side chain (e.g., serine, threonine, tyrosine), and amino acids having a sulfur-containing side chain (e.g., cysteine and methionine). The preservative substitution of the amino acid may be preferably substitution between aspartic acid and glutamic acid, substitution among arginine, lysine, and histidine, substitution between tryptophan and phenylalanine, substitution between phenylalanine and valine, substitution among leucine, isoleucine, and alanine, and substitution between glycine and alanine.

[0052] Examples of the antibody used in the present invention or the antibody comprising any one Fe region selected from the group consisting of (A) to (C) include chimeric antibodies (e.g., rituximab, basiliximab, infliximab, cetuximab, siltuximab, dinutuximab, and alisertiximab), humanized antibodies (e.g., daclizumab, palivizumab, trastuzumab, alemtuzumab, omalizumab, efalizumab, bevacizumab, natalizumab (IgG4), tocilizumab, eculizumab (IgG2), mogamulizumab, pertuzumab, obinutuzumab, vedolizumab, pembrolizumab (IgG4), mepolizumab, elotuzumab, daratumumab, ixekizumab (IgG4), reslizumab (IgG4), and atezolizumab), and human antibodies (e.g., adalimumab, panitumumab, golimumab, ustekinumab, canakinumab, ofatumumab, denosumab (IgG2), ipilimumab, belimumab, raxibacumab, ramucicromab, nivolumab (IgG4), secukinumab, evolocumab (IgG2), alirocumab, necitumumab, brodalumab (IgG2), olaratumab, and dupilumab (IgG4)) (cases not referring to the IgG subtype indicate that they are IgG1).

[0053] Examples of the affinity substance to an antibody described above include peptides (comprising oligopeptides, polypeptides, and proteins), small compounds, nucleic acids, nucleic acid-peptide conjugates, peptide-small compound conjugates, and nucleic acid-small compound conjugates.

[0054] In a specific embodiment, the affinity substance to an antibody described above may be a peptide (comprising an oligopeptide, a polypeptide, and a protein, which may be a glycoprotein). As such a peptide, for example, the following peptides have been reported:

(1) an IgG binding peptide having an affinity to a specific region (CH2 region) of human IgG in general (that is, human IgG1, IgG2, IgG3, and IgG4. Hereinafter the same) (e.g., refer to WO 2016/186206, WO 2013/027796, and WO 2008/054030, all of which are incorporated herein by reference in their entirety);

(2) a protein A Mimetic (PAM) peptide having an affinity to a specific region (CH2 region) of human IgG in general (e.g., refer to Fassina G et al., JOURNAL OF MOLECULAR RECOGNITION, 1996, VOL. 6, 564-569, which is incorporated herein by reference in its entirety);

(3) EPIHRSTLTALL (SEQ ID NO: 25) having an affinity to a specific region (CH2 region) of human IgG in general (e.g., refer to Ehrlich G. K et al., J. Biochem. Biophys. Methods, 2001, VOL. 49, 443-454, which is incorporated herein by reference in its entirety);

(4) (NH2-Cysl-X1-X2-X3-X4)2-Lys-Gly-OH having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Ruvo Met al., ChemBioChem, 2005, VOL. 6, 1242-1253, which is incorporated herein by reference in its entirety);

(5) FARLVSSIRY (SEQ ID NO: 26), FGRLVS- SIRY (SEQ ID NO: 27), and TWKTSRISIF (SEQ ID NO:

28) having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Krook Met al., Journal of Immunological Methods, 1998, VOL. 221, 151-157, which is incorporated herein by reference in its entirety);

(6) QSYP (SEQ ID NO: 29) having an affinity to a specific region of human IgG in general (e.g., refer to Jacobs J.M. et al., Bio. Techniques, 2003, VOL. 34, 132-141, which is incorporated herein by reference in its entirety);

(7) HWRGWV (SEQ ID NO: 30), HYFKFD (SEQ ID NO: 31), and HFRRHL (SEQ ID NO: 32) having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Carbonell R. G. et al., Journal of Chromatography A, 2009, VOL. 1216, 910-918, which is incorporated herein by reference in its entirety);

(8) DAAG (SEQ ID NO: 33) having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Lund L. N. et al., Journal of Chromatography A, 2012, VOL. 1225, 158-167, which is incorporated herein by reference in its entirety); (9) Fe-I, Fe-II, and Fe-III having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Warren L. Delano et al., Science, 2000, VOL. 287, 1279-1283; WO 2001/045746, which is incorporated herein by reference in its entirety); and

(10) NARKFYKG (SEQ ID NO: 34) and NKFRGKYK (SEQ ID NO: 35) having an affinity to a specific region (Fe region) of human IgG in general (e.g., refer to Biochemical Engineering Journal, 2013, VOL. 79, 33-40, which is incorporated herein by reference in its entirety).

[0055] In another specific embodiment, the affinity substance to an antibody described above may be a substance other than the peptide. Reported examples of such a substance include an aptamer having affinity to a specific region (the CH2 region, especially a side chain of Lys340) of human IgG (e.g., human IgG1 to 4) [e.g., GGUG(C/A) (U/T) motif-containing aptamers such as GGUGCU and GGUGAU] (e.g., refer to WO 2007/004748; Nomura Yet al., Nucleic Acids Res., 2010 November; 38(21): 7822-9; and Miyakawa S et al., RNA., 2008 June; 14(6): 1154-63, which are incorporated herein by reference in its entirety).

[0056] The affinity substance to an antibody described above can be obtained by any known method in the field concerned. The affinity substance to an antibody can be obtained by producing an antibody (e.g., the hybridoma method) using the entire antibody or a partial peptide in the antibody (e.g., when an antibody surface exposed region is known, a partial peptide present in the region) or screening the affinity substance (e.g., the phage display method, the systematic evolution of Ligands with exponential enrichment (SELEX) method, the mRNA display method, the ribosome display method, the cDNA display method, and the yeast display method) from a library from which the affinity substance is available (e.g., peptide libraries, antibody libraries, antibody-forming cell libraries, aptamer libraries, phage libraries, mRNA libraries, and cDNA libraries), for example. When the affinity substance to an antibody is an affinity substance to an Fe region (a soluble region) of an antibody, a partial peptide present in a specific region (e.g., CH1, CH2, and CH3) of the Fe region of various kinds of antibodies (e.g., IgG, IgA, IgM, IgD, and IgE) is used, whereby an affinity substance (e.g., an antibody and an aptamer) capable of selectively binding to any portion in the Fe region of the antibody can be efficiently obtained. The thus obtained affinity substances comprise a mixture of substances relatively strong and weak in affinitive binding ability. However, even an affinity substance weak in affinitive binding ability can strengthen its affinitive binding ability by using it in an excessive amount.

[0057] In a preferred embodiment, the affinity substance to an antibody is a peptide. Such a peptide is preferably a peptide having ability to bind to a constant region of a monoclonal antibody, more preferably a peptide having ability to bind to an Fe region of the monoclonal antibody, and even more preferably a peptide having ability to bind to an Fe region of IgG. The length of the peptide is not particularly limited, but is, for example, a peptide consisting of 10 to 40 (e.g., 10 to 20, 20 to 30, and 30 to 40) amino acid residues. Examples of the amino acid residue constituting the peptide include 20 L-a-amino acid residues constituting natural proteins, stereoisomers thereof (e.g., D-amino acid), and isomers thereof (e.g., (3-amino acid).

[0058] In a specific embodiment, the affinity substance to an antibody described above may be a peptide comprising any of the following amino acid sequences:

A peptide comprising

(a-1-1) an amino acid sequence of FNMQQRRF- YEALHDPNLNEEQRNARIRSIRDD (SEQ ID NO: 11)

(e.g., refer to compounds 22 to 24 and 29) or

(a-1-2) an amino acid sequence of FNMQQRRF- YEALHDPNLNEEQRNARIRSIRDDC (SEQ ID NO: 12)

(an amino acid sequence in which two Ks in the known sequence Z34C are substituted with Rs), wherein any one to three amino acid residues in the sequence, which may be the same or different, are each substituted with one amino acid residue selected from the group consisting of a lysine residue, an aspartic acid residue, and a glutamic acid residue, or

(a-2-1) an amino acid sequence of O-Ala- NMQQRRFYEALHDPNLNEEQRNARIRSIRDD (SEQ ID NO: 13) (e.g., refer to compounds 26 to 28) or

(a-2-2) an amino acid sequence of O-Ala- NMQQRRFYEALHDPNLNEEQRNARIRSIRDDC

(SEQ ID NO: 14) (an amino acid sequence in which two Ks in the known sequence Z34C are substituted with Rs, and an N-terminal Fis substituted with -Ala), wherein any one to three amino acid residues in the sequence, which may be the same or different, are each substituted with one amino acid residue selected from the group consisting of a lysine residue, an aspartic acid residue, and a glutamic acid residue, and

(b) an amino acid sequence having 85% or more identity to each of the amino acid sequences of SEQ ID NOs: 11 to 14.

Such a peptide has ability to bind to an Fe region of a monoclonal antibody.

[0059] The peptide consisting of the amino acid sequence of SEQ ID NO: 12 is obtained by changing two Ks (lysine) of the

26th and 28th counted from the N-terminal to Rs (arginine) in the affinity peptide known as Z34C for peptide reagent synthetic reasons. The compound of the present invention comprising a peptide containing any of the above amino acid sequences is useful for regioselective modification of a specific amino acid residue (e.g., Lys248 residue, Lys246 residue, Lys288, Lys290, or Lys317 according to Eu numbering, or amino acid residues other than these residues) in human IgG Fe. The amino acid sequence of Z34C is FNMQCQRRFYEALHDPNLNEEQRNARIKSIRDDC

(SEQ ID NO: 36) (e.g., refer to Starovasnik, M.A. et al., Structural mimicry of a native protein by a minimized binding domain., Proc. Natl. Acad. Sci. USA., 94, 10080- 10085 (1997), which is incorporated herein by reference in its entirety).

[0060] The affinity peptide can have an affinity to human IgG (e.g., human IgG described above. Preferably human IgG1). When the above affinity peptide comprises two cysteine residues (e.g., positions 5 and 34), the affinity peptide may form a cyclic peptide through a disulfide bond by the two cysteine residues.

[0061] For a position to which a lysine residue, an aspartic acid residue, or a glutamic acid residue (an amino acid residue easily modified with a cross-linking agent) is introduced, any position can be used so long as it has affinity to human IgG such as human IgG1. A person skilled in the art can easily identify such a position. The position to which a lysine residue, an aspartic acid residue, or a glutamic acid residue is introduced may be an amino acid residue other than a cysteine residue. The position to which the amino acid residue capable of being easily modified with a cross-linking agent is introduced is more preferably the amino acid residues at position 1, position 3, position 6, position 7, position 13, position 20, position 24, position 31, and position 32, for example.

[0062] The amino acid sequence having the characteristics of (a) and (b) also preferably has one specific amino acid residue selected from the group consisting of a lysine residue, an aspartic acid residue, and a glutamic acid residue (the amino acid residue capable of being easily modified with a cross-linking agent) (preferably a lysine residue) at a certain position and has variations of the normal 20 amino acid residues forming natural proteins (preferably 17 amino acid residues other than a lysine residue, an aspartic acid residue, and a glutamic acid residue, and more preferably 19 amino acid residues other than a lysine residue) at positions other than the certain position. Such a certain position is not limited to a particular position; examples thereof include position 1, position 3, position 6, position 7, position 13, position 20, position 24, position 31, and position 32. The amino acid sequence having the characteristics of (a) and (b) maintains the two cysteine residues, and these two cysteine residues may bind to each other through a disulfide bond. The amino acid sequence having 85% or more identity to each of the amino acid sequences of SEQ ID NOs: 11 to 14 may comprise one to three (preferably one or two, and more preferably one) modified amino acid residues by one, two, three, or four variations selected from the group consisting of deletion, substitution, addition, and insertion (preferably substitution) of amino acid residues. The variations of amino acid residues may be introduced to one region in the amino acid sequence or intruded to a plurality of different regions.

[0063] The amino acid sequence having the characteristics of (a) and (b) may be more preferably the following (c) or (d).

(c) An amino acid sequence selected from the group consisting of the following amino acid sequences of (1) to (9):

(1) KNMQCQRRFYEALHDPNLNEEQRNARIKSIRDDC (SEQ ID NO: 37)
番号37) ;

(2) FNMQCQKRFYEALHDPNLNEEQRNARIKSIRDDC (SEQ ID NO: 38)
番号38) ;

(3) FNMQCQRRFYEAKHDPNLNEEQRNARIRSI RDDC	(SEQ ID NO: 39)
[REDACTED]	
(4) FNMQCQRRFYEALHDPNLNEEQRKARIRSI RDDC	(SEQ ID NO: 40)
[REDACTED]	
(5) FNMQCQRRFYEALHDPNLNKEQRNARIRSI RDDC	(SEQ ID NO: 41)
[REDACTED]	
(6) FNMQCQRRFYEALHDPNLNEEQRNARIRSI KDDC	(SEQ ID NO: 42)
[REDACTED]	
(7) FNKQCQRRFYEALHDPNLNEEQRNARIRSI RDDC	(SEQ ID NO: 43)
[REDACTED]	
(8) FNMQCKRRFYEALHDPNLNEEQRNARIRSI RDDC	(SEQ ID NO: 44)
[REDACTED]; and	
(9) FNMQCQRRFYEALHDPNLNEEQRNARIRSI RKDC	(SEQ ID NO: 45)
[REDACTED]; or	

(d) an amino acid sequence having 90% or more identity to any of the amino acid sequences of **(1)** to (9) (may be modification of the number of amino acid residues described above) having variations of 19 amino acid residues other than a lysin residue at positions other than the one lysine residue and the two cysteine residues (e.g., position **1**, position 3, position 6, position 7, position 13, position 20, position 24, position 31, and position 32) in any of the amino acid sequences of **(1)** to (9).

[0064] It has been determined that a peptide comprising such an amino acid sequence has ability to bind to an Fe region of IgG. The affinity peptide comprising the amino acid sequence of (d) is preferably a peptide having ability to bind to a constant region of a monoclonal antibody, more preferably a peptide having ability to bind to an Fe region of the monoclonal antibody, and even more preferably a peptide having ability to bind to an Fe region of IgG.

[0064] The affinity peptide may have additional variations of amino acid residues other than the introduction of one amino acid residue easily modified with a cross-linking agent so long as it has 85% or more identity to each of the amino acid sequences of SEQ ID NOs: **11 to 14** or the amino acid sequences of **(1)** to (16). A person skilled in the art can easily identify a position to which the additional variations of amino acids can be introduced. The phenylalanine residue at position **1**, the arginine residue at position 6, the leucine residue at position 13, the glutamic acid residue at position 20, the asparagine residue at position 24, or the arginine residue at position 31 (except the position to which the amino acid residue capable of being easily modified with a cross-linking agent has already been introduced) can also be used, for example. Examples of amino acids that can be tryptophan (W), tyrosine (Y), valine (V), aspartic acid (D), glutamic acid (E), arginine (R), histidine (H), and lysine (L). These 19 amino acids other than lysine may be preferably used. The amino acids may each be an L-body or a D-body; an L-body is preferred (in Examples, the amino acid residues forming the peptides are all L-bodies).

[0065] The degree of percent identity to each of the amino acid sequences of SEQ ID NOs: **11 to 14** or the amino acid sequences of **(1)** to (9) can be determined as described above. The degree of percent identity is preferably 90% or more, 92% or more, more preferably 94% or more, even more preferably 95% or more, and particularly preferably 97% or more (that is, variation of only one amino acid residue is included).

[0066] In another specific embodiment, the affinity sub-stance to an antibody described above is a peptide comprising any of the following amino acid sequences:

Formula	1-1 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -I-I-W-C-(X ₀₋₃) _b	(SEQ ID NO: 15)
Formula	1-2 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -I-V-W-C-(X ₀₋₃) _b	(SEQ ID NO: 16)
Formula	1-3 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -V-V-W-C-(X ₀₋₃) _b	(SEQ ID NO: 17)
Formula	1-4 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -A-V-W-C-(X ₀₋₃) _b	(SEQ ID NO: 18)
Formula	1-5 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -L-L-W-C-(X ₀₋₃) _b	(SEQ ID NO: 19)
Formula	1-6 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -L-I-W-C-(X ₀₋₃) _b	(SEQ ID NO: 20)
Formula	1-7 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -L-V-F-C-(X ₀₋₃) _b	(SEQ ID NO: 21)
Formula	1-8 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -Q-V-W-C-(X ₀₋₃) _b	(SEQ ID NO: 22)
Formula	1-9 : (X ₀₋₃) _a -C-X _a a ₁ -X _a a ₂ -X _a a ₃ -X _a a ₄ -X _a a ₅ - X _a a ₆ -E-V-W-C-(X ₀₋₃) _b	(SEQ ID NO: 23)

wherein

(X₀₋₃)_a is absence, or an arginine residue-glycine residue-asparagine residue, a glycine residue-asparagine residue, an aspartic acid residue, or an asparagine residue,

(X₀₋₃)₆ is absence, or a threonine residue-tyrosine residue-histidine residue, or a threonine residue,

X_aa₁ is an alanine residue,

X_aa₂ is a tyrosine residue, a tryptophan residue, or a histidine residue,

X_aa₃ is a histidine residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, an arginine residue, or a glycine residue,

X_aa₄ is a lysine residue, an aspartic acid residue, or a glutamic acid residue,

X_aa₅ is a glycine residue, a serine residue, an asparagine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, a histidine residue, a threonine residue, a leucine residue, an alanine residue, a valine residue, an isoleucine residue, or an arginine residue, and

X_aa₆ is a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue, or

Formula 2-1: (X₀₋₃)_a-C-(X_aa₁')-(X_aa₂')-(X_aa₃')-

(X_aa₄')-(X_aa₅')-(X_aa₆')-L-V-W-C-(X₀₋₃)₆

(SEQ ID NO: 24)

wherein

(X₀₋₃)_a and (X₀₋₃)₆ are the same as the above (X₀₋₃)_a and (X₀₋₃)₆, respectively,

X_aa₁', X_aa₂', X_aa₃', X_aa₄', X_aa₅', and X_aa₆' are the same as the above X_aa₁, X_aa₂, X_aa₃, X_aa₄, X_aa₅, and X_aa₆, respectively

such a peptide has ability to bind to an Fe region of a monoclonal antibody.

[0067] In the amino acid sequences represented by the above formulae 1-1 to 1-9 and formula 2-1, X_aa₂ is a tyrosine residue, a tryptophan residue, or a histidine residue, and preferably a tyrosine residue or a tryptophan residue.

[0068] In the amino acid sequences represented by the above formulae 1-1 to 1-9 and formula 2-1, X_aa₃ is a histidine residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, an arginine residue, or a glycine residue, and preferably a histidine residue.

[0069] In the amino acid sequences represented by the above formulae **1-1** to 1-9 and formula 2-1, Xaa5 is a glycine residue, a serine residue, an asparagine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, a histidine residue, a threonine residue, a leucine residue, an alanine residue, a valine residue, an isoleucine residue, or an arginine residue, and preferably a glycine residue, a threonine residue, or a leucine residue.

[0070] In the amino acid sequences represented by the above formulae **1-1** to 1-9 and formula 2-1, Xaa6 is a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue, and more preferably a glutamine residue.

[0427] The peptide comprising any of the amino acid sequences represented by the above formulae **1-1** to 1-9 and formula 2-1 is preferably a peptide comprising an amino acid sequence selected from the group consisting of the following:

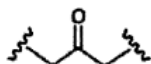
(1')	RGNCAYHKGQ I IWCTYH	(SEQ ID NO: 46)	;
(2')	RGNCAYHKGQ I VWCTYH	(SEQ ID NO: 47)	;
(3')	RGNCAYHKGQ V VWCTYH	(SEQ ID NO: 48)	;
(4')	RGNCAYHKGQ A VWCTYH	(SEQ ID NO: 49)	;
(5')	RGNCAYHKGQ L LWCTYH	(SEQ ID NO: 50)	;
(6')	RGNCAYHKGQ L IWCTYH	(SEQ ID NO: 51)	;
(7')	DCAYHKGQ I VWCT	(SEQ ID NO: 52)	;
(8')	DCAYHKGQ V VWCT	(SEQ ID NO: 53)	;
(9')	DCAYHKGQ A VWCT	(SEQ ID NO: 54)	;
(10')	RGNCAYHKSQ I IWCTYH	(SEQ ID NO: 55)	;
(11')	RGNCAYHKNQ I IWCTYH	(SEQ ID NO: 56)	;
(12')	RGNCAYHKDQ I IWCTYH	(SEQ ID NO: 57)	;
(13')	RGNCAYHKQQ I IWCTYH	(SEQ ID NO: 58)	;
(14')	RGNCAYHKEQ I IWCTYH	(SEQ ID NO: 59)	;
(15')	RGNCAYHKFQ I IWCTYH	(SEQ ID NO: 60)	;
(16')	RGNCAYHKYQ I IWCTYH	(SEQ ID NO: 61)	;
(17')	RGNCAYHKWQ I IWCTYH	(SEQ ID NO: 62)	;
(18')	RGNCAYHKHQ I IWCTYH	(SEQ ID NO: 63)	;
(19')	RGNCAYHKTQ I IWCTYH	(SEQ ID NO: 64)	;
(20')	RGNCAYHKLQ I IWCTYH	(SEQ ID NO: 65)	;
(21')	CAYHKLQ I VWC	(SEQ ID NO: 66)	;
(22')	CAYHKLQ L IWC	(SEQ ID NO: 67)	;
(23')	CAYHKSQ I VWC	(SEQ ID NO: 68)	;
(24')	RGNCAYHKGQ L VFCTYH	(SEQ ID NO: 69)	;
(25')	RGNCAYHKGQ Q VWCTYH	(SEQ ID NO: 70)	;
(26')	RGNCAYHKGQ E VWCTYH	(SEQ ID NO: 71)	;
(27')	CAYHKGQ L VWC	(SEQ ID NO: 72)	;
(28')	RGNCAYHKAQ L VWCTYH	(SEQ ID NO: 73)	;
(29')	RGNCAYHKVQ L VWCTYH	(SEQ ID NO: 74)	;
(30')	RGNCAYHKLQ L VWCTYH	(SEQ ID NO: 75)	;
(31')	RGNCAYHK I Q L VWCTYH	(SEQ ID NO: 76)	;
(32')	RGNCAYHKSQ L VWCTYH	(SEQ ID NO: 77)	;
(33')	RGNCAYHKTQ L VWCTYH	(SEQ ID NO: 78)	;
(34')	RGNCAYHKNQ L VWCTYH	(SEQ ID NO: 79)	;

(3 5')	RGNCA YHKDQLVWCTYH	(SEQ ID NO: 80)
(3 6')	RGNCA YHKQQLVWCTYH	(SEQ ID NO: 81)
(3 7')	RGNCA YHKEQLVWCTYH	(SEQ ID NO: 82)
(3 8')	RGNCA YHKFQLVWCTYH	(SEQ ID NO: 83)
(3 9')	RGNCA YHKRQLVWCTYH	(SEQ ID NO: 84)
(4 0')	RGNCA YHKHQLVWCTYH	(SEQ ID NO: 85)
(4 1')	RGNCA YHKWQLVWCTYH	(SEQ ID NO: 86)
(4 2')	RGNCA YHKYQLVWCTYH	(SEQ ID NO: 87)
(4 3')	RGNCA YFKGQLVWCTYH	(SEQ ID NO: 88)
(4 4')	RGNCA YYKGQLVWCTYH	(SEQ ID NO: 89)
(4 5')	RGNCA YWKGQLVWCTYH	(SEQ ID NO: 90)
(4 6')	RGNCA YRKGQLVWCTYH	(SEQ ID NO: 91)
(4 7')	RGNCA YGKGQLVWCTYH	(SEQ ID NO: 92)
(4 8')	DCAYHKGQLVWC	(SEQ ID NO: 93)
(4 9')	NCA YHKGQLVWC	(SEQ ID NO: 94)
(5 0')	CAYHKGQLVWCT	(SEQ ID NO: 95); and
(5 1')	CAYHKSQLVWC	(SEQ ID NO: 96)

[0072] It has been determined that a peptide comprising such an amino acid sequence has ability to bind to an Fe region of IgG.

[0073] At least two cysteine residues separated from each other in each amino acid sequence of the peptide can form a cyclic peptide through a disulfide bond. Alternatively, in the peptide, the sulfide groups in the two cysteine residues may be coupled with each other through a carbonyl group- containing linker represented by the following:

[Formula 8]



[0074] The broken line portions of the carbonyl group- containing linker represented by the above mean bond portions with the sulfide groups. The linker is more stable than a normal disulfide bond against a reduction reaction and the like. Such a peptide can be prepared by a method described in WO 2016/186206, which is incorporated herein by reference in its entirety, for example.

[0075] The compound of the present invention comprising a peptide containing any of the above amino acid sequences is useful for regioselective modification of a specific amino acid residue (e.g., Lys248 residue, Lys246 residue, Lys288, Lys290, or Lys317 according to Eu numbering, or amino acid residues other than these residues) in human IgG Fe. An amino acid forming the peptide may each be an L-body or a D-body; an L-body is preferred (in Examples, the amino acid residues forming the peptides are all L-bodies).

[0076] The peptide may have a specific amino acid residue modified with a cross-linking agent. Examples of such a specific amino acid residue include a lysine residue, an aspartic acid residue, and a glutamic acid residue; preferred is a lysine residue. Examples of the cross-linking agent include cross-linking agents comprising preferably two or more (DSG) and disuccinimidyl suberate (DSS); cross-linking agents comprising preferably two or more imide acid portions such as dimethyl adipimidate-2HC1 (DMA), dimethyl pimelimidate-2HC1 (DMP), and dimethyl suberimidate- 2HC1 (DMS); and cross-linking agents having an SS bond such as dimethyl 3,3'-dithiobispropionimidate-2HC1 (DTBP) and dithiobis(succinimidyl propionate) (DSP) (e.g., WO 2016/186206, which is incorporated herein by reference in its entirety).

[0077] When the affinity substance to an antibody is a peptide, a terminal amino group and a terminal carboxy group of the peptide may be protected. Examples of a protecting group for the N-terminal amino group include an alkylcarbonyl group (an acyl group) (e.g., an acetyl group, a propoxy group, and a butoxycarbonyl group such as a tert-butoxycarbonyl group), an alkyloxycarbonyl group (e.g., a fluorenylmethoxycarbonyl group), an aryloxy carbonyl group, and an arylalkyl(arylalkyl)oxycarbonyl group (e.g., a benzyloxycarbonyl group). The protecting group for the N-terminal amino group is preferably an acetyl group. Examples of a protecting group for the C-terminal carboxy group include a group capable of forming an ester or an amide. Examples of the group capable of forming an ester or an amide include an alkyloxy group (e.g., methyloxy, ethyloxy, propyloxy, butyloxy, pentyloxy, and hexyloxy), an aryloxy group (e.g., phenyloxy and naphthyloxy), an aralkyloxy group (e.g., benzyloxy), and an amino group. The protecting group for the C-terminal carboxy group is preferably an amino group.

[0078] 1-3. Divalent Group Comprising Leaving Group (L)

In Formula (I), L is a divalent group comprising a leaving group.

[0079] The leaving group has ability to be cleaved and eliminated from E by a reaction between a nucleophilic group in an antibody and an electrophilic group contained in the electrophilic group-comprising divalent group (E). Such a leaving group is common technical knowledge in the field concerned (e.g., Fujishima, S. et al J. Am. Chem. Soc, 2012, 134, 3961-3964 (described above); Chem. Sci. 2015 3217-3224; Nature Chemistry volume 8, pages 542-548 (2016), all of which are incorporated herein by reference in their entireties). The leaving group is not particularly limited so long as it has ability to be cleaved from E by the above reaction, but examples thereof include (1) a group selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl), and (2) heteroarylene.

[0080] Examples of C₁₋₆ alkyl include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, isobutyl, t-butyl, pentyl, and hexyl. C₁₋₆ alkyl is preferably C₁₋₄ alkyl.

[0081] The group selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)-, which are examples of the leaving group, is the following (1) or (2):

(1) a group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-

-O-, -N(OR)-, -N(R)-, or -O-N(R)-; or

(2) a group comprising -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, or -O-N(R)-.

[0082] The group that enhances ability of $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$ to be eliminated is a group that enhances ability of $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$ to be eliminated from an electrophilic group when the group is present adjacent to $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$ as compared to when the group is not present adjacent to $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$. In other words, the group that enhances ability of $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$ to be eliminated is a group having ability to withdraw an electron of an oxygen atom, a sulfur atom, a selenium atom, or a nitrogen atom.

[0083] Preferred example of the group that enhances ability of $-O-$, $-S-$, $-Se-$, $-SO_2-O-$, $-SO_2-N(R)-$, $-SO_2-$, $-C\equiv C-CH_2-O-$, $-N(OR)-$, $-N(R)-$, or $-O-N(R)-$ to be eliminated include an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone. The number of electron-withdrawing groups that may be comprised in each of the arylene and the heteroarylene is 1 or more (e.g., 1 to 3, preferably 1 or 2). Examples of the electron-withdrawing group include a halogen atom, a halogen atom-substituted alkyl (e.g., trifluoromethyl), a boronic acid residue, mesyl, tosyl, triflate, nitro, cyano, a phenyl group, and a keto group (e.g., acyl).

[0084] The "arylene" in the "arylene optionally substituted with an electron-withdrawing group" is preferably C_{6-24} arylene, more preferably C_{6-18} arylene, even more preferably C_{6-14} arylene, and most preferably C_{6-10} arylene. The arylene optionally substituted with an electron-withdrawing group may be further substituted or is not necessarily substituted with a substituent other than the electron-withdrawing group. The number of carbon atoms does not comprise the number of carbon atoms of the electron-withdrawing group and the other substituents. Examples of the arylene include phenylene, naphthylene, and anthracenylene.

[0085] The "heteroarylene" in the "heteroarylene optionally substituted with an electron-withdrawing group" is preferably C_{1-21} heteroarylene, more preferably C_{1-15} heteroarylene, even more preferably C_{1-9} heteroarylene, and most preferably C_{1-6} heteroarylene. The heteroarylene optionally substituted with an electron-withdrawing group may be further substituted or is not necessarily substituted with a substituent other than the electron-withdrawing group. The number of carbon atoms does not comprise the number of carbon atoms of the electron-withdrawing group and the other substituents. The heteroarylene comprises one or more (for example, 1 to 5, preferably 1 to 4, more preferably 1 to 3) heteroatoms selected from the group consisting of a nitrogen atom, an oxygen atom, and a sulfur atom as ring-constituting atoms. Examples of the heteroarylene include pyrrolediy, furandiy, thiophenediy, pyridinediy, pyridazinediy, pyrimidinediy, pyrazinediy, triazinediy, pyrazolediy, imidazolediy, thiazolediy, isothiazolediy, oxazolediy, isoxazolediy, triazolediy, tetrazolediy, indolediy, purinediy, anthraquinonediy, carbazolediy, fluorenediy, quinolinediy, isoquinolinediy, quinazolinediy, and phthalazinediy.

[0086] The optionally condensed 2,5-diketopyrrolidine, the optionally condensed 2,6-diketopiperidine, the optionally condensed 2-ketopyrrolidine, the optionally condensed 2-ketopiperidine, and 2-pyridone may be substituted or are not necessarily substituted with a substituent such as an electron-withdrawing group.

[0087] The heteroarylene, which is an example of the leaving group, is a heteroarylene having a low electron density (that is, less than 1). The heteroarylene, which is a leaving group, is preferably a heteroarylene containing a nitrogen atom as a ring-constituting atom. The heteroarylene containing a nitrogen atom as a ring-constituting atom is preferably C₂₁ heteroarylene containing a nitrogen atom as a ring-constituting atom, more preferably C₁₋₁₅ heteroarylene containing a nitrogen atom as a ring-constituting

atom, and even more preferably C₁₋₉ heteroarylene containing a nitrogen atom as a ring-constituting atom. The heteroarylene, which is a leaving group, may be substituted or is not necessarily substituted with a substituent such as an electron-withdrawing group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the heteroarylene which is a leaving group and contains a nitrogen atom as a ring-constituting atom include imidazolediyl, triazolediyl, tetrazolediyl, and 2-pyridonediy (that is, 2-hydroxypyridinediy).

[0088] A group such as the "arylene optionally substituted with an electron-withdrawing group", which is an example of the group that enhances ability of -O-, -S-, -Se-, -S(=O)-, -S(=O)-N(R)-, -S(=O)-, -C≡C-CH₂-, -N(OR)-, -N(R)-, or -O-N(R)- to be eliminated, and the "heteroarylene", which is an example of the leaving group, may each have, for example, one to five, preferably one to three, more preferably one or two substituents, or do not each necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when such a group as described above has a substituent, examples of the substituent include the following:

(i) a halogen atom;

(ii) a monovalent hydrocarbon group;

(iii) aralkyl;

(iv) a monovalent heterocyclic group;

(v) *Ra*-O-, *Ra*-C(=O)-, *Ra*-O-----C(=O)-,

and *Ra*-C(=O)-O- wherein *Ra* indicates a hydrogen atom or a monovalent hydrocarbon group; (vi) NR₆Rc-, NRbRc-, -C(=O)-, NRbRc-, -C(=O)-O-, and Rb C

(=O)-NRC- wherein R₆ and R_e are the same as or different from each other, and each indicate a hydrogen atom or a monovalent hydrocarbon group; and

(vii) a nitro group, a sulfuric acid group, a sulfonic acid group, a cyano group, and a carboxy group.

[0089] Examples of the halogen atom include a fluorine atom, a chlorine atom, a bromine atom, and an iodine atom.

[0090] Examples of the monovalent hydrocarbon group include a monovalent chain hydrocarbon group, a monovalent alicyclic hydrocarbon group, and a monovalent aromatic hydrocarbon group.

[0091] The monovalent chain hydrocarbon group means a hydrocarbon group comprising only a chain structure and does not comprise any cyclic structure in a main chain thereof. Note that the chain structure may be linear or branched. Examples of the monovalent chain hydrocarbon group include alkyl, alkenyl, and alkynyl. Alkyl, alkenyl, and alkynyl may be linear or branched.

[0092] The alkyl is preferably C₁₋₁₂ alkyl, more preferably C₁₋₆ alkyl, and even more preferably C₁₋₄ alkyl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₁₋₁₂ alkyl include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, isobutyl, t-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and dodecyl.

[0094] The alkenyl is preferably C₂₋₁₂ alkenyl, more preferably C₂₋₆ alkenyl, and even more preferably C₂₋₄ alkenyl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₂₋₁₂ alkenyl include vinyl, propenyl, and n-butenyl.

[0095] The monovalent chain hydrocarbon group is preferably alkyl.

[0096] The monovalent alicyclic hydrocarbon group means a hydrocarbon group comprising only alicyclic hydrocarbon as a cyclic structure and not comprising any aromatic ring, in which the alicyclic hydrocarbon may be monocyclic or polycyclic. Note that the monovalent alicyclic hydrocarbon group is not necessarily required to comprise only an alicyclic hydrocarbon but may comprise a chain structure in part thereof. Examples of the monovalent alicyclic hydrocarbon group include cycloalkyl, cycloalk-enyl, and cycloalkynyl, which may be monocyclic or polycyclic.

[0097] Cycloalkyl is preferably C₃₋₁₂ cycloalkyl, more preferably C₃₋₆ cycloalkyl, and even more preferably C₅₋₆ cycloalkyl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₃₋₁₂ cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0098] The cycloalkenyl is preferably C₃₋₁₂ cycloalkenyl, more preferably C₃₋₆ cycloalkenyl, and even more preferably C₅₋₆ cycloalkenyl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₃₋₁₂ cycloalkenyl include cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl.

[0099] The cycloalkynyl is preferably C₃₋₁₂ cycloalkynyl, more preferably C₃₋₆ cycloalkynyl, and even more preferably C₅₋₆ cycloalkynyl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₃₋₁₂ cycloalkynyl include cyclopropynyl, cyclobutynyl, cyclopentynyl, and cyclohexynyl.

[0100] The monovalent alicyclic hydrocarbon group is preferably cycloalkyl.

[0101] The monovalent aromatic hydrocarbon group means a hydrocarbon group comprising an aromatic cyclic structure. Note that the monovalent aromatic hydrocarbon group is not necessarily required to comprise only an aromatic ring and may comprise a chain structure or alicyclic hydrocarbon in part thereof, in which the aromatic ring may be monocyclic or polycyclic. The monovalent aromatic hydrocarbon group is preferably C₆₋₁₂ aryl, more preferably C₆₋₁₀ aryl, and even more preferably C₆ aryl. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of C₆₋₁₂ aryl include phenyl and naphthyl.

[0102] The monovalent aromatic hydrocarbon group is preferably phenyl.

[0103] Among these, the monovalent hydrocarbon group is preferably alkyl, cycloalkyl, and aryl and more preferably alkyl.

[0104] Aralkyl refers to arylalkyl. The definitions, examples, and preferred examples of aryl and alkyl in arylalkyl are as described above. The aralkyl is preferably C₃₋₁₅ aralkyl. Examples of such an aralkyl include benzoyl, phenethyl, naphthylmethyl, and naphthylethyl.

[0105] The monovalent heterocyclic group refers to a group obtained by removing one hydrogen atom from a heterocycle of a heterocyclic compound. The monovalent heterocyclic group is a monovalent aromatic heterocyclic group or a monovalent nonaromatic heterocyclic group. The monovalent heterocyclic group preferably comprises one or more selected from the group consisting of an oxygen atom, a sulfur atom, a nitrogen atom, a phosphorus atom, a boron atom, and a silicon atom and more preferably comprises one or more selected from the group consisting of an oxygen atom, a sulfur atom, and a nitrogen atom as a hetero atom contained in the heterocyclic group.

[0106] The monovalent aromatic heterocyclic group is preferably a C₁₋₁₅ aromatic heterocyclic group, more preferably a C₁₋₉ aromatic heterocyclic group, and even more preferably a C₁₋₆ aromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the monovalent aromatic heterocyclic group include pyrrolyl, furanyl, thiophenyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, indolyl, purinyl, anthraquinolyl, carbazonyl, fluorenyl, quinolinyl, isoquinolinyl, quinazolinyl, and phthalazinyl.

[0107] The monovalent nonaromatic heterocyclic group is preferably a C₂₋₁₅ nonaromatic heterocyclic group, more preferably a C₂₋₉ nonaromatic heterocyclic group, and even more preferably a C₂₋₆ nonaromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the monovalent nonaromatic heterocyclic group include oxiranyl, aziridinyl, azetidiny, oxetanyl, thietanyl, pyrrolidinyl, dihydrofuran, tetrahydrofuran, dioxolanyl, tetrahydrothiophenyl, pyrrolinyl, imidazolidinyl, oxazolidinyl, piperidinyl, dihydropyran, tetrahydropyran, tetrahydrothiopyran, morpholinyl, thiomorpholinyl, piperazinyl, dihydrooxazinyl, tetrahydrooxazinyl, dihydropyrimidinyl, and tetrahydropyrimidinyl.

[0108] Among these, the monovalent heterocyclic group is preferably a five-membered or six-membered heterocyclic group.

[0109] The substituent may be preferably the following:

(i') a halogen atom;

(ii') C₁₋₁₂ alkyl, phenyl, or naphthyl;

(iii') C₃₋₁₅ aralkyl;

(iv') a five-membered or six-membered heterocyclic group;

(v') $Ra-O-$, $Ra-C(=O)-$, $Ra-O---C(=O)-$, or $Ra-C(=O)-O-$ wherein Ra indicates a hydrogen atom or C₁₋₂ alkyl;

(vi') $NRbRc-$, $NRbRc-C(=O)-$, $NRbRc---C(=O)-O-$, or $RbC(=O)-NRc-$ wherein Rb and Re

are the same as or different from each other, and each indicate a hydrogen atom or C₁₂ alkyl; or

(vii') the same groups as those enumerated in (vii).

[0110] The substituent may be more preferably the following:

- (i'') a halogen atom;
- (ii'') C₁₋₁₂ alkyl;
- (iii'') *Ra-O-*, *Ra-C(=O)-*, *Ra-O---C(=O)-*, or *Ra-C(=O)-O-* (wherein *Ra* indicates a hydrogen atom or C₁₋₁₂ alkyl);
- (iv'') *NRbRc-*, *NRbRc-C(=O)-*, *NRbRc---C(=O)-O-*, or *Rb-C(=O)-NRc-* wherein *Rb* and *Re* are the same as or different from each other, and each indicate a hydrogen atom or C₁₋₁₂ alkyl; or
- (v'') the same groups as those enumerated in (vii).

[0111] The substituent may be even more preferably the following:

- (i''') a halogen atom;
- (ii''') C₁₋₆ alkyl;
- (iii''') *Ra-O-*, *Ra-C(=O)-*, *Ra-O C(=O)-*, or *Ra-C(=O)-O-* (wherein *Ra* indicates a hydrogen atom or C₁₋₆ alkyl);
- (iv''') *NRbRc-*, *NRbRc-C(=O)-*, *NRbRc C(=O)-O-*, or *Rb-C(=O)-NRc-* wherein *Rb* and *Re* are the same as or different from each other, and each indicate a hydrogen atom or C₁₋₆ alkyl; or
- (v''') the same groups as those enumerated in (vii).

[0112] The substituent may be particularly preferably the following:

- (i''') a halogen atom;
- (ii''') c₁₋₄ alkyl;
- (iii''') *Ra-O-*, *Ra-C(=O)-*, *Ra-O---C(=O)-*, or *Ra-C(=O)-O-* (wherein *Ra* indicates a hydrogen atom or C₁₋₄ alkyl);
- (iv''') *NRbRc-*, *NRbRc-C(=O)-*, *NRbRc---C(=O)-O-*, or *R₆-C(=O)-NRc-* wherein *R₆* and *Re* are the same as or different from each other, and each indicate a hydrogen atom or C₁₋₄ alkyl; or
- (v''') the same groups as those enumerated in (vii).

[0113] More specifically, the leaving group may be any of the following (a) to (c).

(a) ring P-Q- [where ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of *O-*, *-S-*, *-Se-*, *-SO₂-O-*, *-SO₂-N(R)-*, *-SO₂-*, *C≡C-CH₂-O-*, *-N(OR)-*, *-N(R)-*, and *O-N(R)-* (where R is a hydrogen atom or C₁₋₆ alkyl)],

(b) heteroarylene, or

(c) -Q- [where Q is selected from the group consisting of *-O-*, *-S-*, *-Se-*, *-SO₂-O-*, *-SO₂-N(R)-*, *-SO₂-*, *-c≡c-CH₂-O-*, *-N(OR)-*, *-N(R)-*, and *-O-N(R)-* (where R is a hydrogen atom or c₁₋₆ alkyl)].

[0114] As the leaving group, (a) or (b) is preferable among (a), (b), and (c), and (a) is particularly preferable. Ring Pin (a) is any of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, or 2-pyridone. Among these, an arylene substituted with an electron-withdrawing group, a heteroarylene substituted with an electron-withdrawing group, 2,5-diketopyrrolidine, and 2,6-diketopiperidine are more preferable, and an arylene substituted with an electron-withdrawing group, 2,5-diketopyrrolidine, and 2,6-diketopiperidine are even more preferable.

[0115] Ring Pin (a) is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone. The details of these groups are the same as those described as preferred examples of the group that enhances ability of -N(R)-, -N(OR)-, -O-, -S-, or -Se- to be eliminated.

[0116] Q in (a) is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl). Among these, Q is preferably -O-, -S-, -SO₂-O-, or -SO₂-N(R)-, more preferably -O- or -S-, and even more preferably -O-.

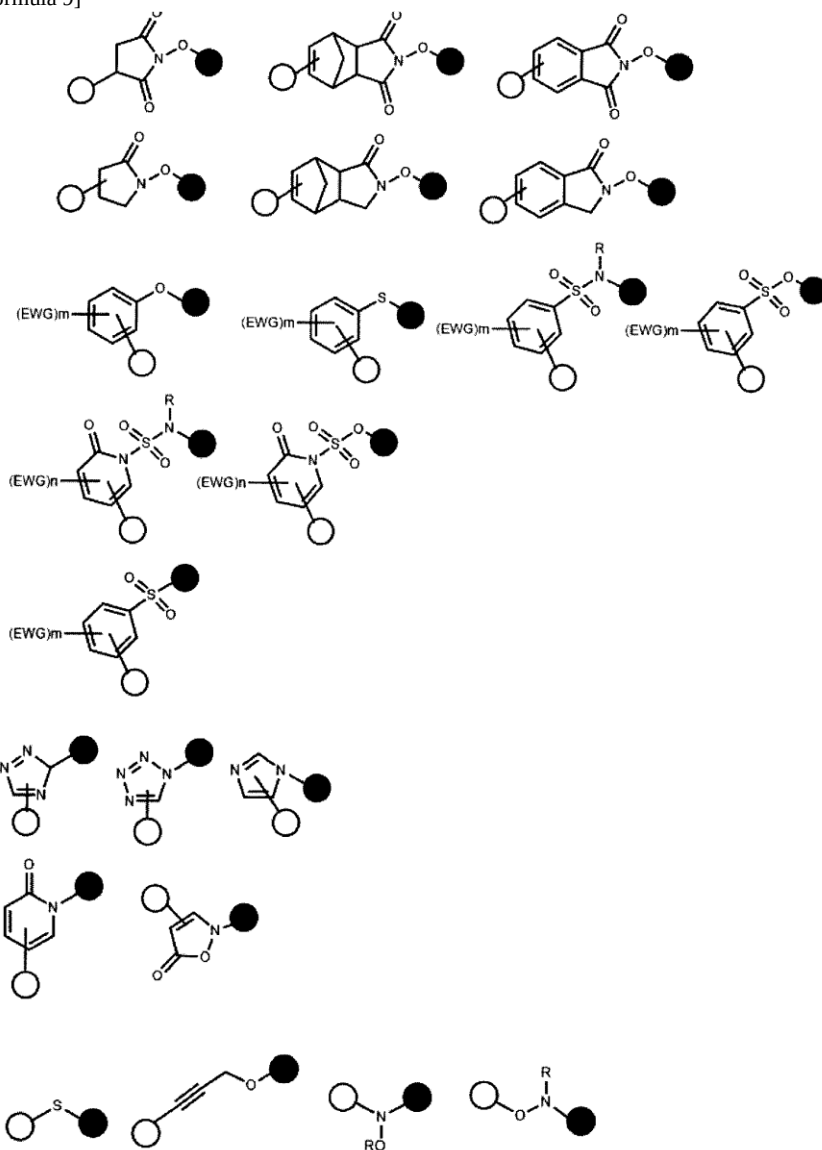
[0117] (b) is a heteroarylene, and is preferably imidazolediyl, triazolediyl, tetrazolediyl, or 2-pyridonediyl (=2-hydroxypyridinediyl), and more preferably imidazolediyl or 2-pyridonediyl.

[0118] Q in (c) is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl).

Q is preferably -S-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, or -O-N(R)-, and more preferably -S-, -N(OR)-, or -O-N(R)-.

[0119] More specifically, the preferable leaving group may be selected from the group consisting of the following structural formulae:

[Formula 9]



(where EWG is an electron-withdrawing group,

m is an integer of **0 to 4**,

n is an integer of **0 to 3**,

R is a hydrogen atom or C₁₋₆alkyl,

a ° is a bond to L₁, and a

• is a bond to E₁).

The details of the electron-withdrawing group and

C₁₋₆ alkyl are as described above. m is preferably an integer of **1 to 4**, and more preferably **1, 2, or 3**. n is preferably an

integer of **1 to 3**, and more preferably **1 or 2**.

[0120] The divalent group comprising a leaving group, represented by *Lis* a divalent group consisting of the above leaving group, or a divalent group comprising another divalent group in addition to the above leaving group. Examples of such another divalent group include a divalent hydrocarbon group, a divalent heterocyclic group, $-C(=O)-$, $-NRL-$ (*RL* indicates a hydrogen atom or the above-described substituent), $-O-$, $-S-$, $C(=S)-$, and a group consisting of a combination of two or more (e.g., two to eight, preferably two to six, and more preferably two to four) of these. The divalent hydrocarbon group and the divalent heterocyclic group may each have, for example, one to five, preferably one to three, more preferably one or two substituents, or do not each necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the above group has a substituent, examples and preferred examples of the substituent are the same as those described above.

[0121] The divalent hydrocarbon group is a linear, branched, or cyclic divalent hydrocarbon group and preferably a linear or branched divalent hydrocarbon group. Examples of the divalent hydrocarbon group include alkylene, alkenylene, alkynylene, and arylene.

[0122] The alkylene is preferably C_{1-12} alkylene, more preferably C_{1-6} alkylene, and particularly preferably C_{1-4} alkylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkylene may be any of linear, branched, or cyclic one and is preferably linear alkylene. Examples of such an alkylene include methylene, ethylene, propylene, butylene, pentylene, and hexylene.

[0123] The alkenylene is preferably C_{2-12} alkenylene, more preferably C_{2-6} alkenylene, and particularly preferably C_{2-4} alkenylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkenylene may be any of linear, branched, or cyclic one and is preferably linear alkenylene. Examples of such an alkenylene include ethylenylene, propynylene, butenylenylene, pentenylenylene, and hexenylenylene.

[0124] The alkynylene is preferably C_{2-12} alkynylene, more preferably C_{2-6} alkynylene, and particularly preferably C_{2-4} alkynylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkynylene may be any of linear, branched, or cyclic one and is preferably linear alkynylene. Examples of such an alkynylene include ethynylene, propynylene, butynylene, pentynylene, and hexynylene.

[0125] The arylene is preferably C_{6-24} arylene, more preferably C_{6-18} arylene, even more preferably C_{6-14} arylene, and still even more preferably C_{6-10} arylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the arylene include phenylene, naphthylene, and anthracenylenylene.

[0126] The divalent heterocyclic group is a divalent aromatic heterocyclic group or a divalent nonaromatic heterocyclic group. The divalent heterocyclic group preferably comprises, as a hetero atom forming a heterocycle, one or more selected from the group consisting of an oxygen atom, a sulfur atom, a nitrogen atom, a phosphorous atom, a boron atom, and a silicon atom and more preferably comprises one or more selected from the group consisting of an oxygen atom, a sulfur atom, and a nitrogen atom.

[0127]

The divalent aromatic heterocyclic group is preferably a C_{1_21} divalent aromatic heterocyclic group, more preferably a C_{1_15} divalent aromatic heterocyclic group, even more preferably a C_{1_9} divalent aromatic heterocyclic group, and still even more preferably a C_{1_6} divalent aromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specific examples of the divalent aromatic heterocyclic group include pyrrolediyl, furandiyl, thiophenediyl, pyridinediyl, pyridazinediyl, pyrimidinediyl, pyrazinediyl, triazinediyl, pyrazolediyl, imidazolediyl, thiazolediyl, iso-thiazolediyl, oxazolediyl, isoxazolediyl, triazolediyl, tetrazolediyl, indolediyl, purinediyl, anthraquinonediyl, carbazolediyl, fluorenediyl, quinolinediyl, isoquinolinediyl, quinazolinediyl, and phthalazinediyl.

[0128] The divalent nonaromatic heterocyclic group is preferably a C_{2_21} nonaromatic heterocyclic group, more preferably a C_{2_15} nonaromatic heterocyclic group, even more preferably a C_{2_9} nonaromatic heterocyclic group, and still even more preferably a C_{2_6} nonaromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specifically, examples of the divalent nonaromatic heterocyclic group include pyrroldionediyl, pyrrolinedionediyl, pyrrolinediyl, oxiranediyl, aziridinediyl, azetidinediyl, oxetanediyl, thietanediyl, pyrrolidinediyl, dihydrofurandiyl, tetrahydrofurandiyl, dioxolanediyl, tetrahydrothiophenediyl, pyrrolidinediyl, imidazolidinediyl, oxazolidinediyl, piperidinediyl, dihydropyrandiyl, tetrahydropyrandiyl, tetrahydrothiopyrandiyl, morpholinediyl, thiomorpholinediyl, piperazinediyl, dihydrooxazinediyl, tetrahydrooxazinediyl, dihydropyrimidinediyl, and tetrahydropyrimidinediyl.

[0129] In a specific embodiment, L can be expressed by L-L₂.

[0130] L₁ is a bond or a divalent group. The definitions, examples, and preferred examples of the divalent group represented by L₁ are the same as those of another divalent group when the divalent group comprising a leaving group, represented by L is a divalent group comprising another divalent group in addition to the leaving group.

[0131] L₂ is a leaving group. The definitions, examples, and preferred examples of the leaving group represented by L₂ are the same as those of the leaving group in L.

[0132] The leaving group represented by L₂ is preferably any of the above (a) to (c). Examples and preferred examples of the leaving group represented by L₂ are also the same as those described in the above (a) to (c).

[0133] The length of a main chain of L (divalent group comprising a leaving group) or L₁ (bond or a divalent group)-L₂ (leaving group) that couples A (affinity substance) with E (divalent group comprising an electrophilic group) can be designed as appropriate according to various factors such as the types of an antibody and an affinity substance, and a relationship between a target site of the affinity substance in the antibody and a specific amino acid residue in the antibody to be regioselectively modified. The main chain of L or L₁-L₂ refers to a chain structure coupling A with E and consisting of a plurality of atoms covalently binding to each other, and excludes a hydrogen atom, a branched structure portion, and a substituent. When a compound represented by Formula (I) is brought into contact with an antibody, A first associates with the antibody. Next, a nucleophilic group in a side chain of a specific amino acid residue to be modified in the antibody (for example, an amino group in a side chain of a lysine residue) present near an antibody association site reacts with an electrophilic group in E. As a result, the leaving group contained in L or L₁ can be eliminated from E while the nucleophilic group binds to the electrophile. At this time, when there is no other amino acid residue of the same type as a specific amino acid residue to be modified near a space

between the antibody association site and the specific amino acid residue, the electrophilic group in E can regioselectively bind to a nucleophilic group in the side chain of the specific amino acid residue to be modified in the antibody without strictly controlling the length of the main chain. It is understood that even when another specific amino acid residue of the same type as the specific amino acid residue is present in such regions, the electrophilic group in E can regioselectively bind to the specific amino acid residue by controlling the length of the main chain.

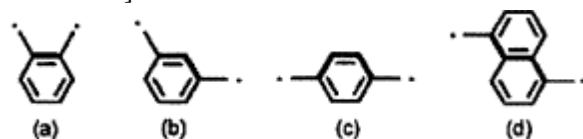
[0134] The length of the main chain of L or L-L₂ coupling A with E is not particularly limited so long as it can regioselectively modify a specific amino acid residue in an antibody. For example, when a specific amino acid residue in human IgG Fe (e.g., Lys248 residue, Lys246 residue, Lys288, Lys290, or Lys317 according to Eu numbering, or amino acid residues other than these residues) is regioselectively modified, the length preferably consists of 20 or fewer atoms. The length of the main chain of L or L₁-L₂ may be preferably 1 or more, more preferably 2 or more, even more preferably 3 or more, and particularly preferably 4 or more or 5 or more. The length of the main chain of L or L₁-L₂ may be preferably 50 or less, more preferably 30 or less, even more preferably 20 or less, and particularly preferably 15 or less or 10 or less. More specifically, the length of the main chain of L or L₁-L₂ may be preferably 1 to 50, more preferably 1 to 30, even more preferably 1 to 20,

and particularly preferably 1 to 15 or 1 to 10. Alternatively, the length of the main chain of L or L₁-L₂ may be preferably 2 to 30, even more preferably 3 to 20, and particularly preferably 4 to 15 or 5 to 10.

[0135] When the main chain has a structure comprising no cyclic structure, the number of atoms of the main chain can be determined by counting the number of atoms in a chain structure (excluding the number of hydrogen atoms, and the number of atoms of branched structure portions and sub-stituent s).

[0136] On the other hand, when the main chain has a structure containing a cyclic structure, in view of defining the length of the main chain, the number of atoms of the main chain can be counted for convenience's sake. Specifically, the number of atoms of the main chain in such a case can be determined by counting the number of atoms of the shortest route connecting two bonds in the cyclic structure in addition to the number of atoms in a chain structure comprising no divalent cyclic structure in the main chain (excluding the number of hydrogen atoms, and the number of atoms of branched structure portions and substituents) (e.g., refer to the (a) to (d) thick routes below).

[Formula 10]



• is a bond.

In the case of (a), the shortest route is the thick route, and thus the number of atoms in the divalent cyclic structure counted as the number of atoms of the main chain is two.

In the case of (b), the shortest route is the thick route, and thus the number of atoms in the divalent cyclic structure counted as the number of atoms of the main chain is three.

In the case of (c), any route is the shortest route (the same distance), and thus the number of atoms in the divalent cyclic structure counted as the number of atoms of the main chain is four.

In the case of (d), the route of the condensed site is the shortest route, and thus the number of atoms in the divalent cyclic structure counted as the number of atoms of the main chain is four.

[0137] The main chain of L or L-L₂ may be preferably a chain structure in which the "other divalent group" in L or the "divalent group" in L₁ does not comprise a divalent cyclic structure. Consequently, the "other divalent group" in L or the "divalent group" in L₁ may be a divalent linear or branched hydrocarbon group, -C(=O)-, -NRL- (RL indicates a hydrogen atom or the above-described substituent), -O-, -S-, C(=S)-, or a group consisting of a

combination of two or more (e.g., two to eight, preferably two to six, and more preferably two to four) of these. The divalent linear or branched hydrocarbon group may have, for example, one to five, preferably one to three, more preferably one or two substituents, or does not necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the group as described above has a substituent, examples and preferred examples of such a substituent are the same as those of the substituent which may be comprised in the heteroarylene, which is an example of the leaving group.

[0138] 1-4. Divalent Group Comprising Electrophilic Group (E)

In Formula (I), Lis a divalent group comprising an electrophilic group (i) coupled with the leaving group and

(ii) having ability to react with a nucleophilic group in the antibody.

[0139] Examples of the nucleophilic group in an antibody include NH_2 in a side chain of a lysine residue, OH in a side chain of a tyrosine residue, OH in a side chain of a serine residue, OH in a side chain of a threonine residue, and SH in a side chain of a cysteine residue. The nucleophilic group in an antibody is preferably NH_2 in a side chain of a lysine residue or OH in a side chain of a tyrosine residue, and more preferably NH_2 in a side chain of a lysine residue.

[0140] The electrophilic group comprised in E can be any electrophilic group capable of being coupled with a leaving group and having ability to react with the nucleophilic group in an antibody as described above. A group selected from the group consisting of -C(=O)- , $\text{-SO}_2\text{-}$, and $\text{-CH}_2\text{-}$ is preferable. As the electrophilic group, $\text{-CH}_2\text{-}$ can also be used depending on an electronic balance with an adjacent group (e.g., leaving group, L_2 , or E_2). For example, when a tosyl group is used as the leaving group, $\text{-CH}_2\text{-}$ can be suitably used as the electrophilic group (Tsukiji et al., Nature Chemical Biology, Vol. 5, No. 5, May 2009, which is incorporated herein by reference in its entirety). The electrophilic group is more preferably -C(=O)- or $\text{-SO}_2\text{-}$, and even more preferably -C(=O)- .

[0141] The divalent group comprising an electrophilic group, represented by E is a divalent group consisting of the above electrophilic group, or a divalent group comprising another divalent group in addition to the above electrophilic group. Examples of such another divalent group include a divalent hydrocarbon group, a divalent heterocyclic group,

-C(=O)- , -NRE- (*RE* indicates a hydrogen atom or the above-described substituent), -O- , -S- , -C(=S)- , and a group consisting of a combination of two

or more (e.g., two to eight, preferably two to six, and more preferably two to four) of these. The divalent hydrocarbon group and the divalent heterocyclic group may each have, for example, one to five, preferably one to three, more preferably one or two substituents, or do not each necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the group as described above has a substituent, examples and preferred examples of such a substituent are the same as those of the substituent which may be comprised in the heteroarylene, which is an example of the leaving group. E

(and E_1 - E_2 - E_3 described below) can be designed so as to be free of a peptide portion having a problem of potential immunogenicity and being easily hydrolyzed in the blood.

[0142] The divalent hydrocarbon group is a linear, branched, or cyclic divalent hydrocarbon group and preferably a linear or branched divalent hydrocarbon group. Examples of the divalent hydrocarbon group include alkylene, alkenylene, alkynylene, and arylene.

[0143] The alkylene is preferably C1-12 alkylene, more preferably C1-6 alkylene, and particularly preferably C1-4 alkylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkylene may be any of Linear, branched, or cyclic one and is preferably linear alkylene. Examples of such an alkylene include methylene, ethylene, propylene, butylene, pentylene, and hexylene.

[0144] The alkenylene is preferably C2-12 alkenylene, more preferably C2-6 alkenylene, and particularly preferably C2-4 alkenylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkenylene may be any of linear, branched, or cyclic one and is preferably linear alkenylene. Examples of such an alkenylene include ethylenylene, propynylene, butenylenylene, pentenylenylene, and hexenylenylene.

[0145] The alkynylene is preferably C2-12 alkynylene, more preferably C2-6 alkynylene, and particularly preferably C2-4 alkynylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkynylene may be any of linear, branched, or cyclic one and is preferably linear alkynylene. Examples of such an alkynylene include ethynylene, propynylene, butynylene, pentynylene, and hexynylene.

[0146] The arylene is preferably C6-24 arylene, more preferably C6-18 arylene, even more preferably C6-14 arylene, and still even more preferably C6-10 arylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the arylene include phenylene, naphthylene, and anthracenylenylene. **[0147]** The divalent heterocyclic group is a divalent aromatic heterocyclic group or a divalent nonaromatic heterocyclic group. The divalent heterocyclic group preferably comprises, as a hetero atom forming a heterocycle, one or more selected from the group consisting of an oxygen atom, a sulfur atom, a nitrogen atom, a phosphorous atom, a boron atom, and a silicon atom and more preferably comprises one or more selected from the group consisting of an oxygen atom, a sulfur atom, and a nitrogen atom.

[0148] The divalent aromatic heterocyclic group is preferably a C1-21 divalent aromatic heterocyclic group, more preferably a C1-15 divalent aromatic heterocyclic group, even more preferably a C1-9 divalent aromatic heterocyclic group, and still even more preferably a C1-6 divalent aromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specific examples of the divalent aromatic heterocyclic group include pyrrolediyl, furandiyl, thiophenediyl, pyridinediyl, pyridazinediyl, pyrimidinediyl, pyrazinediyl, triazinediyl, pyrazolediyl, imidazolediyl, thiazolediyl, isothiazolediyl, oxazolediyl, isoxazolediyl, triazolediyl, tetrazolediyl, indolediyl, purinediyl, anthraquinonediyl, carbazolediyl, fluorenediyl, quinolinediyl, isoquinolinediyl, quinazolinediyl, and phthalazinediyl.

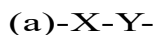
[0149] The divalent nonaromatic heterocyclic group is preferably a C2-21 nonaromatic heterocyclic group, more preferably a C2-15 nonaromatic heterocyclic group, even more preferably a C2-9 nonaromatic heterocyclic group, and still even more preferably a C2-6 nonaromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specifically, examples of the divalent nonaromatic heterocyclic group include pyrroldionediyl, pyrrolinedionediyl, pyrrolinediyl, oxiranediyl, aziridinediyl, azetidinediyl, oxetanediyl, thietanediyl, pyrrolidinediyl, dihydrofurandiyl, tetrahydrofurandiyl, dioxolanediy, tetrahydrothiophenediyl, pyrrolinediyl, imidazolidinediyl, oxazolidinediyl, piperidinediyl, dihydropyrandiyl, tetrahydropyrandiyl, tetrahydrothiopyrandiyl, morpholinediyl, thiomorpholinediyl, piperazinediyl, dihydrooxazinediyl, tetrahydrooxazinediyl, dihydropyrimidinediyl, and tetrahydropyrimidinediyl.

[0150] In a specific embodiment, E can be expressed by E1-E2-E3.

[0151] In Formula (I), E₁ is an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody. The definitions, examples, and preferred examples of the electrophilic group of E₁ are the same as those of the electrophilic group in E.

[0152] E₂ is (a) or (b) below:

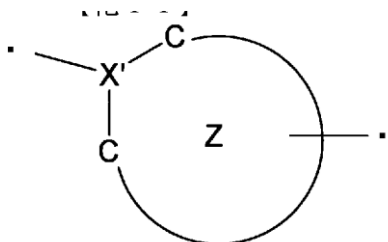
E₂ is a group represented by



wherein X which binds to E₁ is C(R₁)(R₂) where R₁ and R₂ are each independently a hydrogen atom or C₁₋₆ alkyl, N(R₃) where R₃ is a hydrogen atom or C₁₋₆ alkyl, O, S, or Se, and

Y which binds to E₃ is C(R₄)(R_s) where R₄ and R_s are each independently a hydrogen atom or C₁₋₆ alkyl; or **[0559]** (b) the following formula (i):

[Formula 11]



wherein the ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond).

[0153] When E₂ is the above (a), C₁₋₆ alkyl in R₁ to R_s is the same as that described above.

[0154] When E₂ is the above (a), X which binds to E₁ is preferably C(R₁)(R₂), N(R₃), O, S, or Se, more preferably C(R₁)(R₂), N(R₃), O, or S, even more preferably C(R₁)(R₂), N(R₃), or O, still even more preferably C(R₁)(R₂) or N(R₃), and most preferably C(R₁)(R₂) in view of improving the reactivity and stability of the compound of the present invention.

[0155] X in E₂ can be defined in relation to a leaving group (e.g., refer to L₁ and L₂). When X is an atom or a group such as N(R₃), O, S, or Se, not only the leaving group but X can also be eliminated from the electrophilic group. However, when a predetermined amount of a compound represented by Formula (I) is used for a reaction with an antibody, the reaction can be controlled such that X in at least part of the compound represented by Formula (I) is not eliminated from the electrophilic group.

Therefore, in the present invention, an atom or a group as described above can be used as X. X can be preferably an atom or a group that is less likely to be eliminated than the leaving group (that is, an atom or a group having a pKa value larger than a pKa value of the leaving group) in view of improving the efficiency of a target reaction between the compound represented by Formula (I) and an antibody, and further improving the yield of an antibody having a bioorthogonal functional group or bioorthogonal functional groups. Consequently, X in E₂ preferably has ability to be eliminated equal to or less than the leaving group in view of more selectively eliminating the leaving group and suppressing the elimination of X in E₂ to improve the efficiency of a target reaction between the compound represented by Formula (I) and an antibody and to further improve the yield of an antibody having a bioorthogonal functional group or bioorthogonal functional groups. Such X may vary depending on the type of leaving group (e.g., -N(R)-, -N(OR)-, -O-, -S-, -Se-, or heteroarylene), presence or absence of a group comprising a group that enhances ability of a leaving group present adjacent to the leaving group to be eliminated, and the type thereof, but the outline of X is as follows:

(1) When the leaving group is -N(R)- or -N(OR)-, X is preferably C(R) (R) or N(R), and more preferably C(R₁) (R₂).

(2) When the leaving group is -O-, X is preferably C(R₁) (R₂), N(R₃), or O, more preferably C(R₁) (R₂) or N(R₃), and even more preferably C(R₁) (R₂).

(3) When the leaving group is -S-, X is preferably C(R₁) (R₂), N(R₃), O, or S, more preferably C(R₁) (R₂), N(R₃), or O, even more preferably C(R₁) (R₂) or N(R₃), and still even more preferably C(R₁) (R₂).

(4) When the leaving group is -Se-, X is preferably C(R₁) (R₂), N(R₃), O, S, or Se, more preferably C(R₁) (R₂), N(R₃), O, or S, even more preferably C(R₁) (R₂), N(R₃), or O, still even more preferably C(R₁) (R₂) or N(R₃), and most preferably C(R₁) (R₂).

(5) When the leaving group is heteroarylene, X is preferably C(R₁) (R₂), N(R₃), O, S, or Se, more preferably C(R₁) (R₂), N(R₃), O, or S, even more preferably C(R₁) (R₂), N(R₃), or O, still even more preferably C(R₁) (R₂) or N(R₃), and most preferably C(R₁) (R₂).

[05156] When E₂ is the above (b), the ring-constituting atom X' which binds to E₁ comprised in the ring Z is a carbon atom or a nitrogen atom.

[0157] When the ring-constituting atom X' which binds to E₁ is a carbon atom, the ring Z is a divalent cyclic group in which all of the ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms. The divalent cyclic group may have e.g., one to five, preferably one to three, and more preferably one or two substituents, or does not necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the divalent cyclic group has a substituent, examples and preferred examples of such a substituent are the same as those of the substituent which may be comprised in the heteroarylene, which is an example of the leaving group. Examples of such a divalent cyclic group include a cyclic divalent hydrocarbon group (e.g., arylene, cyclic alkylene, cyclic alkenylene, or cyclic alkynylene), and a divalent heterocyclic group (e.g., a divalent aromatic heterocyclic group or a divalent nonaromatic heterocyclic group).

[0158] The divalent hydrocarbon group is a cyclic divalent hydrocarbon group, and examples of the divalent hydrocarbon group include cyclic alkylene, cyclic alkenylene, cyclic alkynylene, and arylene.

[0159] The cyclic alkylene is preferably C₃₋₁₂ alkylene, more preferably C₃₋₁₀ alkylene, and particularly preferably C_{5-e} alkylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of such an alkylene include cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, cycloheptylene, cyclooctylene, cyclononylene, and cyclodekylene.

[0160] The cyclic alkenylene is preferably C₃₋₁₂ alkenylene, more preferably C₃₋₁₀ alkenylene, and particularly preferably C₅₋₈ alkenylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of such an alkenylene include cyclopropenylene, cyclobutenylene, cyclopentenylene, cyclohexenylene, cycloheptenylene, cyclooctenylene, cyclononenylene, and cyclodekenylene.

[0161] The cyclic alkynylene is preferably C₆₋₁₂ alkynylene, more preferably C₇₋₁₂ alkynylene, and particularly preferably C₈₋₁₂ alkynylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Alkynylene may be any of linear, branched, or cyclic one and is preferably linear alkynylene. Examples of such an alkynylene include cyclohexynylene, cycloheptynylene, cyclooctynylene, cyclononynylene, cyclodekynylene, cycloundekynylene, and cyclododekynylene.

[0162] The arylene is preferably C₆₋₂₄ arylene, more preferably C₆₋₁₈ arylene, even more preferably C₆₋₁₄ arylene, and still even more preferably C₆₋₁₀ arylene. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the arylene include phenylene, naphthylene, and anthracenylene.

[0163] The divalent heterocyclic group is a divalent aromatic heterocyclic group or a divalent nonaromatic heterocyclic group. The divalent heterocyclic group preferably comprises, as a heteroatom forming a heterocycle, one or more selected from the group consisting of an oxygen atom, a sulfur atom, a nitrogen atom, a phosphorous atom, a boron atom, and a silicon atom and more preferably comprises one or more selected from the group consisting of an oxygen atom, a sulfur atom, and a nitrogen atom.

[0164] The divalent aromatic heterocyclic group is preferably a C₃₋₂₁ divalent aromatic heterocyclic group, more preferably a C₃₋₁₅ divalent aromatic heterocyclic group, even more preferably a C₃₋₉ divalent aromatic heterocyclic group, and still even more preferably a C₃₋₆ divalent aromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specifically, examples of the divalent aromatic heterocyclic group include pyrrolediy, furandiy, thiophenediy, pyridinediy, pyridazinediy, pyrimidinediy, pyrazolediy, isothiazolediy, isoxazolediy, indolediy, anthraquinonediyl, carbazolediy, fluorenediy, quinolinediy, isoquinolinediy, quinazolinediy, and phthalazinediy.

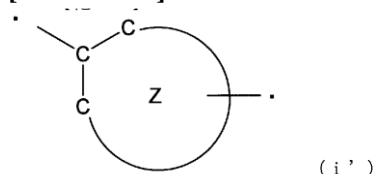
[0165] The divalent nonaromatic heterocyclic group is preferably a C₃₋₂₁ nonaromatic heterocyclic group, more preferably a C₃₋₁₅ nonaromatic heterocyclic group, even more preferably a C₃₋₉ nonaromatic heterocyclic group, and still even more preferably a C₃₋₆ nonaromatic heterocyclic group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. More specifically, examples of the divalent nonaromatic heterocyclic group include pyrrolinedionediyl, pyrrolinediy, azetidinediy, oxetanediyl, thietanediyl, pyrrolidinediy, dihydrofurandiy, tetrahydrofurandiy, tetrahydrothiophenediy, pyrrolinediy, piperidinediy, dihydropyrandiy, tetrahydropyrandiy, tetrahydrothiopyrandiy, piperazinediy, dihydrooxazinediy¹, tetrahydrooxazinediy¹, dihydropyrimidinediy, and tetrahydropyrimidinediy.

[0166]

When the ring-constituting atom X' which binds to E₁ is a nitrogen atom, the ring Z is a divalent heterocyclic group in which the ring-constituting atom X' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms. Such a divalent heterocyclic group is a divalent heterocyclic group comprising a nitrogen atom as a ring-constituting atom. The divalent aromatic heterocyclic group comprising a nitrogen atom as a ring-constituting atom is preferably a C_{3_21} divalent heterocyclic group, more preferably a C_{3_15} divalent heterocyclic group, even more preferably a C_{3_9} divalent heterocyclic group, and still even more preferably a C_{3_6} divalent heterocyclic group. The divalent heterocyclic group may have e.g., one to five, preferably one to three, and more preferably one or two substituents, or does not necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the divalent heterocyclic group has a substituent, examples and preferred examples of such a substituent are the same as those of the substituent which may be comprised in the heteroarylene, which is an example of the leaving group. The number of carbon atoms does not comprise the number of carbon atoms of the substituent. Examples of the divalent heterocyclic group comprising a nitrogen atom as a ring-constituting atom include a divalent aromatic heterocyclic group comprising a nitrogen atom as a ring-constituting atom and a divalent nonaromatic heterocyclic group comprising a nitrogen atom as a ring-constituting atom. Examples of the divalent aromatic heterocyclic group comprising a nitrogen atom as a ring-constituting atom include pyrrolediyl, imidazolediyl, indolediyl, purinediyl, and carbazolediyl. Examples of the divalent nonaromatic heterocyclic group comprising a nitrogen atom as a ring-constituting atom include pyrroledionediyl, pyrrolinedionediyl, pyrrolinediyl, aziridinediyl, azetidinediyl, pyrrolidinediyl, pyrrolinediyl, imidazolidinediyl, piperidinediyl, morpholinediyl, thiomorpholinediyl, piperazinediyl, dihydropyrimidinediyl, and tetrahydropyrimidinediyl.

[0167] (b) The group represented by the above formula (i) is preferably (b') a group represented by the following formula (i'):

[Formula 12]



(where the ring Z is a divalent cyclic group in which all of a ring-constituting atom which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms. • is a bond). The definitions, examples, and preferred examples of the divalent cyclic group for the ring Z in the group represented by the above Formula (i') are the same as those of the divalent cyclic group for the ring Z in the group represented by the above Formula (i).

[0168] E₃ is a divalent group when E₂ is -X-Y-, and is a bond or a divalent group when E₂ is a group represented by Formula (i). The divalent group represented by E₃ is the same as another divalent group when the divalent group comprising an electrophilic group, represented by E is a divalent group comprising another divalent group in addition to the electrophilic group.

[0169] The length of a main chain of E (divalent group comprising an electrophilic group) or E₁ (electrophilic group)-E₂ (the above (a) or (b))-E₃ (bond or divalent group), coupling L (divalent group comprising a leaving group) with B (bioorthogonal functional group) cannot be involved in regioselective modification of the position of a specific amino acid residue in an antibody in a reaction between the compound represented by Formula (I) and the antibody, but can be involved in a distance between an antibody and a bioorthogonal functional group in the antibody having the bioorthogonal functional group, formed after the reaction. The main chain of E or E₁-E₂-E₃ refers to a chain structure coupling L with B and consisting of a plurality of atoms covalently binding to each other, and excludes a hydrogen atom, a branched structure portion, and a substituent. Consequently, the length of the main chain of E or E₁-E₂-E₃ can be designed as appropriate in view of adjusting the distance.

[0170]

The length of the main chain of E or E₁-E₂-E₃ coupling L with B is not particularly limited, but may be a length consisting of three or more atoms.

[0171] When the main chain has a structure comprising no cyclic structure, the number of atoms of the main chain can be determined by counting the number of atoms in a chain structure (excluding the number of hydrogen atoms, and the number of atoms of branched structure portions and substituents).

[0172] On the other hand, when the main chain has a structure containing a cyclic structure, in view of defining the length of the main chain, the number of atoms of the main chain can be counted for convenience's sake. Specifically, the number of atoms of the main chain in such a case can be determined, as described above, by counting the number of atoms of the shortest route connecting two bonds in the cyclic structure in addition to the number of atoms in a chain structure comprising no divalent cyclic structure in the main chain (excluding the number of hydrogen atoms, and the number of atoms of branched structure portions and substituents).

[0173] The main chain of E or E₁-E₂-E, may be preferably a chain structure in which the "other divalent group" in E or the "divalent group" in E₃ does not comprise a divalent cyclic structure. Consequently, the "other divalent group" in E or the "divalent group" in E₃ may be a divalent linear or branched hydrocarbon group, -C(=O)-, -NRE- (RE indicates a hydrogen atom or the above-described substituent), -O-, -S-, C(=S)-, or a group consisting of a combination of two or more (e.g., two to eight, preferably two to six, and more preferably two to four) of these. The divalent linear or branched hydrocarbon group may have, for example, one to five, preferably one to three, more preferably one or two substituents, or does not necessarily have a substituent. It is preferable not to have such a substituent in view of synthesizing a compound having a simple chemical structure. On the other hand, when the group as described above has a substituent, examples and preferred examples of such a substituent are the same as those of the substituent which may be comprised in the heteroarylene, which is an example of the leaving group.

[0174]

1-5. Bioorthogonal Functional Group (B)

In Formula (I), B is a bioorthogonal functional

[0175] The bioorthogonal functional group refers to a group that does not react with biological components (e.g., amino acids, nucleic acids, lipids, sugars, and phosphoric acids) or has a low reaction rate to biological components but selectively reacts with components other than biological components. The bioorthogonal functional group is well known in the technical field concerned (e.g., refer to Sharpless K. B. et al., *Angew. Chem. Int. Ed.* 40, 2004 (2015); Bertozzi C.R. et al., *Science* 291, 2357 (2001); Bertozzi C. R. et al., *Nature Chemical Biology* 1, 13 (2005), all of which are incorporated herein by reference in their entireties).

[0176] When the target of the affinity substance is an antibody (protein), the bioorthogonal functional group is a bioorthogonal functional group to a protein. The bioorthogonal functional group to proteins is a group that does not react with side chains of 20 natural amino acid residues forming proteins and reacts with certain functional groups. The 20 natural amino acid residues forming proteins are alanine (A), asparagine (N), cysteine (C), glutamine (Q), glycine (G), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), proline (P), serine (S), threonine (T), tryptophan (W), tyrosine (Y), valine (V), aspartic acid (D), glutamic acid (E), arginine (R), histidine (H), and lysine (L). Among these 20 natural amino acid residues, glycine, which has no side chain (that is, has a hydrogen atom), and alanine, isoleucine, leucine, phenylalanine, and valine, which have a hydrocarbon group as a side chain (that is, comprise no hetero atom selected from the group consisting of a sulfur atom, a nitrogen atom, and an oxygen atom in their side chains) are inactive to normal reactions. Consequently, the bioorthogonal functional group to proteins is a functional group incapable of reacting with, in addition to the side chains of these amino acids having side chains inactive to normal reactions, side chains of asparagine, glutamine, methionine, proline, serine, threonine, tryptophan, tyrosine, aspartic acid, glutamic acid, arginine, histidine, and lysine.

[0178] More specifically, the bioorthogonal functional group may correspond to any one chemical structure selected from the group consisting of the following:

[illegible]

R1fl one or a plurality of R1gs, and one or a plurality of R1hs are the same as or different from each other, and are each an atom or a group selected from the group consisting of (a) to (g) or an electron-withdrawing group, and

- is a bond.

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a) a hydrogen atom or a halogen atom;

(b) a monovalent hydrocarbon group;

(c) an aralkyl;

(d) a monovalent heterocyclic group;

(e) $Ra-O-$, $Ra-C(=O)-$, $Ra-O-----C(=O)-$,

or $Ra-C(=O)-O-$ wherein Ra indicates a hydrogen atom or a monovalent hydrocarbon group;

(f) NR_6Rc- , $NRbRc-C(=O)-$, $NRbRc-C(=O)-O-$, or $Rb-C$

$(=O)-NRC-$ wherein R_6 and Rc are the same as or different from each other, and each indicate a hydrogen atom or a monovalent hydrocarbon group; and

(g) a nitro group, a sulfuric acid group, a sulfonic acid group, a cyano group, and a carboxy group.

[0180] The definitions, examples, and preferred examples of the halogen atom, the monovalent hydrocarbon group, the aralkyl, the monovalent heterocyclic group, and the monovalent hydrocarbon group in Ra to Re , in (a) to (g) are the same as those in the above (i) to s(vii). The atom or group selected from the group consisting of (a) to (g) is particularly preferably the atom or group of (a) or (b).

[0181] The bioorthogonal functional group may be preferably a group selected from the group consisting of an azide residue, a thiol residue, an alkyne residue, a maleimide residue, and a disulfide residue among the bioorthogonal functional groups described above in view of improving reaction efficiency or the like.

Examples of the electron-withdrawing group include those described above, in which preferred are a halogen atom, a boronic acid residue, mesyl, tosyl, and triflate.

[0182] 1-6. Preferred Structure of Compound Represented by Formula (I)

In a preferred embodiment, the compound represented by Formula (I) may be a compound represented by the following Formula (I-1):



wherein

A and B are the same those as in Formula (I),

L_1 is a bond or a divalent group,

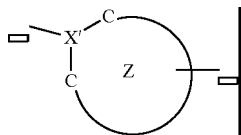
L_2 is a leaving group,

E_1 is an electrophilic group (i) coupled with a leaving group and (ii) having ability to react with a nucleophilic group in an antibody,

E_2 is a group represented by (a)-X-Y-[where X which binds to E_1 is $C(R_1)(R_2)$ (where R_1 and R_2 are each independently a hydrogen atom or C_{1-6} alkyl), $N(R_3)$ (where R_3 is a hydrogen atom or C_{1-6} alkyl), O, S, or Se, and Y which binds to E_3 is $C(R_4)(R_5)$ (where R_4 and R_5 are each independently a hydrogen atom or C_{1-6} alkyl), or (b) the following formula (i):

[Formula 14]

(i)

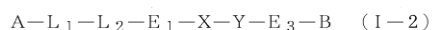


(where the ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E_1 and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X' which binds to E_1 is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms. • is a bond),

E₃ is a divalent group when E₂ is -X-Y-, and is a bond or a divalent group when E₂ is a group represented by Formula (i), and the leaving group has ability to be cleaved and eliminated from E₁ by a reaction between the nucleophilic group and the electrophilic group].

In Formula (I-1), the leaving group represented by L₂ is preferably any of the above (a) to (c). Examples and preferred examples of the leaving group represented by L₂ are also the same as those described in the above (a) to (c).

[0184] In a preferred specific embodiment, the compound represented by Formula (I-1) may be a compound represented by the following Formula (I-2):



wherein

A, Li, X, Y, and B are the same as those in Formula (I-1),

L₂ is

(a) ring P-Q- [where ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, C≡C-CH₂-O-, -N(OR)-, -N(R)-, and O-N(R)-

(where R is a hydrogen atom or C₁₋₆ alkyl),

(b) heteroarylene, or

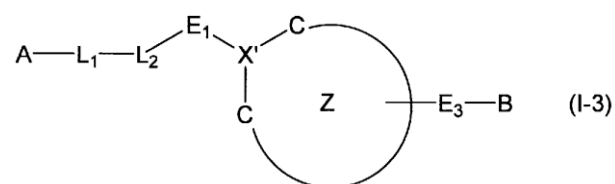
(c) -Q- [where Q is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl)],

E₁ is selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-, and

E₃ is a divalent group.

[0185] In another preferred specific embodiment, the compound represented by Formula (I-1) may be a compound represented by the following Formula (I-3):

[Formula 15]



wherein

A, Li, ring Z, ring-constituting atom X', and B are the same as those in the above Formula (I-1),

L₂ is (a) ring P-Q- [where ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and

Q is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -c≡c-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl),

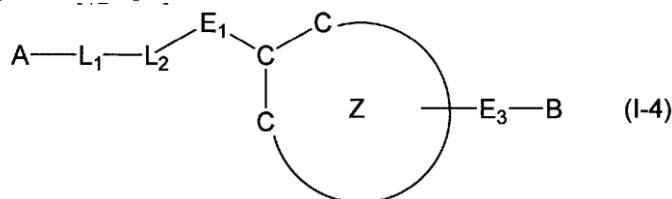
(b) heteroarylene, or

(c) -Q- [where Q is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -c≡c-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl)],
E₁ is selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-, and
E₃ is a bond or a divalent group.

[0186]

The compound represented by formula (I-3) may be preferably a compound represented by the following Formula (I-4):

[Formula 16]



wherein

A, Li, and B are the same as those in the above Formula (I-1),

L₂ is (a) ring P-Q [where ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6-diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of

-O-,
-S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-,
-c≡c-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)-
(R)- (where R is a hydrogen atom or C₁₋₆ alkyl),

(b) heteroarylene, or

(c) -Q- [where Q is selected from the group consisting of -O-, -S-, -Se-, -SO₂-O-, -SO₂-N(R)-, -SO₂-, -c≡c-CH₂-O-, -N(OR)-, -N(R)-, and -O-N(R)- (where R is a hydrogen atom or C₁₋₆ alkyl)],

E₁ is selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-,

ring Z is a divalent cyclic group in which all of a ring-constituting atom which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, and

E₃ is a bond or a divalent group.

[0187] In the compounds represented by the above Formulae (I-1) to (I-4), the definitions, examples, and preferred examples of A, L₁, L₂, E₁, E₂, E₃, B, -X-Y-, C₁₋₆ alkyl in R₁ to R₅, and the group represented by Formula (i) (e.g., a divalent cyclic group or a divalent heterocyclic group) are the same as those described above. The definitions, examples, and preferred examples of (a) to (c) each of which is L₂, ring Z (e.g., a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X' which binds to E₁ is a nitrogen atom, and ring-carbon atoms), ring P, and a group such as Q (e.g., C₁₋₆ in R) are also the same as those described above.

[0188] 1-7. Method of Production

The compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof can be prepared as appropriate. The compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof is represented by Formula (I), preferably Formula (I-1), more preferably Formula (I-2), (I-3), or (I-4).

[0189] For the affinity substance to an antibody (A), one having any functional group can be selected as appropriate. Consequently, using a reactive group capable of reacting with the functional group, the affinity substance is reacted with a structural unit represented by L-E-B, whereby a structural unit represented by A-L-E-B can be prepared. Such a reaction can be conducted in an appropriate reaction system such as an organic solvent system or an aqueous solution system at an appropriate temperature (e.g., about 15° C. to 200° C.), for example. The reaction system may comprise an appropriate catalyst. The reaction time is e.g., 1 minute to 20 hours, preferably 10 minutes to 15 hours, more preferably 20 minutes to 10 hours, and even more preferably 30 minutes to 8 hours.

[0190] In the reaction system, a molar ratio (Y/X) of the structural unit (Y) represented by L-E-B to the affinity substance (X) is not limited to a particular ratio because it varies in accordance with the types of the structural unit and the affinity substance, the number of sites in the affinity substance to be modified with the structural unit, and the like. However, the molar ratio (Y/X) is e.g., 0.1 to 50, preferably 0.5 to 40, more preferably 1 to 35, even more preferably 2 to 25, and particularly preferably 3 to 15.

[0191] Determination of the formation of the compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof, which depends on its specific raw materials and the molecular weight of a product, can be performed by electrophoresis, chromatography (e.g., gel permeation chromatography, ion-exchange chromatography, reversed phase column chromatography, and HPLC), or mass spectrometry, for example, and preferably mass spectrometry. The compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof can be purified as appropriate by any method such as chromatography (e.g., the pieces of chromatography described above and affinity chromatography).

[0192] 1-8. Others

In the inventions described below [e.g., inventions represented by Formulae (II) and (III), formulae having subordinate concepts thereof, and partial structural formulae], any symbols (e.g., A, L, E, and B) and the details of terms expressed in relation to the symbols (e.g., definitions, examples, and preferred examples) are common to those of the invention of the compound represented by the above Formula (I) or a formula having a subordinate concept thereof, or a salt thereof. Specific groups (e.g., a bioorthogonal functional group, a divalent group, and a substituent) that can define the inventions described below and any technical elements such as specific values (e.g., definitions, examples, and preferred examples) can also be common to those described above. Consequently, these matters can be quoted as appropriate in the inventions described below without any special reference. Similarly, the technical elements of a specific invention described in the inventions described below can be quoted as appropriate as the technical elements of the present invention and other inventions.

[0193] 2. Method for producing antibody having bioorthogonal functional group

The present invention provides a method for producing an antibody having a bioorthogonal functional group or bioorthogonal functional groups or a salt thereof, the method comprising the following.

(1) Reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody, B is a bioorthogonal functional group, and the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):



wherein

E and B are the same as those in Formula (I), and

Ab is an antibody, or a salt thereof.

[0194] The antibody used in the method for producing an antibody having a bioorthogonal functional group or bioorthogonal functional groups is the same as the above-described antibody. The antibody is preferably a monoclonal antibody. Examples of the isotype of the monoclonal antibody include IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA, IgD, IgE, and IgY. The monoclonal antibody is a full-length antibody or an antibody fragment (e.g., F(ab')₂, Fab', Fab, Fv, and a single-chain antibody); the full-length antibody is preferred. The antibody is particularly preferably a human antibody, a humanized antibody, or a chimeric antibody having human IgG (e.g., IgG1, IgG2, IgG3, and IgG4) in a constant region.

[0195] Ab in Formula (II) is the same as the above-described antibody and covalently binds to the electrophilic group in E. Examples of the nucleophilic group in the antibody to be subjected to covalent bond with the electrophilic group in E include NH₂ in a side chain of a lysine residue, OH in a side chain of a tyrosine residue, OH in a side chain of a serine residue, OH in a side chain of a threonine residue, and SH in a side chain of a cysteine residue.

[0196] E in Formula (II) is the same as E in the above Formula (I). E can be represented by E₁-E₂-E₃. E₁, E₂, and E₃ are the same as those described above. As described above, E and E₁-E₂-E₃ can be designed so as to be free of a peptide portion having a problem of potential immunogenicity and being easily hydrolyzed in the blood. In this case, the antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by Formula (II) can be used for preparing an antibody having a functional substance or functional substances not having such a problem.

[0197] The electrophilic group in E and the electrophilic group in E₁ covalently bind to the nucleophilic group in the antibody. Examples of the electrophilic group covalently binding to the nucleophilic group in the antibody include: NH-C(=O)-, NH-SO₂-, and NH-CH₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH₂ in a side chain of a lysine residue); O-C(=O)-, O-SO₂-, and O-CH₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is OH in a side chain of a tyrosine residue, a serine residue, or a threonine residue); and S-C(=O)- and S-CH₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is SH in a side chain of a cysteine residue). The electrophilic group covalently binding to the nucleophilic group in the antibody is preferably NH-C(=O)- or NH-SO₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH₂ in a side chain of a lysine residue), O-C(=O)- or O-SO₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is OH in a side chain of a tyrosine residue), more preferably NH-C(=O)- or NH-SO₂- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH₂ in a side chain of a lysine residue), and even more preferably NH-C(=O)- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH₂ in a side chain of a lysine residue).

[0198] B in Formula (II) is the same as B in the above Formula (I).

[0199] The antibody having a bioorthogonal functional group or bioorthogonal functional groups produced by the production method of the present invention is preferably an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups. When the compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof is represented by Formula (I), it is possible to produce an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by Formula

(II). The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups is preferably an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in a constant region, and more preferably an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in an Fe region.

[0200] In the present specification, "regioselective" or "regioselectivity" refers to a state in which even though a specific amino acid residue is not present locally at a specific region in the antibody, a certain structural unit capable of binding to the specific amino acid residue in the antibody is present locally at a specific region in the antibody. Consequently, expressions related to regioselectivity such as "regioselectively having," "regioselective binding," and "binding with regioselectivity" mean that the binding rate or the possession rate of a certain structural unit in the target region comprising one or more specific amino acid residues is higher at a significant level than the possession rate or the binding rate of the structural unit in the non-target region comprising a plurality of amino acid residues homogeneous with respect to the specific amino acid residues in the target region. Such regioselective binding or possession can be achieved by the present invention, which enables the certain structural unit to preferentially react with the specific amino acid residues in the target region in the antibody, not by randomly reacting the certain structural unit with the specific amino acid residues in the antibody but by using a compound comprising an affinity substance to an antibody.

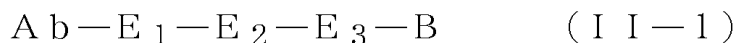
[0201] More specifically, when the antibody (Ab) comprises one or more specific amino acid residues (e.g., a lysine residue, a tyrosine residue, a threonine residue, a serine residue, or a cysteine residue) in a target region consisting of 1 to 50 continuous amino acid residues [e.g., a region consisting of amino acid residues at positions 246 to 248 in the human IgG Fe region, (b) a region consisting of amino acid residues at positions 288 to 290 in the human IgG Fe region, or (c) a region consisting of an amino acid residue at position 317 in the human IgG Fe region] and comprises five or more of the specific amino acid residues in a non-target region other than the target region, a partial structure other than the antibody can bind to one or more specific amino acid residues comprised in the target region with 30% or more regioselectivity. The regioselectivity may be e.g., preferably 40% or more, more preferably 50% or more, even more preferably 60% or more, and particularly preferably 70% or more, 80% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 100%.

[0202] The antibody having a bioorthogonal functional group or bioorthogonal functional groups produced by the production method of the present invention can have the bioorthogonal functional group (that is, a structural unit represented by E-B) depending on the number of heavy chains. The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups can be produced by first associating the compound (A-L-E-B) represented by Formula (I) with a constant region of an antibody heavy chain via the affinity substance to an antibody (A), and then reacting an electrophilic group in E with a nucleophilic group in a side chain of a specific amino acid residue near the association site (constant region of the same heavy chain as the antibody heavy chain). Consequently, by using an antibody having a plurality of (e.g., 1 to 8, preferably 1 to 4, more preferably 2) antibody heavy chains in the of structural units having a subordinate concept thereof) in the same target region of the plurality of antibody heavy chains. For example, by using an antibody having two antibody heavy chains (e.g., IgG, IgD, IgE, F(ab')₂ antibody, Fe region protein, or Fe fusion protein) in the production method of the present invention, it is possible to produce an antibody regioselectively having two structural units represented by E-B in the same target region of the two antibody heavy chains. That is, in the antibody having a bioorthogonal functional group or bioorthogonal functional groups, the modification mode by the bioorthogonal functional group can be the same between a plurality of (e.g., two) heavy chains.

[0203] The production method of the present invention may comprise subjecting a formed antibody to a specific treatment to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups and further modified. Examples of such a specific treatment include an antibody fragmentation treatment (e.g., a treatment with a specific protease such as papain or pepsin).

[0204] When the compound represented by Formula (I) or a salt thereof is used in the production method of the present invention, an antibody having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II) can be produced.

[0205] In a preferred embodiment, when a compound represented by Formula (I-1) is used as the compound represented by Formula (I) in the production method of the present invention, an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-1) can be produced:



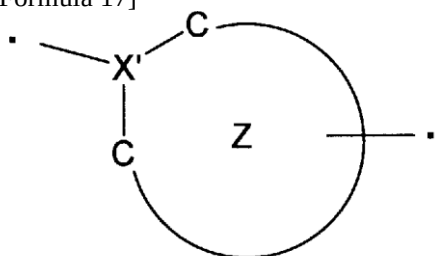
wherein

Ab is an antibody,

E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a)-X-Y-[where X which binds to E₁ is C(R₁) (R₂) (where R₁ and R₂ are each independently a hydrogen atom or C₁₋₆ alkyl), N(R₃) (where R₃ is a hydrogen atom or C₁₋₆ alkyl), O, S, or Se, and Y which binds to E₃ is C(R₄) (R₅) (where R₄ and R₅ are each independently a hydrogen atom or C₁₋₆ alkyl), or (b) the following formula (i):

[Formula 17]

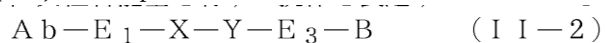


(where the ring Z is a divalent cyclic group in constituting atom X' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms. • is a bond),

E₃ is a divalent group when E₂ is -X-Y-, and is a bond or a divalent group when E₂ is a group represented by Formula (i), and

B is a bioorthogonal functional group].

[0206] In a preferred specific embodiment, when a compound represented by Formula (I-2) is used as the compound represented by Formula (I) in the production method of the present invention, an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-2) can be produced:



wherein

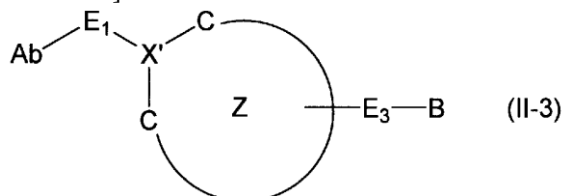
Ab, X, Y, and B are the same as those in Formula (II-1),

E₁ is a group selected from the group consisting of -C(=O)-, -SO₂-, and CH₂-, and

E₃ is a divalent group.

[0207] In another preferred specific embodiment, when a compound represented by Formula (I-3) is used as the compound represented by Formula (I-1) in the production method of the present invention, an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-3) can be produced.

[Formula 18]



wherein

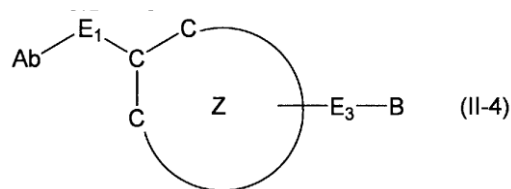
Ab, ring-constituting atom X', ring Z, and B are the same as those in Formula (II-1),

E₁ is a group selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-, and

E₃ is a bond or a divalent group.

[0208] When a compound represented by Formula (I-4) is used as the compound represented by Formula (I-3) in the production method of the present invention, an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-4) can be preferably produced:

[Formula 19]



Z, and B are the same as those in Formula (II-1),

E₁ is a group selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-, and

E₃ is a bond or a divalent group.

[0209] Any symbols (e.g., E, E_i, E₂, E₃, and B) in Formula (II), (II-1), (II-2), (II-3) or (II-4) and the details of terms (e.g., antibody, electrophilic group, and bioorthogonal functional group) expressed in relation to the symbols (e.g., definitions, examples, and preferred examples) are common to those of the above Formula (I) or a formula having a subordinate concept thereof.

[0210] The compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof has the electrophilic group in E or the electrophilic group in E₁, and therefore can react with the antibody. Such a reaction can be conducted as appropriate under a condition incapable of causing denaturation or decomposition (e.g., cleavage of an amide bond) of proteins (a mild condition). Such a reaction can be conducted in an appropriate reaction system such as a buffer at room temperature (e.g., about 15° C. to 30° C.), for example. The pH of the buffer is e.g., 5 to 9, preferably 5.5 to 8.5, and more preferably 6.0 to 8.0. The buffer may comprise an appropriate catalyst. The reaction time is e.g., 1 minute to 20 hours, preferably 10 minutes to 15 hours, more preferably 20 minutes to 10 hours, and even more preferably 30 minutes to 8 hours. For the details of such a reaction, refer to G. J. L. Bemardes et al., Chem. Rev., 115, 2174 (2015); G. J. L. Bemardes et al., Chem. Asian. J., 4,630 (2009); B. G. Davies et al., Nat. Commun., 5, 4740 (2014); A. Wagner et al., Bioconjugate. Chem., 25,825 (2014), all of which are incorporated herein by reference in their entireties, for example.

[0211] In the reaction system, a molar ratio (Y/X) of the compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof (Y) to the antibody (X) is not limited to a particular ratio because it varies in accordance with the types of the compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof, and the antibody, the number of sites in the antibody to be modified by the compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof (e.g., DAR), and the like. However, the molar ratio (Y/X) is e.g., to 100, preferably 0.5 to 80, more preferably 1 to 70, even more preferably 2 to 50, and particularly preferably 3 to 30.

[0212] Determination of the formation of the antibody having a bioorthogonal functional group or bioorthogonal functional groups, which depends on its specific raw materials and the molecular weight of a product, can be performed by electrophoresis, chromatography (e.g., gel permeation chromatography, ion-exchange chromatography, reversed phase column chromatography, and HPLC), or mass spectrometry, for example, and preferably mass spectrometry. Determination of regioselectivity can be performed by peptide mapping, for example. Peptide mapping can be performed by protease (e.g., trypsin and chymotrypsin) treatment and mass spectrometry, for example. For the protease, an endoprotease is preferred. Examples of such an endoprotease include trypsin, chymotrypsin, Glu-C, Lys-N, Lys-C, and Asp-N. Determination of the number of bioorthogonal functional groups that the antibody having a bioorthogonal functional group or bioorthogonal functional groups has can be performed by electrophoresis, chromatography, or mass spectrometry, for example, and preferably mass spectrometry. The antibody having a bioorthogonal functional group or bioorthogonal functional groups can be purified as appropriate by any method such as chromatography (e.g., the pieces of chromatography described above and affinity chromatography).

[0213] 3. Method for producing antibody having functional substance using compound having affinity substance to antibody and bioorthogonal functional group, or salt thereof

The present invention provides a method for producing an antibody having a functional substance or functional substances or a salt thereof, the method comprising the following.

(1) Reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody

to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):

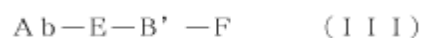


wherein

E and B are the same as those in the above Formula (I), and

Ab is an antibody, or a salt thereof, and

(2) reacting the antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the above Formula (II), or a salt thereof with a functional substance via the bioorthogonal functional group to form an antibody having a functional substance or functional substances, represented by the following Formula (III):



wherein Ab is the same as that in Formula (II),

E is the same as that in Formula (I),

B' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and

F is a functional substance, or a salt thereof.

[0214] Step (1) can be performed in a similar manner to the method for producing an antibody having a bioorthogonal functional group or bioorthogonal functional groups.

[0215] The functional substance (F) used in step (2) is not limited to a particular substance so long as it is a substance imparting any function to the antibody; examples thereof include drugs, labelling substances, and stabilizers; preferred are drugs and labelling substances. The functional substance may be a single functional substance or a substance in which two or more functional substances are coupled with each other.

[0216] The drug may be a drug to any disease. Examples of such a disease include cancer (e.g., lung cancer, stomach cancer, colon cancer, pancreatic cancer, renal cancer, liver cancer, thyroid cancer, prostatic cancer, bladder cancer, ovarian cancer, uterine cancer, bone cancer, skin cancer, a brain tumor, and melanoma), autoimmune diseases and inflammatory diseases (e.g., allergic diseases, articular rheumatism, and systemic lupus erythematosus), brain or nerve diseases (e.g., cerebral infarction, Alzheimer's disease, Parkinson disease, and amyotrophic lateral sclerosis), infectious diseases (e.g., microbial infectious diseases and viral infectious diseases), hereditary rare diseases (e.g., hereditary spherocytosis and nondystrophic myotonia), eye diseases (e.g., age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa), diseases in the bone and orthopedic field (e.g., osteoarthritis), blood diseases (e.g., leukosis and purpura), and other diseases (e.g., diabetes, metabolic diseases such as hyperlipidemia, liver diseases, renal diseases, lung diseases, circulatory system diseases, and digestive system diseases). The drug may be a drug that treats or prevents a certain disease, or a drug that alleviates side effects associated with use of the antibody to a target protein of the certain disease.

[0217] More specifically, the drug is an anti-cancer agent. Examples of the anti-cancer agent include chemotherapeutic agents, toxins, and radioisotopes or substances comprising them. Examples of chemotherapeutic agents include DNA injuring agents, antimetabolites, enzyme inhibitors, DNA intercalating agents, DNA cleaving agents, topoisomerase inhibitors, DNA binding inhibitors, tubulin binding inhibitors, cytotoxic nucleosides, and platinum compounds.

Examples of toxins include bacteriotoxins (e.g., diphtheria toxin) and phytotoxins (e.g., ricin). Examples of radioisotopes include radioisotopes of a hydrogen atom (e.g., ^3H), radioisotopes of a carbon atom (e.g., ^{14}C), radioisotopes of a phosphorous atom (e.g., ^{32}P), radioisotopes of a sulfur atom (e.g., ^{35}S), radioisotopes of yttrium (e.g., ^{90}Y), radioisotopes of technetium (e.g., $^{99\text{m}}\text{Tc}$), radioisotopes of indium (e.g., ^{111}In), radioisotopes of an iodine atom (e.g., ^{123}I , ^{125}I , ^{129}I , and ^{131}I), radioisotopes of samarium (e.g., ^{153}Sm), radioisotopes of rhenium (e.g., ^{186}Re), radioisotopes of astatine (e.g., ^{211}At), and radioisotopes of bismuth (e.g., ^{212}Bi).

[0218] Examples of labelling substances include enzymes (e.g., peroxidase, alkaline phosphatase, luciferase, and α -galactosidase), affinity substances (e.g., streptavidin, biotin, digoxigenin, and aptamer), fluorescent substances (e.g., fluorescein, fluorescein isothiocyanate, rhodamine, green fluorescent protein, and red fluorescent protein), luminescent substances (e.g., luciferin, aequorin, acridinium ester, tris(2,2'-bipyridyl)ruthenium, and luminol), and radioisotopes (e.g., those described above) or substances comprising them.

[0219]

[0219] The functional substance is a large compound, a middle compound, or a small compound and is preferably a small compound. The small compound refers to compounds with a molecular weight of 1,500 or lower. The small compound is a natural compound or a synthesized compound. The molecular weight of the small compound may be 1,200 or lower, 1,000 or lower, 900 or lower, 800 or lower, 700 or lower, 600 or lower, 500 or lower, 400 or lower, or 300 or lower. The molecular weight of the small compound may be 30 or higher, 40 or higher, or 50 or higher. The small compound may be any of the drugs or labelling substances described above. Examples of the small compound include amino acids, oligopeptides, vitamins, nucleosides, nucleotides, oligonucleotides, monosaccharides, oligosaccharides, lipids, fatty acids, and salts thereof.

[0220] The functional substance has various functional groups corresponding to its structure. When the functional substance has a functional group easily reacting with the bioorthogonal functional group, the functional group of the functional substance and the bioorthogonal functional group can be reacted with each other as appropriate. The functional group easily reacting with the bioorthogonal functional group can vary depending on a specific type of the bioorthogonal functional group. A person skilled in the art can select an appropriate functional group as the functional group easily reacting with the bioorthogonal functional group as appropriate (e.g., Boutureira et al., Chem. Rev., 2015, 115, 2174-2195, which is incorporated herein by reference in its entirety). Examples of the functional group easily reacting with the bioorthogonal functional group include, but are not limited to, an azide residue when the bioorthogonal functional group is an alkyne residue, a hydrazine residue when the bioorthogonal functional group is an aldehyde residue or a ketone residue, and a maleimide residue and a disulfide residue when the bioorthogonal functional group is a thiol residue. For example, when the bioorthogonal functional group is an alkyne residue and the functional group easily reacting with the bioorthogonal functional group is an azide residue (or vice versa), the divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group may be a divalent group comprising a triazole residue (which may be condensed with another ring or is not necessarily condensed therewith); when the bioorthogonal functional group is an aldehyde residue or a ketone residue and the functional group easily reacting with the bioorthogonal functional group is a hydrazine residue (or vice versa), the divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group may be a divalent group comprising a hydrazone residue; when the bioorthogonal functional group is a thiol residue and the functional group easily reacting with the bioorthogonal functional group is a maleimide residue or a disulfide residue (or vice versa), the divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group may be a divalent group comprising a thiosuccinimide residue or a divalent group comprising a disulfide residue (e.g., Boutureira et al., Chem. Rev., 2015, 115, 2174-2195, which is incorporated herein by reference in its entirety). The divalent group comprising a triazole residue (which may be condensed with another ring or is not necessarily condensed therewith), the divalent group comprising a hydrazone residue, the divalent group comprising a thiosuccinimide residue, or the divalent group comprising a disulfide residue is a preferred example of the divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group.

[0221] On the other hand, when the functional substance has no functional group easily reacting with the bioorthogonal functional group, a substance derivatized with a desired functional group can be used as the functional substance. When the functional substance is a soluble protein, for example, a substance derivatized so as to have a functional group that the soluble protein does not naturally have can be used.

[0222] Derivatization is a common technical knowledge in the field concerned (e.g., WO 2004/010957, United States Patent Application Publication No. 2006/0074008, and United States Patent Application Publication No. 2005/0238649, all of which are incorporated herein by reference in their entireties). Derivatization may be performed using the cross-linking agent described above, for example. Alternatively, derivatization may be performed using a specific linker having a desired functional group. Such a linker may be able to separate the functional substance and the antibody from each other through the cleavage of the linker under an appropriate condition (e.g., intracellular or extracellular), for example. Examples of such a linker include peptidyl linkers decomposed by specific proteases [e.g., intracellular proteases (e.g., proteases present in lysosome or endosome) and

extracellular proteases (e.g., secretory proteases)] (e.g., U.S. Pat. No. 6,214,345; Dubowchik et al., Pharm. Therapeutics 83: 67-123 (1999), all of which are incorporated herein by reference in their entireties) and linkers capable of being cleaved at local acidic sites present in living bodies (e.g., U.S. Pat. Nos. 5,622,929, 5,122,368, and 5,824,805, all of which are incorporated herein by reference in their entireties). The linker may be self-immolative (e.g., WO 02/083180, WO 04/043493, and WO 05/112919, all of which are incorporated herein by reference in their entireties). In the present invention, the derivatized functional substance can also be referred to simply as the "functional substance."

[0223] Ab in Formula (III) is the same as the above-described antibody and covalently binds to the electrophilic group in E. Examples of the nucleophilic group in the antibody to be subjected to covalent bond with the electrophilic group in E include NH_2 in a side chain of a lysine residue, OH in a side chain of a tyrosine residue, OH in a side chain of a serine residue, OH in a side chain of a threonine residue, and SH in a side chain of a cysteine residue.

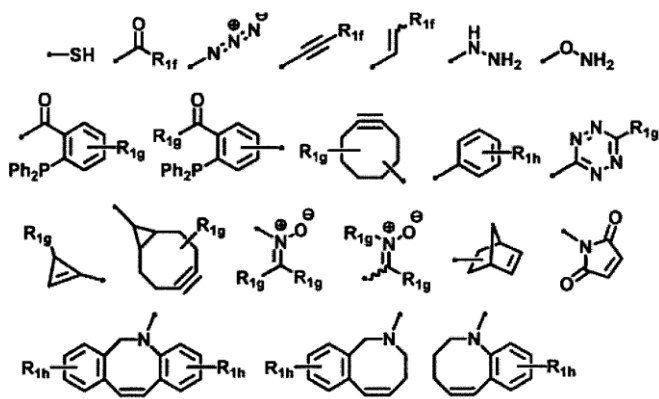
[0224] E in Formula (III) is the same as E in the above Formula (I). E can be represented by $\text{E}_1\text{-E}_2\text{-E}_3$. E_1 , E_2 , and E_3 are the same as those described above. As described above, E and $\text{E}_1\text{-E}_2\text{-E}_3$ can be designed so as to be free of a peptide portion having a problem of potential immunogenicity and being easily hydrolyzed in the blood. In this case, the antibody having a functional substance or functional substances, represented by Formula (III) can be suitably used as pharmaceuticals.

[0225] The electrophilic group in E and the electrophilic group in E_1 covalently bind to the nucleophilic group in the antibody. Examples of the electrophilic group covalently binding to the nucleophilic group in the antibody include: NH-C(=O)- , $\text{NH-SO}_2\text{-}$, and $\text{NH-CH}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH_2 in a side chain of a lysine residue); O-C(=O)- , $\text{O-SO}_2\text{-}$, and $\text{O-CH}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is OH in a side chain of a tyrosine residue, a serine residue, or a threonine residue); and S-C(=O)- and $\text{S-CH}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is SH in a side chain of a cysteine residue). The electrophilic group covalently binding to the nucleophilic group in the antibody is preferably NH-C(=O)- or $\text{NH-SO}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH_2 in a side chain of a lysine residue), O-C(=O)- or $\text{O-SO}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is OH in a side chain of a tyrosine residue), more preferably NH-C(=O)- or $\text{NH-SO}_2\text{-}$ (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH_2 in a side chain of a lysine residue), and even more preferably NH-C(=O)- (when the nucleophilic group in the antibody to be subjected to covalent bond with E is NH_2 in a side chain of a lysine residue).

[0226] In a specific embodiment, when the functional substance has a functional group easily reacting with the bioorthogonal functional group, or when the functional substance is derivatized so as to have a functional group easily reacting with the bioorthogonal functional group, the functional group easily reacting with the bioorthogonal functional group may be selected from the group consisting of an azide residue, an aldehyde residue, a thiol residue, an alkyne residue, an alkene residue, a halogen residue, a tetrazine residue, a nitron residue, a hydroxyamine residue, a nitrile residue, a hydrazine residue, a ketone residue, a boronic acid residue, a cyanobenzothiazole residue, an allyl residue, a phosphine residue, a maleimide residue, a disulfide residue, a thioester residue, an α -halocarbonyl residue, an isonitrile residue, a sydnone residue, and a selenium residue.

[0227] In a more specific embodiment, when the functional substance has a functional group easily reacting with the bioorthogonal functional group, or when the functional substance is derivatized so as to have a functional group easily reacting with the bioorthogonal functional group, the functional group easily reacting with the bioorthogonal functional group may be selected from the group consisting of the groups illustrated below:

[Formula 20-1]



R₁ is one or a plurality of Rigs, and one or a plurality of R₁hs are the same as or different from each other, and are each an atom or a group selected from the group consisting of the above (i) to (vii) or an electron-withdrawing group, and • is a bond to the functional substance.

[0229] The divalent group comprising a portion formed by a reaction between a functional substance and a bioorthogonal functional group, which is not limited to a particular group, may be a divalent group comprising a residue corresponding to any one chemical structure selected from the group consisting of the following:

[illegible]

[0231] When the antibody (Ab) comprises one or more specific amino acid residues (e.g., a lysine residue, a tyrosine residue, a

threonine residue, a serine residue, or a cysteine residue) in a target region consisting of 1 to 50 continuous amino acid residues [e.g., a region consisting of amino acid residues at positions 246 to 248 in the human IgG Fe region,

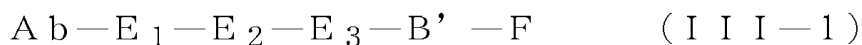
(b) a region consisting of amino acid residues at positions 288 to 290 in the human IgG Fe region, or (c) a region consisting of an amino acid residue at position 317 in the human IgG Fe region] and comprises five or more of the specific amino acid residues in a non-target region other than the target region, a partial structure other than the antibody can bind to one or more specific amino acid residues comprised in the target region with 30% or more regioselectivity. The regioselectivity may be e.g., preferably 40% or more, more preferably 50% or more, even more preferably 60% or more, and particularly preferably 70% or more, 80% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 100%.

[0232] The antibody having a functional substance or functional substances produced by the production method of the present invention can have a functional substance (that is, a structural unit represented by E-B'-F) depending on the number of heavy chains. This is because the antibody having a functional substance or functional substances can be produced from an antibody having a bioorthogonal functional group or bioorthogonal functional groups that can have a structural unit represented by E-B depending on the number of heavy chains. Consequently, by using an antibody having a plurality of (e.g., 1 to 8, preferably 1 to 4, more preferably 2) antibody heavy chains in the production method of the present invention, it is possible to produce an antibody regioselectively having a plurality of structural units represented by E-B'-F (or a plurality of structural units having a subordinate concept thereof) in the same target region of the plurality of antibody heavy chains. For example, by using an antibody having two antibody heavy chains (e.g., IgG, IgD, IgE, F(ab')₂ antibody, Fe region protein, or Fe fusion protein) in the production method of the present invention, it is possible to produce an antibody regioselectively having two structural units represented by E-B'-F in the same target region of the two antibody heavy chains. That is, in the antibody having a functional substance or functional substances, the modification mode by the functional substance can be the same between a plurality of (e.g., two) heavy chains.

[0233] The production method of the present invention may comprise subjecting a formed antibody to a specific treatment to form an antibody having a functional substance or functional substances and further modified. Examples of such a specific treatment include an antibody fragmentation treatment (e.g., a treatment with a specific protease such as papain or pepsin).

[0234] When the compound represented by Formula (I) or a salt thereof is used in the production method of the present invention, in step (1), an antibody having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II) can be produced, and then in step (2), an antibody having a functional substance or functional substances represented by the above Formula (III) can be produced.

[0235] In a preferred embodiment, when the compound represented by Formula (I-1) is used as the compound represented by Formula (I) in the production method of the present invention, in step (1), an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the above Formula (II-1) can be produced, and then in step (2), an antibody having a functional substance or functional substances, represented by the above Formula (III-1) can be produced.



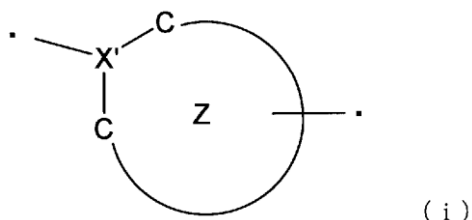
wherein

Ab is an antibody,

E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a)-X-Y-[where X which binds to E₁ is C(R₁) (R₂) (where R₁ and R₂ are each independently a hydrogen atom or C₁₋₆ alkyl), N(R₃) (where R₃ is a hydrogen atom or C₁₋₆ alkyl), O, S, or Se, and Y which binds to E₃ is C(R₄) (R_s) (where R₄ and R_s are each independently a hydrogen atom or C₁₋₆ alkyl), or (b) the following formula (i):

[Formula 21]

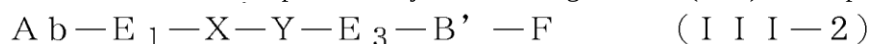


(where the ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms. • is a bond),

E₃ is a divalent group when E₂ is -X-Y-, and is a bond or a divalent group when E₂ is a group represented by Formula (i),

B' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and F is a functional substance.)

[0236] In a preferred specific embodiment, when the compound represented by Formula (I-2) is used as the compound represented by Formula (I-1) in the production method of the present invention, in step (1), an antibody having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-2) can be produced, and then in step (2), an antibody having a functional substance or functional substances, represented by the following Formula (III-2) can be produced:



wherein

Ab, X, Y, B', and F are the same as those in the above Formula (III-1),

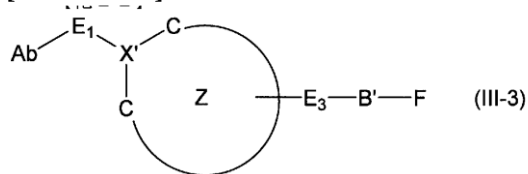
E₁ is a group selected from the group consisting of

-C(=O)-, -SO₂-, and CH₂-, and

E₃ is a divalent group.

[02237] In another preferred specific embodiment, when the compound represented by Formula (I-3) is used as the compound represented by Formula (I-1) in the production method of the present invention, in step (1), an antibody having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-3) can be produced, and then in step (2), an antibody having a functional substance or functional substances, represented by the following Formula (III-3) can be produced:

[Formula 22]



[wherein

Ab, ring-constituting atom X', ring Z, B', and F are the same as those in Formula (III-1),

E₁ is a group selected from the group consisting of

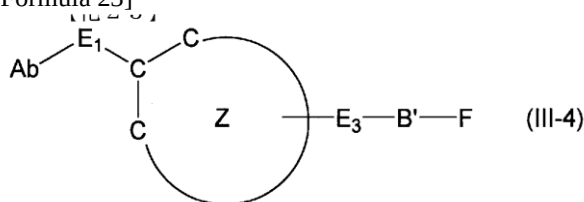
-C(=O)-, -SO₂-, and CH₂-, and

E₃ is a bond or a divalent group.

[0238]

[0238] When the compound represented by Formula (I-4) is used as the compound represented by Formula (I-3) in the production method of the present invention, preferably, in step (1), an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-4) can be produced, and then in step (2), an antibody having a functional substance or functional substances, represented by the following Formula (III-4) can be produced.

[Formula 23]



wherein

Ab, ring Z, B', and F are the same as those in Formula (III-1),

E₁ is a group selected from the group consisting of -C(=O)-, -SO₂-, and -CH₂-, and

E₃ is a bond or a divalent group.

[0239] Any symbols (e.g., E, Ei, E, E, and B) in Formula (III), (III-1), (III-2), (III-3) or (III-4) and the details of terms (e.g., antibody, electrophilic group, and bioorthogonal functional group) expressed in relation to the symbols (e.g., definitions, examples, and preferred examples) are common to those of the above Formula (I) or a formula having a subordinate concept thereof.

[0240] The antibody having a bioorthogonal functional group or bioorthogonal functional groups can react with the functional substance via the bioorthogonal functional group. Such a reaction can be conducted as appropriate under a condition incapable of causing denaturation or decomposition (e.g., cleavage of an amide bond) of proteins (a mild condition) as described above.

[0241] In the reaction system, a molar ratio (ZN) of the functional substance (Z) to the antibody (Y) having a bioorthogonal functional group or bioorthogonal functional groups is not limited to a particular ratio because it varies in accordance with the types of the bioorthogonal functional group, the functional substance, and the antibody, the number of sites in the antibody to be modified (e.g., DAR), and the like. However, the molar ratio (ZN) is e.g., 0.1 to 100, preferably 0.5 to 80, more preferably 1 to 70, even more preferably 2 to 50, and particularly preferably 3 to 30.

[0242] Determination of the formation of the antibody having a functional substance or functional substances, which depends on its specific raw materials and the molecular weight of a product, can be performed by electrophoresis, chromatography (e.g., gel permeation chromatography, ion-exchange chromatography, reversed phase column chromatography, and HPLC), or mass spectrometry, for example, and preferably mass spectrometry. Determination of regioselectivity can be performed by peptide mapping, for example. Peptide mapping can be performed by protease (e.g., trypsin and chymotrypsin) treatment and mass spectrometry, for example. For the protease, an endoprotease is preferred. Examples of such an endoprotease include trypsin, chymotrypsin, Glu-C, Lys-N, Lys-C, and Asp-N. Determination of the number of functional substances that the antibody having a functional substance or functional substances has can be performed by electrophoresis, chromatography, or mass spectrometry, for example, and preferably mass spectrometry. The antibody having a functional substance or functional substances can be purified as appropriate by any method such as chromatography (e.g., the pieces of chromatography described above and affinity chromatography).

[0243] 4. Antibody regioselectively having bioorthogonal functional group or functional substance, or salt thereof

The present invention provides an antibody regioselectively having a bioorthogonal functional group or a functional substance, or a salt thereof.

[0244] The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a salt thereof is an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups represented by Formula (II-1). As the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-1), the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-2) or (II-3) is preferably provided. As the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-3), the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups represented by the above Formula (II-4) is more preferably provided. Any symbols (e.g., E, Ei, E₂, E₃, and B) in Formula (II), (II-1), (II-2), (II-3) or (II-4) and the details of terms (e.g., antibody, electrophilic group, and bioorthogonal functional group) expressed in relation to the symbols (e.g., definitions, examples, and preferred examples) are common to those of the above Formula (I) or a formula having a subordinate concept thereof.

[0245] The antibody regioselectively having a functional substance or functional substances or a salt thereof is an antibody regioselectively having a functional substance or functional substances represented by the above Formula (III-1). As the antibody regioselectively having a functional substance or functional substances represented by the above Formula (III-1), the antibody regioselectively having a functional substance or functional substances represented by the above Formula (III-2) or (III-3) is preferably provided. As the antibody regioselectively having a functional substance or functional substances represented by the above Formula (III-3), the antibody regioselectively having a functional substance or functional substances represented by the above Formula (III-4) is more preferably provided. Any symbols (e.g., E, Ei, E₂, E₃, and B) in Formula (III), (III-1), (III-2),

(III-3) or (III-4) and the details of terms (e.g., antibody, electrophilic group, and bioorthogonal functional group) expressed in relation to the symbols (e.g., definitions, examples, and preferred examples) are common to those of the above Formula (I) or a formula having a subordinate concept thereof.

[0246] The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances is the same as the above-described antibody. Such an antibody is preferably a monoclonal antibody. Examples of the isotype of the monoclonal antibody include IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA, IgD, IgE, and IgY. The monoclonal antibody is a full-length antibody or an antibody fragment (e.g., F(ab')₂, Fab', Fab, Fv, and a single-chain antibody); the full-length antibody is preferred. Such an antibody is particularly preferably a human antibody, a humanized antibody, or a chimeric antibody having human IgG (e.g., IgG1, IgG2, IgG3, and IgG4) in a constant region.

[0247] The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances is preferably an antibody having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances only in a constant region of the antibody, and more preferably an antibody having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances only in an Fe region of the antibody.

[0248] When the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances comprises one or more specific amino acid residues (e.g., a lysine residue, a tyrosine residue, a threonine residue, a serine residue, or a cysteine residue) in a target region consisting of 1 to 50 continuous amino acid residues [e.g., (a) a region consisting of amino acid residues at positions 246 to 248 in the human IgG Fe region, (b) a region consisting of amino acid residues at positions 288 to 290 in the human IgG Fe region, or (c) a region consisting of an amino acid residue at position 317 in the human IgG Fe region] and comprises five or more of the specific amino acid residues in a non-target region other than the target region, the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances can have the bioorthogonal functional group or the functional substance with 30% or more regioselectivity in the one or more specific amino acid residues comprised in the target region. The regioselectivity may be e.g., preferably 40% or more, more preferably 50% or more, even more preferably 60% or more, and particularly preferably 70% or more, 80% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more, or 100%.

[0249]

[0249] The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances can have the bioorthogonal functional group (that is, a structural unit represented by E-B) or the functional substance (that is, a structural unit represented by E-B'-F) depending on the number of heavy chains. When the antibody having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances has a plurality of (e.g., 1 to 8, preferably 1 to 4, more preferably 2) antibody heavy chains, the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances can regioselectively have a bioorthogonal functional group or a functional substance in the same target region of the plurality of antibody heavy chains. That is, in the antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a functional substance or functional substances, the modification mode by the bioorthogonal functional group or the functional substance can be the same between a plurality of (e.g., two) heavy chains.

[0250] 5. Salt

In the present invention, examples of the salt include salts with inorganic acids, salts with organic acids, salts with inorganic bases, salts with organic bases, and salts with amino acids. Examples of salts with inorganic acids include salts with hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, and nitric acid. Examples of salts with organic acids include salts with formic acid, acetic acid, trifluoroacetic acid, lactic acid, tartaric acid, fumaric acid, oxalic acid, maleic acid, citric acid, succinic acid, malic acid, benzenesulfonic acid, and p-toluenesulfonic acid. Examples of salts with inorganic bases include salts with alkali metals (e.g., sodium and potassium), alkaline-earth metals (e.g., calcium and magnesium), other metals such as zinc and aluminum, and ammonium. Examples of salts with organic bases include salts with trimethylamine, triethylamine, propylenediamine, ethylenediamine, pyridine, ethanolamine, monoalkyl ethanolamine, dialkyl ethanolamine, diethanolamine, and triethanolamine. Examples of salts with amino acids include salts with basic amino acids (e.g., arginine, histidine, lysine, and ornithine) and acidic amino acids (e.g., aspartic acid and glutamic acid). The salt is preferably a salt with an inorganic acid (e.g., hydrogen chloride) or a salt with an organic acid (e.g., trifluoroacetic acid).

[0251] 6. Uses

The compound of the present invention having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof is useful for regioselective modification of an antibody by the bioorthogonal functional group, for example. Consequently, the present invention provides a reagent of regioselectively modifying an antibody by a bioorthogonal functional group, the reagent comprising a compound having an affinity substance to an antibody and a bioorthogonal functional group, or a salt thereof.

[0252] The regioselective modifying reagent of the present invention may be provided in a form of a composition further comprising other components. Examples of such other compounds include solutions and stabilizers (e.g., antioxidants and preservatives). Among solutions, aqueous solutions are preferred. Examples of aqueous solutions include water (e.g., distilled water, sterilized distilled water, purified water, and a physiological saline solution) and buffers (e.g., an aqueous phosphoric acid solution, a Tris-hydrochloric acid buffer, a carbonic acid-bicarbonic acid buffer, an aqueous boric acid solution, a glycine-sodium hydroxide buffer, and a citric acid buffer); buffers are preferred. The pH of solutions is e.g., 5.0 to 9.0 and preferably 5.5 to 8.5. The regioselective modifying reagent of the present invention can be provided in a liquid form or a powder form (e.g., freeze-dried powder).

[0253] The antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups or a salt thereof is useful as an intermediate in preparation of an antibody regioselectively having a functional substance or functional substances or a salt thereof, for example.

[0254] The antibody regioselectively having a functional substance or functional substances or a salt thereof is useful as pharmaceuticals or reagents (e.g., diagnostic reagents and reagents for research), for example, and particularly as pharmaceuticals. It is reported that when the number of bonds and the bond positions of a drug of an antibody drug conjugate (ADC) are changed, pharmacokinetics, a releasing rate of the drug, and effects change. Given these circumstances, next-generation ADCs are required to control the number and positions of a drug to be conjugated. It is believed that when the number and positions are constant, the problems of expected efficacy, variations in conjugate medicines, and lot difference, or what is called regulation, will be solved. The antibody or salt thereof of the present invention regioselectively having a functional substance or functional substances can solve such a problem of regulation. Consequently, the antibody or salt thereof of the present invention regioselectively having a functional substance or functional substances may be provided in the form of a pharmaceutical composition. Such a pharmaceutical composition may comprise a pharmaceutically allowable carrier in addition to the antibody or salt thereof regioselectively having a functional substance or functional substances. Examples of the

pharmaceutically allowable carrier include, but are not limited to, excipients such as sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate, and calcium carbonate; binders such as cellulose, methylcellulose, hydroxypropylcellulose, polypropylpyrrolidone, gelatin, gum arabic, polyethylene glycol, sucrose, and starch; disintegrators such as starch, carboxymethylcellulose, hydroxypropyl starch, sodium hydrogencarbonate, calcium phosphate, and calcium citrate; lubricants such as magnesium stearate, Aerosil, talc, sodium lauryl sulfate; aromatics such as citric acid, menthol, glycyl lysine ammonium salts, glycine, and orange powder; preservatives such as sodium benzoate, sodium hydrogen sulfite, methylparaben, and propylparaben; stabilizers such as citric acid, sodium citrate, and acetic acid, suspensions such as methylcellulose, polyvinylpyrrolidone, and aluminum stearate; dispersants such as surfactants; diluents such as water, a physiological saline solution, and orange juice; and base waxes such as cacao butter, polyethylene glycol, and refined kerosene. The antibody or salt thereof of the present invention regioselectively having a functional substance or functional substances may also have any modification (e.g., PEGylation) achieving stability.

[0255] Examples of preparations suitable for oral administration include liquid medicines dissolving an effective amount of a ligand in a diluted solution such as water, a physiological saline solution, or orange juice; capsules, sachets, and tablets comprising an effective amount of a ligand as a solid or granules; suspension medicines suspending an effective amount of an active ingredient in an appropriate dispersion medium; and emulsions dispersing a solution dissolving an effective amount of an active ingredient in an appropriate dispersion medium to be emulsified.

[0256] The pharmaceutical composition is suitable for nonoral administration (e.g., intravenous injection, hypodermic injection, intramuscular injection, local injection, and intraperitoneal administration). Examples of the pharmaceutical composition suitable for such nonoral administration include aqueous or nonaqueous, isotonic, aseptic injection medicines, which may comprise an antioxidant, a buffer, a bacteriostat, a tonicity agent, or the like. Examples thereof also include aqueous or nonaqueous, aseptic suspension medicines, which may comprise a suspension, a solubilizing agent, a thickener, a stabilizer, an antiseptic, or the like.

[0257] The dose of the pharmaceutical composition, which varies by the type and activity of an active ingredient, the severity of diseases, an animal type as a subject to be dosed, the drug receptivity, body weight, and age of a subject to be dosed, or the like, can be set as appropriate.

[0258] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

[0259] Example 1: Synthesis of IgG1 Fe Affinity Substance

(1-1) Synthesis of affinity peptide to antibody **[0895]** All the peptides illustrated below, which are affinity substances to an antibody, were prepared by similar methods. A compound having an N-terminal capped with an acetyl group (compounds 1, 2, and 32 to 53) and a compound having an N-terminal capped with 3-(triphenylmethylthio) propionic acid (compounds 3, 31, and 54) were prepared by solid phase peptide synthesis using a Rink amide resin by Fmoc method, and stirred for three hours in a solution of trifluoroacetic acid:water:triisopropylsilane:ethanedithiol=94:2.5:1.0:2.5, thereby performing cutting out from the resin and deprotection. The resin was removed by filtration, diethyl ether was added for precipitation, and the diethyl ether was removed by decantation to obtain a peptide as crude crystals. This peptide was purified by preparative HPLC to obtain an affinity peptide as a product.

[0260]

[Formula 24]



Compound 1

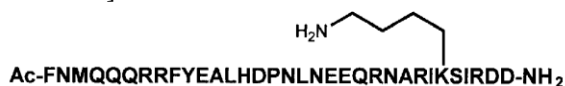
(Amino acid sequence of peptide portion: SEQ ID NO: 5)

[0261]

MS (ESI) m/z: z=3 1392[M+3H]³⁺, z=4 1044[M+ 4H]⁴⁺

[0262]

[Formula 25]



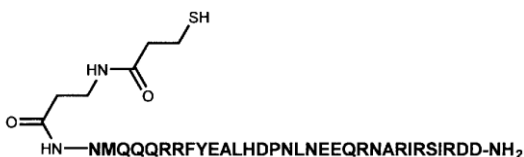
Compound 2

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0263]

MS (ESI) m/z: z=3 1392[M+3H]³⁺, z=4 1044[M+ 4H]⁴⁺

[Formula 26]



Compound 3

(Amino acid sequence of peptide portion: SEQ ID NO: 7)

[0265]

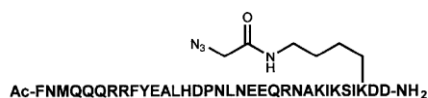
MS (ESI)m/z: z=3 1478 [M+3H]³⁺, z=4 1108 [M+4H]⁴⁺

[0266] (1-2) Synthesis of Affinity Peptide Azide Adduct to Antibody

The peptide-azide adduct (compounds 4 and 5) illustrated below was synthesized as follows. Using an amino acid in which only a Lys residue to be functionalized later with a mtt group, a compound having an N-terminal capped with an acetyl group was prepared by solid phase peptide synthesis with a Rink amide resin by Fmoc method. The product was stirred in a solution of dichloromethane trifluoroacetic acid:triisopropylsilane=90:5:5 for one hour to deprotect only the mtt group. The resin was washed with DMF, then dissolved in triethylamine (50 molar equivalents), azidoacetyl acid NHS ester (10 molar equivalents), and DMF 4 mL), and added. The resulting mixture was stirred for 16 hours. The solution was removed, and then the product was stirred in a solution of trifluoroacetic acid: water:triisopropylsilane=95:2.5:2.5 for one hour, thereby performing cutting out from the resin and deprotection. The resin was removed by filtration, diethyl ether was added for precipitation, and the diethyl ether was removed by decantation to obtain a peptide as crude crystals. This peptide was purified by preparative HPLC to obtain an affinity peptide azide adduct as a product.

[0267]

[Formula 27]



Compound 4

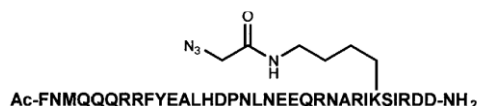
(Amino acid sequence of peptide portion: SEQ ID NO: 8)

[0268]

MS(ESI) m/z: z=3 1401[M+3H]³⁺, z=4 1051[M+ 4H]⁴⁺

[0269]

[Formula 28]



Compound 5

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0270] MS(ESI) m/z: z=3 1420[M+3H]³⁺, z=4 1065[M+ 4H]⁴⁺

[0271]

Example 2: Synthesis of Antibody-Modifying Linker and Coupling Thereof with IgG1 Fe-Affinity Peptide Azide Adduct

(2-1) Synthesis of Imidazolylcarbonyl Compound

(2-1-1) Synthesis of Imidazolylcarbonyl Compound (compound 6)



Compound 6

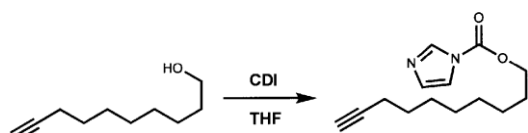
[0272] 3-Butyn-1-ol (0.100 g, 1.32 mmol) and carbonyl- d11imidazole (263 mg, 1.62 mmol) were dissolved in a THF solvent and stirred at room temperature for one hour. The reaction solution was diluted with ethyl acetate and was washed with water and brine. Thereafter, sodium sulfate was added thereto, and the resulting mixture was allowed to stand for five minutes. Sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a crude product, which was then purified by silica gel column chromatography. The fraction containing the product was collected and concentrated under reduced pressure to obtain 1H-imidazole-1-carboxylic acid- 3-butynyl ester (0.180 g, 1.10 mmol) corresponding to compound 6.

[0273] ¹H NMR (400 MHz, Chloroform-d) δ 2.08 (t, *I*=2.7Hz, 1H), 2.73 (td, *I*=6.6, 2.7 Hz, 2H), 4.54 (t, *I*=6.6 Hz, 2H), 7.11 (s, 1H), 7.43 (s, 1H), 8.18 (s, 1H).

[0274] MS(ESI) m/z: 165[M+Na]⁺

[0275] (2-1-2) Synthesis of Imidazolylcarbonyl Compound (compound 7)

[Formula 30]



Compound 7

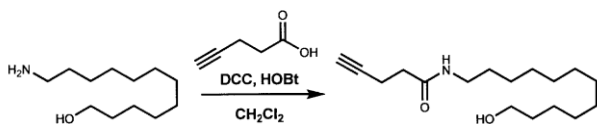
[0276] 9-Decyn-1-ol (0.100 g, 0.745 mmol) and carbonyl-diimidazole (148 mg, 0.916 mmol) were dissolved in a THF solvent and stirred at room temperature for one hour. The reaction solution was diluted with ethyl acetate and was washed with water and brine. Thereafter, sodium sulfate was added thereto, and the resulting mixture was allowed to stand for five minutes. Sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a crude product, which was then purified by silica gel column chromatography. The fraction containing the product was collected and concentrated under reduced pressure to obtain 1H-imidazole-1-carboxylic acid-9-butynyl ester (0.152 g, 0.612 mmol) corresponding to compound 7.

[0277] ¹H NMR (400 MHz, Chloroform-d) δ 1.33-1.50 (m, 10H), 1.82 (m, 2H), 1.96 (t, $J=2.6$ Hz, 1H) 2.21 (td, $J=6.8, 2.6$ Hz, 2H), 4.43 (t, $J=6.8$ Hz, 2H) 7.10 (s, 1H), 7.45 (brs, 1H), 8.16 (s, 1H).

[0278] MS(ESI) m/z : 271[M+Na]

[0279] (2-1-3) Synthesis of Imidazolylcarbonyl Compound (compound 9)

[Formula 31]



Compound 8

[0280] N,N'-dicyclohexylcarbodiimide (113 mg, 0.546 mmol), 1-hydroxybenzotriazole monohydrate (78.1 mg, 0.546 mmol), and 4-pentynoic acid (53.6 mg, 0.546 mmol) were dissolved in a dichloromethane solvent, and stirred at room temperature for one. Thereafter, 12-amino-dodecanol (0.100 g, 0.497 mmol) was added thereto, and the resulting mixture was stirred at room temperature for four hours. The reaction solution was diluted with ethyl acetate and was washed with water and brine. Thereafter, sodium sulfate was added thereto, and the resulting mixture was allowed to stand for five minutes. Sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain 12-(pent-4-yn-1-oxo) aminododecan-1-ol (0.102 g, 0.363 mmol) corresponding to compound 8.

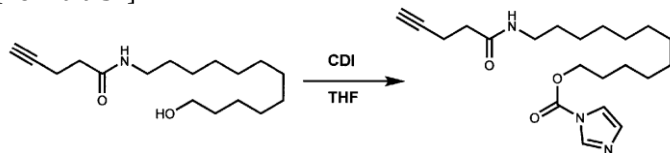
[0281]

MS(ESI) m/z : 281[M+H]⁺

[0282]

[Formula32]

[Formula 32]



Compound 9

[0283] 12-(Pent-4-ynamido) dodecan-1-ol (25.0 mg, 0.089 mmol) and carbonyldiimidazole (29.1 mg, 0.178 mmol) were dissolved in a THF solvent and stirred at room temperature for one hour. The reaction solution was diluted with ethyl acetate and was washed with water and brine. Thereafter, sodium sulfate was added thereto, and the resulting mixture was allowed to stand for five minutes. Sodium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a crude product, which was then purified by silica gel column chromatography. The fraction containing the product was collected and concentrated under reduced pressure to obtain 1H-imidazole-1-carboxylic acid-12-(pent-4-yn-1-oxo) aminododecanyl ester (12.0 mg, 0.032 mmol) corresponding to compound 9.

[0284] ¹H NMR (400 MHz, Chloroform-d) δ 1.23-1.50 (m, 20H), 1.81 (t, J=2.6 Hz, 1H), 2.36 (m, 2H), 2.53 (td, J=6.8, 2.6 Hz, 2H), 3.23 (t, J=6.8 Hz, 2H), 4.41 (t, J=6.8 Hz, 2H), 5.64 (brs, 1H), 7.07 (s, 1H), 7.43 (brs, 1H), 8.13 (s, 1H).

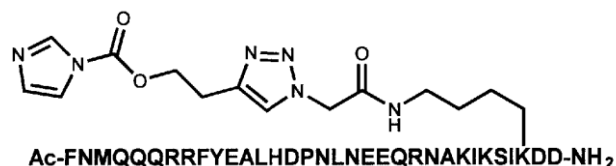
[0285] MS(ESI) m/z: 398[M+Na]⁺

[0286] (2-2) Synthesis of Imidazolylcarbonyl Compound and Coupling Thereof with Affinity Peptide Azide Compound

The affinity peptide azide compound (compound 4) synthesized in Example 1 was coupled with the imidazolyl-carbonyl compounds (compounds 6, 7, and 9) synthesized in Example 2 by similar methods. The peptide azide compound was dissolved in 100 mM phosphate buffer (pH 7.0), and aminoguanidine hydrochloride (20 molar equivalents), sodium ascorbate (20 molar equivalents), and 100 mg/mL imidazolylcarbonyl compound (3 molar equivalents) in dimethyl sulfoxide solution were added thereto. A separately prepared aqueous solution of copper sulfate monohydrate (4 molar equivalents) and tris(3-hydroxypropyl)triazolylmethylamine (20 molar equivalents) was added to this reaction mixture, and the resulting mixture was stirred. The reaction was monitored by LC-MS, and disappearance of the raw materials was determined. Thereafter, the product was concentrated and diluted with water repeatedly four times by ultrafiltration (Amicon Ultra, 3K MWCO), and the resulting aqueous solution was freeze-dried to obtain a peptide-imidazolylcarbonyl compound (compounds 10, 11, and 12) illustrated below.

[0287]

[Formula 33]



Compound 10

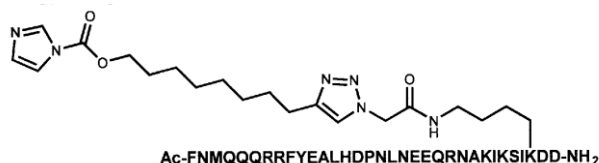
(Amino acid sequence of peptide portion: SEQ ID NO: 8)

[0288]

MS (ESI) m/z : $z=3$ 1455 $[M+3H]^3+$, $z=4$ 1092 $[M+4H]^4+$

[0289]

[Formula 34]



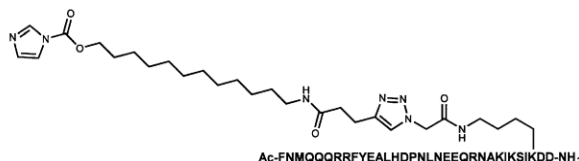
Compound 11

(Amino acid sequence of peptide portion: SEQ ID NO: 8)

[0290] MS (ESI) m/z : $z=3$ 1483 $[M+3H]^3+$, $z=4$ 1113 $[M+4H]^4+$

[0291]

[Formula 35]



Compound 12

(Amino acid sequence of peptide portion: SEQ ID NO: 8)

[0292] MS (ESI) m/z : $z=3$ 1525 $[M+3H]^3+$, $z=4$ 1144 $[M+4H]^4+$

[0293]

[Reference Example 1: Verification of Difference in Reactivity Due to Difference in Number of Atoms from Affinity Peptide to Antibody-Modifying Group (Electrophilic Group) by Model Experiment]

(1-1) Synthesis of IgG Antibody Trastuzumab-Peptide Conjugate

20 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co. Ltd.) was dissolved in 2.0 μ L of 100 mM HEPES buffer (pH 7.2), 20 molar equivalents of the peptide-imidazolylcarbonyl compound (compounds 10, 11, and 12) synthesized in Example 2 (2-2) was added to the antibody, and the resulting mixture was stirred at 37° C. for four hours. The reaction solution was subjected to water substitution by ultrafiltration (Amicon Ultra, 3K MWCO) to remove the peptide reagent, and the reaction was thereby stopped, thus obtaining an IgG antibody trastuzumab-peptide conjugate.

[0294] (1-2) Analysis of IgG Antibody Trastuzumab-Peptide Conjugate by SDS-PAGE

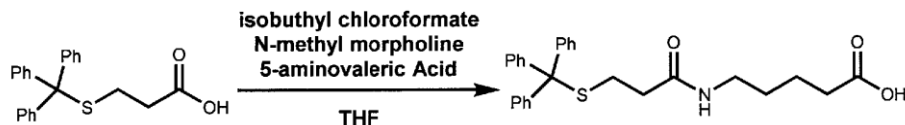
The three types of IgG antibody trastuzumab-peptide conjugates obtained in (1-1) using three types of peptide-imidazolylcarbonyl compounds (compounds 10, 11, and 12) were subjected to SDS-PAGE (Mini-PROTEAN TGX gel, 4-20%, Bio-RAD; under reduction conditions; stained with Coomassie Brilliant Blue G-250 Stain). A result thereof is illustrated in FIG. 2. From this result, it was found that the modification reaction of the antibody progressed when compounds 10 and 11 were used, and did not progress when compound 12 was used.

[0295] Example 3: Synthesis of Antibody Affinity Reagent Loaded with Maleimide

(3-1) Synthesis of protected thiol compound

(3-1-1) Synthesis of Protected Thiol Compound (compound 14)

[Formula 36]



Compound 13

[0296] 3-(Triphenylmethylthio) propionic acid (100 mg, 0.287 mmol) was dissolved in THF, isobutyl chlorocarbonate (42.7 μ L, 0.316 mmol) and N-methylmorpholine (69.4 μ L, 0.631 mmol) were added thereto at 0° C., and the resulting mixture was stirred for 30 minutes to prepare a corresponding mixed anhydride. 5-Aminovaleric acid (33.6 mg, 0.287 mmol) was dissolved in a 1 M aqueous sodium hydroxide solution at room temperature, and then the THF solution of the mixed anhydride described above was added dropwise thereto at room temperature. The resulting mixture

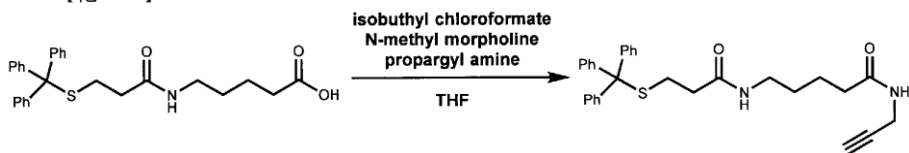
was stirred for 16 hours at room temperature. Thereafter, the reaction solution was washed with water and ethyl acetate, and an aqueous phase was collected. A 6 M aqueous hydrochloric acid solution was added to the aqueous phase to adjust the pH within the system to 3.0, then separatory extraction with ethyl acetate was performed, then an organic phase was washed with brine, and then magnesium sulfate anhydride was added thereto, and allowed to stand for five minutes. Magnesium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a crude product, which was then purified by silica gel column chromatography. The fraction containing the product was collected and concentrated under reduced pressure to obtain 5-[3-(triphenylmethyl) sulfanyl-propyl-1-oxo] amino-hexanoic acid (110 mg, 0.246 mmol) corresponding to compound 13.

[0297] ^1H NMR (400 MHz, Chloroform- d) δ 1.66 (m, 3H), 2.03 (t, $J=7.3$ Hz, 2H), 2.39 (t, $J=7.3$ Hz, 2H), 2.52 (t, $J=7.0$ Hz, 2H), 3.22 (q, $J=6.6$ Hz, 2H), 5.37 (s, 1H), 7.19-7.36 (m, 9H), 7.40-7.49 (m, 6H).

[0298] MS(ESI) m/z : 470[M+Na]

[0299]

[Formula 37]



Compound 14

[0300] 5-(3-Triphenylmethyl-propyl-1-oxo) amino- hexanoic acid (20.2 mg, 0.045 mmol) was dissolved in THF, isobutyl chlorocarbonate (6.70 μ L, 0.050 mmol) and N-methylmorpholine (10.9 μ L, 0.0991 mmol) were added thereto at 0° C., and the resulting mixture was stirred for 30 minutes to prepare a corresponding mixed anhydride. Prop- argylamine (16.6 μ L, 0.226 mmol) was dissolved in a 1 M aqueous sodium hydroxide solution at room temperature, and then the THF solution of the mixed anhydride described above was added dropwise thereto at room temperature. The resulting mixture was stirred for 16 hours at room temperature. Thereafter, the reaction solution was washed with water and ethyl acetate, and an aqueous phase was collected. A 6 M aqueous hydrochloric acid solution was added to the aqueous phase to adjust the pH within the system to 3.0, then separatory

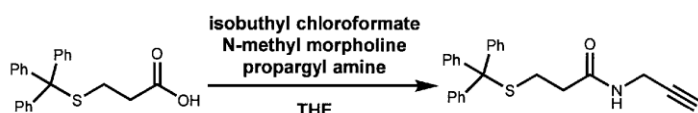
extraction with ethyl acetate was performed, then an organic phase was washed with brine, and then magnesium sulfate anhydride was added thereto, and allowed to stand for five minutes. Magnesium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain a crude product, which was then purified by silica gel column chromatography. The fraction containing the product was collected and concentrated under reduced pressure to obtain N-propynyl-5-(3-triphenylmethylsulfanyl-propyl-1-oxo) amino-hexanamide (20.0 mg, 0.041 mmol) corresponding to compound 14.

[0301] ^1H NMR (400 MHz, Chloroform-d) δ 1.52 (dt, $J=8.2, 6.5$ Hz, 2H), 1.59-1.72 (m, 2H), 2.02-2.09 (m, 2H), 2.17-2.28 (m, 2H) 2.52 (t, $J=7.2$ Hz, 2H), 3.21 (brs, 2H), 4.01 (dd, $J=5.3, 2.6$ Hz, 2H), 6.04 (s, 1H), 7.19-7.35 (m, 9H), 7.40-7.49 (m, 6H).

[0302] MS(ESI) m/z : 507[M+H] $^+$

[0303] (3-1-2) Synthesis of Protected Thiol Compound (Compound 15)

[Formula 38]



Compound 15

[0304] 3-(Triphenylmethylthio) propionic acid (100 mg, 0.287 mmol) was dissolved in THF, isobutyl chlorocarbonate (42.7 μL , 0.316 mmol) and N-methylmorpholine (69.4 μL , 0.631 mmol) were added thereto at 0° C., and the resulting mixture was stirred for 30 minutes to prepare a corresponding mixed anhydride. Propargylamine (105 μL , 1.43 mmol) was dissolved in a 1 M aqueous sodium hydroxide solution at room temperature, and then the THF solution of the mixed anhydride described above was added dropwise thereto at room temperature. The resulting mixture was stirred for 16 hours at room temperature. Thereafter, the reaction solution was washed with water and ethyl acetate, and an aqueous phase was collected. A 6 M aqueous hydrochloric acid solution was added to the aqueous phase to adjust the pH within the system to 3.0, then separatory extraction with ethyl acetate was performed, then an organic phase was washed with brine, and then magnesium sulfate anhydride was added thereto, and allowed to stand for five minutes. Magnesium sulfate was removed by filtration, and the resulting solution was concentrated under reduced pressure to obtain N-propynyl-3-triphenylmethylsulfanyl-propanamide (110 mg, 0.286 mmol) corresponding to compound 15.

[0305] ^1H NMR (400 MHz, Chloroform-d) δ 2.01 (t, $J=7.1$ Hz, 2H), 2.24 (t, $J=2.8$ Hz, 1H), 2.53 (t, $J=7.6$ Hz, 2H), 3.99

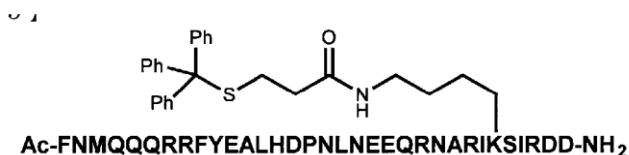
(m, 2H), 5.40 (brs, 1H), 7.19-7.35 (m, 9H), 7.40-7.49 (m, 6H).

[0306] MS(ESI) m/z : 408[M+Na] $^+$

[0307] (3-2) Coupling of Affinity Peptide with Protected Thiol Compound

(3-2-1) Synthesis of Peptide-Protected Thiol Coupled Compound (Compound 16)

[Formula 39]



Compound 16

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0308] Compound 5 synthesized in Example 1 was dissolved in N,N'-dimethylformamide. 3-(Triphenylmethyl-thio) propionic acid (5 molar equivalents), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (15 molar equivalents), and 1-hydroxybenzotriazole (15 molar equivalents) were dissolved in N,N'-dimethylformamide and added to the system. The resulting mixture was stirred at room temperature for 12 hours and then purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining a peptide-protected thiol coupled compound (compound 16) as a product.

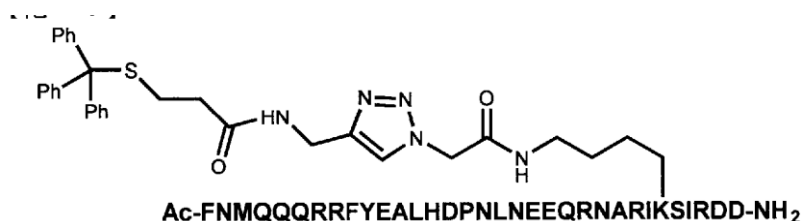
[0309] MS (ESI) m/z : $z=3$ 1502[M+3H]³⁺, $z=4$ 1125[M+4H]⁴⁺

[0310] (3-2-2) Synthesis of Peptide-Protected Thiol Coupled Compound (Compounds 17 and 18)

The affinity peptide azide compound (compound 5) synthesized in Example 1 was coupled with the protected thiol compounds (compounds 17 and 18) synthesized in (4-1-1) and (4-1-2) by similar methods. The peptide azide compound was dissolved in 100 mM phosphate buffer (pH 7.0), and aminoguanidine hydrochloride (20 molar equivalents), sodium ascorbate (20 molar equivalents), and 100 mg/mL protected thiol compound (3 molar equivalents) dimethyl sulfoxide solution were added thereto. A separately prepared aqueous solution of copper sulfate monohydrate (4 molar equivalents) and tris(3-hydroxypropyltriazolylmethyl) amine (20 molar equivalents) was added to this reaction mixture, and the resulting mixture was stirred. The reaction was monitored by LC-MS, and disappearance of the raw materials was determined. Thereafter, the product was concentrated and diluted with water repeatedly four times by ultrafiltration (Amicon Ultra-4, 3K MWCO), and the resulting aqueous solution was freeze-dried to obtain a peptide-protected thiol coupled compound (compounds 17 and 18).

[0311]

[Formula 40]



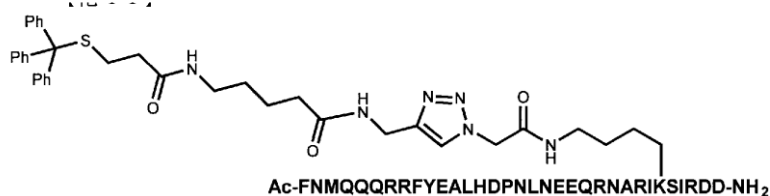
Compound 17

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0312] MS(ESI) m/z : $z=3$ 1548[M+3H]³⁺, $z=4$ 1161[M+4H]⁴⁺

[0313]

[Formula 41]



Compound 18

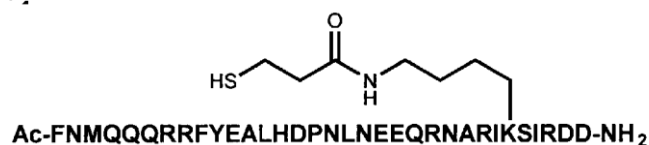
(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0314] MS(ESI) m/z : $z=3$ 1581[M+3H]³⁺, $z=4$ 1186[M+4H]⁴⁺

[0315] (3-3) Synthesis of Peptide-Protected Thiol Coupled Compound (Compounds 19, 20, and 21)
The affinity peptide-protected thiol coupled compound (compounds 16, 17, and 18) synthesized in (3-2) was stirred with a trifluoroacetic acid:dichloromethane=1:1 solution for one hour to remove a triphenylmethyl group. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining a thiol-peptide coupled compound (compounds 19, 20, and 21) as a product.

[0316]

[Formula 42]



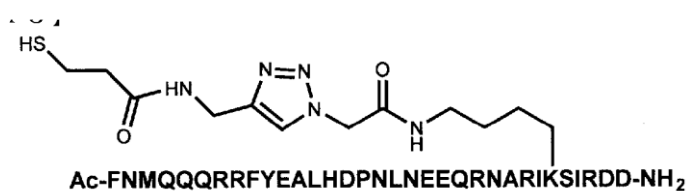
Compound 19

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0317] MS(ESI) m/z : $z=3$ 1454[M+3H]³⁺, $z=4$ 1091[M+4H]⁴⁺

[0318]

[Formula 43]



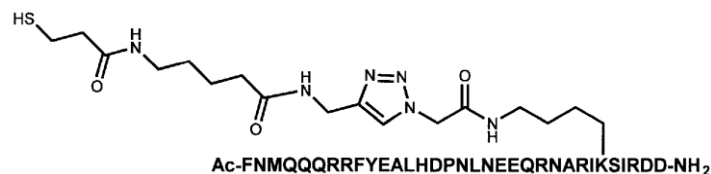
Compound 20

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0319] MS(ESI) m/z : $z=3$ 1468[M+3H]³⁺, $z=4$ 1111[M+4H]⁴⁺

[0320]

[Formula 44]



Compound 21

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

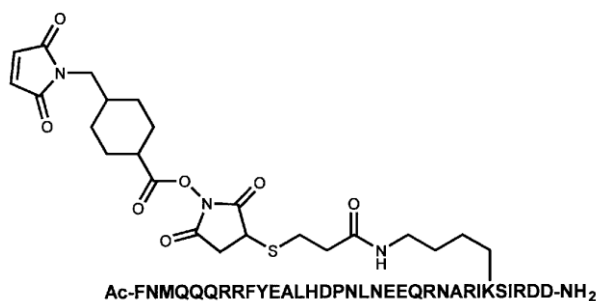
[0321] m/z: z=3 1501[M+3H]³⁺, z=4 1126[M+4H]⁴⁺

[0322] (3-4) Synthesis of Antibody Affinity Peptide Reagent Loaded with Maleimide (1)

The thiol-peptide coupled compound (compounds 19, 20, and 21) synthesized in (3-3) was dissolved in dimethyl sulfoxide, a dimethyl sulfoxide solution of N-hydroxymaleimide (20 molar equivalent) was added thereto, and the resulting mixture was stirred for one hour. Disappearance of the raw materials was determined by LC-MS. Thereafter, trans-4-[(2,5-dihydro-1H-pyrroli-1-yl) methyl] cyclohexanecarboxylic acid (40 molar equivalents), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (30 molar equivalents), and 1-hydroxybenzotriazole (30 molar equivalents) were added thereto, and the resulting mixture was stirred for four hours. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining a maleimide-affinity peptide reagent (compounds 22, 23, and 24) as a product.

[0323]

[Formula 45]



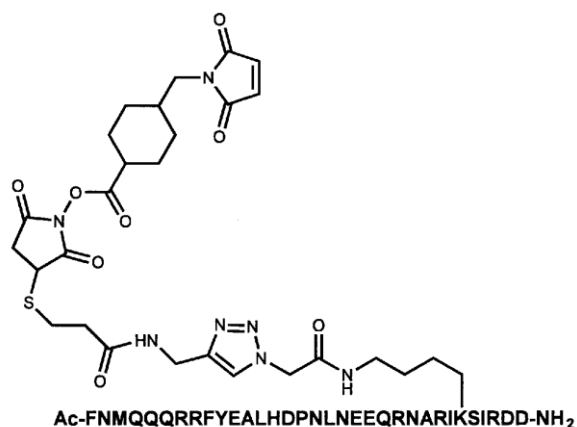
Compound 22

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0324] MS (ESI) m/z: z=3 1532 [M+3H]³⁺, z=4 1150 [M+4H]⁴⁺

[0325]

[Formula 46]



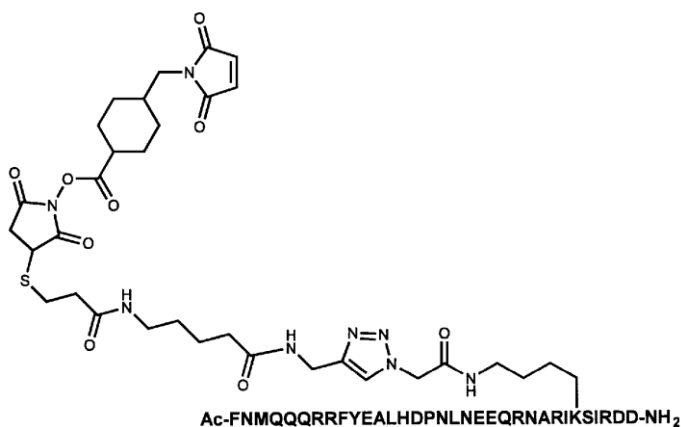
Compound 23

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0324] MS (ESI) m/z: z=3 1578 [M+3H]³⁺, z=4 1184 [M+4H]⁴⁺

[0327]

[Formula 47]



Compound 24

(Amino acid sequence of peptide portion: SEQ ID NO: 6)

[0328]

MS (ESI) m/z: z=3 1611 [M+3H]³⁺, z=4 1208 [M+4H]⁴⁺

[0329] Example 4: Introduction of Regioselective Maleimide to Anti-HER2 IgG Antibody Trastuzumab and Analysis Thereof

(4-1) Synthesis of Peptide Reagent Having Thiol

Compound 25 below was synthesized by solid phase peptide synthesis using 2-chlorotrityl resin by Fmoc method. After the solid phase synthesis, the product was stirred in a solution of trifluoroacetic acid:water:triisopropylsilane=95:2.5:2.5 for one hour, thereby performing cutting out from the resin and deprotection. The resin was removed by filtration, diethyl ether was added for precipitation, and the diethyl ether was removed by decantation to obtain a peptide as crude crystals. This peptide

was purified by preparative HPLC to obtain compound 25 below as a product. Compound 25 is not an affinity peptide but a model peptide compound instead of a medicine, which should be introduced via a maleimide group to an antibody regioselectively having maleimide, which is a bioorthogonal functional group.

[0330]

[Formula 48]



Compound 25

(Amino acid sequence of peptide portion: SEQ ID NO: 9)

[0331] MS (ESI) m/z : $z=2$ 1482[M+2H]²⁺, $z=3$ 988[M+3H]³⁺

[0332] (4-2) Regiospecific Modification of IgG Antibody Trastuzumab with Maleimide-Affinity Peptide Reagent

The maleimide-affinity peptide reagent (compound 22,23,24) synthesized in (4-4) of Example 4 was dissolved in N,N'-dimethylformamide to be 2.18 mM. 20.0 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co., Ltd.) was dissolved in 14.8 μ L of 50 mM HEPES buffer (pH 7.2), the peptide reagent (10 to 40 molar equivalents) was added to the antibody, and the resulting mixture was stirred at 37° C. for four hours. The reaction solution was subjected to solvent substitution to a 20 mM acetate buffer (pH 4.5) by ultrafiltration (Amicon Ultra, 10K MWCO). By repeating the same operation three times, the affinity peptide reagent was removed to obtain maleimide-modified IgG antibody trastuzumab.

[0333] (4-3) Conjugation of Maleimide-Modified IgG Antibody Trastuzumab with Peptide Reagent Having Thiol

The maleimide-modified IgG antibody trastuzumab synthesized in (5-2) of Example 5 was subjected to solvent substitution to a 50 mM HEPES buffer (pH 7.2) by ultrafiltration (Amicon Ultra, 10K MWCO) to return the pH in the system to neutrality. An aqueous solution of compound 25 adjusted to 10 mg/mL (80 molar equivalents to the antibody) was added thereto, and the resulting mixture was stirred for 16 hours. The reaction solution was subjected to water substitution by ultrafiltration (Amicon Ultra, 10K MWCO) to remove the peptide reagent, and the reaction was thereby stopped, thus obtaining an IgG antibody trastuzumab-peptide conjugate.

[0334] (4-4) Analysis of IgG Antibody Trastuzumab-Peptide Conjugate by SDS-PAGE

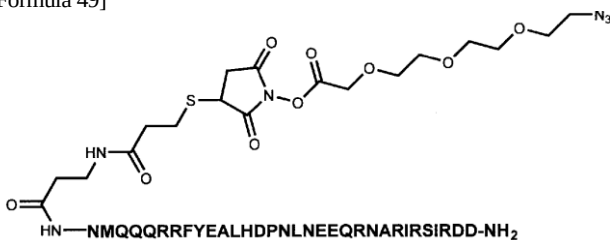
The IgG antibody trastuzumab-peptide conjugate obtained in (4-3) was subjected to SDS-PAGE (Mini-PROTEAN TGX gel, 4 to 20%, Bio-RAD; under reduction conditions; stained with Coomassie Brilliant Blue G-250 Stain). A result thereof is illustrated in FIG. 3. From this result, it was found that the modification reaction of the antibody progressed when compounds 22 and 23 were used, and did not progress when compound 24 was used in the maleimide regioselective introduction reaction (5-2).

(4-5) Determination of Heavy Chain Selectivity of Maleimide-Modified IgG Antibody Trastuzumab Under Reduction Conditions by ESI-TOFMS Analysis

[0335] Example 5: Synthesis of Antibody-Modifying Reagent Loaded with Azide

(5-1) Synthesis of Antibody-Modifying Reagent (Comparative Example Compound) Having Hetero Atom at beta-Position of Antibody-Modifying Group (Electrophilic Group)

[Formula 49]



Compound 26

(Amino acid sequence of peptide portion: SEQ ID NO: 7)

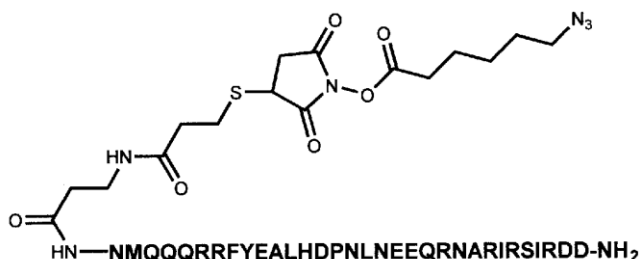
[0336] The antibody affinity peptide (compound 2) synthesized in Example 1 was dissolved in dimethyl sulfoxide, a dimethyl sulfoxide solution of N-hydroxymaleimide (20 molar equivalents) was added thereto, and the resulting mixture was stirred for one hour. Disappearance of the raw materials was determined by LC-MS. Thereafter, 11-azido- 3,6,9-trioxaundecanoic acid (40 molar equivalents), 1-ethyl- 3-(3-dimethylaminopropyl) carbodiimide hydrochloride (32 molar equivalents), and 1-hydroxybenzotriazole (32 molar equivalents) were added thereto, and the resulting mixture was stirred for two hours. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining an azide-PEG-affinity peptide reagent (compound 26) as a product.

[0337] MS (ESI) m/z : $z=3$ 1501 $[M+3H]^3+$, $z=4$ 1125 $[M+4H]^4+$

[0338] (5-2) Synthesis of Antibody-Modifying Reagent Having Carbon Atom or Hetero Atom at beta-Position of Antibody-Modifying Group (Electrophilic Group)

(5-2-1) Synthesis of Antibody-Modifying Reagent (Compounds 27, 59, and 60) by Coupling of Azide Reagent with Affinity Peptide

[Formula 50]



Compound 27

(Amino acid sequence of peptide portion: SEQ ID NO: 7)

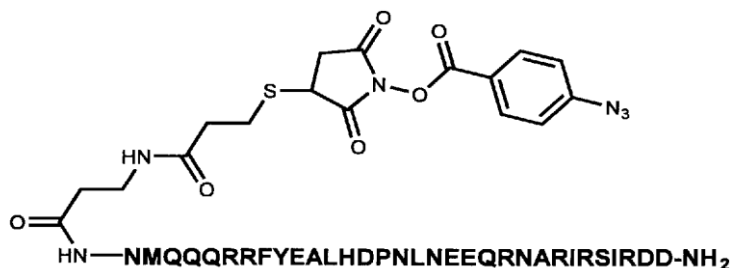
[0339] The antibody affinity peptide (compound 2) synthesized in Example 1 was dissolved in dimethyl sulfoxide, a dimethyl sulfoxide solution of N-hydroxymaleimide (20 molar equivalents) was added thereto, and the resulting mixture was stirred for one hour. Disappearance of the raw materials was determined by LC-MS. Thereafter, 11-azido- 3,6,9-trioxaundecanoic acid (40 molar equivalents), 1-ethyl- 3-(3-dimethylaminopropyl) carbodiimide hydrochloride (32 molar equivalents), and 1-hydroxybenzotriazole (32 molar equivalents) were added thereto, and the resulting mixture was stirred for two hours. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining an azide-PEG-affinity peptide reagent (compound 27) as a product.

[0340]

MS (ESI) m/z : $z=3$ 1501 $[M+3H]^3+$, $z=4$ 1125 $[M+4H]^4+$

[0341] (5-2-2) Synthesis of Antibody-Modifying Reagent (Compounds 28 and 61) by Coupling of Azide Reagent with Affinity Peptide

[Formula 51]



Compound 28

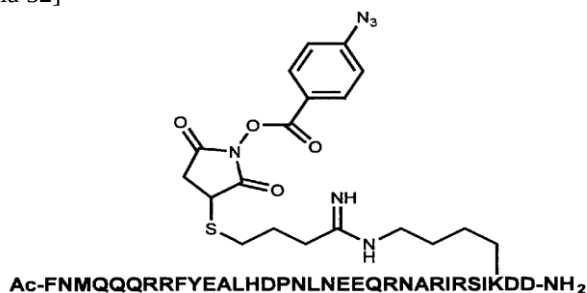
(Amino acid sequence of peptide portion: SEQ ID NO: 7)

[0342] The antibody affinity peptide (compound 3) synthesized in Example 1 was dissolved in dimethyl sulfoxide, a dimethyl sulfoxide solution of N-hydroxymaleimide (20 molar equivalents) was added thereto, and the resulting mixture was stirred for one hour. Disappearance of the raw materials was determined by LC-MS. Thereafter, 4-azido-benzoic acid (40 molar equivalents), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (32 molar equivalents), and 1-hydroxybenzotriazole (32 molar equivalents) were added thereto, and the resulting mixture was stirred for two hours. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining an azide-phenyl-affinity peptide reagent (compound 28) as a product.

[0343] MS (ESI) m/z : $z=3$ 1478[M+3H]³, $z=4$ 1108[M+4H]⁴

[0344] (5-2-3) Synthesis of Antibody-Modifying Reagent (Compounds 29 and 62 to 83) by Coupling of Azide Reagent with Affinity Peptide

[Formula 52]



Compound 29

(Amino acid sequence of peptide portion: SEQ ID NO: 5)

[0345] The antibody affinity peptide (compound 3) synthesized in Example 1 was dissolved in dimethylsulfoxide, 2-iminothiolane hydrochloride (10 molar equivalents) and diisopropylethylamine (15 molar equivalents) were added thereto, and the resulting mixture was stirred for one hour. Disappearance of the raw materials was determined by LC-MS. Thereafter, a dimethyl sulfoxide solution of N-hydroxymaleimide (20 molar equivalent) was added thereto, and the resulting mixture was stirred for one hour. The progress of the reaction was determined by LC-MS. Thereafter, 4-azidobenzoic acid (40 molar equivalents), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (32 molar equivalents), and 1-

hydroxybenzotriazole (32 molar equivalents) were added thereto, and the resulting mixture was stirred for two hours. The completion of the reaction was determined by LC-MS, and then the product was purified by preparative HPLC. A fraction containing a target product was determined by ESI-MS, followed by freeze-drying, thus obtaining an azide-phenyl-affinity peptide reagent (compound 29) as a product.

[0346] MS (ESI) m/z : $z=3$ 1512 $[M+3H]^3+$, $z=4$ 1134 $[M+4H]^4+$

[0347] Example 6: Regiospecific Modification of IgG1 Fe with IgG1 Fe Affinity Peptide Reagent and Analysis by ESI-TOFMS

(6-1) Antibody Modification Reaction with Antibody-Modifying Reagent Having Carbon Atom at Beta Position of Antibody-Modifying Group (Electrophilic Group)

(6-1-1) Antibody Modification Reaction Using Affinity Peptide Reagent (Compound 27)

loaded with azide (compound 27) synthesized in Example 5 was dissolved in N,N'-dimethylformamide to be 0.22 mM. 200 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co., Ltd.) was dissolved in 1200 μ L of 50 mM HEPES buffer (pH 7.2), 20 molar equivalents of 0.22 mM peptide reagent was added to the antibody, and the resulting mixture was stirred at 37°C. for 16 hours. 50 μ L of a 10 mg/mL aqueous Lys solution was added to the reaction solution, and the resulting mixture was stirred for 30 minutes to stop the reaction. The reaction mixture was purified with Protein A (Aspire Protein A Tips, Thermo), and the eluate was substituted with 9.57 mM PBS buffer (pH 7.0) and concentrated by ultrafiltration (Amicon Ultra, !OK MWCO) to obtain azide-modified IgG antibody trastuzumab.

[0348] (6-1-2) Antibody Modification Reaction Using Affinity Peptide Reagent (compounds 28 and 61) (azide-modified antibody 3, 4)

The affinity peptide reagent loaded with azide (compound 28) synthesized in Example 5 was dissolved in N,N'-dimethylformamide to be 0.22 mM. 200 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co., Ltd.) was dissolved in 1200 μ L of 50 mM HEPES buffer (pH 7.2), 20 molar equivalents of 0.22 mM peptide reagent was added to the antibody, and the resulting mixture was stirred at 37°C. for 16 hours. 50 μ L of a 10 mg/mL aqueous Lys solution was added to the reaction solution, and the resulting mixture was stirred for 30 minutes to stop the reaction. The reaction mixture was purified with Protein A (Aspire Protein A Tips, Thermo), and the eluate was substituted with 9.57 mM PBS buffer (pH 7.0) and concentrated.

[0349] (6-1-3) Antibody Modification Reaction Using Affinity Peptide Reagent (Compound 29)

The affinity peptide reagent loaded with azide (compound 29) synthesized in Example 5 was dissolved in N,N'-dimethylformamide to be 0.22 mM. 200 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co., Ltd.) was dissolved in 1200 μ L of 50 mM HEPES buffer (pH 7.2), 20 molar equivalents of 0.22 mM peptide reagent was added to the antibody, and the resulting mixture was stirred at 37°C. for 16 hours. 50 μ L of a 10 mg/mL aqueous Lys solution was added to the reaction solution, and the resulting mixture was stirred for 30 minutes to stop the reaction. The reaction mixture was purified with Protein A (Aspire Protein A Tips, Thermo), and the eluate was substituted with 9.57 mM PBS buffer (pH 7.0) and concentrated by ultrafiltration (Amicon Ultra, !OK MWCO) to obtain azide-modified IgG antibody trastuzumab.

[0350] (6-2) Determination of Heavy Chain Selectivity of Azide-Modified IgG Antibody Trastuzumab Under Reduction Conditions by ESI-TOFMS Analysis

(6-2-1) Determination of heavy chain selectivity of azide-modified trastuzumab under reduction conditions by ESI-TOFMS analysis when compound 27 is used in modification reaction

To the azide-modified trastuzumab (azide-modified antibody 1) synthesized in (6-1-2) of Example 6, 2.0 μ L of a 7 mM tris(2-carboxyethyl) phosphine hydrochloride solution (100 equivalents to the antibody) was added, and the resulting mixture was allowed to stand at room temperature for 20 minutes. The mass was measured by ESI-TOFMS; for the raw material trastuzumab used as a control, heavy chain peaks were observed at 50595 and 50757, and a light chain peak was observed at 23440. For a reaction product, peaks were observed at 50714 and 50876 with alkyl azide introduced to the heavy chain, and a light chain peak was observed at 23440, the same as that of the raw material. FIG. 5 illustrates a result of ESI-TOFMS analysis.

[0352] (6-2-3) Determination of Heavy Chain Selectivity of Azide-Modified Trastuzumab Under Reduction Conditions by ESI-TOFMS Analysis when Compound 29 is Used in Modification Reaction

To the azide-modified trastuzumab (azide-modified antibody 5) synthesized in (6-1-3) of Example 6, 2.0 μ L of a 7 mM tris(2-carboxyethyl) phosphine hydrochloride solution (100 equivalents to the antibody) was added, and the resulting mixture was allowed to stand at room temperature for 20 minutes. The mass was measured by ESI-TOFMS; for the raw material trastuzumab used as a control, heavy chain peaks were observed at 50595 and 50757, and a light chain peak was observed at 23440. For a reaction product, peaks were observed at 50714 and 50876 with aminebenzoic acid introduced to the heavy chain, and a light chain peak was observed at 23440, the same as that of the raw material. Note that the azidobenzoic acid-introduced compound was reduced to an aminebenzoic acid-introduced compound by the pretreatment for the measurement.

[0353]

Comparative Example 1: Modification Reaction of IgG1 Fe with Peptide Reagent Having Hetero Atom at α -Position and Analysis by ESI-TOFMS

The peptide reagent having a heteroatom at the β -position (compound 26) synthesized in Example 5 was dissolved in N,N'-dimethylformamide to be 0.22 mM. 200 μ g of anti-HER2 IgG antibody trastuzumab (Chugai Pharmaceutical Co., Ltd.) was dissolved in 1200 μ L of 50 mM HEPES buffer (pH 7.2), 120 μ L of 0.22 mM peptide reagent (20 molar equivalents to the antibody) was added, and the resulting mixture was stirred at 37 $^{\circ}$ C. for 16 hours. 50 μ L of a 10 mg/mL aqueous Lys solution was added to the reaction solution, and the resulting mixture was stirred for 30 minutes to stop the reaction. This solution was subjected to solvent substitution to a PBS solution by ultrafiltration (Amicon Ultra, Wako Pure Chemical Industries, Ltd.). Thereafter, 2.0 μ L of 7 mM determined that an antibody modification reaction with compound 26 having a hetero atom at a α -position of an antibody-modifying group (electrophilic group) had not progressed.

[0354] From this result, it was found that the modification reaction of the antibody progressed when compounds 27, 28, and 29 were used, and did not progress when compound 26 was used in the azide regioselective introduction reaction (6-2).

[0355]**Example 7: Conjugation of Regiospecific Azide-Introduced Trastuzumab with Arbitrary Compound and ESI-TOFMS****Analysis of Product****(7-1) Conjugation of Regiospecific Azide-Introduced Trastuzumab with Cy3-Cycloalkyne****(7-1-1) Conjugation of Regiospecific Azide-Introduced Trastuzumab with Cy3-DBCO and ESI-TOFMS Analysis**

The azidobenzoic acid-introduced trastuzumab synthesized in (6-1-2) of Example 6 was dissolved in 20 mM PBS buffer to be 0.3 mM, then 20 μ L of dibenzocyclooctyne- Cy3 manufactured by Sigma Aldrich (0.94 mM, dissolved in DMF) was added thereto, and the resulting mixture was stirred at room temperature for 12 hours. The reaction solution was subjected to water substitution by ultrafiltration (Amicon Ultra, 10K MWCO) to stop the reaction. The mass was measured by ESI-TOFMS; for the trastuzumab, a peak was observed at 148060, for the azide-introduced trastuzumab synthesized in (6-1-2), a peak was observed at 148489, and for a reaction product, a peak was observed at 150487 with two molecules of Cy3 introduced (FIG. 6).

[0356]**(7-1-2) ESI-TOFMS Analysis Under Reduction Conditions**

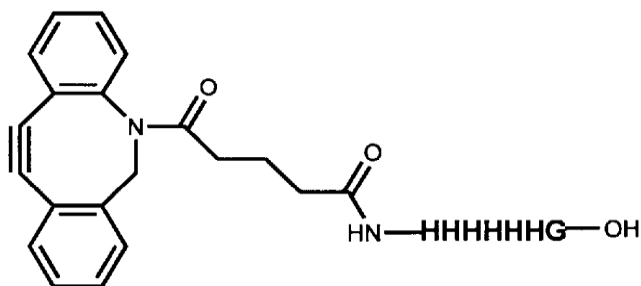
To the trastuzumab-Cy3 conjugate PBS solution synthesized in (7-1-1), 2 μ L of a 7 mM tris(2-carboxyethyl) phosphine hydrochloride solution (100 equivalents to the trastuzumab-Cy3 conjugate) was added, and the resulting mixture was stirred at room temperature for 20 minutes. The mass was measured by ESI-TOFMS; for the raw material azide-introduced trastuzumab synthesized in (6-1-1) of Example 6, heavy chain peaks were observed at 50714 and 50876, and a light chain peak was observed at 23440, whereas for the trastuzumab-Cy3 conjugate, peaks were observed at 51722 and 51885 with Cy3 introduced to the heavy chain, and a light chain peak was observed at 23440, the same as that of the raw material (FIG. 20). Note that the azidobenzoic acid-introduced compound was reduced to an aminebenzoic acid-introduced compound by a reduction reaction of 7 mM tris(2-carboxyethyl) phosphine salt, which was a pretreatment for the measurement.

[0357] (7-2) Conjugation of Regiospecific Azide-Introduced Trastuzumab with Peptide-Cycloalkyne**(7-2-1) Synthesis of Peptide-Cycloalkyne**

A peptide portion of compound 30 below was synthesized by solid phase peptide synthesis using 2-chlorotrityl resin by Fmoc method. After the solid phase synthesis, 1-hydroxybenzotriazole (2 molar equivalents), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (2 molar equivalents), triethylamine (2 molar equivalents), and 5-(5,6-dihydro-11,12-didehydridibenzo [b,f][b,f]azocin-5(6H)-yl)-oxopentanoic acid (2.2 molar equivalents) were added at any time, and the resulting mixture was stirred for 19 hours. The product was stirred in a solution of trifluoroacetic acid:water:triisopropylsilane=95:2.5:2.5 for one hour, thereby performing cutting out from the resin and deprotection, and triethylamine was added to the filtrate after cutting for neutralization. The resin was removed by filtration, diethyl ether was added for precipitation, and the diethyl ether was removed by decantation to obtain a peptide as crude crystals. This peptide was purified by preparative HPLC to obtain compound 30 below as a product.

[0358]

[Formula 53]



Compound 30

(Amino acid sequence of peptide portion: SEQ ID NO: 50)

[0359] MS (ESI) m/z : 1200[M+H]⁺

[0360] (7-2-2) Conjugation of Regiospecific Azide-Introduced Trastuzumab with Peptide-Cycloalkyne and ESI-TOFMS Analysis

The azidobenzoic acid-introduced trastuzumab synthesized in (6-1-1) of Example 6 was dissolved in 20 mM PBS buffer to be 0.3 mM. Thereafter, 20 μ L of a 0.94 mM aqueous solution of the peptide-cycloalkyne (compound 29) synthesized in (7-2-1) of Example 7 was added thereto, and the resulting mixture was stirred at room temperature for 12 hours. The reaction solution was subjected to water substitution by ultrafiltration (Amicon Ultra, 10K MWCO) to stop the reaction. The mass was measured by ESI-TOFMS; for the trastuzumab, a peak was observed at 148060, for the azide-introduced trastuzumab synthesized in (6-1-1), a peak was observed at 148489, and for a reaction product, a peak was observed at 150912 with two peptides introduced (FIG. 8).

[0361] (7-2-3) ESI-TOFMS Analysis Under Reduction Conditions

[0362] Example 8: Determination of Regiospecific Modification of Bioorthogonal Functional Group-Introduced Trastuzumab

(8-1) Glycolysis of Bioorthogonal Functional Group-Introduced Trastuzumab

(8-1-1) Glycolysis of Azidobenzoic Acid-Introduced Trastuzumab (1)

Using PNGase F (NEW ENGLAND BioLabs, catalog number P0704), glycolysis and post-treatment were performed on the azidobenzoic acid-introduced trastuzumab (azide-modified antibody 3) synthesized in (6-2-2) of Example 6 according to manufacturer's protocol and the technique of patent (WO 2017/191817.

[0363] (8-1-2) Glycolysis of Azidobenzoic Acid-Introduced Trastuzumab (2)

Using PNGase F (NEW ENGLAND BioLabs, catalog number P0704), glycolysis and post-treatment were performed on the azidobenzoic acid-introduced trastuzumab (azide-modified antibody 5) synthesized in (6-1-3) of Example 6 according to manufacturer's protocol and the technique of patent (WO 2017/191817, which is incorporated herein by reference in its entirety).

[0364] (8-2) Trypsin Treatment of Azidobenzoic Acid-Introduced Trastuzumab

(8-2-1) Trypsin Treatment of Glycosylated Azidobenzoic Acid-Introduced Trastuzumab (1)

To a 1.5 mL low-adsorption micro test tube, 3.4 µL of a sample solution of the glycosylated azidobenzoic acid- introduced trastuzumab obtained in (8-1-1) and 6.6 µL of 50 mM ammonium hydrogencarbonate buffer were added to be 10 µL in total. A 50 mM ammonium hydrogencarbonate buffer and 10 µL of a 20 mM aqueous dithiothreitol solution dissolved in 20% trifluoroethanol were added thereto. The resulting mixture was heated at 65° C. for one hour, then 10 µL of a 50 mM aqueous iodoacetamide solution was added thereto, and the resulting mixture was reacted in a dark place at room temperature for 30 minutes while being shaken at 300 rpm. After the reaction, 40 µL of a 50 mM ammonium hydrogencarbonate buffer was added thereto, the resulting mixture was stirred, 10 µL of a 20 ng/µL aqueous trypsin solution was added thereto, and the resulting mixture was subjected to enzyme digestion at 37° C. for 18 hours. After the digestion, 2 µL of a 20% aqueous trifluoroacetic acid solution was added thereto to stop the reaction. Thereafter, the solution was diluted 10 times with a 50 mM ammonium hydrogen carbonate buffer or 0.5% trifluoroacetate. The diluted solution was used for LC-MS/MS measurement.

[0365] (8-2-2) Trypsin Treatment of Glycosylated Azidobenzoic Acid-Introduced Trastuzumab (2)

To a 1.5 mL low-adsorption micro test tube, 10 µL of a sample solution of the glycosylated azidobenzoic acid-introduced trastuzumab obtained in (8-1-2), a 50 mM ammonium hydrogencarbonate buffer, and 10 µL of a 20 mM aqueous dithiothreitol solution dissolved in 20% trifluoroethanol were added. The resulting mixture was heated at 65° C. for one hour, then 10 µL of a 50 mM aqueous iodoacetamide solution was added thereto, and the resulting mixture was reacted in a dark place at room temperature for 30 minutes while being shaken at 300 rpm. After the reaction, 40 µL of a 50 mM ammonium hydrogencarbonate buffer was added thereto, the resulting mixture was stirred, 10 µL of a 20 ng/µL aqueous trypsin solution was added thereto, and the resulting mixture was subjected to enzyme digestion at 37° C. for 18 hours. After the digestion, 2 µL of a 20% aqueous trifluoroacetic acid solution was added thereto to stop the reaction. Thereafter, the solution was diluted 10 times with a 50 mM ammonium hydrogen carbonate buffer or 0.5% trifluoroacetate. The diluted solution was used for LC-MS/MS measurement.

[0366] (8-3) LC-MS/MS Measurement Conditions for Trastuzumab (Analyzer)

Nano HPLC: EASY-nLC 1000 (Thermo Fisher Scientific)

Mass Spectrometer: Tribrid Mass Spectrometer Orbitrap Fusion (Thermo Fisher Scientific)

[0367] (HPLC Analysis Conditions)

Trap column: Acclaim PepMap (registered trade- mark) 100, 75 µm x 2 cm, (Thermo Fisher Scientific) Analysis column: ESI-column (NTCC-360/75-3-125, 75 µm x 12.5 cm, 3 µm (Nikkyo Technos Co., Ltd.))

Mobile Phase A: a 0.1% aqueous formate solution **[1273]** Mobile Phase B: a 0.1% formate acetonitrile solution

Loading solution: a 0.1% aqueous trifluoroacetic acid solution

Flow rate: 300 nL/min

Sample injection amount: 0.3-1 µL

Gradient condition (B %): 2% (0.0 minute to 0.5 minute), 2%-30% (0.5 minute to 23.5 minutes), 30%-75% (23.5 minutes to 25.5 minutes), and 75% (25.5 minutes to 35.0 minutes).

[0368] (Mass Spectrometer Analysis Conditions)

Ionization: ESI, Positive mode

Scan type: Data Dependent Acquisition

Activation Type: Collision Induced Dissociation (CID)

Data acquisition was performed using Xcalibur 3.0 (Thermo Fisher Scientific) and Thermo Orbitrap Fusion Tune Application 2.0 (Thermo Fisher Scientific) as accompanying software.

[0369] (8-4) Analysis Conditions for Modified Site of Trastuzumab, Denosumab, and Dupilumab

Modified site analysis of an LC-MS/MS measurement result was performed using BioPharma Finder 3.0 (Thermo Fisher Scientific).

[0370] In analysis by Proteome Discoverer, Sequest HT was used as the study engine, and the precursor ion was set to 350-5000Da. In addition, Trypsin was used as the digestive enzyme, and the Maximum Missed Cleavage Sites were rendered as 3. Further the Mass Tolerances for the precursor and fragment ions were respectively set at 5ppm and 0.5Da. As modification of cysteine residue with iodoacetamide, Carbamidomethyl (+57.021Da) was set in static modification. For Static Modification, Carbamidomethyl (+57.021 Da) was set as modification of a cysteine residue with iodoacetamide. For Dynamic Modifications, oxidations of methionine (+15.995Da) and modification of lysine residues (azidobenzoic acid derivative (+119.037Da) and amine benzoic acid derivative (+119.037Da) were established. Further, a filter was placed so that Peptide Confidence was rendered only high.

In addition, in order to perform reductive alkylation during digestion, the azidobenzoic acid derivative was reduced to render an amine benzoic acid derivative.

[0371]

Further, as amino acid sequence data to search for modification sites, 1-2 shown in FIG.10 were used.

[0372] (8-5) Analysis Result of Modified Site of Digest by LC-MS/MS

(8-5-1) Analysis Result of Modified Site of Trypsin Digest by LC-MS/MS (1)

After analysis using LC-MS/MS, an MS spectrum of the peptide fragment of THTCPPCPA-PELLGGPSVFLFPPKPKDTLMISR (SEQ ID NO: 11), which is a peptide consisting of 33 amino acid residues comprising a modified site to a lysine residue by trypsin digestion of azide-modified trastuzumab (aminebenzoic acid-introduced compound (+119.037 Da)) obtained in (11- 2-1) (measured value: m/z 945.73328; theoretical value: 945.73350; and tetravalent) was observed (FIG. 11); and from a CID spectrum, a product ion of m/z 1125.49 (theoretical value: 1125.11) corresponding to divalent y19 indicating modification of a lysine residue at position 246 or 248 in EU numbering of the heavy chain was determined (FIG. 12).

[0373] (8-5-2) Analysis Result of Modified Site of Trypsin Digest by LC-MS/MS (2)

[1292] After analysis using LC-MS/MS, an MS spectrum of the peptide fragment of FNWYVDGVEVHNAKTKPR (SEQ ID NO: 12), which is a peptide consisting of 18 amino acid residues comprising a modified site to a lysine residue by trypsin digestion of azide-modified trastuzumab (aminebenzoic acid-introduced compound (+119.037 Da)) obtained in (11-2-2) (measured value: m/z 760.38287; theoretical value: 760.38390; and trivalent) was observed (FIG. 13), and from a CID spectrum, a product ion of m/z 1009.83 (theoretical value: 1009.52) corresponding to divalent y16 indicating modification of a lysine residue at position 288 or 290 in EU numbering of the heavy chain was determined (FIG. 14).

[0374]

(Summary)

The following is a summary of the results:

Table 1

Amino Acid Sequence	SEQ NO
FNMQQQRRFYALHDPNLNEEQRNARIRSIKDD	5
FNMQQQRRFYALHDPNLNEEQRNARIKSIRDD	6
β -Ala-NMQQRRFYALHDPNLNEEQRNARIRSIKDD	7
FNMQQQRRFYALHDPNLNEEQRNAKIKSIKDD	8

[0376] In addition, it has been shown that position-selective modification of specific amino acid residues is possible for antibodies, including lysine residues at positions 246 and/or 248 (e.g., Example 8 (8-5-1)), and lysine residues at positions 288 and/or 290 (e.g., Example 8 (8-5-2)).

[0377] Furthermore, the affinity substance for an antibody and the compound having a bioorthogonal functional group represented by the following formula (I) are described with some examples as shown in Tables 2 and 3 below.

[herein,

A is an affinity substance for an antibody,

L is a divalent group containing a leaving group,

E is (i) linked to the leaving group and (ii) a divalent group containing an electrophilic group capable of reacting with a nucleophilic group in the antibody,

B is a bioorthogonal functional group,

and the leaving group is capable of being cleaved from E and released by a reaction between the nucleophilic group and the electrophilic group.]

[0378]

[Table 2]

Table 2. Affinity substances for antibodies, and relationship between compounds with bio-orthogonal functional groups and antibodies with bio-compatible functional groups capable of manufacturing these.

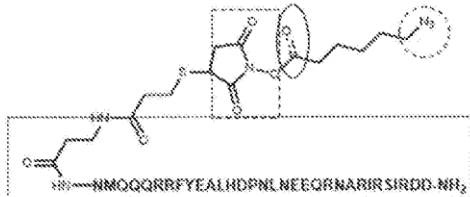
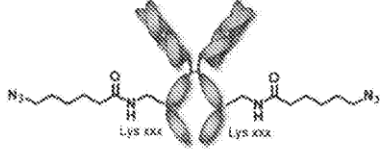
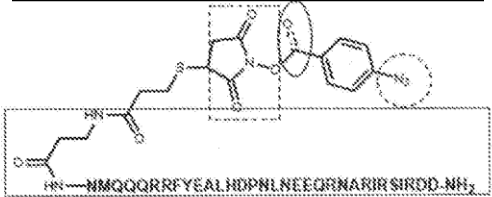
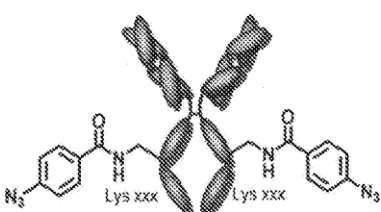
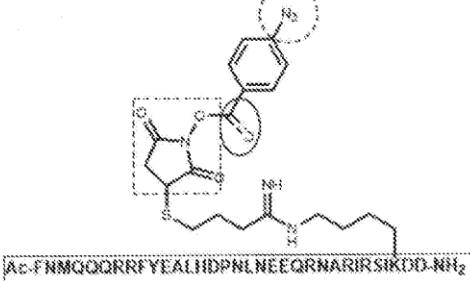
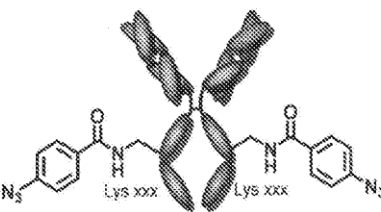
Example	Compound and specific structure	Possible to create using compound on left. Antibody having biocompatible orthogonal functional group
3-4	Ac-FNNMQQRBFYEALHDPNINEEQRNARIKSRDD-NH₂ Above amino acid group: SEQ NO 6	
3-4	Ac-FNNMQQRBFYEALHDPNINEEQRNARIKSRDD-NH₂ Above amino acid group: SEQ NO 6	

*The NH-alkyl moiety extending from the antibody is derived from the side chain of the lysine residue in the antibody (the same below).

[0379]

[Table]

Table 3. Affinity substances for antibodies, and relationship between compounds with bio-orthogonal functional groups and antibodies with bio-compatible functional groups capable of manufacturing these (Version 2).

Example	Compound and specific structure	Possible to create using compound on left. Antibody having biocompatible orthogonal functional group
	Affinity substance (A) Leaving group (L2) Electrophilic group (E1) Bio-orthogonal functional group (B)	
5-2-1	 Above amino acid group: SEQ NO 7	
5-2-2	 Above amino acid group: SEQ NO 7	
5-2-3	 Above amino acid group: SEQ NO 5	

【0 3 8 0】 The above shows that it is possible to site-selectively modify antibodies using compounds with affinity substances for antibodies, cleavable moieties, and reactive groups by appropriately adjusting factors such as the type of affinity substance, the length of the linker between the affinity substance and the reactive group, and the position in the affinity substance where the linker is introduced.

[Industrial Applicability]

【0 3 8 1】 The present invention is useful, for example, for the production of position-selectively modified antibodies.

[Sequence Table]

SEQUENCE LISTING

<110> Ajinomoto Co., Inc.

<120> Compounds and salts thereof comprising an affinity substance to an antibody, and a bioorthogonal group

<130> PAMA-30455

<160> 96

<170> PatentIn version 3.5

<210> 1

<211> 230

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc region of human IgG1

<220>

<221> misc_feature

<222> (18).. (19)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (21).. (21)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (140).. (140)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (142).. (142)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (177).. (177)

<223> Xaa can be any naturally occurring amino acid

<400> 1

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
1 5 10 15

Glu Xaa Xaa Gly Xaa Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
20 25 30

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
35 40 45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65 70 75 80

Asp Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
85 90 95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
100 105 110

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
115 120 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Xaa Glu Xaa Thr Lys
130 135 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
165 170 175

Xaa Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
180 185 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
195 200 205

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 210 215 220

Leu Ser Leu Ser Pro Gly
 225 230

<210> 2
 <211> 449
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Heavy chain of Trastuzumab (humanized IgG1 monoclonal antibody)

<400> 2

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
 20 25 30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
 100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asp Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
 325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
 355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
 420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 435 440 445

Gly

<210> 3
 <211> 237
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Fc region of human IgG1 antibody

<400> 3

Ile Glu Gly Arg Met Asp Pro Lys Ser Cys Asp Lys Thr His Thr Cys
 1 5 10 15

Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu
		20						25					30		
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu
	35						40					45			
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys
	50					55					60				
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
65					70					75					80
Pro	Arg	Glu	Glu	Gln	Tyr	Asp	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu
				85					90					95	
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys
			100					105					110		
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys
		115					120					125			
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser
	130					135						140			
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys
145					150					155					160
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln
				165					170					175	
Pro	Glu	Asn	Asn	Tyr	Lys	Ala	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly
			180						185					190	
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln
		195					200						205		
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn
	210					215					220				

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 225 230 235

<210> 4
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Light chain of Trastuzumab (humanized IgG1 monoclonal antibody)

<400> 4

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

Phe Asn Arg Gly Glu Cys
 210

<210> 5
 <211> 33
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 5

Phe Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
 1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Lys Asp
 20 25 30

Asp

<210> 6
 <211> 33
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 6

Phe Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Lys Ser Ile Arg Asp
20 25 30

Asp

<210> 7

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> beta-alanine

<400> 7

Xaa Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp

<210> 8
<211> 33
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 8

Phe Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Lys Ile Lys Ser Ile Lys Asp
20 25 30

Asp

<210> 9
<211> 25
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 9

Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His Leu Pro Asn Glu Glu
1 5 10 15

Gln Arg Gly Gly Gly Gly Ser Cys Gly
20 25

<210> 10
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> Model for peptide drug

<400> 10

His His His His His His Gly
1 5

<210> 11

<211> 33

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 11

Phe Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp

<210> 12

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<400> 12

Phe Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 13
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> beta-alanine

<400> 13

Xaa Asn Met Gln Gln Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp

<210> 14
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, and the amino acid residue at C-terminus may be aminated.

<220>
<221> MISC_FEATURE
<222> (1)..(1)

<223> beta-alanine

<400> 14

Xaa Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 15

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> absent, or arginine

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> absent, or glycine

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> absent, aspartic acid, or asparagine

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> alanine

<220>

<221> MISC_FEATURE

<222> (6).. (6)
 <223> tyrosine, tryptophan, or histidine

 <220>
 <221> MISC_FEATURE
 <222> (7).. (7)
 <223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or
 glycine

 <220>
 <221> MISC_FEATURE
 <222> (8).. (8)
 <223> lysine

 <220>
 <221> MISC_FEATURE
 <222> (9).. (9)
 <223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic
 acid, phenylalanine, tyrosine, tryptophan, histidine, threonine,
 leucine, alanine, valine, isoleucine, or arginine

 <220>
 <221> MISC_FEATURE
 <222> (10).. (10)
 <223> glutamine, glutamic acid, asparagine, or aspartic acid

 <220>
 <221> MISC_FEATURE
 <222> (15).. (15)
 <223> absent, or threonine

 <220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

 <220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

 <400> 15

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Ile Ile Trp Cys Xaa Xaa
 1 5 10 15

Xaa

<210> 16
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
 <221> MISC_FEATURE
 <222> [1].. [1]
 <223> absent, or arginine

<220>
 <221> MISC_FEATURE
 <222> [2].. [2]
 <223> absent, or glycine

<220>
 <221> MISC_FEATURE
 <222> [3].. [3]
 <223> absent, aspartic acid, or asparagine

<220>
 <221> MISC_FEATURE
 <222> [5].. [5]
 <223> alanine

<220>
 <221> MISC_FEATURE
 <222> [6].. [6]
 <223> tyrosine, tryptophan, or histidine

<220>
 <221> MISC_FEATURE
 <222> [7].. [7]
 <223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or glycine

<220>
 <221> MISC_FEATURE
 <222> [8].. [8]
 <223> lysine

<220>

<221> MISC_FEATURE
 <222> (9).. (9)
 <223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan, histidine, threonine, leucine, alanine, valine, isoleucine, or arginine

<220>
 <221> MISC_FEATURE
 <222> (10).. (10)
 <223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>
 <221> MISC_FEATURE
 <222> (15).. (15)
 <223> absent, or threonine

<220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

<220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

<400> 16

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Ile Val Trp Cys Xaa Xaa
 1 5 10 15

Xaa

<210> 17
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
 <221> MISC_FEATURE

<222> (1).. (1)
 <223> absent, or arginine

<220>
 <221> MISC_FEATURE
 <222> (2).. (2)
 <223> absent, or glycine

<220>
 <221> MISC_FEATURE
 <222> (3).. (3)
 <223> absent, aspartic acid, or asparagine

<220>
 <221> MISC_FEATURE
 <222> (5).. (5)
 <223> alanine

<220>
 <221> MISC_FEATURE
 <222> (6).. (6)
 <223> tyrosine, tryptophan, or histidine

<220>
 <221> MISC_FEATURE
 <222> (7).. (7)
 <223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or glycine

<220>
 <221> MISC_FEATURE
 <222> (8).. (8)
 <223> lysine

<220>
 <221> MISC_FEATURE
 <222> (9).. (9)
 <223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan, histidine, threonine, leucine, alanine, valine, isoleucine, or arginine

<220>
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 <223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>
 <221> MISC_FEATURE
 <222> (15).. (15)
 <223> absent, or threonine

<220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

<220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

<400> 17

Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Val	Val	Trp	Cys	Xaa	Xaa
1			5					10					15	

Xaa

<210> 18
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
 <221> MISC_FEATURE
 <222> (1).. (1)
 <223> absent, or arginine

<220>
 <221> MISC_FEATURE
 <222> (2).. (2)
 <223> absent, or glycine

<220>
 <221> MISC_FEATURE
 <222> (3).. (3)
 <223> absent, aspartic acid, or asparagine

<220>
 <221> MISC_FEATURE

<222> (5).. (5)
 <223> alanine

 <220>
 <221> MISC_FEATURE
 <222> (6).. (6)
 <223> tyrosine, tryptophan, or histidine

 <220>
 <221> MISC_FEATURE
 <222> (7).. (7)
 <223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or
 glycine

 <220>
 <221> MISC_FEATURE
 <222> (8).. (8)
 <223> lysine

 <220>
 <221> MISC_FEATURE
 <222> (9).. (9)
 <223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic
 acid, phenylalanine, tyrosine, tryptophan, histidine, threonine,
 leucine, alanine, valine, isoleucine, or arginine

 <220>
 <221> MISC_FEATURE
 <222> (10).. (10)
 <223> glutamine, glutamic acid, asparagine, or aspartic acid

 <220>
 <221> MISC_FEATURE
 <222> (15).. (15)
 <223> absent, or threonine

 <220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

 <220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

 <400> 18

 Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Ala Val Trp Cys Xaa Xaa
 1 5 10 15

Xaa

<210> 19
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> absent, or arginine

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> absent, or glycine

<220>
<221> MISC_FEATURE
<222> (3)..(3)
<223> absent, aspartic acid, or asparagine

<220>
<221> MISC_FEATURE
<222> (5)..(5)
<223> alanine

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> tyrosine, tryptophan, or histidine

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or glycine

<220>

<221> MISC_FEATURE

<222> [8].. [8]

<223> lysine

<220>

<221> MISC_FEATURE

<222> [9].. [9]

<223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan, histidine, threonine, leucine, alanine, valine, isoleucine, or arginine

<220>

<221> MISC_FEATURE

<222> [10].. [10]

<223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>

<221> MISC_FEATURE

<222> [15].. [15]

<223> absent, or threonine

<220>

<221> MISC_FEATURE

<222> [16].. [16]

<223> absent, or tyrosine

<220>

<221> MISC_FEATURE

<222> [17].. [17]

<223> absent, or histidine

<400> 19

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Leu Leu Trp Cys Xaa Xaa
1 5 10 15

Xaa

<210> 20

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues

may be linked by disulfide bridge.

<220>
<221> MISC_FEATURE
<222> (1).. (1)
<223> absent, or arginine

<220>
<221> MISC_FEATURE
<222> (2).. (2)
<223> absent, or glycine

<220>
<221> MISC_FEATURE
<222> (3).. (3)
<223> absent, aspartic acid, or asparagine

<220>
<221> MISC_FEATURE
<222> (5).. (5)
<223> alanine

<220>
<221> MISC_FEATURE
<222> (6).. (6)
<223> tyrosine, tryptophan, or histidine

<220>
<221> MISC_FEATURE
<222> (7).. (7)
<223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or glycine

<220>
<221> MISC_FEATURE
<222> (8).. (8)
<223> lysine

<220>
<221> MISC_FEATURE
<222> (9).. (9)
<223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan, histidine, threonine, leucine, alanine, valine, isoleucine, or arginine

<220>
<221> MISC_FEATURE
<222> (10).. (10)
<223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>
 <221> MISC_FEATURE
 <222> [15].. (15)
 <223> absent, or threonine

<220>
 <221> MISC_FEATURE
 <222> [16].. (16)
 <223> absent, or tyrosine

<220>
 <221> MISC_FEATURE
 <222> [17].. (17)
 <223> absent, or histidine

<400> 20

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Leu Ile Trp Cys Xaa Xaa
 1 5 10 15

Xaa

<210> 21
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
 <221> MISC_FEATURE
 <222> [1].. (1)
 <223> absent, or arginine

<220>
 <221> MISC_FEATURE
 <222> [2].. (2)
 <223> absent, or glycine

<220>
 <221> MISC_FEATURE

<222> (3).. (3)
 <223> absent, aspartic acid, or asparagine

 <220>
 <221> MISC_FEATURE
 <222> (5).. (5)
 <223> alanine

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 <220>
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 glycine

 <220>
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 <223> lysine

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 leucine, alanine, valine, isoleucine, or arginine

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 <223> absent, or threonine

 <220>
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 <223> absent, or tyrosine

 <220>
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 <222> (17).. (17)
 <223> absent, or histidine

<400> 21

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Leu Val Phe Cys Xaa Xaa
1 5 10 15

Xaa

<210> 22

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> absent, or arginine

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> absent, or glycine

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> absent, aspartic acid, or asparagine

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> alanine

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> tyrosine, tryptophan, or histidine

<220>

<221> MISC_FEATURE

<222> (7).. (7)
 <223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or
 glycine

 <220>
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 <222> (8).. (8)
 <223> lysine

 <220>
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 <222> (9).. (9)
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 leucine, alanine, valine, isoleucine, or arginine

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 <220>
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 <223> absent, or threonine

 <220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

 <220>
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 <222> (17).. (17)
 <223> absent, or histidine

 <400> 22

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Gln Val Trp Cys Xaa Xaa
 1 5 10 15

Xaa

<210> 23
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<220>
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<223> absent, or arginine

<220>
<221> MISC_FEATURE
<222> (2).. (2)
<223> absent, or glycine

<220>
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<223> absent, aspartic acid, or asparagine

<220>
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<223> alanine

<220>
<221> MISC_FEATURE
<222> (6).. (6)
<223> tyrosine, tryptophan, or histidine

<220>
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<222> (7).. (7)
<223> histidine, phenylalanine, tyrosine, tryptophan, arginine, or glycine

<220>
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<220>
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 <222> (10).. (10)
 <223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>
 <221> MISC_FEATURE
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 <223> absent, or threonine

<220>
 <221> MISC_FEATURE
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 <223> absent, or tyrosine

<220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

<400> 23

Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Val	Trp	Cys	Xaa	Xaa
1			5					10						15	

Xaa

<210> 24
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<220>
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 <223> absent, or arginine

<220>
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<222> (2).. (2)
 <223> absent, or glycine

<220>
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 <222> (3).. (3)
 <223> absent, aspartic acid, or asparagine

<220>
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<220>
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 <222> (6).. (6)
 <223> tyrosine, tryptophan, or histidine

<220>
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 <222> (7).. (7)
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<220>
 <221> MISC_FEATURE
 <222> (8).. (8)
 <223> lysine

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 <222> (9).. (9)
 <223> glycine, serine, asparagine, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan, histidine, threonine, leucine, alanine, valine, isoleucine, or arginine

<220>
 <221> MISC_FEATURE
 <222> (10).. (10)
 <223> glutamine, glutamic acid, asparagine, or aspartic acid

<220>
 <221> MISC_FEATURE
 <222> (15).. (15)
 <223> absent, or threonine

<220>
 <221> MISC_FEATURE
 <222> (16).. (16)
 <223> absent, or tyrosine

<220>
 <221> MISC_FEATURE
 <222> (17).. (17)
 <223> absent, or histidine

<400> 24

Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Val	Trp	Cys	Xaa	Xaa
1			5						10					15	

Xaa

<210> 25
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to CH2 region of human IgG

<400> 25

Glu	Pro	Ile	His	Arg	Ser	Thr	Leu	Thr	Ala	Leu	Leu
1			5				10				

<210> 26
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG

<400> 26

Phe	Ala	Arg	Leu	Val	Ser	Ser	Ile	Arg	Tyr
1			5				10		

<210> 27
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG

<400> 27

Phe Gly Arg Leu Val Ser Ser Ile Arg Tyr
1 5 10

<210> 28

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG

<400> 28

Thr Trp Lys Thr Ser Arg Ile Ser Ile Phe
1 5 10

<210> 29

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to specific region of human IgG

<400> 29

Gln Ser Tyr Pro
1

<210> 30

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG

<400> 30

His Trp Arg Gly Trp Val
1 5

<210> 31
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG

<400> 31

His Tyr Phe Lys Phe Asp
1 5

<210> 32
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG

<400> 32

His Phe Arg Arg His Leu
1 5

<210> 33
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG

<400> 33

Asp Ala Ala Gly
1

<210> 34
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG

<400> 34

Asn Ala Arg Lys Phe Tyr Lys Gly
1 5

<210> 35

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG

<400> 35

Asn Lys Phe Arg Gly Lys Tyr Lys
1 5

<210> 36

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 36

Phe Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Lys Ile Lys Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 37

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Lysine residue at N-terminus may be acetylated and two cysteine

residues may be linked by disulfide bridge

<400> 37

Lys Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 38

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 38

Phe Asn Met Gln Cys Gln Lys Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 39

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 39

Phe	Asn	Met	Gln	Cys	Gln	Arg	Arg	Phe	Tyr	Glu	Ala	Lys	His	Asp	Pro
1			5					10						15	

Asn	Leu	Asn	Glu	Glu	Gln	Arg	Asn	Ala	Arg	Ile	Arg	Ser	Ile	Arg	Asp
		20					25						30		

Asp Cys

<210> 40
 <211> 34
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Phenylalanine residue at N-terminus may be acetylated and two cysteine residues may be linked by disulfide bridge

<400> 40

Phe	Asn	Met	Gln	Cys	Gln	Arg	Arg	Phe	Tyr	Glu	Ala	Leu	His	Asp	Pro
1			5					10						15	

Asn	Leu	Asn	Glu	Glu	Gln	Arg	Lys	Ala	Arg	Ile	Arg	Ser	Ile	Arg	Asp
		20					25						30		

Asp Cys

<210> 41
 <211> 34
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Phenylalanine residue at N-terminus may be acetylated and two cysteine residues may be linked by disulfide bridge

<400> 41

Phe	Asn	Met	Gln	Cys	Gln	Arg	Arg	Phe	Tyr	Glu	Ala	Leu	His	Asp	Pro
1			5					10						15	

Asn Leu Asn Lys Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 42

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 42

Phe Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Lys Asp
20 25 30

Asp Cys

<210> 43

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 43

Phe Asn Lys Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 44
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 44

Phe Asn Met Gln Cys Lys Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Asp
20 25 30

Asp Cys

<210> 45
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> Phenylalanine residue at N-terminus may be acetylated and two
cysteine residues may be linked by disulfide bridge

<400> 45

Phe Asn Met Gln Cys Gln Arg Arg Phe Tyr Glu Ala Leu His Asp Pro
1 5 10 15

Asn Leu Asn Glu Glu Gln Arg Asn Ala Arg Ile Arg Ser Ile Arg Lys
20 25 30

Asp Cys

<210> 46
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 46

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 47
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 47

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Ile Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 48
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid

residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 48

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Val Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 49

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 49

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Ala Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 50

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 50

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Leu Leu Trp Cys Thr Tyr
1 5 10 15

His

<210> 51
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 51

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Leu Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 52
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 52

Asp Cys Ala Tyr His Lys Gly Gln Ile Val Trp Cys Thr
1 5 10

<210> 53
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino

acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 53

Asp	Cys	Ala	Tyr	His	Lys	Gly	Gln	Val	Val	Trp	Cys	Thr
1				5					10			

<210> 54

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 54

Asp	Cys	Ala	Tyr	His	Lys	Gly	Gln	Ala	Val	Trp	Cys	Thr
1				5					10			

<210> 55

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 55

Arg	Gly	Asn	Cys	Ala	Tyr	His	Lys	Ser	Gln	Ile	Ile	Trp	Cys	Thr	Tyr
1				5					10				15		

His

<210> 56

<211> 17

<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 56

Arg Gly Asn Cys Ala Tyr His Lys Asn Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 57
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 57

Arg Gly Asn Cys Ala Tyr His Lys Asp Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 58
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 58

Arg Gly Asn Cys Ala Tyr His Lys Gln Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 59

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 59

Arg Gly Asn Cys Ala Tyr His Lys Glu Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 60

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 60

Arg Gly Asn Cys Ala Tyr His Lys Phe Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 61
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 61

Arg Gly Asn Cys Ala Tyr His Lys Tyr Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 62
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 62

Arg Gly Asn Cys Ala Tyr His Lys Trp Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 63
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 63

Arg Gly Asn Cys Ala Tyr His Lys His Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 64

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 64

Arg Gly Asn Cys Ala Tyr His Lys Thr Gln Ile Ile Trp Cys Thr Tyr
1 5 10 15

His

<210> 65

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 65

Arg Gly Asn Cys Ala Tyr His Lys Leu Gln Ile Ile Trp Cys Thr Tyr
 1 5 10 15

His

<210> 66
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 66

Cys Ala Tyr His Lys Leu Gln Ile Val Trp Cys
 1 5 10

<210> 67
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 67

Cys Ala Tyr His Lys Leu Gln Leu Ile Trp Cys
 1 5 10

<210> 68
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid

residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 68

Cys Ala Tyr His Lys Ser Gln Ile Val Trp Cys
1 5 10

<210> 69

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 69

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Leu Val Phe Cys Thr Tyr
1 5 10 15

His

<210> 70

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 70

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Gln Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 71
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 71

Arg Gly Asn Cys Ala Tyr His Lys Gly Gln Glu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 72
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 72

Cys Ala Tyr His Lys Gly Gln Leu Val Trp Cys
1 5 10

<210> 73
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 73

Arg Gly Asn Cys Ala Tyr His Lys Ala Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 74

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 74

Arg Gly Asn Cys Ala Tyr His Lys Val Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 75

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 75

Arg Gly Asn Cys Ala Tyr His Lys Leu Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 76
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 76

Arg Gly Asn Cys Ala Tyr His Lys Ile Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 77
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 77

Arg Gly Asn Cys Ala Tyr His Lys Ser Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 78
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 78

Arg Gly Asn Cys Ala Tyr His Lys Thr Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 79

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 79

Arg Gly Asn Cys Ala Tyr His Lys Asn Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 80

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 80

Arg Gly Asn Cys Ala Tyr His Lys Asp Gln Leu Val Trp Cys Thr Tyr

1 5 10 15

His

<210> 81
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 81

Arg Gly Asn Cys Ala Tyr His Lys Gln Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 82
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 82

Arg Gly Asn Cys Ala Tyr His Lys Glu Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 83

<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 83

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1 5 10 15

His

<210> 84
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 84

Arg Gly Asn Cys Ala Tyr His Lys Arg Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 85
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues

may be linked by disulfide bridge.

<400> 85

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His

<210> 86

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 86

Arg	Gly	Asn	Cys	Ala	Tyr	His	Lys	Trp	Gln	Leu	Val	Trp	Cys	Thr	Tyr
1				5					10					15	

His

<210> 87

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 87

Arg	Gly	Asn	Cys	Ala	Tyr	His	Lys	Tyr	Gln	Leu	Val	Trp	Cys	Thr	Tyr
1				5					10					15	

His

<210> 88
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 88

Arg Gly Asn Cys Ala Tyr Phe Lys Gly Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 89
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 89

Arg Gly Asn Cys Ala Tyr Tyr Lys Gly Gln Leu Val Trp Cys Thr Tyr
1 5 10 15

His

<210> 90
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 90

Arg	Gly	Asn	Cys	Ala	Tyr	Trp	Lys	Gly	Gln	Leu	Val	Trp	Cys	Thr	Tyr
1				5				10					15		

His

<210> 91

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 91

Arg	Gly	Asn	Cys	Ala	Tyr	Arg	Lys	Gly	Gln	Leu	Val	Trp	Cys	Thr	Tyr
1				5				10					15		

His

<210> 92

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 92

Arg Gly Asn Cys Ala Tyr Gly Lys Gly Gln Leu Val Trp Cys Thr Tyr
 1 5 10 15

His

<210> 93
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 93

Asp Cys Ala Tyr His Lys Gly Gln Leu Val Trp Cys
 1 5 10

<210> 94
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 94

Asn Cys Ala Tyr His Lys Gly Gln Leu Val Trp Cys
 1 5 10

<210> 95
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide having an affinity to Fc region of human IgG. The amino

acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 95

Cys	Ala	Tyr	His	Lys	Gly	Gln	Leu	Val	Trp	Cys	Thr
1				5					10		

<210> 96

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Peptide having an affinity to Fc region of human IgG. The amino acid residue at N-terminus may be acetylated, the amino acid residue at C-terminus may be aminated and two cysteine residues may be linked by disulfide bridge.

<400> 96

Cys	Ala	Tyr	His	Lys	Ser	Gln	Leu	Val	Trp	Cys
1				5					10	

[Document Name] Claims

[Claim 1]

A compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or
a salt thereof.

[Claim 2]

The compound or salt thereof according to claim 1,
wherein the affinity substance is peptide.

[Claim 3]

The compound or salt thereof according to claim 2,
wherein the affinity substance is peptide.

[Claim 4]

The compound or salt thereof according to claim 2 or 3,
wherein the peptide is a peptide capable of binding to the Fc region of a monoclonal antibody.

[Claim 5]

The compound or salt thereof according to claim 4,
wherein the peptide is a peptide having binding ability to the Fc domain of IgG.

[Claim 6]

The compound or salt thereof according to any one of claims 2 to 5,
wherein the peptide has 10 to 40 amino acid residues.

[Claim 7]

The compound or salt thereof according to any one of claims 2 to 6,
wherein the peptide comprises (a-1-1) an amino acid sequence of FNMQQRRFYALHDPNLNNEEQRNAR I RS I RDD (SEQ ID NO: 11) or (a-1-2) an amino acid sequence of FNMQQRRFYALHDPNLNNEEQRNAR I RS I RDD C (SEQ ID NO: 12),
any one to three amino acid residues in the sequence are substituted with one amino acid residue each selected from the group consisting of lysine residues, aspartic acid residues, and glutamic acid residues, which may be the same or different, or
(a-2-1) an amino acid sequence of { β -A I a-NMQQRRFYALHDPNLNNEEQRNAR IR SIRDD (SEQ ID NO: 13) or (a-2-2) an amino acid sequence of { β -A I a-NMCCRRFYALHDPNLNNEEQRNAR IR SIRDDC (SEQ ID NO: 14);
any one to three amino acid residues in the sequence are substituted with one amino acid residue each selected from the group consisting of lysine residues, aspartic acid residues, and glutamic acid residues, which may be the same or different, and
[the compound or salt] comprises (b) an amino acid sequence having 90% or more identity to each of the amino acid sequences of SEQ ID NOs: 11 to 14,

[Claim 8]

The compound or salt thereof according to any one of claims 2 to 6,
wherein the peptide comprises:

Formula 1-1: (X₀₋₃)_a-C-Xaa1-Xaa2-Xaa3-Xaa4-Xaa5- Xaa6-I-I-W-C- (X₀₋₃)_b (SEQ ID NO: 15)

Formula 1-2: (X₀₋₃)_a-C-Xaa 1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa6-I-V-W-C- (X₀₋₃)_b (SEQ ID NO: 16)

Formula 1-3: $(X_{0-3})_a$ -C-Xaa1-Xaa2-Xaa3-Xaa4-Xaa5- Xaa 6-V-V-W-C- $(X_{0-3})_b$ (SEQ ID NO: 17)
 Formula 1-4: $(X_{0-3})_a$ -C-Xaa1-Xaa2-Xaa3-Xaa4-Xaa5- Xaa6-A-V-W-C- $(X_{0-3})_b$ (SEQ ID NO: 18)
 Formula 1-5: $(X_{0-3})_a$ -C-Xaa 1-Xaa2-Xaa3-Xaa4-Xaa5- X a a 6-L-L-W-C- $(X_{0-3})_b$ (SEQ ID NO: 19)
 Formula 1 -6: $(X_{0-3})_a$ -C-X a a 1 -X a a 2-X aa 3-X a a 4-X a a 5-Xaa6-L-I-W-C- $(X_{0-3})_b$ (Sequence number 20)
 Formula 1-7: $(X_{0-3})_a$ -C-Xaa 1-Xaa2-Xaa3-Xaa4-Xaa5- Xa a 6-L-V-F-C- $(X_{0-3})_b$ (Sequence No. 21)
 Formula 1-8: $(X_{0-3})_a$ -C-Xaa1-Xaa2-Xaa3-Xaa4-Xaa5- Xa a 6-Q-V-W-C- $(X_{0-3})_b$ (Sequence number 22)
 Formula 1-9: $(X_{0-3})_a$ -C-Xaa1-Xaa2-Xaa3-Xaa4-Xaa5- Xa a 6-E-V-W-C- $(X_{0-3})_b$ (Sequence number 23);
 wherein

$(X_{0-3})_a$, is absence, or an arginine residue-glycine residue-asparagine residue, an aspartic acid residue, a glycine residue-asparagine residue, or an asparagine residue,

$(X_{0-3})_b$, is absence, or a threonine residue-tyrosine residue-histidine residue, or a threonine residue,

Xaa1 is an alanine residue,

Xaa2 is a tyrosine residue, a tryptophan residue, or a histidine residue,

Xaa3 is a histidine residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, an arginine residue, or a glycine residue,

Xaa4 is a lysine residue, an aspartic acid residue, or a glutamic acid residue,

Xaa5 is a glycine residue, a serine residue, an asparagine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a phenylalanine residue, a tyrosine residue, a tryptophan residue, a histidine residue, a threonine residue, a leucine residue, an alanine residue, a valine residue, an isoleucine residue, or an arginine residue,

Xaa6 is a glutamine residue, a glutamic acid residue, an asparagine residue, or an aspartic acid residue, and

Formula 2-1: $(X_{0-3})_a$ -C-(Xaa1')-(Xaa2')-(Xaa3')-(Xaa4')-(Xaa5')-(Xaa6')-L-V-W-C- $(X_{0-3})_b$
 (SEQ ID NO: 24)

wherein

$(X_{0-3})_a$ and $(X_{0-3})_b$ are the same as the above $(X_{0-3})_a$ and $(X_{0-3})_b$, respectively, and

Xaa1', Xaa2', Xaa3', Xaa4', Xaa5', and Xaa6' are the same as the above Xaa1, Xaa2, Xaa3, Xaa4, Xaa5, and Xaa6, respectively.

[Claim 9]

The compound or salt thereof according to any one of claims 1 to 8,
 wherein the leaving group is (1) a group selected from the group consisting of -O-, -S-, -Se-,
 -SO₂-O-, -SO₂-N(R)-, -SO₂-, -C≡C-CH₂-O-, -N(OR)-, -N(R)-, and
 -O-N(R)- where R is a hydrogen atom or C₁₋₆ alkyl, or (2) heteroarylene.

[Claim 10]

The compound or salt thereof according to any one of claims 1 to ,
 wherein the nucleophilic group is selected from the group consisting of NH₂ in a side chain of a lysine residue,
 OH in a side chain of a tyrosine residue, OH in a side chain of a serine residue, OH in a side chain of a threonine
 residue, and SH in a side chain of a cysteine residue.

[Claim 11]

The compound or salt thereof according to any one of claims 1 to 10, wherein the electrophilic group is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and $-CH_2-$.

[Claim 12]

The compound or salt thereof according to any one of claims 1 to 11, wherein the bioorthogonal functional group is selected from the group consisting of an azide residue, an aldehyde residue, a thiol residue, an alkyne residue, an alkene residue, a halogen residue, a tetrazine residue, a nitron residue, a hydroxylamine residue, a nitrile residue, a hydrazine residue, a ketone residue, a boronic acid residue, a cyanobenzothiazole residue, an allyl residue, a phosphine residue, a maleimide residue, a disulfide residue, a thioester residue, an α -halocarbonyl residue, an isonitrile residue, a sydnone residue, and a selenium residue.

[Claim 13]

The compound or salt thereof according to any one of claims 1 to 12, wherein the bioorthogonal functional group is selected from the group consisting of an azide residue, a thiol residue, an alkyne residue, a maleimide residue, and a disulfide residue.

[Claim 14]

The compound or salt thereof according to any one of claims 1 to 13, wherein the compound represented by the above Formula (I) is a compound represented by Formula (I-1) below:

A-L1-L2-E1-E2-E3-B (I-1)

wherein

A and B are the same as those in the above Formula (I),

L₁ is a bond or a divalent group,

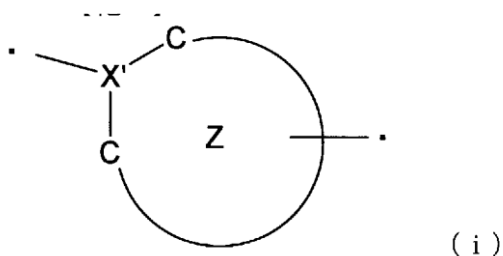
L₂ is a leaving group,

E₁ is an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

E₂ is a group represented by (a) $-X-Y-$ wherein X which binds to E₁, is C (R₁) (R₂) (where R₁, and R₂ are each independently a hydrogen atom or C1-6 alkyl, N (R₃) where R₃ is a hydrogen atom or C1-6 alkyl), O, S, or Se, and Y which binds to E₃ is C (R₄) (R₅) (where R₄ and R₅, are each independently a hydrogen atom or C1-6 alkyl, or

E₂ is a group represented by (b) the following formula (i):

[Formula 1]



where ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁, and ring constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring-constituting atom X' which binds to E₁, is a nitrogen atom, and ring constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond,

when E₂ is $-X-Y-$, E₃ is a divalent group, and when E₂ is a group represented by Formula (i), E₃ is a bond or a divalent group, and

the leaving group has ability to be cleaved and eliminated from E₁, by a reaction between the nucleophilic group and the electrophilic group.

[Claim 15]

The compound or salt thereof according to claim 14,

wherein the L_2 is

(a) ring P-Q- wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{Se}-$, $-\text{SO}_2-\text{O}-$, $-\text{SO}_2-\text{N}(\text{R})-$, $-\text{SO}_2-$, $-\text{C}\equiv\text{C}-\text{CH}_2-\text{O}-$, $-\text{N}(\text{OR})-$, $-\text{N}(\text{R})-$, and $-\text{O}-\text{N}(\text{R})-$

where R is a hydrogen atom or C_{1-6} alkyl,

(b) heteroarylene, or

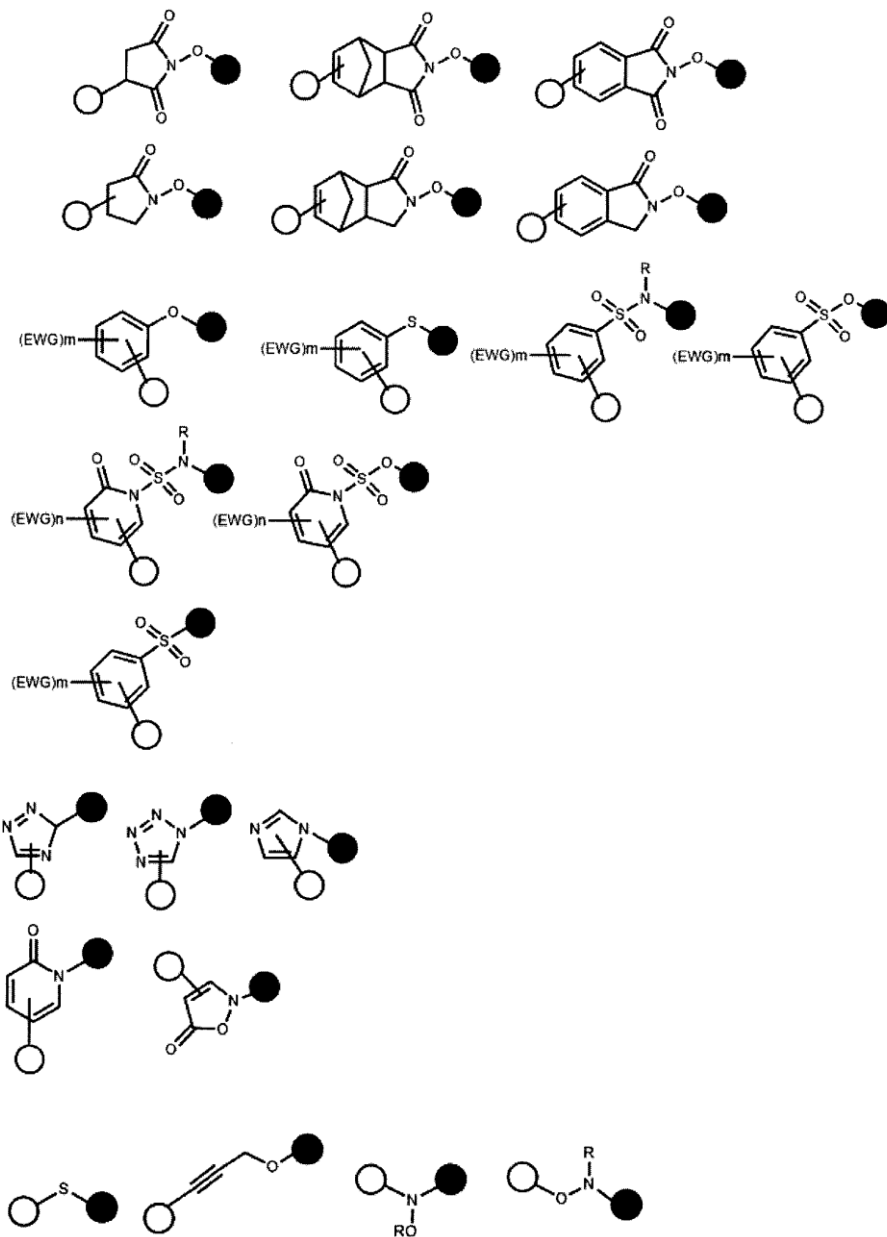
(c) -Q-

wherein Q is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{Se}-$, $-\text{SO}_2-\text{O}-$, $-\text{SO}_2-\text{N}(\text{R})-$, $-\text{SO}_2-$, $-\text{C}\equiv\text{C}-\text{CH}_2-\text{O}-$, $-\text{N}(\text{OR})-$, $-\text{N}(\text{R})-$, and $-\text{O}-\text{N}(\text{R})-$ where R is a hydrogen atom or C_{1-6} alkyl.

[Claim 16]

The compound or salt thereof according to any one of claims 14 to 15,
wherein the L2 is a group selected from the group consisting of the following structural formulae:

[Formula 2]



where EWG is an electron-withdrawing group,

m is an integer of 0 to 4,

n is an integer of 0 to 3,

R is a hydrogen atom or C1-6 alkyl,

◦ is a bond to L₁, and

• is a bond to E₁.

[Claim 17]

The compound or salt thereof according to any one of claims 1 to 16,
wherein a main chain of L₁ or L₁-L₂ coupling A with E consists of 20 or less atoms.

[Claim 18]

The compound or salt thereof according to any one of claims 14 to 17,
wherein the compound represented by the above Formula (I-1) is a compound represented by the following Formula (I-2):

A-L₁-L₂-E₁-X-Y-E₃-B (I-2)

(wherein

A, L₁, X, Y, and B are the same as those in the above Formula (I-1),

L₂ is

(a) ring P—Q— wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)— (R)— where R is a hydrogen atom or C₁-6 alkyl,

(b) heteroarylene, or

(c) —Q— wherein Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)— (R)—, where R is a hydrogen atom or C₁-6 alkyl,

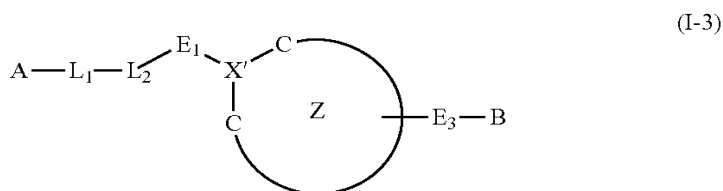
E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and —CH₂—, and E₃ is a divalent group.

[Claim 19]

The compound or salt thereof according to any one of claims 14 to 17,

Wherein the compound represented by the above Formula (I-1) is a compound represented by the following Formula (I-3):

[Formula 3]



wherein

A, L₁, ring Z, and B are the same as those in the above Formula (I-1),

L₂, is

(a) ring P-Q- wherein ring P is selected from the group consisting of an arylene optionally substituted with an electron-withdrawing group, a heteroarylene optionally substituted with an electron-withdrawing group, optionally condensed 2,5-diketopyrrolidine, optionally condensed 2,6 diketopiperidine, optionally condensed 2-ketopyrrolidine, optionally condensed 2-ketopiperidine, and 2-pyridone, and Q is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)—, where R is a hydrogen atom or C₁-6 alkyl,

(b) heteroarylene, or

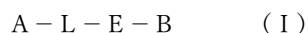
(c) -Q- wherein is selected from the group consisting of —O—, —S—, —Se—, —SO₂—O—, —SO₂—N(R)—, —SO₂—, —C≡C—CH₂—O—, —N(OR)—, —N(R)—, and —O—N(R)—, where R is a hydrogen atom or C₁-6. alkyl,

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and $-CH_2-$, and

E₃ is a bond or a divalent group.

[Claim 20]

A reagent of regioselectively modifying an antibody by a bioorthogonal functional group, the reagent comprising a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

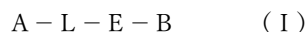
B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof.

[Claim 21]

A method for producing an antibody having a bioorthogonal functional group or bioorthogonal functional groups or a salt thereof, the method comprising:

reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

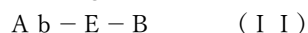
A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):



wherein

E and B are the same as those in the above Formula (I), and

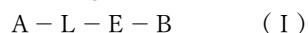
Ab is an antibody, or

a salt thereof.

[Claim 22]

A method for producing an antibody having a functional substance or functional substances or a salt thereof, the method comprising:

(1) reacting a compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):



wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic group and the electrophilic group, or a salt thereof with an antibody to form an antibody having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II):



wherein

E and B are the same as those in the above Formula (I), and Ab is an antibody, or a salt thereof; and

(2) reacting the antibody having a biorthogonal functional group or bioorthogonal functional groups, represented by the above Formula (II), or a salt thereof with a functional substance via the bioorthogonal functional group to form an antibody having a functional substance or functional substances, represented by the following Formula (III):



wherein Ab is the same as that in Formula (II),

E is the same as that in Formula (I),

B' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and

F is a functional substance, or a salt thereof.

[Claim 23]

An antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following Formula (II-1):



wherein

Ab is an antibody,

E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a) -X-Y

wherein X which binds to E₁, is C (R₁) (R₂)

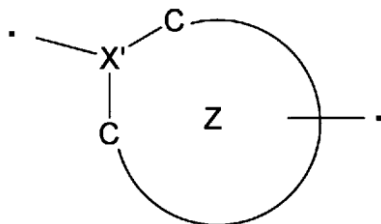
where R₁ and R₂ are each independently a hydrogen atom or C1-6 alkyl, N (R₃) where R₃ is a hydrogen atom or C1-6 alkyl, O, S, or Se, and

Y which binds to E₃ is C (R₄) (R₅)

where R₄ and R₅ are each independently a hydrogen atom or C1-6 alkyl, or

E₂ is a group represented by (b) the following formula (i):

[Formula 4]



wherein ring Z is a divalent cyclic group in which all of a ring-constituting atom X' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring constituting atom X' which binds to E, is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond,

when E₂ is —X—Y—, E₃ is a divalent group, and when E₂ is a group represented by Formula (i), E₃ is a bond or a divalent group, and

B is a bioorthogonal functional group, or

a salt thereof.

[Claim 24]

The antibody or salt thereof according to claim 23,

wherein the antibody is an antibody having a biorthogonal functional group or bioorthogonal functional groups only in a constant region of a monoclonal antibody.

[Claim 25]

The antibody or salt thereof according to claim 23 or 24,

wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in an Fc region of a monoclonal antibody.

[Claim 26]

The antibody or salt thereof according to any of claims 23 to 25,

wherein the antibody is a human IgG regioselectively having a bioorthogonal functional group or bioorthogonal functional groups in a region consisting of amino acid residues at positions 246 to 248 or 288 to 290 in a human IgG Fc region.

[Claim 27]

The antibody or salt thereof according to any of claims 23 to 25,

wherein the antibody represented by the above Formula (II-1) is an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following formula (II-2):



wherein

Ab, X, Y, and B are the same as those in the above Formula (II-1),

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and CH_2 , and

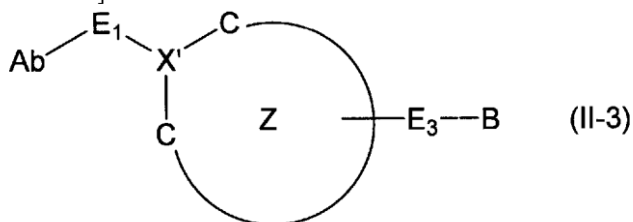
E₃ is a divalent group.

[Claim 28]

The antibody or salt thereof according to any of claims 23 to 26,

Wherein the antibody represented by the above Formula (II-1) is an antibody regioselectively having a bioorthogonal functional group or bioorthogonal functional groups, represented by the following formula (II-3):

[Formula 5]



wherein

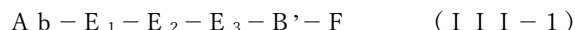
Ab, ring-constituting atom X', ring Z, and B are the same as those in the above Formula (II-1),

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and CH_2 , and

E₃ is a bond or a divalent group.

[Claim 29]

An antibody regioselectively having a functional substance or functional substances, represented by the following Formula (III-1):



wherein

Ab is an antibody,

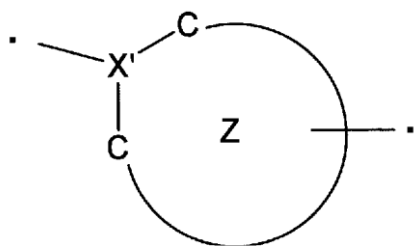
E₁ is an electrophilic group coupled with a nucleophilic group in the antibody,

E₂ is a group represented by (a) $-X-Y-$

wherein X which binds to E₁ is C (R₁) (R₂) where R₁ and R₂ are each independently a hydrogen atom or C₁₋₆ alkyl, N (R₃)

where R₃ is a hydrogen atom or C₁₋₆ alkyl, O, S, or Se, and Y which binds to E₃ is C (R₄) (R₅) where R₄ and R₅ are each independently a hydrogen atom or C₁₋₆ alkyl, or E₂ is a group represented by (b) the following formula (i):

[Formula 6]



wherein ring Z is a divalent cyclic group in which all of a ring-constituting atom X ' which binds to E₁ and ring-constituting atoms on both sides thereof are carbon atoms, or a divalent heterocyclic group in which the ring constituting atom X ' which binds to E₁ is a nitrogen atom, and ring-constituting atoms on both sides of the nitrogen atom are carbon atoms, and • is a bond, E₂ is a divalent group when E₃ is —X—Y—, and is a bond or a divalent group when E₂ is a group represented by Formula (i), B ' is a divalent group comprising a portion formed by a reaction between the functional substance and the bioorthogonal functional group, and F is a functional substance, or a salt thereof.

[Claim 30]

The antibody or salt thereof according to claim 29,

wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in a constant region of a monoclonal antibody.

[Claim 31]

The antibody or salt thereof according to claim 29 or 30,

wherein the antibody is an antibody having a bioorthogonal functional group or bioorthogonal functional groups only in an Fc region of a monoclonal antibody.

[Claim 32]

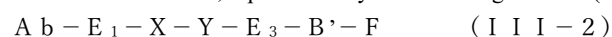
The antibody or salt thereof according to any of claim 29 to 31,

wherein the antibody is a human IgG regioselectively having a functional substance or functional substances in a region consisting of amino acid residues at positions 246 to 248 or 288 to 290 in a human IgG Fc region.

[Claim 33]

The antibody or salt thereof according to any of claim 29 to 32,

wherein the antibody represented by the above Formula (III-1) is an antibody regioselectively having a functional substance or functional substances, represented by the following formula (III-2):



wherein

Ab, X, Y, B ', and F are the same as those in the above Formula (III-1),

E₁ is selected from the group consisting of —C(=O)—, —SO₂—, and CH₂, and

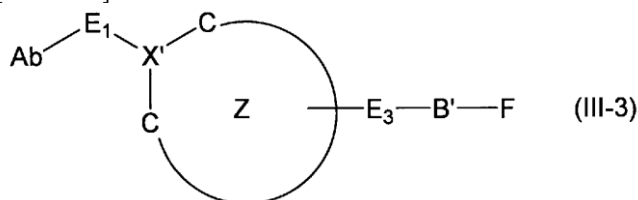
E₃ is a divalent group.

[Claim 34]

The antibody or salt thereof according to any of claim 29 to 32,

Wherein the antibody represented by the above Formula (III-1) is an antibody regioselectively having a functional substance or functional substances, represented by the following formula (III-3):

[Formula 7]



wherein

Ab, ring-constituting atom X', ring Z, B', and F are the same as those in the above Formula (III-1),

E₁ is selected from the group consisting of $-C(=O)-$, $-SO_2-$, and CH_2 , and

E₃ is a divalent group.

[Document Name] Abstract

[Abstract]

[Problem] To develop technology to enable the modification of antibodies, in particular the site-selective modification of antibodies.

[Solution Means] A compound having an affinity substance to an antibody and a bioorthogonal functional group, represented by the following Formula (I):

A-L-E-B (I)

wherein

A is an affinity substance to an antibody,

L is a divalent group comprising a leaving group,

E is a divalent group comprising an electrophilic group (i) coupled with the leaving group and (ii) having ability to react with a nucleophilic group in the antibody,

B is a bioorthogonal functional group, and

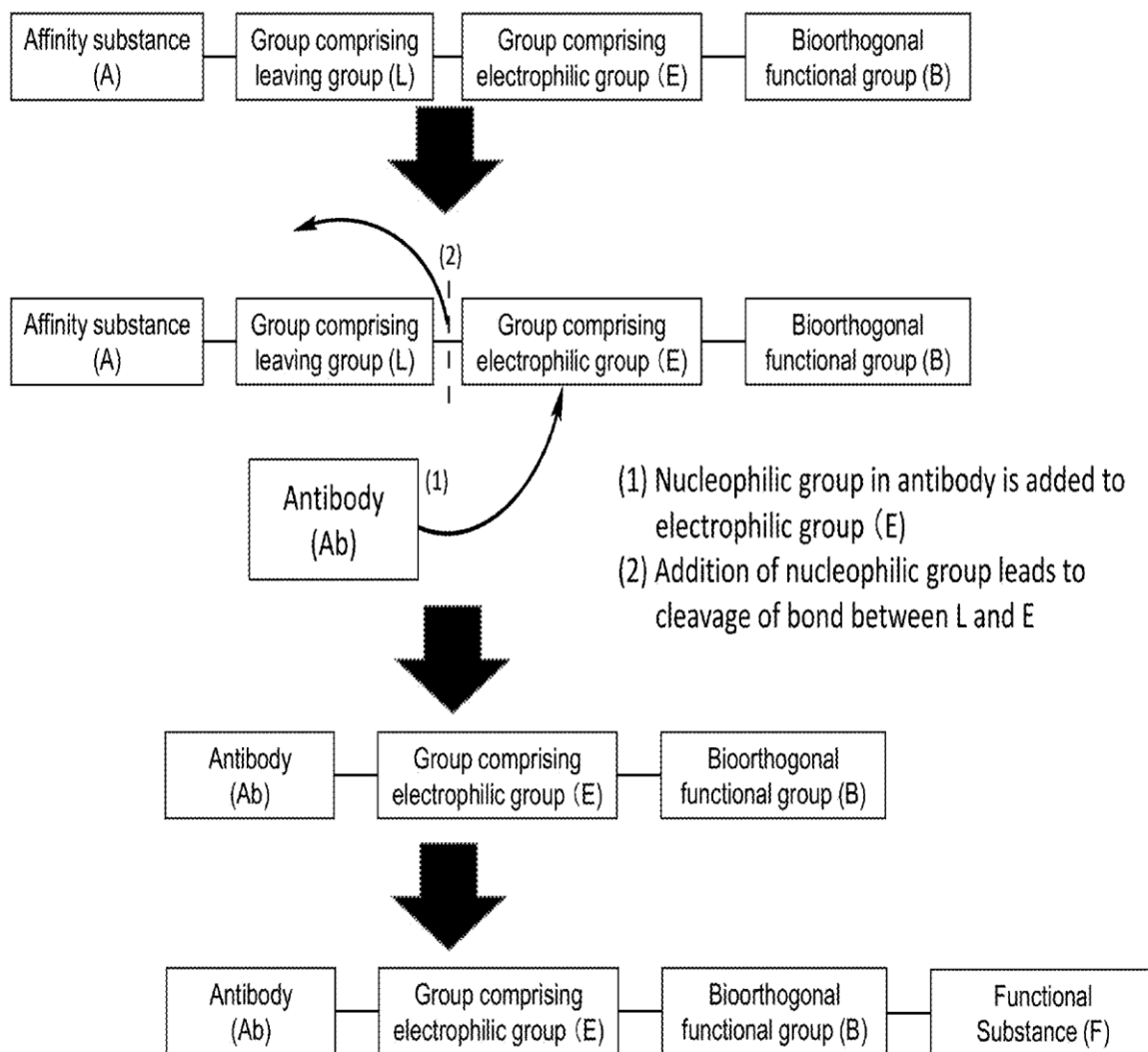
the leaving group has ability to be cleaved and eliminated from E by a reaction between the nucleophilic

group and the electrophilic group, or

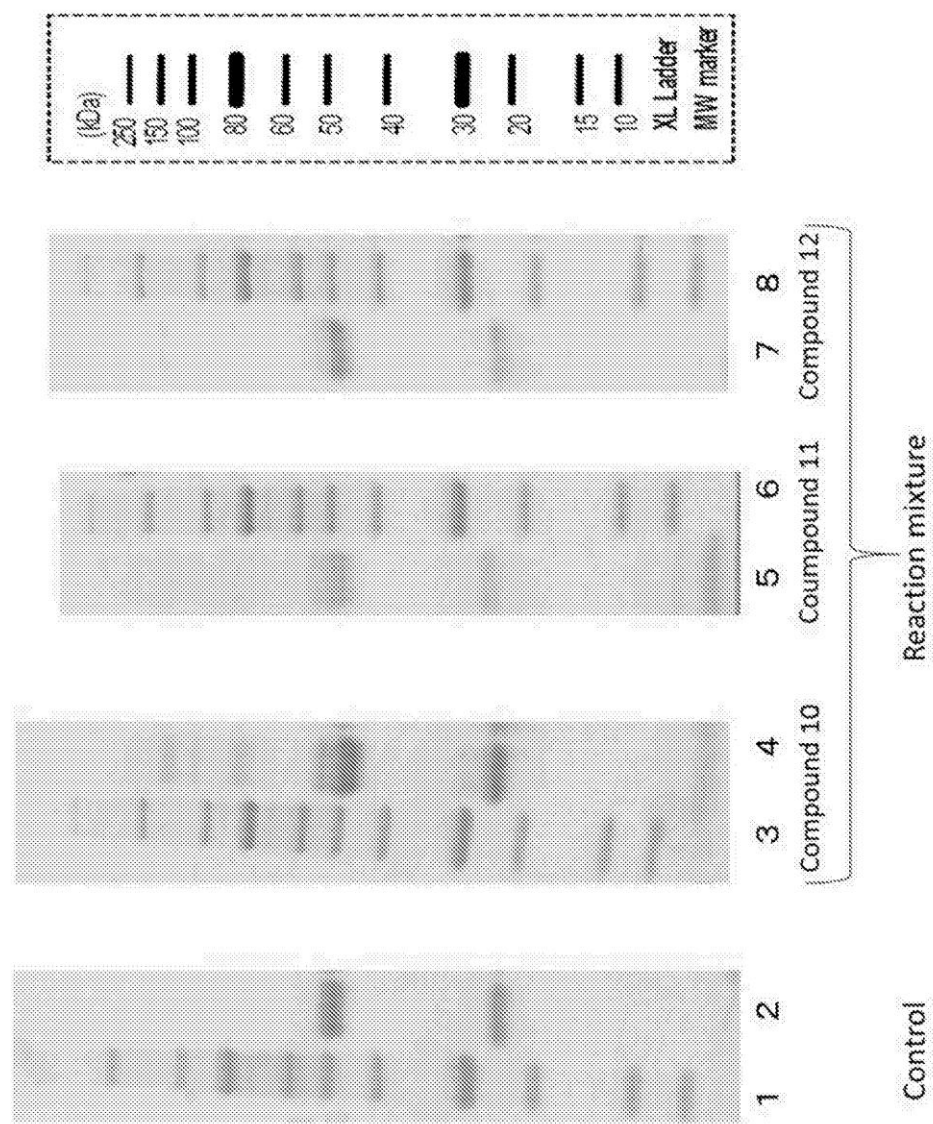
a salt thereof.

[Selected Drawing] FIG. 1

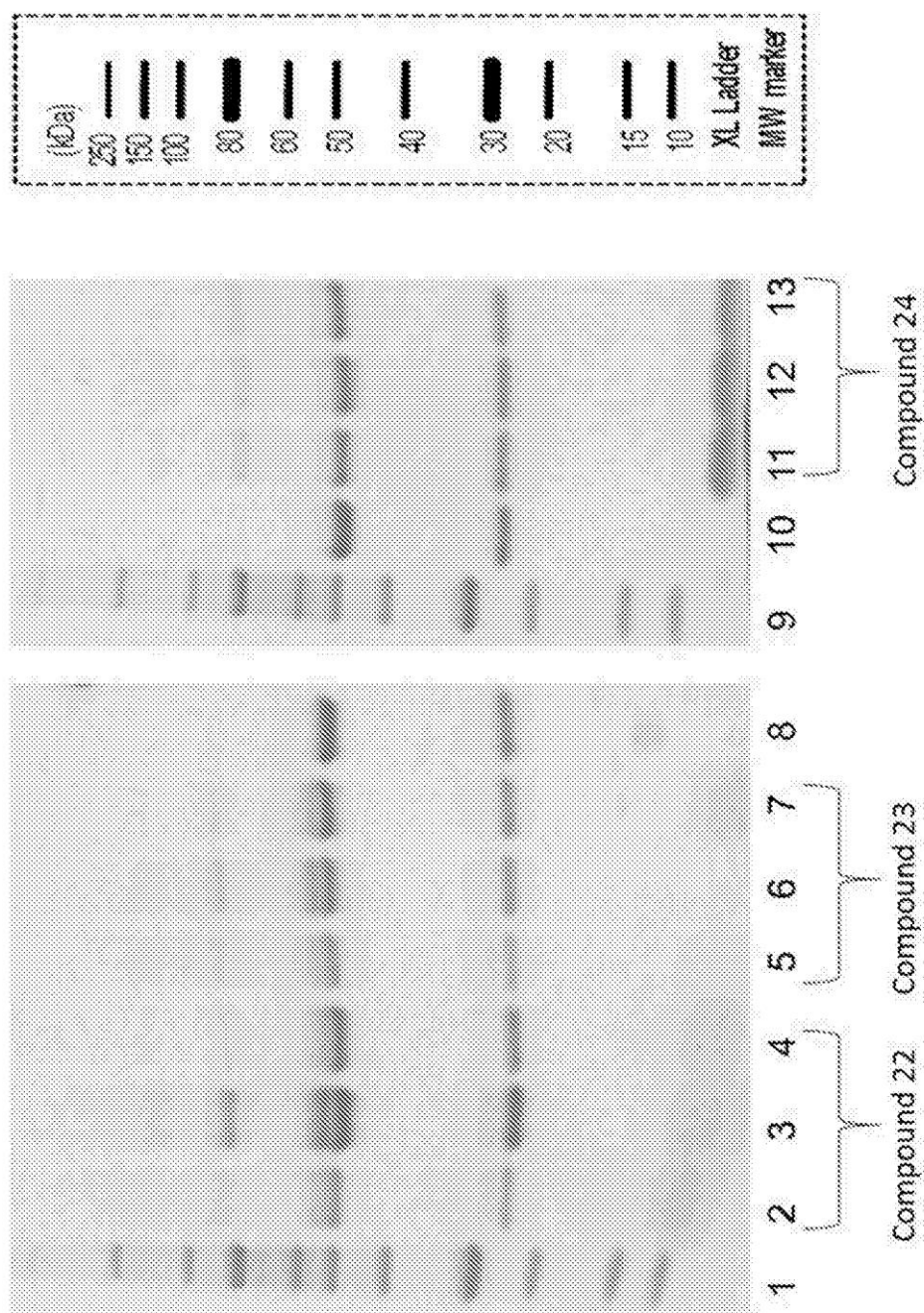
[FIG. 1]



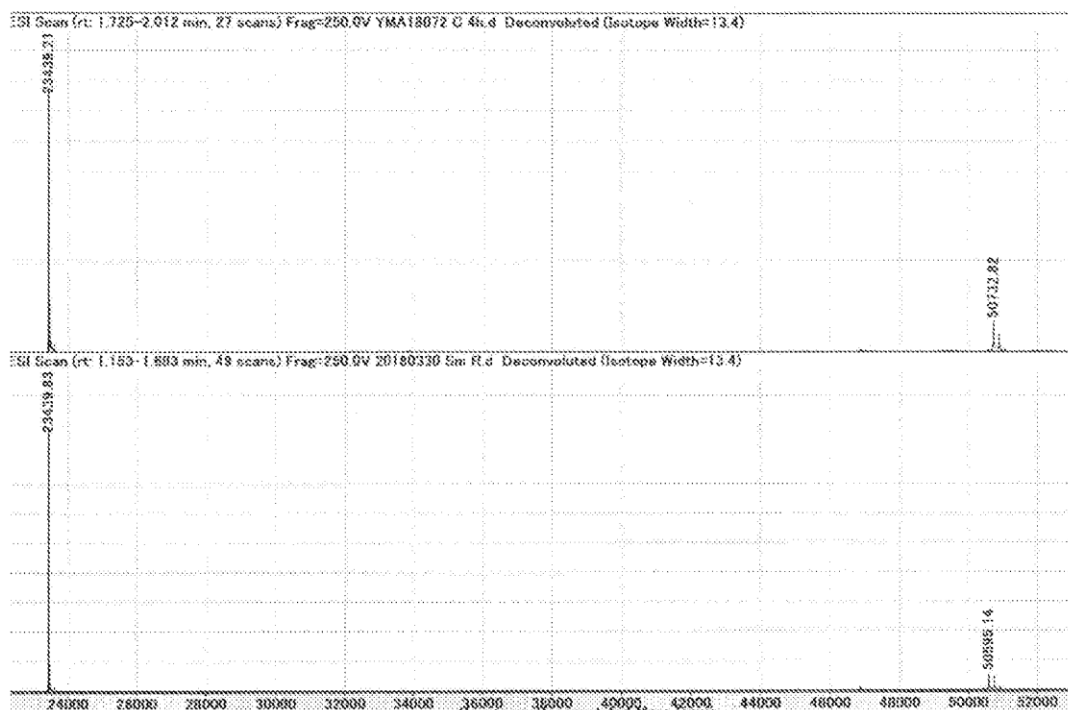
[FIG. 2]



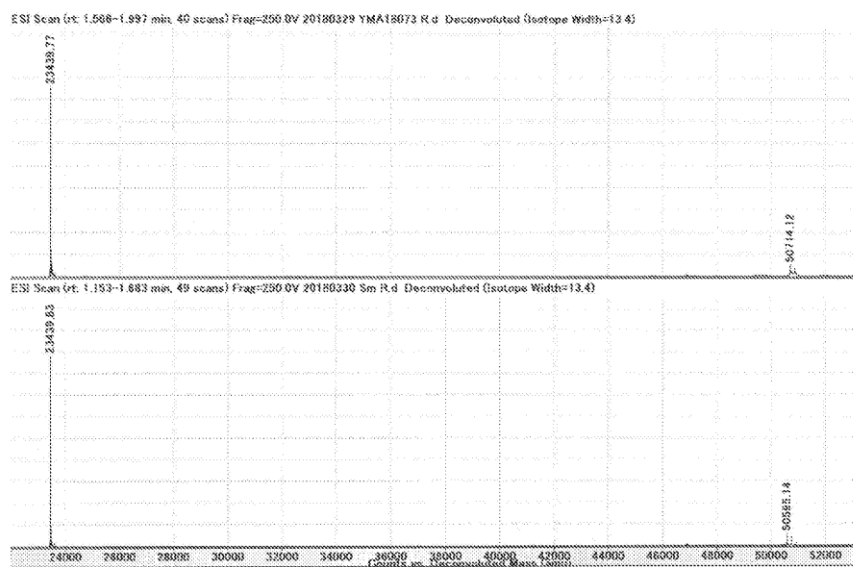
[FIG. 3]



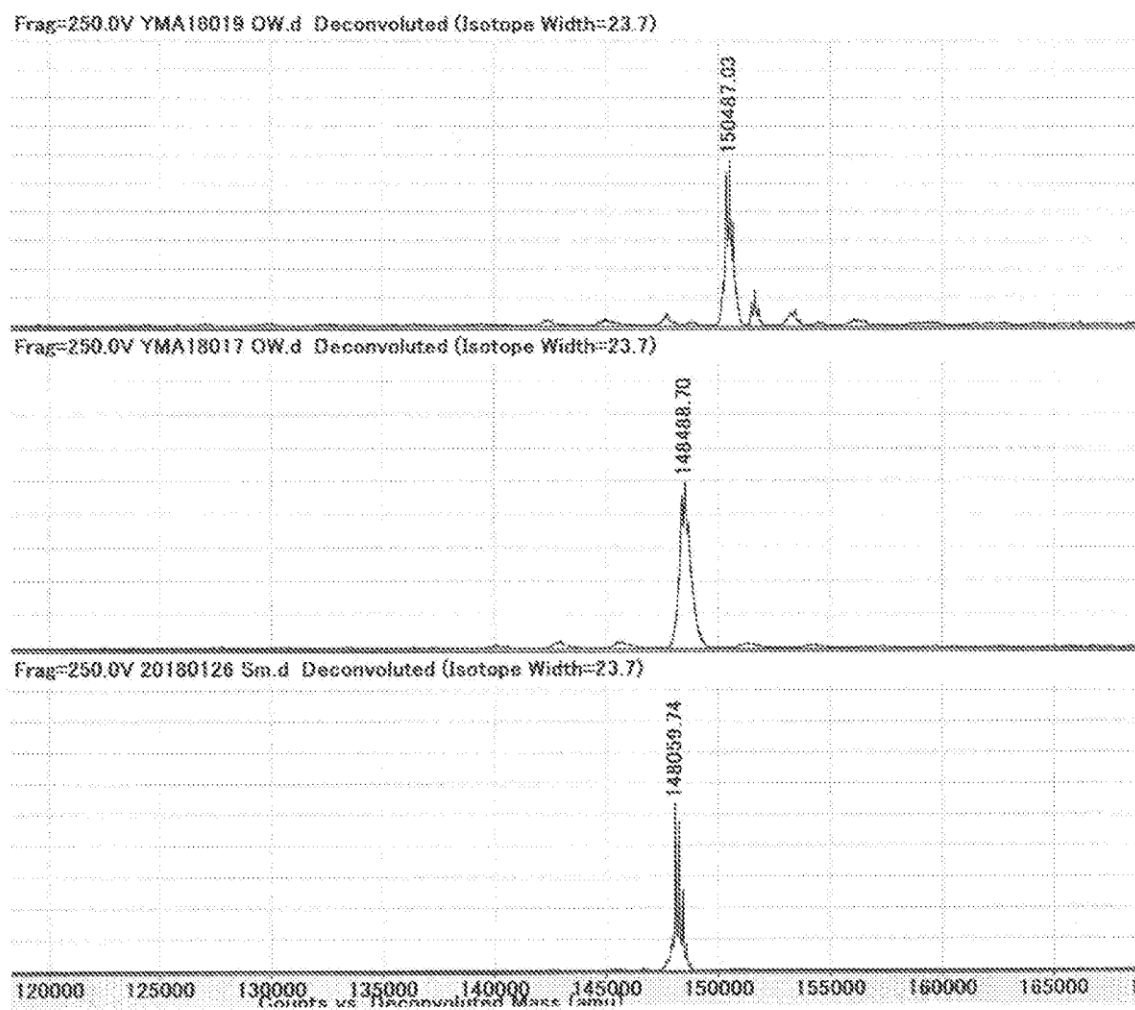
[FIG. 4]



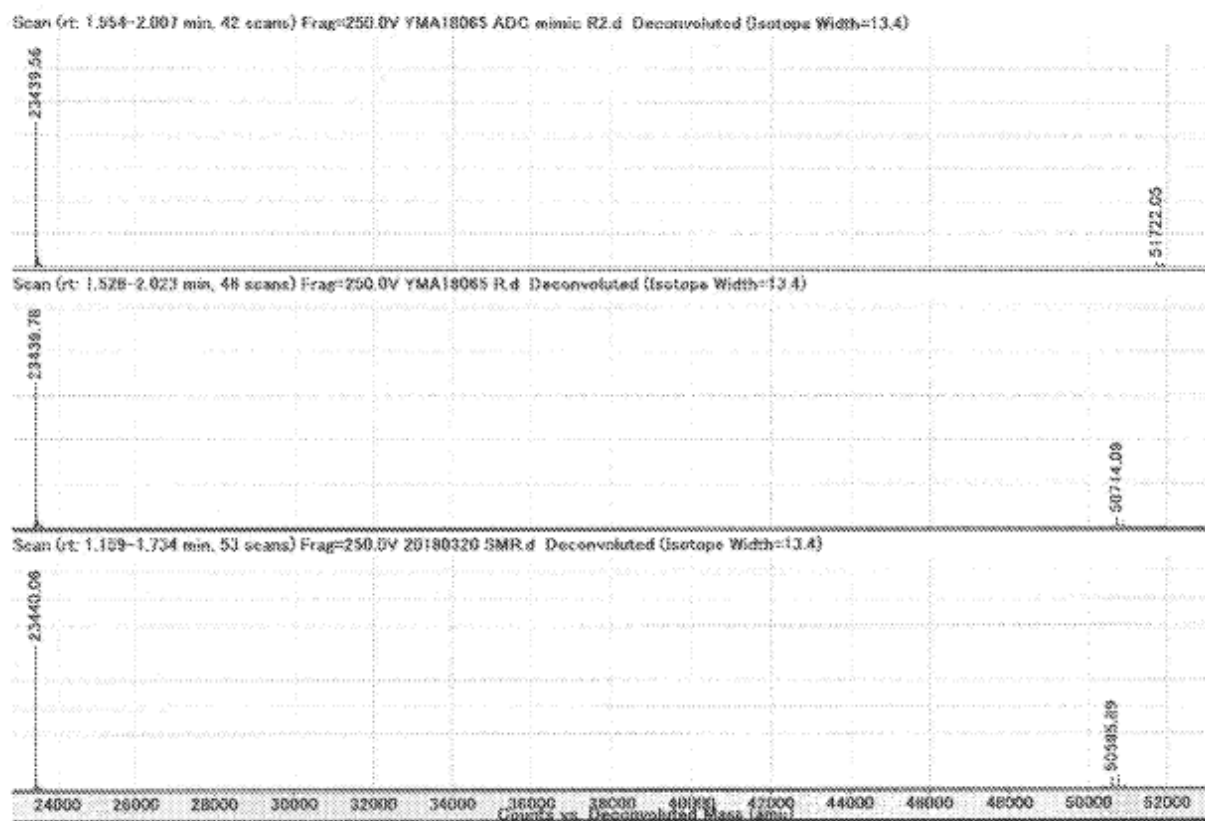
[FIG. 5]



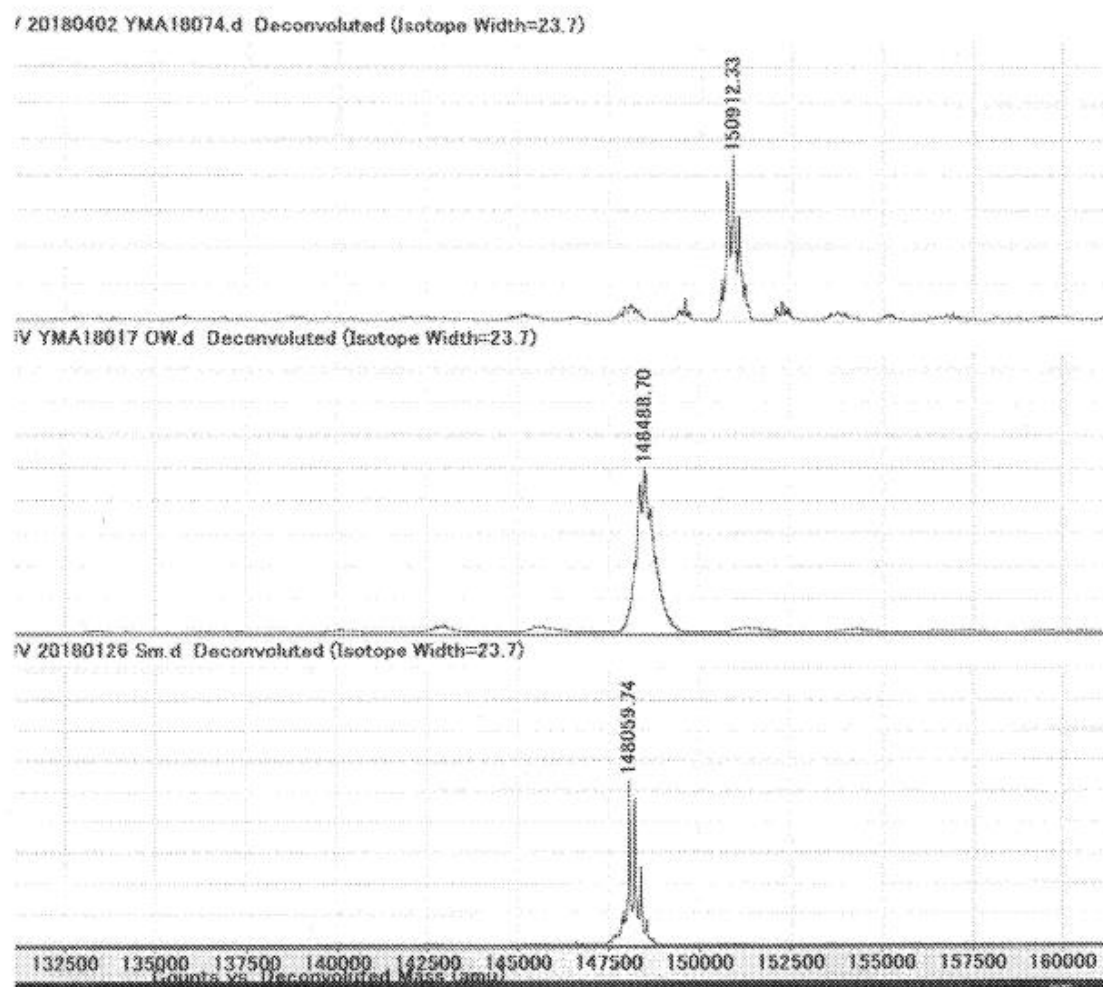
[FIG. 6]



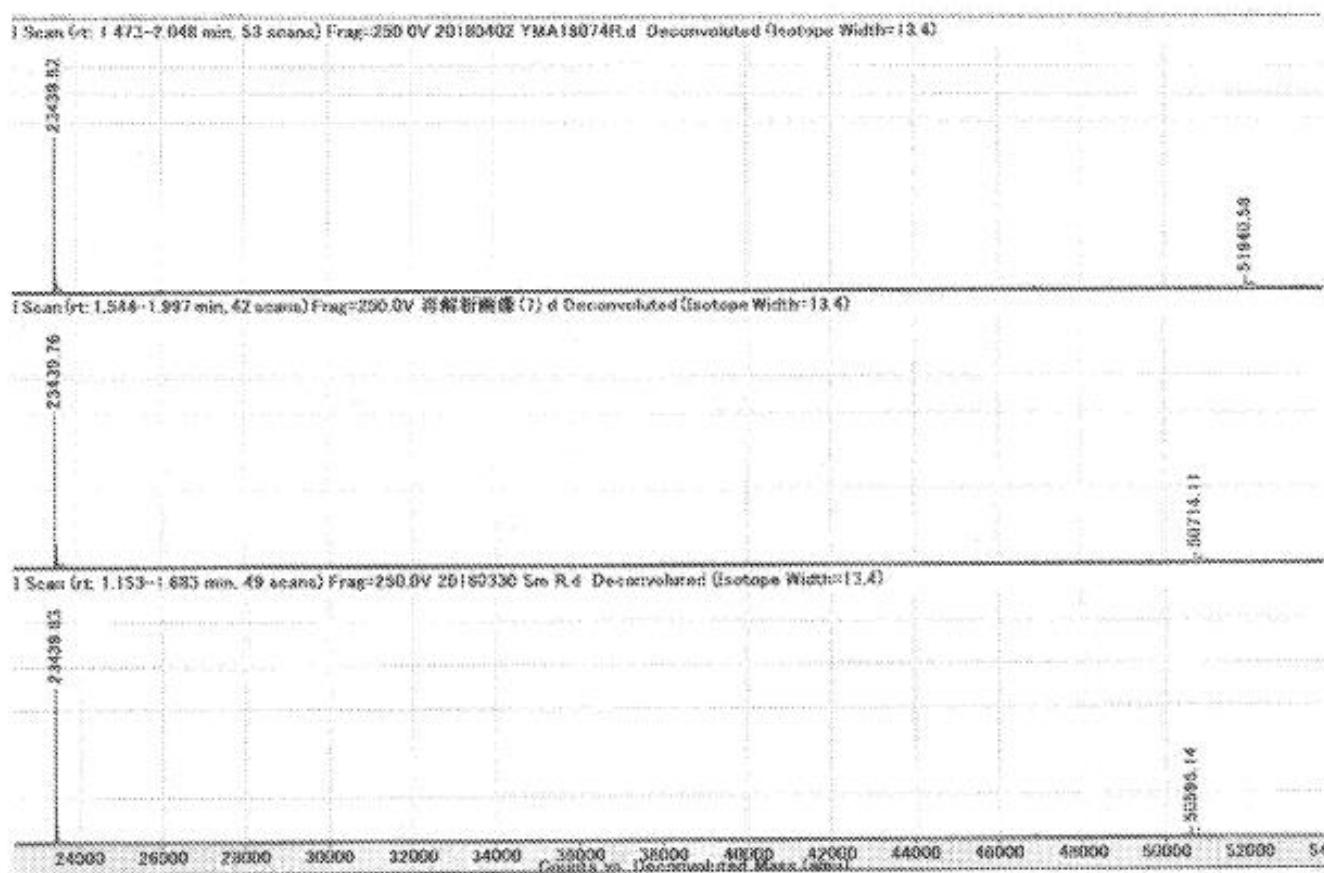
[FIG. 7]



[FIG. 8]



[FIG. 9]



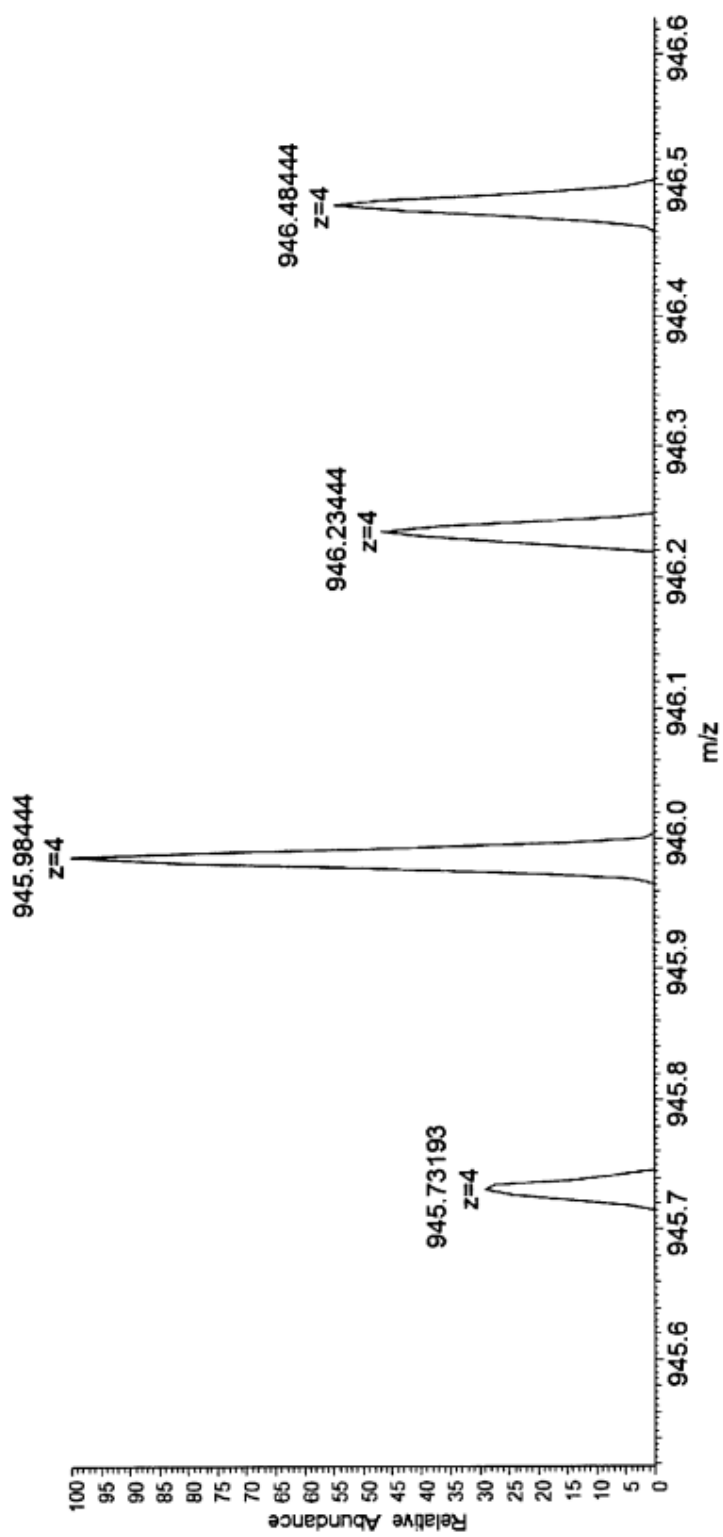
(1) Amino acid sequence of heavy chain of trastuzumab

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA
PGKGLEWVARIIYPTNGYTRYADSVKGRFTISADTSKNTAY
LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYDSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
LSDSGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYT
QKSLSLS PG (SEQ ID NO: 2)

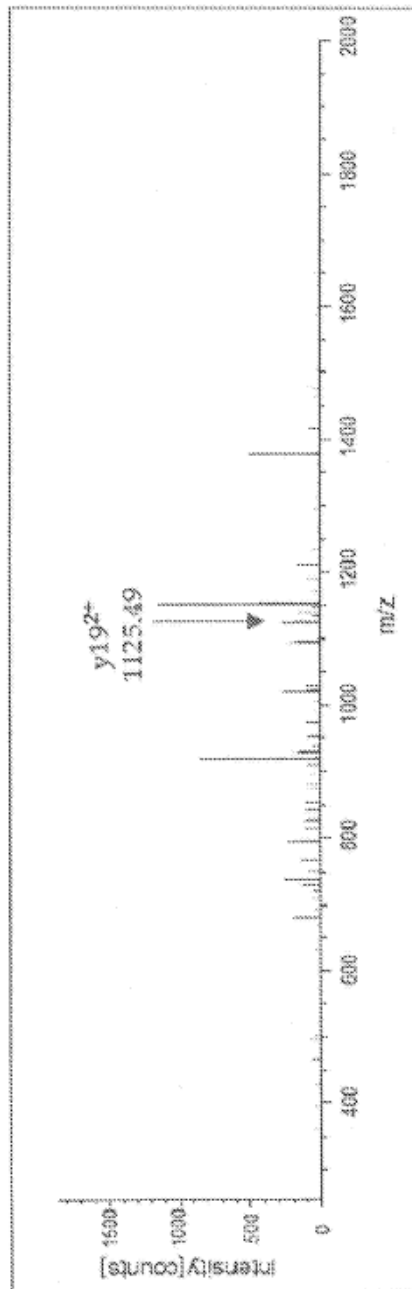
(2) Amino acid sequence of light chain of trastuzumab

DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKP
GKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQP
EDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC (SEQ ID NO: 4)

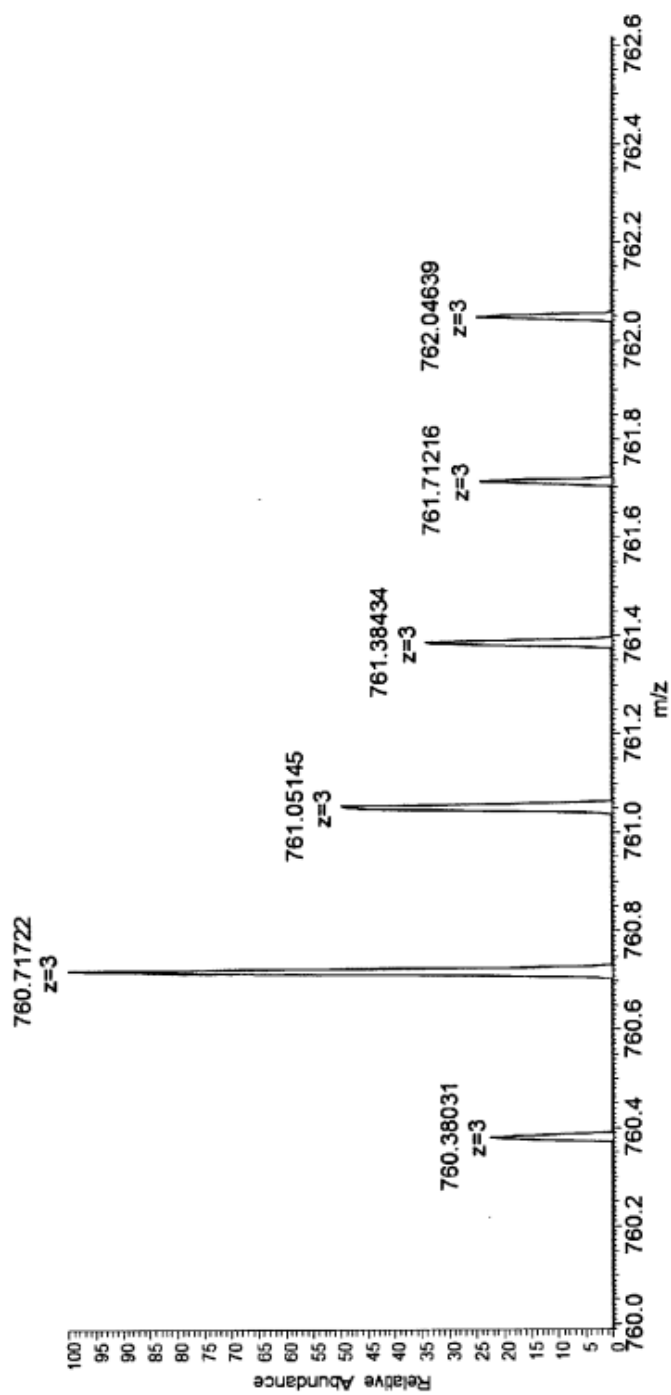
[FIG. 11]



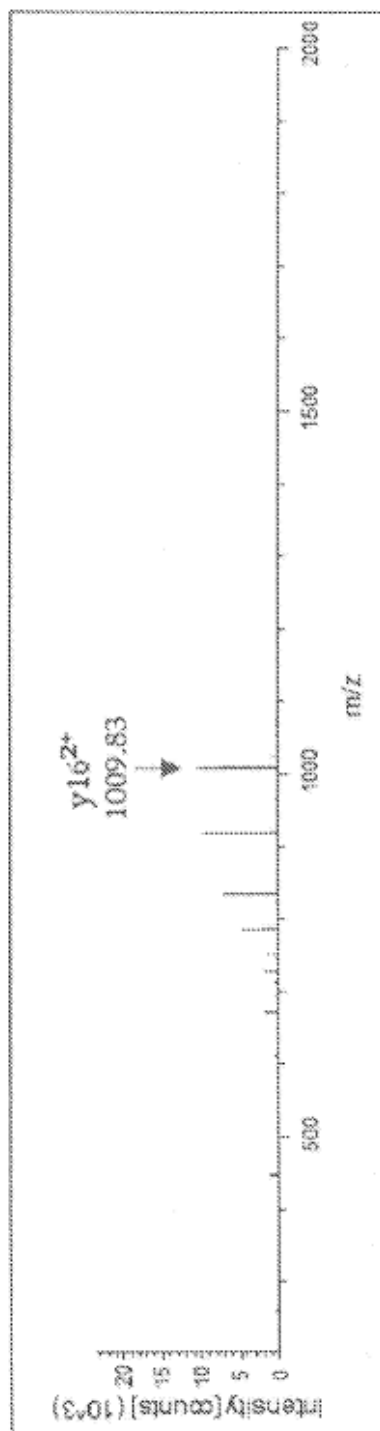
[FIG. 12]



[FIG. 13]



[FIG. 14]



[FIG. 15]

(1) Consensus amino acid sequence between an Fc region in a heavy chain of trastuzumab and an IgG1 Fc region

PKSCDKTHTCPCPAPEXXGXPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
DSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRXEXTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKXTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFS CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 1)

(2) Amino acid sequence of an IgG1 Fc region

IEGRMDPKSCDKTHTCPCPAPEAEGAPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYDSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKATPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 3)