Description

Title: PEPTIDES, PEPTIDE COMPLEXES, PHARMACEUTICAL COMPOSITIONS, CELL CULTURE COMPOSITIONS, AND COMPOSITIONS FOR MEDICAL, DIAGNOSTIC, OR RESEARCH USE

Technical Field

[0001]

The present invention relates to peptides, peptide complexes, pharmaceutical compositions, cell culture compositions, and compositions for medical, diagnostic, or research use.

Background Art

[0002]

Thrombopoietin (TPO) is a cytokine mainly produced in the liver and kidneys, and signal transduction by TPO via the TPO receptor (TPOR or c-MPL) plays an important role in the regulation of megakaryopoiesis and platelet production, and in the maintenance and self-renewal of hematopoietic stem cells. Therefore, research has been conducted to search for TPO substitutes or TPOR agonists that activate the TPO/TPOR signaling pathway for the purpose of treating blood disorders involving megakaryopoiesis and platelet production.

Recombinant TPO was proven to be effective in the treatment of thrombocytopenia, but side effects such as thrombocytopenia and pancytopenia due to the production of neutralizing antibodies against TPO were reported. In recent years, it has been reported that TPOR agonists mainly composed of low-molecular-weight compounds are effective in the treatment of thrombocytopenia (Non Patent Literature 1, Non Patent Literature 2). Among these, some have been approved as therapeutic agents for immune thrombocytopenia, thrombocytopenia caused by hepatitis C, bone marrow failure, and the like, but side effects such as thrombocytosis, thrombosis, bone marrow reticulin fibrosis, and recurrence of thrombocytopenia have also been reported (Non Patent Literature 3).

On the other hand, in addition to treatment methods in which a TPOR agonist is directly administered to a patient, treatment methods have been studied in which megakaryocytes and platelets are efficiently induced to differentiate from iPS cells ex vivo, and platelets are stably supplied. In this case, it has been reported that the use of a low-molecular-weight compound serving as a TPO substitute is effective (Patent Document 1).

It has been reported that, when TPO binds to TPOR, it dimerizes TPOR, transmits signals into cells, and expresses physiological actions (Non Patent Literature 4, Non Patent Literature 5). In recent years, TPO agonist antibodies utilizing such properties of TPOR have been reported (Non Patent Literature 6, Patent Document 2, Patent Document 3).

Citation List

Patent Literature

[0003]

Patent Literature 1: WO2013/051625

Patent Literature 2: WO2002/033072

Patent Literature 3: JP-B-4708190

Non Patent Literature

[0004]

Non Patent Literature 1: Blood 2006, 107, 4300.

Non Patent Literature 2: Experimental Hematology 2005, 33, 85.

Non Patent Literature 3: Drug 2021, 81, 1285.

Non Patent Literature 4: Science 2020, 367, 643.

Non Patent Literature 5: EMBO J. 2011, 30, 4398.

Non Patent Literature 6: PNAS 2021, 118, e2017849118.

Summary of Invention

Technical Problem

[0005]

An object of the present invention is to provide a peptide having a thrombopoietin receptor binding ability, a peptide complex having an intracellular signaling activation ability, or a peptide complex having a TPO/TPOR signal activating ability.

Means for solving the technical problem

[0006]

As a result of extensive studies conducted by the present inventors in order to solve the above problems, the present inventors found that a specific peptide has a thrombopoietin receptor binding ability, that a peptide complex containing a specific peptide has an intracellular signaling activation ability, and that a peptide complex containing a specific peptide has a

TPO/TPOR signal activating ability, thereby completing the present invention.

That is, aspects of the present invention are as follows.

[0007]

[1]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising:

an amino acid sequence represented by Formula A1; or

an amino acid sequence derived from the amino acid sequence represented by Formula A1, wherein a plurality of amino acid residues are substituted, deleted, added, or inserted.

A1: X^1-X^2-X^3-MeG-X^4-X^5-X^6-X^7-df-X^8-X^9-X^{10}-X^{11}-X^{12}-MeC

wherein,

X^1 is any amino acid residue,

X^2 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

X^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members which may be substituted,

X^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^{11} is any amino acid residue, and

X^{12} is any amino acid residue,

wherein, in Formula A1, the amino acid sequence is described from an N-terminus to a C-terminus.

[2]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [1],

wherein, in Formula A1,

X^1 is F, A, Q, E, F4COO, F3Me, F3C, F4Me, F4C, F4OMe, 3Py, Cha, or F4aao,

X^2 is W, W7N, W1Me7N, or W1aa,

X^3 is V, R, Cit, T, alT, or S,

X^4 is W, W1Me, or Nal1,

X^5 is V, T, alT, or dMeS,

X^6 is V, I, alI, Eva, Tbg, or Gcpe,

X^7 is MeG, MeA, Meda, MeKCOpipzaa, dp, EtG, or EtA,

X^8 is R, Q, or KCOpipzaa,

X^9 is V, T, alT, P, Hpr, Hyp, or Mor,

X^{10} is I, V, Gthp, or G4pipaa,

X^{11} is S, E, I, F, W, Y, R, Cit, Atp, KCOpipzaa, or Hgl, and

X^{12} is D, H, R, S, Q, SMe, Cit, E, or Hgl.

[3]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising: an amino acid sequence represented by Formula A2; or an amino acid sequence derived from the amino acid sequence represented by Formula A2, wherein 1 to 12 amino acid residues at one or more of positions 1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, and 14 are substituted, deleted, added or inserted.

A2: F-W-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 1).

[4]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [1],

wherein, in Formula A1,

X^1 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^2 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^4 is an amino acid residue having, in a side chain, an aryl group which may be

substituted,

X^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

X^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group, or a cyclic secondary amino acid residue having 4 to 6 members,

X^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^{11} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, and

X^{12} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

[5]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [4],

wherein, in Formula A1,

X^1 is F or F4Me,

X^2 is W or W7N,

X^3 is V, T, or alT,

X^4 is W or Nal1,

X^5 is V,

X^6 is V, I, alI, or Gcpe,

X^7 is MeG, MeA, Meda, MeKCOpipzaa, or dp,

X^8 is R,

X^9 is V or P,

X^{10} is I or V,

X^{11} is S, KCOpipzaa, or Hgl, and

X^{12} is D.

[6]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [1] to [5], wherein the peptide is a cyclic peptide.

[7]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [1] to [5], wherein the peptide has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in Formula A1 or Formula A2 contained in the peptide, are bound.

[8]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [1] to [5], further comprising an additional amino acid residue.

[9]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [4] or [5], wherein the peptide has TPO receptor binding ability.

[10]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising:

an amino acid sequence represented by Formula B1; or

an amino acid sequence derived from the amino acid sequence represented by Formula B1, wherein a plurality of amino acid residues are substituted, deleted, added, or inserted,

B1: Y^1-Y^2-Y^3-MeG-Y^4-Y^5-Y^6-Y^7-df-R-Y^8-Y^9-Y^{10}-D-MeC

wherein,

Y^1 is any amino acid residue,

Y^2 is any amino acid residue,

Y^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

Y^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

wherein, in Formula B1, the amino acid sequence is described from the N-terminus to the C-terminus.

[11]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [10],

wherein, in Formula B1,

Y^1 is F, F4aao, F3COO, F3aao, 4Py, 3Py, or Cha,

Y^2 is Ahp, W1Me7N, W, or W7N,

Y^3 is V, I, alI, Q, T, TMe, or KCOpipzaa,

Y^4 is W, W1Me, Nal1, or Nal2,

Y^5 is V, Tbg, TMe, alTMe, dMeS, Gthp, G4pipaa, or Cit,

Y^6 is V, Ahp, Nle, I, L, Tbg, IMe, Cbg, Gcpe, Chg, or alTMe,

Y^7 is MeG, EtG, MeA, Meda, dp, MeQ, or Medq,

Y^8 is V, P, Hpr, Tic, TMe, or alTMe,

Y^9 is I, alI, alTMe, dMeS, Gthp, or G4pipaa, and

Y^{10} is S or Hgl.

[12]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [10], wherein the peptide comprises: an amino acid sequence represented by Formula B2; or an amino acid sequence derived from the amino acid sequence represented by Formula B2, wherein 1 to 10 amino acid residues at one or more of positions 1, 2, 3, 5, 6, 7, 8, 11, 12, and 13 are substituted, deleted, added or inserted,

B2: F-Ahp-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 97).

[13]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [10] to [12], wherein the peptide is a cyclic peptide.

[14]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [10] to [12], wherein the peptide has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in Formula B1 or Formula B2 contained in the peptide, are bound.

[15]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [10] to [12], further comprising an additional amino acid residue.

[16]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [10] to [12], wherein the peptide has TPO receptor binding ability.

[17]

A peptide complex, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, comprising a first peptide and a second peptide,

wherein the first peptide and the second peptide are identical to or different from each other, and are each the peptide according to [1] or [10].

[18]

The peptide complex, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof according to [17], wherein the peptide complex comprises the first peptide, the second peptide, and a linker connecting the first peptide and the second peptide, and wherein the second peptide is identical to or different from the first peptide and has an amino acid sequence represented by Formula A1 or an amino acid sequence consisting of a sequence resulting from substitution, deletion, addition, or insertion of a plurality of amino acid residues in the amino acid sequence represented by Formula A1.

[19]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the first peptide and the second peptide have a sequence identity of 90% or more and 100% or less.

[20]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the first peptide and the second peptide are substantially the same peptide.

[21]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the first peptide and the second peptide are each a cyclic peptide.

[22]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the first peptide and the second peptide each have a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC contained in the first peptide or the second peptide are bound.

[23]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the first peptide and the second peptide further comprise additional amino acid residues.

[24]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the C-terminus of the first peptide and the C-terminus of the second peptide are linked via the linker.

[25]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the linker is a PEG linker or a linker consisting of PEG and an amino acid residue.

[26]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18], wherein the peptide complex has intracellular signaling activation ability.

[27]

A pharmaceutical composition comprising the peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [1] to [5] and [10] to [12].

[28]

A pharmaceutical composition comprising the peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18].

[29]

A cell culture composition used for cell culture, comprising the peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18].

[30]

A composition for medical, diagnostic, or research use, comprising the peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to [18].

[31]

A composition for medical, diagnostic, or research use, comprising the peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of [1] to [5] and [10] to [12].

Advantageous Effects of Invention

[0008]

The present invention provides a peptide having a thrombopoietin receptor binding ability, a peptide complex having an intracellular signaling activation ability, or a peptide complex having a TPO/TPOR signal activating ability.

Description of Embodiments

[0009]

Hereinafter, embodiments of the peptide, peptide complex, pharmaceutical composition, composition for cell culture, and composition for medical, diagnostic, or research use of the present invention will be described in detail. However, the present invention is not limited to the embodiments described below, and various modifications can be made without departing from the gist of the present invention.

[0010]

[Abbreviations (General)]

Å as angstrom (unit);

AcOH as acetic acid;

BSA as bovine serum albumin;

Boc as tert-butoxycarbonyl group;

ClAc as chloroacetyl;

ClAcOSu as (2,5-dioxopyrrolidin-1-yl) 2-chloroacetate (CAS No.: 27243-15-8);

cPEG1c as 3,3′-oxydipropionic acid (CAS No.: 5961-83-1);

CV as column volume;

DCM as dichloromethane or methylene chloride;

DIPCI or DIC as N,N′-diisopropylcarbodiimide;

DIPEA or DIEA as N,N-diisopropylethylamine;

DMA as N,N-dimethylacetamide;

Dmb as dimethoxybenzyl group;

DMEM as Dulbecco's Modified Eagle Medium;

DMF as N,N-dimethylformamide;

DMSO as dimethyl sulfoxide;

DODT as 3,6-dioxa-1,8-octanedithiol;

EC50 as 50% effective concentration;

FBS as fetal bovine serum;

Fmoc as 9-fluorenylmethyloxycarbonyl;

Fmoc-Lys(Fmoc)-OH as N^2,N^6-bis((((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine;

g as gram (unit);

HATU as O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate;

HPLC as high performance liquid chromatography;

LC-MS or LC/MS as liquid chromatography mass spectrometer;

M as molar (unit);

MeCN as acetonitrile;

mg as milligram (unit);

min as minute (unit);

mL as milliliter (unit);

mM as millimolar (unit);

mm as millimeter (unit);

Mpe group as O-3-methyl-pent-3-yl group;

NHS as N-hydroxysuccinimide;

NHS-cPEG1c-NHS as bis(2,5-dioxopyrrolidin-1-yl) 3,3′-oxydipropionate (CAS No.: 65869-64-9);

nm as nanometer (unit);

μL as microliter (unit);

OSu as succinimide;

Pbf as 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl group;

PEG as polyethylene glycol;

rpm as revolutions per minute (unit);

Sub as dibenzosuberyl group;

tBu as tert-butyl group;

TEAA as triethylamine acetate;

TFA as trifluoroacetic acid;

TIS as triisopropylsilane;

Trt or Tr as trityl group;

[Abbreviations (Unnatural amino acids)]

3Py: (S)-2-amino-3-(pyridin-3-yl)propanoic acid (CAS No.: 64090-98-8)

4Py: (S)-2-amino-3-(pyridin-4-yl)propanoic acid (CAS No.: 37535-49-2)

Ahp: (S)-2-aminoheptanoic acid (CAS No.: 44902-02-5)

alI: L-alloisoleucine (CAS No.: 1509-34-8)

alT: L-allothreonine (CAS No.: 28954-12-3)

alTMe: O-methyl-L-allothreonine (CAS No.: 104195-80-4)

Atp: (S)-2-amino-3-(tetrahydro-2H-pyran-4-yl)propanoic acid (CAS No.: 1344910-91-3)

Cbg: (S)-2-amino-2-cyclobutylacetic acid (CAS No.: 49607-08-1)

Cha: (S)-2-amino-3-cyclohexylpropanoic acid (CAS No.: 27527-05-5)

Chg: (S)-2-amino-2-cyclohexylacetic acid (CAS No.: 14328-51-9)

Cit: (S)-2-amino-5-ureidopentanoic acid (CAS No.: 372-75-8)

de: D-glutamic acid (CAS No.: 6893-26-1)

df: D-phenylalanine (CAS No.: 673-06-3)

dMeS: (S)-2-amino-3-hydroxy-3-methylbutanoic acid (CAS No.: 2280-27-5)

dp: D-proline (CAS No.: 344-25-2)

EtA: ethyl-L-alanine (CAS No.: 65278-03-7)

EtG: ethylglycine (CAS No.: 627-01-0)

Eva: (S)-2-amino-3-ethylpentanoic acid (CAS No.: 14328-49-5)

F3aao: (S)-2-amino-3-(3-(carboxymethoxy)phenyl)propanoic acid (CAS No.: 2973751-62-9)

F3C: (S)-2-amino-3-(3-chlorophenyl)propanoic acid (CAS No.: 80126-51-8)

F3COO: (S)-3-(2-amino-2-carboxyethyl)benzoic acid (CAS No.: 13861-02-4)

F3Me: (S)-2-amino-3-(m-tolyl)propanoic acid (CAS No.: 114926-37-3)

F4aao: (S)-2-amino-3-(4-(carboxymethoxy)phenyl)propanoic acid (CAS No.: 24558-63-2)

F4C: (S)-2-amino-3-(4-chlorophenyl)propanoic acid (CAS No.: 14173-39-8)

F4COO: (S)-4-(2-amino-2-carboxyethyl)benzoic acid (CAS No.: 126109-42-0)

F4Me: (S)-2-amino-3-(p-tolyl)propanoic acid (CAS No.: 1991-87-3)

F4OMe: (S)-2-amino-3-(4-methoxyphenyl)propanoic acid (CAS No.: 6230-11-1)

G4pipaa: (S)-2-amino-2-(1-(carboxymethyl)piperidin-4-yl)acetic acid (CAS No.: N/A)

Gcpe: (S)-2-amino-2-cyclopentylacetic acid (CAS No.: 2521-84-8)

Gthp: (S)-2-amino-2-(tetrahydro-2H-pyran-4-yl)acetic acid (CAS No.: 811842-25-8)

Hgl: (S)-2-aminoadipic acid (CAS No.: 1118-90-7)

Hpr: (S)-piperidine-2-carboxylic acid (CAS No.: 3105-95-1)

Hyp: (2S,4R)-4-hydroxypyrrolidine-2-carboxylic acid (CAS No.: 51-35-4)

IMe: (S)-2-amino-3,3-dimethylpentanoic acid (CAS No.: 173831-84-0)

KCOpipzaa: N6-(4-(carboxymethyl)piperazine-1-carbonyl)-L-lysine (CAS No.: N/A)

MeA: methyl-L-alanine (CAS No.: 3913-67-5)

MeC: methyl-L-cysteine (CAS No.: 4026-48-6)

Meda: methyl-D-alanine (CAS No.: 29475-64-7)

Medq: methyl-D-glutamine (CAS No.: 862504-01-6)

MeG: methylglycine (CAS No.: 107-97-1)

MeKCOpipzaa: N6-(4-(carboxymethyl)piperazine-1-carbonyl)-N2-methyl-L-lysine (CAS No.: N/A)

MeQ: methyl-L-glutamine (CAS No.: 300560-56-9)

Mor: (S)-morpholine-3-carboxylic acid (CAS No.: 106825-79-0)

Nal1: (S)-2-amino-3-(naphthalen-1-yl)propanoic acid (CAS No.: 55516-54-6)

Nal2: (S)-2-amino-3-(naphthalen-2-yl)propanoic acid (CAS No.: 58438-03-2)

Nle: (S)-2-aminohexanoic acid (CAS No.: 327-57-1)

PEG10c: 1-amino-3,6,9,12,15,18,21,24,27,30-decaoxatritriacontan-33-oic acid (CAS No.: 2170987-85-4)

PEG12c: 1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid (CAS No.: 1415408-69-3)

PEG4c: 1-amino-3,6,9,12-tetraoxapentadecan-15-oic acid (CAS No.: 663921-15-1)

PEG8c: 1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (CAS No.: 756526-04-2)

SMe: O-methyl-L-serine (CAS No.: 32620-11-4)

Tbg: (S)-2-amino-3,3-dimethylbutanoic acid (CAS No.: 20859-02-3)

Tic: (S)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (CAS No.: 74163-81-8)

TMe: O-methyl-L-threonine (CAS No.: 4144-2-9)

W1aa: 1-(carboxymethyl)-L-tryptophan (CAS No.: 773823-50-0)

W1Me: 1-methyl-L-tryptophan (CAS No.: 21339-55-9)

W1Me7N: (S)-2-amino-3-(1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid (CAS No.: 1632971-18-6)

W7N: (S)-2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid (CAS No.: 49758-35-2)

[0011]

[TPO]

Thrombopoietin (TPO) is a ligand of c-MPL, is constitutively produced in the liver and the like, circulates through the bloodstream, is transported to the bone marrow, and stimulates early development such as megakaryopoiesis. The concentration of TPO in blood is regulated by the amount of c-MPL on platelets and megakaryocytes. In addition to regulation by the number of TPO molecules including regulation of deficiency of TPO mRNA in bone marrow, megakaryopoiesis and platelet production may be regulated as a result of regulating the activity of TPO by a proteolytic process that generates truncated forms of the molecule (Stem Cells 1998, 16, 322.).

As one embodiment, the peptide complex of the present invention preferably has an intracellular signaling activation ability, and more preferably has a TPO/TPOR signal activating ability, and therefore can be used as a substitute for naturally occurring TPO.

[0012]

[TPO Receptor]

The TPO receptor is also known as c-MPL, and is a 635-amino-acid protein encoded by the c-MPL gene. The TPO receptor has three functional regions including an extracellular cytokine-binding region, a transmembrane region, and a cytoplasmic region that binds to signaling molecules including Janus kinases (JAKs) and signal transducers and activators of transcription (STATs).

[0013]

[TPO/TPOR Signal Activating Ability]

The TPO/TPOR signal activating ability means an ability to produce an effect similar to an effect produced by naturally occurring TPO. It also means an ability to specifically activate the TPO/TPOR signaling pathway.

The TPO/TPOR signal activating ability can be evaluated by a known method, and can be evaluated, for example, by a binding ability to the TPO receptor and an activating ability of intracellular signaling molecules receiving signals from the TPO receptor.

[0014]

[TPO Receptor Binding Ability]

The TPO receptor binding ability means an ability to specifically bind to the TPO receptor. The binding ability to the TPO receptor can be evaluated by a known method, and can be evaluated, for example, but not limited to, by an SPR method or an ELISA method using a recombinant protein of the TPO receptor. In any evaluation system, when binding to the TPO receptor can be detected according to standard procedures using an optimal concentration of the peptide or peptide complex under optimal conditions, it is said to have a TPO receptor binding ability (or binding activity to TPOR).

[0015]

[Intracellular Signaling Activation Ability]

The intracellular signaling activation ability can be evaluated by phosphorylation of intracellular signaling molecules such as ERK, AKT, and STAT, and further by expression of reporter genes downstream thereof. Non-limiting examples include evaluation using an AlphaLISA SureFire Ultra p-Erk1/2 (Thr202/Tyr204) Assay kit (PerkinElmer), an AlphaLISA SureFire Ultra phospho-STAT5 (Tyr694/699) kit (PerkinElmer), HEK-Blue™ TPO Cells (InvivoGen), and the like. In any evaluation system, when activation of intracellular signaling can be detected according to standard procedures using an optimal concentration of the peptide or peptide complex under optimal conditions, it is said to have an intracellular signaling activation ability.

[0016]

[ERK Activation]

ERK activation is one type of intracellular signal activation, and means an ability to phosphorylate ERK (extracellular signal-regulated kinase), which is an intracellular signaling molecule. ERK is a factor included in the MAPK signaling pathway, and is phosphorylated and activated via MEK by signals from receptors on the cell surface. For example, the peptide complex of the present invention phosphorylates ERK.

[0017]

[STAT5-Dependent Transcriptional Activation]

STAT5-dependent transcriptional activation is one type of intracellular signal activation, and means an ability of STAT5 (signal transducer and activator of transcription), which is an intracellular signaling factor and transcription activator, to activate transcription of a target gene. STAT5 is a factor included in the JAK-STAT signaling pathway, and after being activated via JAK by signals from receptors on the cell surface, translocates into the nucleus and functions as a transcription activator. For example, the peptide complex of the present invention activates transcription of a STAT5-dependent reporter gene.

[0018]

[TPO/TPOR Signaling Pathway]

A signaling pathway generally means a biochemical causal relationship initiated by protein-protein interaction, for example binding of a growth factor to a receptor, resulting in transmission of a signal from one part of a cell to another part of the cell. The TPO/TPOR signaling pathway means a biochemical causal relationship occurring when the above growth factor is TPO.

The TPO receptor is also known as c-MPL, and c-MPL belongs to the type I cytokine receptor family, and as with erythropoietin (EPO) and granulocyte colony-stimulating factor (GCSF) receptors, binding of TPO causes a conformational change of the homodimeric receptor. Subsequently, phosphorylation of the intracellular region of c-MPL and various secondary signaling molecules occurs.

Examples of signaling molecules activated by TPO include Janus kinases (JAKs), signal transducers and activators of transcription (STATs), phosphatidylinositol-3-kinase (PI3K)/Akt, and Ras/mitogen-activated protein kinase (MAPK). Activation of these signaling pathways results in induction of megakaryopoiesis and thrombopoiesis from hematopoietic stem cells.

[0019]

[Conservative amino acid substitution]

In the peptide and peptide complex of the present invention, when 1, 2, or 3 amino acid residues are substituted, deleted, added, or inserted in a specific amino acid sequence, conservative amino acid substitution is preferably carried out.

[0020]

“Conservative amino acid substitution” refers to substitution with a functionally equivalent or similar amino acid.

[0021]

The conservative amino acid substitution in a peptide causes a static change in the amino acid sequence of the peptide. For example, one or two or more amino acids having a same polarity act functionally equivalently in causing a static change in the amino acid sequence of the peptide. In general, a substitution within a certain group can be considered to be conservative in terms of structure and function.

[0022]

However, as is obvious to those skilled in the art, a role of a particular amino acid residue can be determined in the context of a three-dimensional structure of a molecule

containing the particular amino acid.

[0023]

A cysteine residue can take an oxidized (disulfide) form having lower polarity than a reduced (thiol) form.

[0024]

A long aliphatic moiety in an arginine side chain can constitute structurally and functionally important features.

[0025]

Further, an aromatic ring-containing side chain (tryptophan, tyrosine, and phenylalanine) can contribute to ion-aromatic interaction or cation-pi interaction. In such a case, substitution of the amino acids having these side chains with amino acids belonging to an acidic or non-polar group can be structurally and functionally conservative.

[0026]

Residues of proline, glycine, cysteine (disulfide form), and the like may have a direct effect on a three-dimensional structure of the main chain, which often makes it not possible to substitute these amino acids without causing structural distortion.

[0027]

The conservative amino acid substitution includes, as shown below, specific substitution based on side chain similarity (L. Lehninger, Biochemistry, 2nd edition, pp. 73-75, Worth Publisher, New York (1975)) and typical substitution.

[0028]

Further, conservative amino acid substitution is preferably substitution of a natural amino acid with an amino acid belonging to the same group as the group to which the amino acid belongs, in groups in which natural amino acids are classified based on their common side-chain properties, for example, as follows.

[0029]

Hydrophobic (also called nonpolar) amino acids: Amino acids that exhibit hydrophobicity (also called nonpolar properties) and include L-alanine (A), glycine (G), L-valine (V), L-leucine (L), L-isoleucine (I), L-proline (P), L-phenylalanine (F), L-tryptophan (W), L-tyrosine (Y), L-methionine (M).

The hydrophobic amino acids can be further divided into the following groups.

[0030]

Aliphatic amino acids: amino acids having an aliphatic hydrocarbon group or a hydrogen atom in the side chain, including, for example, L-alanine (A or Ala), glycine (G or

Gly), L-valine (V or Val), L-isoleucine (I or Ile), and L-leucine (L or Leu).

[0031]

Aliphatic/branched-chain amino acids: amino acids having a branched fatty acid in the side chain, including, for example, L-valine (V), L-isoleucine (I), and L-leucine (L).

[0032]

Aromatic amino acids: amino acids having an aromatic ring in the side chain, including, for example, L-tryptophan (W), L-tyrosine (Y), and L-phenylalanine (F).

[0033]

Hydrophilic (also called polar) amino acids: Amino acids that exhibit hydrophilicity (polarity) and include L-serine (S), L-threonine (T), L-cysteine (C), L-asparagine (N), L-glutamine (Q), L-aspartic acid (D), L-glutamic acid (E), L-lysine (K), L-arginine (R), and L-histidine (H).

[0034]

The hydrophilic amino acids can be further divided into the following groups.

[0035]

Acidic amino acids: amino acids having a side chain exhibiting acidity, including, for example, L-aspartic acid (D) and L-glutamic acid (E).

[0036]

Basic amino acids: amino acids having a side chain exhibiting basicity, including, for example, L-lysine (K), arginine (R), and L-histidine (H).

[0037]

Neutral amino acids: amino acids having a side chain exhibiting neutrality, including, for example, L-serine (S), L-threonine (T), L-asparagine (N), L-glutamine (Q), and L-cysteine (C).

[0038]

Glycine (G) and L-proline (P) can also be classified into “amino acids affecting the direction of the main chain”.

[0039]

L-cysteine (C) and L-methionine (M), which contain sulfur molecules in the side chain, can also be classified into “sulfur-containing amino acids”.

[0040]

As used herein, “amino acid” includes not only natural amino acids but also unnatural amino acids. Unnatural amino acids include, for example, N-alkyl amino acids in which a natural amino acid described above is N-alkylated; and those modified with lower alkyl groups

(for example, of C1 to C5, preferably C1 to C3, and more preferably C1) in which the nitrogen forming a peptide bond is branched or not branched. Among the N-alkyl amino acids, N-ethyl amino acid, N-butyl amino acid, or N-methyl amino acid is preferable, and N-methyl amino acid is more preferable.

[0041]

Further, unnatural amino acids also include D-type amino acids (also referred to as D-amino acids), chemically modified amino acids such as β-amino acids, γ-amino acids, amino acid variants, amino acid derivatives, and the like; amino acids that are not constituent materials for proteins in vivo, such as norleucine, ornithine, and the like.

[0042]

Furthermore, amino acids in which a functional group is further added to or substituted into the side chain of a natural amino acid (for example, amino acids having substitution or addition in a portion such as an arylene group or alkylene group of the side chain, amino acids in which the number of carbons of the arylene group, alkylene group, or alkyl group of the side chain is increased, amino acids having substitution on the aromatic ring of the side chain, amino acids having a heterocyclic ring, or amino acids having a fused ring) are included.

[0043]

It should be noted that, by addition or substitution of a structure such as a functional group to the side chain of a natural amino acid, properties different from those of the natural amino acid can be imparted. For example, (S)-2-amino-3-(pyridin-4-yl)propanoic acid (4Py) is alanine to which a pyridyl group (pyridine ring) is added to the side chain, but by addition of the pyridyl group, unlike alanine belonging to the nonpolar amino acid group, it exhibits polarity of basicity.

[0044]

That is, unnatural amino acids having similar side-chain properties can be included in the above-mentioned groups obtained by classifying natural amino acids based on their common side-chain properties. For example, methyl-L-arginine (MeR), which is an N-methylated amino acid of arginine belonging to the basic amino acids, is an unnatural amino acid, but since it exhibits basicity, it can be classified as a basic amino acid. In this manner, unnatural amino acids exhibiting side-chain properties similar to those of a certain amino acid can also be included as targets of conservative amino acid substitution.

[0045]

Non-limiting examples of unnatural amino acids include N-methyl amino acids, (S)-2-amino-3-(pyridin-4-yl)propanoic acid (4Py), allothreonine (alT), L-citrulline (Cit), O-

methyl-L-serine (SMe), (2S)-2-amino-3-(oxan-4-yl)propanoic acid (Atp), L-2-aminoadipic acid (Hgl), (S)-2-amino-3-(naphthalen-1-yl)propanoic acid (Nal1), (S)-2-amino-3-(1H-pyrrolo[2,3-c]pyridin-3-yl)propanoic acid (W6N), and (S)-2-amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid (W7N), and the like. It should be noted that N-methyl amino acids can also be classified as N-alkyl amino acids, and can also be classified according to the side-chain properties of the original amino acid that is not N-methylated.

[0046]

[Peptides and Pharmaceutically Acceptable Salts, Esters, or Solvates Thereof]

Examples of pharmaceutically acceptable salts are inorganic acid salts, organic acid salts, inorganic base salts, organic base salts, and acidic and basic amino acid salts. However, the salts are not limited thereto.

[0047]

Examples of the inorganic acid salts are hydrochloride salts, hydrobromide salts, sulfate salts, nitrate salts, and phosphate salts. However, the salts are not limited thereto.

[0048]

Examples of the organic acid salts are acetate salts, succinate salts, fumarate salts, maleate salts, tartrate salts, citrate salts, lactate salts, stearate salts, benzoate salts, methanesulfonate salts, and p-toluenesulfonate salts. However, the salts are not limited thereto.

[0049]

Examples of the inorganic base salts are alkali metal salts such as sodium salts and potassium salts, alkaline earth metal salts such as calcium salts and magnesium salts, aluminum salts, and ammonium salts. However, the salts are not limited thereto.

[0050]

Examples of the organic base salts are diethylamine salts, diethanolamine salts, meglumine salts, and N,N′-dibenzylethylenediamine salts. However, the salts are not limited thereto.

[0051]

Examples of the acidic amino acid salts are aspartate salts and glutamate salts. Examples of the basic amino acid salts are arginine salts, lysine salts, and ornithine salts. However, the salts are not limited thereto.

[0052]

Examples of pharmaceutically acceptable esters are acetate esters and sulfate esters. However, the esters are not limited thereto.

[0053]

Examples of pharmaceutically acceptable solvates are hydrates. However, the solvates are not limited thereto.

[0054]

[Peptide]

The peptide of the present invention may be a pharmaceutically acceptable salt, ester, or solvate. An example of the solvate is a hydrate.

<First Embodiment>

A first embodiment of the peptide of the present invention is a peptide comprising an amino acid sequence represented by formula A1, or an amino acid sequence in which a plurality of amino acid residues are substituted, deleted, added, or inserted in the amino acid sequence represented by formula A1. In formula A1, the amino acid sequence is described from the N-terminal side to the C-terminal side. Examples of the plurality are 1, 2, or 3. When amino acid residues are substituted, it is preferable that amino acid residues other than the 4th MeG, the 9th df, and the 15th MeC are substituted. In this peptide, for example, it is preferable that a peptide complex that is a homodimer exhibits an intracellular signaling activation ability.

[0055]

A1: X^1-X^2-X^3-MeG-X^4-X^5-X^6-X^7-df-X^8-X^9-X^{10}-X^{11}-X^{12}-MeC

[0056]

wherein,

X^1 is any amino acid residue,

X^2 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

X^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group

which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members which may be substituted,

X^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^{11} is any amino acid residue, and

X^{12} is any amino acid residue.

[0057]

X^1 is preferably F, A, Q, E, F4COO, F3Me, F3C, F4Me, F4C, F4OMe, 3Py, Cha, or F4aao.

X^2 is preferably W, W7N, W1Me7N, or W1aa.

X^3 is preferably V, R, Cit, T, alT, or S.

X^4 is preferably W, W1Me, or Nal1.

X^5 is preferably V, T, alT, or dMeS.

X^6 is preferably V, I, alI, Eva, Tbg, or Gcpe.

X^7 is preferably MeG, MeA, Meda, MeKCOpipzaa, dp, EtG, or EtA.

X^8 is preferably R, Q, or KCOpipzaa.

X^9 is preferably V, T, alT, P, Hpr, Hyp, or Mor.

X^{10} is preferably I, V, Gthp, or G4pipaa.

X^{11} is preferably S, E, I, F, W, Y, R, Cit, Atp, KCOpipzaa, or Hgl.

X^{12} is preferably D, H, R, S, Q, SMe, Cit, E, or Hgl.

[0058]

In the peptide of the first embodiment, it is preferable that the peptide is a peptide comprising an amino acid sequence represented by formula A2, or an amino acid sequence in which at least one amino acid residue is substituted, deleted, added, or inserted in an amino acid sequence consisting of 1 to 12 amino acid residues selected from the group consisting of the 1st, 2nd, 3rd, 5th, 6th, 7th, 8th, 10th, 11th, 12th, 13th, and 14th amino acid residues in the amino acid sequence represented by formula A2. In formula A2, the amino acid sequence is described from the N-terminal side to the C-terminal side. Examples of the at least one are 1, 2, or 3. When amino acid residues are substituted, it is preferable that amino acid residues other than the 4th MeG, the 9th df, and the 15th MeC are substituted.

[0059]

A2: F-W-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 1).

[0060]

In a peptide comprising an amino acid sequence represented by formula A1, or an

amino acid sequence in which a plurality of amino acid residues are substituted, deleted, added, or inserted in the amino acid sequence represented by formula A1, preferable amino acid residues are as follows. This peptide preferably has, for example, TPO receptor binding ability. Further, in this peptide, for example, it is preferable that a peptide complex that is a homodimer exhibits an intracellular signaling activation ability.

[0061]

X^1 is preferably an amino acid residue having, in a side chain, an aryl group which may be substituted.

X^2 is preferably an amino acid residue having, in a side chain, an aryl group which may be substituted.

X^3 is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted

X^4 is preferably an amino acid residue having, in a side chain, an aryl group which may be substituted.

X^5 is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group.

X^6 is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group.

X^7 is preferably any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members.

X^8 is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

X^9 is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group, or a cyclic secondary amino acid residue having 4 to 6 members.

X^{10} is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group.

X^{11} is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

X^{12} is preferably an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

[0062]

X^1 is preferably F or F4Me.

X^2 is preferably W or W7N.

X^3 is preferably V, T, or alT.

X^4 is preferably W or Nal1.

X^5 is preferably V.

X^6 is preferably V, I, alI, or Gcpe.

X^7 is preferably MeG, MeA, Meda, MeKCOpipzaa, or dp.

X^8 is preferably R.

X^9 is preferably V or P.

X^{10} is preferably I or V.

X^{11} is preferably S, KCOpipzaa, or Hgl.

X^{12} is preferably D.

[0063]

Any of the above-described peptides is preferably a cyclic peptide.

[0064]

In the peptide of the first embodiment, when the peptide is a cyclic peptide, it preferably has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in formula A1 or formula A2 included in the peptide, are bound. The amino acid residue derived from the chloroacetylated amino acid is preferably the N-terminal amino acid residue. The peptide having a cyclic structure is preferably one from which the chloroacetyl group has been removed.

[0065]

A cyclic peptide refers to a peptide in which two amino acids are bound to each other in the peptide, thereby causing the peptide to be wholly or partially cyclized. Note that in the present application, cyclic peptides include those in which amino acids form a cross-linking structure in the peptide, those in which a cyclic structure is formed by lactam ring formation or a macrocyclization reaction, those having a lasso peptide-like structure, and the like. That is, in the present application, the cyclic peptide may be one in which a part thereof forms a cyclic structure, and it may also have a linear portion.

[0066]

In some cases, a part of the amino acids may be modified for cyclization of the peptide of the present embodiment. The peptide of the present embodiment also includes peptides containing amino acids subjected to such partial modification. As an example of modification for cyclization, a chloroacetyl group may be added to an amino acid located at the N-terminus and bound to a cysteine residue in the peptide to form a cyclic structure. Peptides containing various (natural/unnatural) amino acids to which a chloroacetyl group has been added are also included in the peptides in the present specification.

[0067]

Peptides have problems such as generally poor metabolic stability in vivo and difficulty in penetrating cell membranes due to their large size. In response to such problems, a method of cyclizing the peptide has been adopted. It has been suggested that peptide cyclization improves protease resistance and metabolic stability, in addition to also restricting conformational change, thereby increasing rigidity and improving a membrane penetrating property and affinity to a target protein.

[0068]

Peptide cyclization can be performed according to a publicly known method. For example, a peptide designed to comprise two or more cysteine residues can form a cyclic structure via a disulfide bond after translation, although the invention is not limited thereto. Further, cyclization can also be achieved by synthesizing a peptide having a chloroacetyl group at the N-terminus and placing a cysteine residue in the peptide using a genetic code reprogramming technique according to the method by Goto et al. (Y. Goto et al., Acss Chem. Biol. 3 120-129 (2008)). In this method, a mercapto group spontaneously performs a nucleophilic attack on the chloroacetyl group after translation, leading to cyclization of the peptide via a thioether bond. Cyclization may also be achieved by placing, in the peptide, a combination of other amino acids that are bound to form a cycle by the genetic code reprogramming technique Alternatively, cyclization may be carried out by synthesizing a peptide having a cycloamide at the N-terminus, arranging an L-2-aminoadipic acid residue in the peptide, and bonding them together. Thus, any known cyclization method can be used without particular limitation.

[0069]

In the peptide of the first embodiment, it is preferable to further contain an additional amino acid residue. The additional amino acid residue in this case is not particularly limited.

[0070]

In the peptide of the first embodiment, the additional amino acid residue may be included in a peptide forming a cyclic structure, or an amino acid residue may be further added in a linker form from the cyclic peptide. The number of amide bonds of the peptide or peptide moiety (the number/length of amino acids) is not particularly limited, but the total amino acid residues (meaning the number of amino acid residues included in the peptide forming the cyclic structure, and when amino acid residues are further added in a linker form from the cyclic peptide, those amino acids are not included) is preferably within 20 residues. A preferable peptide length is 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino

acid residues, and preferably 19 or fewer or 18 or fewer amino acid residues. A more preferable peptide length is 13 to 16 amino acid residues, and most preferably 14 or 15 amino acid residues.

[0071]

In the peptide of the first embodiment, it is preferable to have a TPO receptor binding ability.

[0072]

<Second Embodiment>

A second embodiment of the peptide of the present invention is a peptide comprising an amino acid sequence represented by formula B1, or an amino acid sequence in which a plurality of amino acid residues are substituted, deleted, added, or inserted in the amino acid sequence represented by formula B1. In formula B1, the amino acid sequence is described from the N-terminal side to the C-terminal side. Examples of the plurality are 1, 2, or 3. When amino acid residues are substituted, it is preferable that amino acid residues other than the 4th MeG, the 9th df, and the 15th MeC are substituted.

[0073]

B1:Y^1-Y^2-Y^3-MeG-Y^4-Y^5-Y^6-Y^7-df-R-Y^8-Y^9-Y^{10}-D-MeC

[0074]

wherein,

Y^1 is any amino acid residue,

Y^2 is any amino acid residue,

Y^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

Y^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group

which may be substituted,

Y^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

[0075]

Y^1 is preferably F, F4aao, F3COO, F3aao, 4Py, 3Py, or Cha.

Y^2 is preferably Ahp, W1Me7N, W, or W7N.

Y^3 is preferably V, I, alI, Q, T, TMe, or KCOpipzaa.

Y^4 is preferably W, W1Me, Nal1, or Nal2.

Y^5 is preferably V, Tbg, TMe, alTMe, dMeS, Gthp, G4pipaa, or Cit.

Y^6 is preferably V, Ahp, Nle, I, L, Tbg, IMe, Cbg, Gcpe, Chg, or alTMe.

Y^7 is preferably MeG, EtG, MeA, Meda, dp, MeQ, or Medq.

Y^8 is preferably V, P, Hpr, Tic, TMe, or alTMe.

Y^9 is preferably I, alI, alTMe, dMeS, Gthp, or G4pipaa.

Y^{10} is preferably S or Hgl.

[0076]

In the peptide according to the second embodiment, it is preferable that the peptide is a peptide comprising an amino acid sequence represented by Formula B2, or an amino acid sequence in which at least one amino acid residue is substituted, deleted, added, or inserted in an amino acid sequence consisting of 1 to 10 amino acid residues selected from the group consisting of the 1st, 2nd, 3rd, 5th, 6th, 7th, 8th, 11th, 12th, and 13th amino acid residues in the amino acid sequence represented by Formula B2. In Formula B2, the amino acid sequence is described from the N-terminal side to the C-terminal side. At least one example is 1, 2, or 3. When an amino acid residue is substituted, it is preferable that an amino acid residue other than the 4th MeG, the 9th df, and the 15th MeC is substituted.

[0077]

B2: F-Ahp-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 97).

[0078]

The peptide according to the second embodiment is preferably a cyclic peptide.

When the peptide according to the second embodiment is a cyclic peptide, it preferably has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in Formula B1 or Formula B2 contained in the peptide, are bound. The amino acid residue derived from the chloroacetylated amino acid is preferably the N-terminal amino acid residue. The peptide having a cyclic structure is preferably one from which the chloroacetyl group has been removed.

[0079]

The peptide according to the second embodiment preferably further comprises an additional amino acid residue. The additional amino acid residue in this case is not particularly limited.

[0080]

In the peptide according to the second embodiment, the additional amino acid residue may be included in the peptide forming the cyclic structure, or amino acid residues may be further added in a linker form from the cyclic peptide. The number of amide bonds (the number/length of amino acids) in the peptide or peptide moiety is not particularly limited, but the total amino acid residues (meaning the number of amino acid residues included in the peptide forming the cyclic structure, and excluding amino acid residues further added in a linker form from the cyclic peptide) are preferably within 20 residues. A preferable peptide length is 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino acid residues, and preferably 19 or less or 18 or less amino acid residues. A more preferable peptide length is 13 to 16 amino acid residues, and most preferably 14 or 15 amino acid residues.

[0081]

The peptide according to the second embodiment preferably has TPO receptor binding ability.

[0082]

[Peptide Complex]

The peptide complex of the present invention comprises a first peptide and has TPO/TPOR signal activating ability. The peptide complex of the present invention may also have cell proliferation ability derived from the TPO/TPOR signal activating ability.

[0083]

The peptide complex is a peptide, a peptide-containing compound, or a pharmaceutically acceptable salt thereof, comprising the first peptide and another peptide or compound. The peptide complex may comprise one or two or more (three or more or four or more) first peptides.

[0084]

The peptide complex may further comprise one or two or more partial peptides different from the first peptide. In the peptide complex, it is preferable that the first peptide and the partial peptide are bound via a linker. The peptide complex is preferably a homomultimer containing only peptides having the same amino acid sequence. The peptide complex may be a heteromultimer containing peptides having different amino acid sequences. The peptide

complex is preferably a homodimer having a first peptide and a second peptide having the same amino acid sequence, wherein the first peptide and the second peptide are bound via a linker. As shown in the Examples, the first peptide and the second peptide exhibit intracellular signaling activation ability by adopting a peptide complex structure via a linker.

[0085]

The peptide complex of the present invention may comprise a first peptide, a second peptide, and a linker connecting the first peptide and the second peptide. In this case, the second peptide may be the same as or different from the first peptide.

[0086]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the first peptide is the peptide according to the first embodiment, and the second peptide is the peptide according to the first embodiment or the peptide according to the second embodiment, preferably the peptide according to the first embodiment.

[0087]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the sequence identity between the first peptide and the second peptide is preferably 90% or more and 100% or less.

[0088]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the first peptide and the second peptide are preferably substantially the same peptide.

[0089]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the first peptide and the second peptide are preferably each cyclic peptides.

[0090]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the first peptide and the second peptide preferably each have a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC contained in the first peptide or the second peptide are bound. The amino acid residue derived from the chloroacetylated amino acid is preferably the N-terminal amino acid residue. The peptide having a cyclic structure is preferably one from which the chloroacetyl group has been removed.

[0091]

One embodiment of the peptide complex of the present invention is a peptide complex comprising a first peptide and a second peptide, wherein the first peptide and the second peptide may further comprise additional amino acid residues. In this case, the additional amino acid residues are preferably included in the peptide forming the cyclic structure.

[0092]

The peptide complex preferably comprises the first peptide, the second peptide, and a linker connecting the first peptide and the second peptide.

[0093]

The linker may be a structure connecting a plurality of peptides to each other in the peptide complex. Examples of the linker include amino acid linkers (peptide linkers), chemical linkers, fatty acid linkers, nucleic acid linkers, and sugar chain linkers, and may also be, for example, a complex such as a chemical linker and a peptide linker.

[0094]

An example of the chemical linker is a PEG (Polyethyleneglycol) linker.
The PEG linker may be a linker consisting of 1 to 36 ethylene glycol units.

[0095]

Further, the linker may also be a fatty acid linker containing a divalent chemical moiety derived from a fatty acid. The amino acid (peptide) linker is a linker containing at least one amino acid, and examples thereof include glycine-rich peptides such as peptides having the sequence [G-G-G-G-S]n (wherein n is 1, 2, 3, 4, 5, or 6), as described in U.S. Patent No. 7,271,149, and serine-rich peptide linkers described in U.S. Patent No. 5,525,491.

[0096]

Non-limitingly, the addition of the linker may change the physical properties (for example, solubility) of the peptide. Furthermore, the linker may be a combination of the above linkers. For example, glycine (G)-L-lysine (K) may be bound as an amino acid linker, and further, a PEG linker may be bound to the terminal end of the side chain of the Lys. Further, the linker may have a structure in which amino acids and PEG are alternately bound, such as PEG-amino acid-PEG. Here, PEG means a PEG linker.

[0097]

Another example of the linker is a linker in which 1 to 6 amino acids are added to the PEG linker portion. This linker may have a structure in which amino acids are added to one end of the PEG linker portion, or may have a structure in which amino acids are added to both ends of the PEG linker portion. A preferable example of the amino acid added to the PEG portion is

L-lysine (K), but is not limited to L-lysine (K), and may be another amino acid.

[0098]

Regarding the addition position of the linker, the linker may be added anywhere in the peptide. For example, it may be positioned at the C-terminus of the peptide and bound to L-cysteine (C) or methyl-L-cysteine (MeC), which binds to the first amino acid to form a cyclic structure, or may be bound to an amino acid contained in the cyclic peptide. Although not limiting, the linker is preferably bound to L-cysteine (C) or methyl-L-cysteine (MeC) positioned at the C-terminus, or bound to a side chain of an amino acid contained in the cyclic peptide.

[0099]

For example, when two cyclic peptide structures in which the first amino acid of the amino acid sequences represented by SEQ ID NOS: 1 to 145 is bound to methyl-L-cysteine (MeC), which is the 15th amino acid, form a dimer structure from methyl-L-cysteine (MeC) via the structure shown in Table 3, the linker structure can be said to be the structure shown in Table 3. The dimer is preferably one in which the C-terminus of the first peptide and the C-terminus of the second peptide are bound via a linker.

[0100]

In the peptide complex of the present embodiment, the first peptide and the second peptide are peptides consisting of the amino acid sequence represented by Formula A1 or the amino acid sequence represented by Formula B1, and are preferably peptides consisting of the amino acid sequence represented by Formula A1.

[0101]

A1:X^1-X^2-X^3-MeG-X^4-X^5-X^6-X^7-df-X^8-X^9-X^{10}-X^{11}-X^{12}-MeC

[0102]

wherein, in Formula A1,

X^1 is F, A, Q, E, F4COO, F3Me, F3C, F4Me, F4C, F4OMe, 3Py, Cha, or F4aao,

X^2 is W, W7N, W1Me7N, or W1aa,

X^3 is V, R, Cit, T, alT, or S,

X^4 is W, W1Me, or Nal1,

X^5 is V, T, alT, or dMeS,

X^6 is V, I, alI, Eva, Tbg, or Gcpe,

X^7 is MeG, MeA, Meda, MeKCOpipzaa, dp, EtG, or EtA,

X^8 is R, Q, or KCOpipzaa,

X^9 is V, T, alT, P, Hpr, Hyp, or Mor,

X^{10} is I, V, Gthp, or G4pipaa,

X^{11} is S, E, I, F, W, Y, R, Cit, Atp, KCOpipzaa, or Hgl, and

X^{12} is D, H, R, S, Q, SMe, Cit, E, or Hgl.

[0103]

B1:Y^1-Y^2-Y^3-MeG-Y^4-Y^5-Y^6-Y^7-df-R-Y^8-Y^9-Y^{10}-D-MeC

[0104]

wherein, in Formula B1,

Y^1 is F, F4aao, F3COO, F3aao, 4Py, 3Py, or Cha,

Y^2 is Ahp, W1Me7N, W, or W7N,

Y^3 is V, I, alI, Q, T, TMe, or KCOpipzaa,

Y^4 is W, W1Me, Nal1, or Nal2,

Y^5 is V, Tbg, TMe, alTMe, dMeS, Gthp, G4pipaa, or Cit,

Y^6 is V, Ahp, Nle, I, L, Tbg, IMe, Cbg, Gcpe, Chg, or alTMe,

Y^7 is MeG, EtG, MeA, Meda, dp, MeQ, or Medq,

Y^8 is V, P, Hpr, Tic, TMe, or alTMe,

Y^9 is I, alI, alTMe, dMeS, Gthp, or G4pipaa, and

Y^{10} is S or Hgl.

[0105]

The peptide complex of the present embodiment has intracellular signaling activation ability, and preferably has TPO/TPOR signal activating ability.

[0106]

[Pharmaceutical Composition]

Another embodiment of the present invention is a pharmaceutical composition. One aspect of the pharmaceutical composition of the present embodiment is a pharmaceutical composition comprising at least one selected from the group consisting of the above-described peptides and pharmaceutically acceptable salts, esters, or solvates thereof (hereinafter, for simplicity, also simply referred to as “the peptide of the present invention”). Another aspect is a pharmaceutical composition comprising at least one selected from the group consisting of the above-described peptide complexes and pharmaceutically acceptable salts, esters, or solvates thereof (hereinafter, for simplicity, also simply referred to as “the peptide complex of the present invention”). Still another aspect is a pharmaceutical composition comprising at least one selected from the group consisting of the above-described peptides and peptide complexes and pharmaceutically acceptable salts, esters, or solvates thereof.

[0107]

The pharmaceutical composition preferably contains an effective amount of the peptide of the present invention or the peptide complex of the present invention as an active ingredient.

The diseases targeted by the pharmaceutical composition refer to any diseases caused by, exacerbated by, or otherwise associated with an increase or decrease in the expression or activity of the TPO receptor, TPO/TPOR signal transduction, or any other intracellular signaling cascade activated via the TPO receptor. Examples include, but are not limited to, thrombocytopenic syndrome, aplastic anemia, and the like.

[0108]

A route of administration of the pharmaceutical composition is not particularly limited, and the medical composition may be administered either orally or parenterally. Examples of parenteral administration include administration by injection such as intramuscular injection, intravenous injection, or subcutaneous injection, transdermal administration, transmucosal administration (nasal, buccal, ocular, pulmonary, vaginal, or rectal administration), and the like.

[0109]

Considering that peptides are an easily metabolizable and excretable substance, the above-mentioned pharmaceutical composition can be subjected to various modifications. For example, adding polyethylene glycol (PEG) or a sugar chain to the polypeptide can prolong the residence time of the peptide in the blood and reduce antigenicity of the peptide. Further, an emulsion, a nanoparticle, a nanosphere, or the like prepared using a biodegradable polymer compound such as polylactic acid-glycol (PLGA), porous hydroxyapatite, a liposome, a surface-modified liposome, emulsions prepared with unsaturated fatty acids, nanoparticles, nanospheres, and the like may be used as a sustained-release base in which to encapsulate the polypeptide. In the case of transdermal administration, a weak electric current can be applied to the skin surface to allow the pharmaceutical composition to penetrate the stratum corneum (an iontophoresis method).

[0110]

For the above-mentioned pharmaceutical composition, the active ingredient may be used as it is, or the pharmaceutical composition may be formulated by adding a pharmaceutically acceptable carrier, excipient, additive, or the like to the active ingredient. Examples of the dosage form include a liquid agent (e.g., an injected liquid), a dispersant, a suspension, a tablet, a pill, a powder, a suppository, a powdered drug, a fine granule, a granule, a capsule, a syrup, a troche, an inhalant, an ointment, an eye drop, a nasal drop, an ear drop, a cataplasm, and the like.

[0111]

The formulation can be performed by a conventional method by using, for example, an excipient, a binder, a disintegrant, a lubricant, a solubilizing agent, a dissolution adjuvant, a colorant, a flavoring agent, a stabilizer, an emulsifier, an absorption promoter, a surfactant, a pH adjuster, an antiseptic, an antioxidant, or the like as appropriate.

[0112]

Examples of components to be used in the formulation include, but are not limited to, purified water, saline, a phosphate buffer, dextrose, glycerol, a pharmaceutically acceptable organic solvent such as ethanol, animal or vegetable oil, lactose, mannitol, glucose, sorbitol, crystalline cellulose, hydroxypropyl cellulose, starch, corn starch, silicic anhydride, magnesium aluminum silicate, collagen, polyvinyl alcohol, polyvinylpyrrolidone, a carboxyvinyl polymer, carboxymethylcellulose sodium, sodium polyacrylate, sodium alginate, water-soluble dextran, sodium carboxymethyl starch, pectin, methyl cellulose, ethyl cellulose, xanthan gum, gum arabic, tragacanth, casein, agar, polyethylene glycol, diglycerol, glycerol, propylene glycol, vaseline, paraffin, octyldodecyl myristate, isopropyl myristate, a higher alcohol, stearyl alcohol, stearic acid, human serum albumin, trehalose, polysorbate and the like.

[0113]

The pharmaceutical composition can include an absorption promoter to improve the absorption of a poorly absorbed pharmaceutical. Examples of a substance that can be used as such an absorption promoter include a surfactant such as a polyoxyethylene lauryl ether, sodium lauryl sulfate, or a saponin; a bile acid salt such as glycocholic acid, deoxycholic acid, or taurocholic acid; a chelating agent such as EDTA or salicylic acids; a fatty acid such as caproic acid, capric acid, lauric acid, oleic acid, linoleic acid, or a mixed micelle; an enamine derivative; an N-acyl collagen peptide; an N-acyl amino acid; a cyclodextrin; a chitosan; a nitric oxide donor; and the like.

[0114]

The pill or the tablet can also be coated with sugar or a gastro-soluble or enteric substance. The injection liquid can comprise distilled water for injection, normal saline, propylene glycol, polyethylene glycol, vegetable oil, an alcohol, or the like. Further, a wetting agent, an emulsifier, a dispersant, a stabilizer, a solubilizing agent, a dissolution adjuvant, an antiseptic, or the like can be added.

[0115]

The pharmaceutical composition of the present invention may be administered in combination with other pharmaceuticals or treatments useful for the aforementioned disease.

[0116]

When administered to a mammal (e.g., a human, a mouse, a rat, a guinea pig, a rabbit, a dog, a horse, a monkey, a pig, a sheep, etc.), particularly a human, the dose to be administered of the pharmaceutical composition of the present invention is not particularly limited, and varies depending on a symptom, age, sex, weight, and sensitivity of a patient, an administration method, a dosing interval, a type of active ingredient, and a type of formulation. For example, the pharmaceutical composition can be administered once or split over several times at a dose of 30 μg to 1000 mg, 100 μg to 500 mg, or 100 μg to 100 mg. When administering by injection, the medical composition may be administered once or split over several times at a dose of 1 μg/kg to 3000 μg/kg or 3 μg/kg to 1000 μg/kg, depending on a weight of a patient.

[0117]

[Cell Culture Composition]

Another embodiment of the present invention is a cell culture composition used for cell culture, comprising the peptide complex described above.

[0118]

In one aspect, since the peptide complex has an intracellular signal activation ability, preferably a TPO/TPOR signal activation ability, it can also be used as a medium reagent or additive for cell culture, preferably as a medium reagent or additive for the culture of mammalian cells, more preferably human cells.

[0119]

The medium is not particularly limited as long as it is a medium for culturing cells or tissues. The medium may be a serum-containing medium, and is preferably a serum-free medium or a low-serum medium.

[0120]

The culture medium additive may be in the form of a solution or in the form of a dried solid (for example, a solid form, powder form, or the like). When it is in the form of a solution, it may be used as it is as a culture medium, or a culture medium may be prepared by diluting it with a solvent and, if necessary, adding the additives described above thereto. Examples of the solvent used for dilution include water, buffer solutions, physiological saline, media used for various cell and tissue cultures, and the like, and these may be used alone or in combination of two or more kinds thereof.

[0121]

When the culture medium additive is in the form of a dried solid, it may be dissolved in a solvent such as water, buffer solutions, physiological saline, media used for various cell

and tissue cultures, and the like, and, if necessary, the additives described above may be added thereto, and the resulting preparation may be used as a culture medium.

[0122]

The content of the peptide complex of the present invention in the medium for culturing the cells or tissues, or in the medium for cells obtained therefrom, may be, for example, a final concentration of about 0.01 to about 10000 nmol/L, preferably about 0.1 to about 1000 nmol/L, more preferably about 0.5 to about 1000 nmol/L, and still more preferably about 1 to about 100 nmol/L, based on the total amount of the composition or the total amount of the medium.

[0123]

[Composition for Medical, Diagnostic, or Research Use]

Another embodiment of the present invention is a composition for medical, diagnostic, or research use.

The composition for medical, diagnostic, or research use according to the present embodiment is a pharmaceutical composition comprising at least one selected from the group consisting of the peptide described above and pharmaceutically acceptable salts, esters, or solvates thereof (hereinafter, for simplicity, also referred to simply as “the peptide of the present invention”). Another aspect is a composition comprising at least one selected from the group consisting of the peptide complex described above and pharmaceutically acceptable salts, esters, or solvates thereof (hereinafter, for simplicity, also referred to simply as “the peptide complex of the present invention”).

[0124]

<Composition for Medical Use >

The composition for medical use according to the present embodiment (hereinafter also simply referred to as the “medical composition”) comprises at least one selected from the group consisting of the peptide of the present invention and the peptide complex of the present invention.

[0125]

The medical composition preferably contains, as an active ingredient, an effective amount of the peptide of the present invention or the peptide complex of the present invention.

The diseases targeted by the medical composition refer to any diseases caused by, exacerbated by, or otherwise associated with an increase or decrease in the expression or activity of the TPO receptor, or any diseases caused by, exacerbated by, or otherwise associated with an increase or decrease in TPO/TPOR signal transduction or any other intracellular signal

transduction cascade activated via the TPO receptor. Examples thereof include, but are not limited to, thrombocytopenic syndrome, aplastic anemia, and the like.

[0126]

A route of administration of the medical composition is not particularly limited, and the medical composition may be administered either orally or parenterally. Examples of parenteral administration include administration by injection such as intramuscular injection, intravenous injection, or subcutaneous injection, transdermal administration, transmucosal administration (nasal, buccal, ocular, pulmonary, vaginal, or rectal administration), and the like.

[0127]

Considering that peptides are an easily metabolizable and excretable substance, the above-mentioned medical composition can be subjected to various modifications. For example, adding polyethylene glycol (PEG) or a sugar chain to the polypeptide can prolong the residence time of the peptide in the blood and reduce antigenicity of the peptide. Further, an emulsion, a nanoparticle, a nanosphere, or the like prepared using a biodegradable polymer compound such as polylactic acid-glycol (PLGA), porous hydroxyapatite, a liposome, a surface-modified liposome, emulsions prepared with unsaturated fatty acids, nanoparticles, nanospheres, and the like may be used as a sustained-release base in which to encapsulate the polypeptide. In the case of transdermal administration, a weak electric current can be applied to the skin surface to allow the pharmaceutical composition to penetrate the stratum corneum (an iontophoresis method).

[0128]

For the above-mentioned medical composition, the active ingredient may be used as it is, or the medical composition may be formulated by adding a pharmaceutically acceptable carrier, excipient, additive, or the like to the active ingredient. Examples of the dosage form include a liquid agent (e.g., an injected liquid), a dispersant, a suspension, a tablet, a pill, a powder, a suppository, a powdered drug, a fine granule, a granule, a capsule, a syrup, a troche, an inhalant, an ointment, an eye drop, a nasal drop, an ear drop, a cataplasm, and the like.

[0129]

The formulation can be performed by a conventional method by using, for example, an excipient, a binder, a disintegrant, a lubricant, a solubilizing agent, a dissolution adjuvant, a colorant, a flavoring agent, a stabilizer, an emulsifier, an absorption promoter, a surfactant, a pH adjuster, an antiseptic, an antioxidant, or the like as appropriate.

[0130]

Examples of components to be used in the formulation include, but are not limited to,

purified water, saline, a phosphate buffer, dextrose, glycerol, a pharmaceutically acceptable organic solvent such as ethanol, animal or vegetable oil, lactose, mannitol, glucose, sorbitol, crystalline cellulose, hydroxypropyl cellulose, starch, corn starch, silicic anhydride, magnesium aluminum silicate, collagen, polyvinyl alcohol, polyvinylpyrrolidone, a carboxyvinyl polymer, carboxymethylcellulose sodium, sodium polyacrylate, sodium alginate, water-soluble dextran, sodium carboxymethyl starch, pectin, methyl cellulose, ethyl cellulose, xanthan gum, gum arabic, tragacanth, casein, agar, polyethylene glycol, diglycerol, glycerol, propylene glycol, vaseline, paraffin, octyldodecyl myristate, isopropyl myristate, a higher alcohol, stearyl alcohol, stearic acid, human serum albumin, trehalose, polysorbate and the like.

[0131]

The medical composition can include an absorption promoter to improve the absorption of a poorly absorbed pharmaceutical. Examples of a substance that can be used as such an absorption promoter include a surfactant such as a polyoxyethylene lauryl ether, sodium lauryl sulfate, or a saponin; a bile acid salt such as glycocholic acid, deoxycholic acid, or taurocholic acid; a chelating agent such as EDTA or salicylic acids; a fatty acid such as caproic acid, capric acid, lauric acid, oleic acid, linoleic acid, or a mixed micelle; an enamine derivative; an N-acyl collagen peptide; an N-acyl amino acid; a cyclodextrin; a chitosan; a nitric oxide donor; and the like.

[0132]

The pill or the tablet can also be coated with sugar or a gastro-soluble or enteric substance. The injection liquid can comprise distilled water for injection, normal saline, propylene glycol, polyethylene glycol, vegetable oil, an alcohol, or the like. Further, a wetting agent, an emulsifier, a dispersant, a stabilizer, a solubilizing agent, a dissolution adjuvant, an antiseptic, or the like can be added.

[0133]

The medical composition of the present invention may be administered in combination with other pharmaceuticals or treatments useful for the aforementioned disease.

[0134]

When administered to a mammal (e.g., a human, a mouse, a rat, a guinea pig, a rabbit, a dog, a horse, a monkey, a pig, a sheep, etc.), particularly a human, the dose to be administered of the medical composition of the present invention is not particularly limited, and varies depending on a symptom, age, sex, weight, and sensitivity of a patient, an administration method, a dosing interval, a type of active ingredient, and a type of formulation. For example, the medical composition can be administered once or split over several times at a dose of 30 μg

to 1000 mg, 100 μg to 500 mg, or 100 μg to 100 mg. When administering by injection, the medical composition may be administered once or split over several times at a dose of 1 μg/kg to 3000 μg/kg or 3 μg/kg to 1000 μg/kg, depending on a weight of a patient.

[0135]

<Composition for Diagnostic Use >

The composition for diagnostic use according to the present embodiment (hereinafter also simply referred to as the “diagnostic composition”) comprises at least one selected from the group consisting of the peptide of the present invention and the peptide complex of the present invention.

[0136]

The peptide binds to the TPO receptor. Accordingly, it may also be used as a diagnostic agent for detecting the TPO receptor. The diagnostic agent may be a detection agent for detecting the expression level of the TPO receptor, and when used as a detection agent, the peptide of the present invention may be detectably labeled. In this manner, the peptide or a composition comprising the same can be used as a diagnostic agent for detecting the TPO receptor.

[0137]

<Composition for Research Use >

The composition for research use according to the present embodiment (hereinafter also simply referred to as the “research composition”) comprises at least one selected from the group consisting of the peptide of the present invention and the peptide complex of the present invention.

[0138]

In one aspect, the peptide and/or the peptide complex binds to the TPO receptor. Accordingly, it can be preferably used for research involving the TPO receptor.

[0139]

The research composition according to the present embodiment is preferably used in a test method for testing at least one of the following with respect to the peptide, the peptide complex, or a complex comprising the peptide or the peptide complex further bound to a substance via a linker.

a) Solubility in a solvent.

b) Binding ability to the TPO receptor.

c) Toxicity to cells and/or tissues.

d) Toxicity to experimental animals.

[0140]

Regarding the test method, the test of solubility in a solvent of the peptide, the peptide complex, or a complex comprising the peptide or the peptide complex further bound to a substance via a linker may be a measurement of solubility. In the measurement of solubility, the solvent is not particularly limited and may be freely selected according to the purpose. Further, regarding the method for measuring solubility, known methods can be appropriately selected according to the type of solvent.

[0141]

The test of binding ability to the TPO receptor may be a measurement of binding ability to the TPO receptor, and although not limited thereto, known methods can preferably be used, such as surface plasmon resonance (SPR) assay, Scatchard analysis, and/or competitive binding assay methods such as radioimmunoassay (RIA), enzyme immunoassay (EIA), and sandwich competitive assay.

[0142]

The test of toxicity to cells and/or tissues may be any known toxicity evaluation test using cells and/or tissues, e.g., in-vitro. Cells and tissues may be, but are not limited to, cells and/or tissues for which toxicity evaluation studies are usually performed for pharmaceutical products.

[0143]

The test method for toxicity in experimental animals may be any known toxicity evaluation test using experimental animals. The experimental animals may be any animal that is normally used, and examples thereof include, but are not limited to, mouse, rat, guinea pig, gerbil, hamster, ferret, rabbit, dog, cat, pig, goat, horse, cow, bird (e.g., chicken, quail, etc.), monkey, primate other than human (e.g., capuchin monkey, marmoset, rhesus monkey, etc.), and the like. The above toxicity evaluation tests are not particularly limited, but may be safety-related tests usually conducted in non-clinical tests of pharmaceuticals, including, for example, general toxicity tests (single-dose toxicity test/repeated-dose toxicity test), genotoxicity tests (Ames test/chromosomal aberration test/In vitro micronucleus test), carcinogenicity tests, reproductive and developmental toxicity tests (ICH-I, II, III), local irritation tests (eye irritation test, skin irritation test, etc.), other toxicity tests (skin sensitization test, phototoxicity test, antigenicity test), chemical analysis and bioanalysis (TK/PK), and the like.

[0144]

[Nucleic Acid]

Still another embodiment of the present invention is a nucleic acid encoding the

peptide or peptide complex described above.

The nucleic acid according to the present embodiment may be natural or unnatural. Further, the nucleic acid includes, but is not limited to, DNA, RNA, and chimeras thereof. The nucleic acid according to the present embodiment can be designed and produced by known methods based on the amino acid sequence of the peptide or peptide complex described above.

[0145]

[Peptide Drug Conjugate (PDC)]

Still another embodiment of the present invention is a complex (peptide drug conjugate, PDC) comprising the peptide or peptide complex described above, a desired substance to be delivered to the TPO receptor, and a linker for binding the peptide or peptide complex and the substance.

The peptide or the peptide complex binds to the TPO receptor. Accordingly, the peptide or peptide complex is capable of delivering a substance to the TPO receptor.

The substance may be any substance desired by a person skilled in the art, as long as it is a substance desired to be delivered to the TPO receptor.

The substance is not particularly limited, and examples thereof include compounds, peptides, RI (radioisotopes), proteins, nucleic acids, and DDSs (drug delivery systems).

The compound is not particularly limited, but is preferably a low-molecular-weight compound or a medium-molecular-weight compound, and examples thereof include known low-molecular-weight drugs.

The peptide is not particularly limited, but is preferably a peptide that binds to a target in the body and exhibits some effect, and examples thereof include cyclic peptides.

The RI is not particularly limited, but may be a compound labeled with a radioisotope, such as a low-molecular-weight compound, medium-molecular-weight compound, or antibody labeled with a radioisotope, and examples thereof include compounds for PET (positron emission tomography) examination.

The protein is not particularly limited, and may be any protein exhibiting a useful function in the body, such as an antibody or enzyme. Examples thereof include enzymes used in enzyme replacement therapy.

Nucleic acid: may be DNA, RNA, or chimeras thereof, and is not particularly limited. Examples thereof include nucleic acid pharmaceuticals.

The molecule used in the DDS is not particularly limited, but known molecules used in DDSs such as liposomes or micelles are preferred. The DDS molecule may further contain a compound such as a pharmaceutical internally.

Further, the substance desired to be delivered to the TPO receptor may be a complex of the substances listed above.

[Examples]

[0146]

Hereinafter, the present invention will be described in detail based on Examples; however, the present invention is not limited to these Examples. Those skilled in the art can easily make modifications and changes to the present invention based on the description of the present specification, and such modifications and changes are included within the technical scope of the present invention.

[0147]

Chemical synthesis

All raw materials, building blocks, reagents, acids, bases, solid phase resins, and solvents used in the chemical synthesis in the following examples are either commercially available products used as is, or products that can be synthesized by those skilled in the art using organic chemical methods. Note that for amino acids comprising protective groups, commercially available products were used as is unless otherwise specified.

Elongation of the peptide chain on the solid phase resin was performed using the resin described in each example as a starting raw material normally used under peptide coupling reaction conditions and Fmoc removal reaction conditions. The reactions were carried out using a peptide automatic synthesizer, namely, Biotage Syro I, Biotage Syro II, CEM Corporation Liberty Blue, CEM Corporation Liberty Blue HT12, or CEM Corporation Liberty Prime, in accordance with the manufacturer's manuals.

As the resin used, NovaPEG Rink Amide resin or Seiber Amide resin was used, and the amount used ranged from 5 mg to 2 g depending on each peptide.

The reagent cocktail used for side-chain deprotection and cleavage from the solid-phase resin was 4 mL to 50 mL depending on each peptide, and solutions having the following compositions were used.

A: TFA/H_2O/TIS/DODT (92.5/2.5/2.5/2.5)

B: TFA/H_2O/TIS/DODT (90/2.5/2.5/5)

The general Fmoc amino acids used are listed below, and side-chain protecting groups are shown in parentheses.

[0148]

[Table 1-1]

Ｆｍｏｃ Amino Acid	ＣＡＳ No.
Ｆｍｏｃ－３Ｐｙ－ＯＨ	１７５４５３－０７－３
Ｆｍｏｃ－４Ｐｙ－ＯＨ	１６９５５５－９５－７
Ｆｍｏｃ－Ａｈｐ－ＯＨ	１１９７０２０－２２－６
Ｆｍｏｃ－Ａｌａ－ＯＨ	３５６６１－３９－３
Ｆｍｏｃ－ａｌＩ－ＯＨ	２５１３１６－９８－０
Ｆｍｏｃ－ａｌＴ（ｔＢｕ）－ＯＨ	２０１４８１－３７－０
Ｆｍｏｃ－ａｌＴＭｅ－ＯＨ	１２７９０２９－７０－７
Ｆｍｏｃ－Ａｒｇ（Ｐｂｆ）－ＯＨ	１５４４４５－７７－９
Ｆｍｏｃ－Ａｓｐ（Ｍｐｅ）－ＯＨ	１８０６７５－０８－５
Ｆｍｏｃ－Ａｔｐ－ＯＨ	３６８８６６－３４－６
Ｆｍｏｃ－Ｃｂｇ－ＯＨ	１３９１６３０－３１－１
Ｆｍｏｃ－Ｃｈａ－ＯＨ	１３５６７３－９７－１
Ｆｍｏｃ－Ｃｈｇ－ＯＨ	１６１３２１－３６－４
Ｆｍｏｃ－Ｃｉｔ－ＯＨ	１３３１７４－１５－９
Ｆｍｏｃ－ｄｅ（ｔＢｕ）－ＯＨ	１０４０９１－０８－９
Ｆｍｏｃ－ｄｆ－ＯＨ	８６１２３－１０－６
Ｆｍｏｃ－ｄＭｅＳ－ＯＨ	１２１７６０３－４１－２
Ｆｍｏｃ－ｄｐ－ＯＨ	１０１５５５－６２－８
Ｆｍｏｃ－ＥｔＡ－ＯＨ	８４０００－０９－９
Ｆｍｏｃ－ＥｔＧ－ＯＨ	１６２５４５－２９－１
Ｆｍｏｃ－Ｅｖａ－ＯＨ	１３１０６８０－４７－７
Ｆｍｏｃ－Ｆ３ａａｏ（ｔＢｕ）－ＯＨ	２９７３７５３－９５－４
Ｆｍｏｃ－Ｆ３Ｃ－ＯＨ	１９８５６０－４４－０
Ｆｍｏｃ－Ｆ３ＣＯＯ（ｔＢｕ）－ＯＨ	２１０２８２－３３－０
Ｆｍｏｃ－Ｆ３Ｍｅ－ＯＨ	２１１６３７－７４－０
Ｆｍｏｃ－Ｆ４ａａｏ（ｔＢｕ）－ＯＨ	１８１９５１－９２－８

[Table 1-2]

Ｆｍｏｃ Amino Acid	ＣＡＳ No.
Ｆｍｏｃ－Ｆ４Ｃ－ＯＨ	１７５４５３－０８－４
Ｆｍｏｃ－Ｆ４ＣＯＯ（ｔＢｕ）－ＯＨ	１８３０７０－４４－２
Ｆｍｏｃ－Ｆ４Ｍｅ－ＯＨ	１９９００６－５４－７
Ｆｍｏｃ－Ｆ４ＯＭｅ－ＯＨ	７７１２８－７２－４
Ｆｍｏｃ－Ｇ４ｐｉｐａａ（ｔＢｕ）－ＯＨ	２９７３７５３－１４－７
Ｆｍｏｃ－Ｇｃｐｅ－ＯＨ	２２０４９７－６１－０
Ｆｍｏｃ－Ｇｌｎ（Ｔｒｔ）－ＯＨ	１３２３２７－８０－１
Ｆｍｏｃ－Ｇｌｕ（ｔＢｕ）－ＯＨ	７１９８９－１８－９
Ｆｍｏｃ－Ｇｌｙ－ＯＨ	２９０２２－１１－５
Ｆｍｏｃ－Ｇｌｙ（ＤＭＢ）－ＯＨ	１６６８８１－４２－１
Ｆｍｏｃ－Ｇｔｈｐ－ＯＨ	３６８８６６－３１－３
Ｆｍｏｃ－Ｈｇｌ（ｔＢｕ）－ＯＨ	１５９７５１－４７－０
Ｆｍｏｃ－Ｈｉｓ（Ｔｒｔ）－ＯＨ	１０９４２５－５１－６
Ｆｍｏｃ－Ｈｉｓ（Ｂｏｃ）－ＯＨ	８１３７９－５２－４
Ｆｍｏｃ－Ｈｐｒ－ＯＨ	８６０６９－８６－５
Ｆｍｏｃ－Ｈｙｐ（ｔＢｕ）－ＯＨ	１２２９９６－４７－８
Ｆｍｏｃ－Ｉｌｅ－ＯＨ	７１９８９－２３－６
Ｆｍｏｃ－ＩＭｅ－ＯＨ	１２２７７５０－７３－３
Ｆｍｏｃ－ＫＣＯｐｉｐｚａａ（ｔＢｕ）－ＯＨ	２６８０６０７－３４－３
Ｆｍｏｃ－ＫＣＯｐｉｐｚａａ（Ｍｐｅ）－ＯＨ	２９７３７５３－３８－５
Ｆｍｏｃ－Ｌｅｕ－ＯＨ	３５６６１－６０－０
Ｆｍｏｃ－Ｌｙｓ（Ｂｏｃ）－ＯＨ	７１９８９－２６－９
Ｆｍｏｃ－Ｌｙｓ（Ｆｍｏｃ）－ＯＨ	７８０８１－８７－５
Ｆｍｏｃ－ＭｅＡ－ＯＨ	８４０００－０７－７
Ｆｍｏｃ－ＭｅＣ（Ｔｒｔ）－ＯＨ	９４４７９７－５１－７
Ｆｍｏｃ－Ｍｅｄａ－ＯＨ	１３８７７４－９２－２
Ｆｍｏｃ－Ｍｅｄｑ（Ｔｒｔ）－ＯＨ	２０４４７１０－８９－４
Ｆｍｏｃ－ＭｅＧ－ＯＨ	７７１２８－７０－２
Ｆｍｏｃ－ＭｅＫＣＯｐｉｐｚａａ（ｔＢｕ）－ＯＨ	２９７３７５２－８４－８

[Table 1-3]

Fmoc Amino Acid	CAS No.
Fmoc－MeQ（Trt）－OH	163205-13-8
Fmoc－Mor－OH	281655-37-6
Fmoc－Nal1－OH	96402-49-2
Fmoc－Nal2－OH	112883-43-9
Fmoc－Nle－OH	77284-32-3
Fmoc－Pro－OH	71989-31-6
Fmoc－PEG10c－OH	2101563-45-3
Fmoc－PEG12c－OH	1952360-91-6
Fmoc－PEG4c－OH	557756-85-1
Fmoc－PEG8c－OH	756526-02-0
Fmoc－Phe－OH	35661-40-6
Fmoc－Ser（tBu）－OH	71989-33-8
Fmoc－Ser（Trt）－OH	111061-56-4
Fmoc－SMe－OH	159610-93-2
Fmoc－Tbg－OH	132684-60-7
Fmoc－Thr（tBu）－OH	71989-35-0
Fmoc－Thr（Trt）－OH	133180-01-5
Fmoc－Tic－OH	136030-33-6
Fmoc－TMe－OH	928063-81-4
Fmoc－Trp（Boc）－OH	143824-78-6
Fmoc－Tyr（tBu）－OH	71989-38-3
Fmoc－Val－OH	68858-20-8
Fmoc－W1aa（tBu）－OH	1629658-33-8
Fmoc－W1Me－OH	1334509-86-2
Fmoc－W1Me7N－OH	1813528-10-7
Fmoc－W7N－OH	737007-45-3

[0149]

As a purification method for the obtained crude peptide, unless otherwise specified, any one of the following reverse-phase preparative purification systems A)/B)/C)/D)/E) was used.

A): Shimadzu Corporation prep-HPLC system (LC-20AP, SPD-M20A, CTO-20AC, and CBM-20A);

B): Waters Corporation AutoPurification System;

C): Waters Corporation AutoPurification System with SQD;

D): Waters Corporation Preparative HPLC System;

E): YMC Co., Ltd. Contichrom CUBE

[0150]

Unless otherwise specified, any one of the following columns a)-k) was used.

a) Kinetex EVO C18 30 × 150 mm

b) XBridge C18 5 μm 19 × 150 mm

c) XBridge C18 5 μm 30 × 150 mm

d) XBridge C18 5 μm 50 × 150 mm

e) XBridge C18 5 μm 50 × 250 mm

f) XSelect C18 5 μm 19 × 150 mm

g) XSelect C18 5 μm 30 × 150 mm

h) XSelect CSH PrepC18 5 μm OBD 50 × 250 mm

i) XSelect Fluoro-Phenyl 5 μm 10 × 150 mm

j) YMC-Actus Triart Prep C18-S 10 μm 20 × 250 mm

k) YMC-Triart Prep C18-S 10 μm 10 × 250 mm

[0151]

Structural determination of the chemically synthesized peptide was carried out by confirming, by ESI-MS (+) in mass spectrometric analysis, the molecular weight calculated in consideration of the amino acids used according to the target sequence and the building blocks used as necessary. “ESI-MS (+)” indicates electrospray ionization mass spectrometry performed in positive ion mode. The detected mass was reported in “m/z” units. Compounds having a molecular weight of approximately greater than 1000 were frequently detected as multivalent ions.

[0152]

Basic Analytical Instruments and Basic Conditions

For mass spectrometric analysis of the peptides synthesized in the following Examples, the following basic analytical instruments and basic conditions were used unless otherwise specified. Gradient B (%) analysis was carried out using any one of the conditions x/y/z.

[0153]

Instrument: Waters Corporation AutoPurification System-SQD2 single quadrupole mass spectrometer

Column: Kinetex EVO C18 1.7 μm 2.1 × 50 mm, 100 Å

Column temperature: 60°C

Mobile phase A: 0.025% TFA in H_2O

Mobile phase B: 0.025% TFA in MeCN

Flow rate: 0.6 mL/min

Wavelength: 220 nm

Gradient B (%):

x: 5-95%/2.10 min, 95-95%/0.75 min;

[0154]

Instrument: Shimadzu Corporation LC/MS system (LC-20ADXR, CTO-20AC, SPD-M20A, SIL-20AXR, CBM-20A and LCMS-2020)

Column: Kinetex EVO C18 2.6 μm 2.1 × 150 mm, 100 Å

Column temperature: 60°C

Mobile phase A: 0.025% TFA in H_2O

Mobile phase B: 0.025% TFA in MeCN

Flow rate: 0.5 mL/min

Wavelength: 225 nm PDA

Gradient B (%):

y: 20-60%/7.15 min, 60-95%/0.3 min, 95-95%/1.55 min;

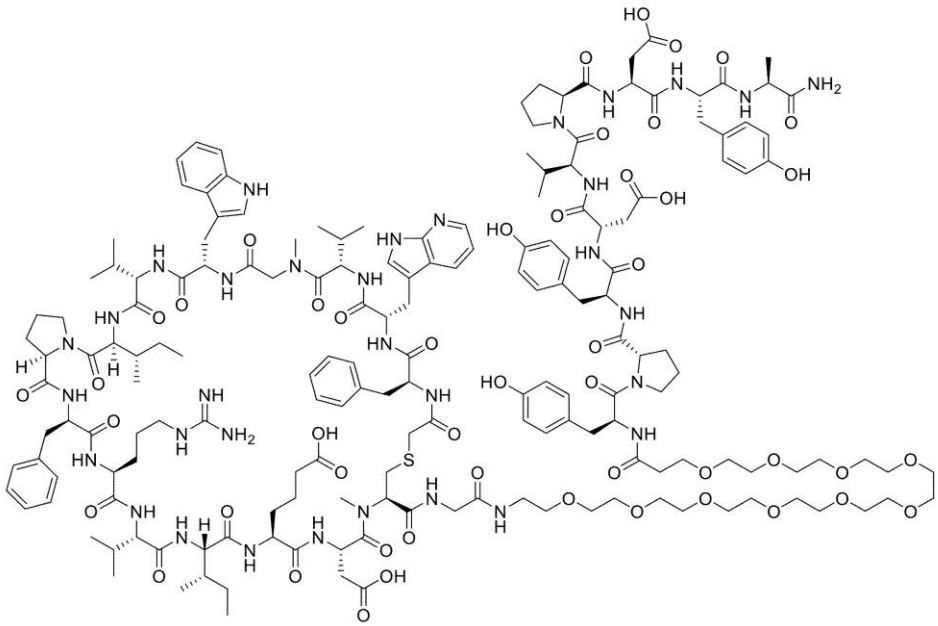
z: 40-80%/7.15 min, 80-95%/0.3 min, 95-95%/1.55 min;

[Example 1]

[0155]

Synthesis of monomer structure number 58-2 (for the constitution, refer to the table below. The same applies hereinafter.)

[Chemical Formula 1]



[0156]

Using Sieber amide resin, synthesis of the target peptide was initiated from removal of the Fmoc group according to the general method described above. At that time, Biotage Syro II was used as the solid-phase synthesizer, and synthesis was carried out in accordance with the

manufacturer's manual. For introduction of each residue, Fmoc-AA/HATU/DIEA (8.4 equivalents/8 equivalents/16.8 equivalents) relative to 1 equivalent of resin was used, and reaction was carried out twice for 20 minutes each at 75°C in DMF. However, for the 3rd residue, 6th residue, 7th residue, 11th residue, and 12th residue, reaction was carried out twice for 30 minutes each at 75°C. For the 10th residue and 15th residue, reaction was carried out twice for 30 minutes each at 50°C. Introduction of Fmoc-PEG10c at the 17th residue was carried out once for 60 minutes at 25°C. Further, when introducing Fmoc-W7N-OH and Fmoc-Hgl(tBu)-OH, solutions obtained by dissolving those amino acids in NMP were used.

Removal of the Fmoc group was carried out by reacting with a 20% piperidine solution in DMF at room temperature for 5 minutes, followed by removal of the solution, and then reacting again with a 20% piperidine solution in DMF at room temperature for 5 minutes.

Introduction of the chloroacetyl group was carried out by removing the Fmoc group of the α-amino group from the solid-phase resin retaining the Fmoc-protected peptide obtained in the previous step by the method described above, then adding a DMF solution of ClAcOSu (20 equivalents) to the solid-phase resin and shaking at room temperature for 60 minutes.

For side-chain deprotection and cleavage from the solid-phase resin, reagent cocktail-A (a mixture of TFA/H_2O/TIS/DODT in a volume ratio of 92.5:2.5:2.5:2.5) was added to a reaction vessel containing the solid-phase resin, followed by thorough shaking, and then shaking at room temperature for 60 minutes. The reaction solution was recovered by filtration through a frit. When this filtrate was added to cooled excess diisopropyl ether, a cloudy precipitate formed, and this mixture was centrifuged, and the solution was decanted. The obtained solid was washed again with a cooled mixed solvent of diethyl ether/hexane (1/1), and then dried for 60 minutes. The obtained solid was used in the subsequent cyclization reaction.

The peptide cyclization reaction was carried out by dissolving the peptide in DMSO/H_2O (9/1) such that the final concentration of the peptide was 1.25 mM based on the number of moles of the solid-phase resin, adding triethylamine (21 equivalents), and allowing the mixture to stand at room temperature for 3 hours. The obtained reaction solution was concentrated under reduced pressure using Genevac EZ-2 Elite, after which DMSO was added such that the peptide concentration became 12.5 mM.

The obtained crude product was subjected to solid-phase extraction using a Gilson column (column: Gilson ASPEC C18 50 mg 1 mL): (1) the column was washed with extraction solution A (0.1% TFA in 95% MeCN/H2O, 0.3 mL). (2) The column was equilibrated with extraction solution B (0.1% TFA in 5% MeCN/H_2O, 0.3 mL). (3) 0.02 mL of the above solution was loaded onto the column. (4) The column was washed with extraction solution B (0.4 mL).

(5) Extraction was carried out with extraction solution A (0.4 mL). The obtained extract was concentrated under reduced pressure using EZ-2 Elite.

The purity of one of the main peaks of the target product was calculated from the area ratio of the LC/MS (UV wavelength 220 nm) chromatogram under the following analytical conditions, and was 65%.

Analytical conditions: retention time = 1.47 min; column: Kinetex EVO C18 1.7 μm 2.1 × 50 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (% B conc): 5-95% over 2.10 min, then 95-95% over 0.75 min; flow rate: 0.6 mL/min

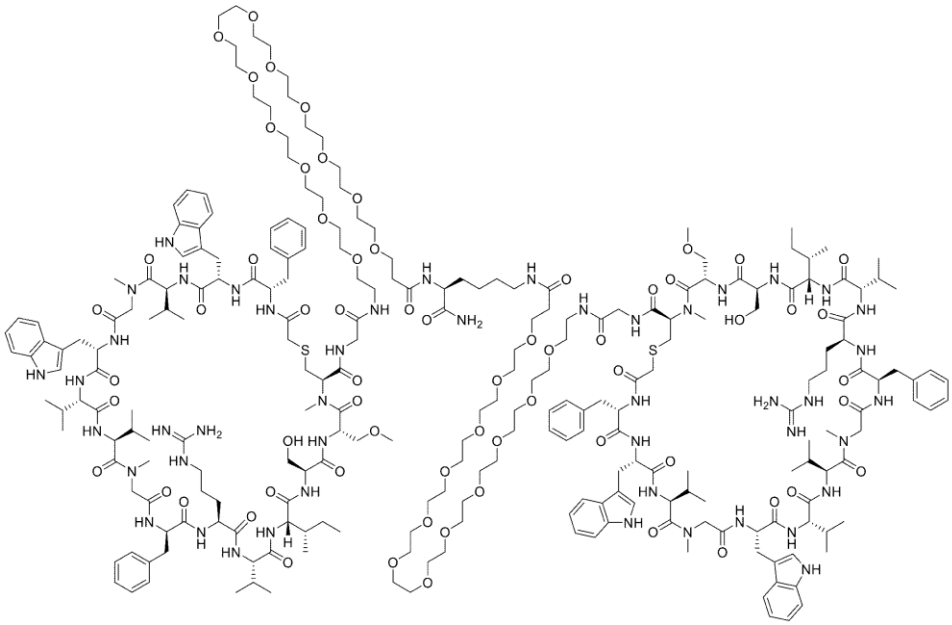
ESI-MS (+) observed value m/z = 1202 (M+3H)3+.

[Example 2]

[0157]

Synthesis of dimer structure number 18

[Chemical Formula 2]



[0158]

Using NovaPEG Rink Amide resin, the target peptide was synthesized by the aforementioned general method starting from removal of the Fmoc group. At that time, Syro II manufactured by Biotage was used as a solid-phase synthesizer, and the synthesis was carried out according to the manufacturer's manual. For introduction of each residue, Fmoc-AA/HATU/DIEA (12.6 equivalents/11.22 equivalents/25.2 equivalents) relative to 1 equivalent of resin was used, and the reaction was carried out twice for 20 minutes at 75°C in DMF.

However, for the 3rd residue, 8th residue, 10th residue, 12th residue, 13th residue, 14th residue, and 15th residue, the reaction was carried out twice for 30 minutes at 50°C. Introduction of Fmoc-PEG10c at the 17th residue was carried out once for 60 minutes at 25°C. In addition, when introducing Fmoc-SMe-OH, a solution obtained by dissolving the amino acid in NMP was used.

Removal of the Fmoc group was performed by reacting with a DMF solution of 20% piperidine at room temperature for 5 minutes, followed by removal of the solution, and then reacting again with a DMF solution of 20% piperidine at room temperature for 15 minutes.

Introduction of the chloroacetyl group was carried out under conditions in which, after removing the Fmoc group of the α-amino group from the solid-phase resin retaining the Fmoc-protected peptide obtained in the previous step by the aforementioned method, chloroacetic acid (0.3 M DMF solution, 12.6 equivalents), HCTU (0.28 M DMF solution, 12 equivalents), and DIEA (1.05 M DMF solution, 25 equivalents) were added to the solid-phase resin and shaken at room temperature for 30 minutes, and this operation was carried out twice consecutively.

For side-chain deprotection and cleavage from the solid-phase resin, reagent cocktail-A (a mixture of TFA/H_2O/TIS/DODT at a volume ratio of 92.5:2.5:2.5:2.5) was added to a reaction vessel containing the solid-phase resin and shaken well, followed by shaking at room temperature for 60 minutes. The reaction solution was collected by filtration through a frit. When this filtrate was added to an excess amount of cooled diisopropyl ether, a cloudy precipitate formed, and this mixture was centrifuged, followed by decantation of the solution. The obtained solid was washed again with a cooled mixed solvent of diisopropyl ether/hexane (1/1), and then dried for 60 minutes. The obtained solid was used in the next cyclization reaction.

For the peptide cyclization reaction, the peptide was dissolved in DMSO such that the final concentration of the peptide was 2.5 mM based on the number of moles of the solid-phase resin, and after adding triethylamine (10 equivalents) and stirring, the mixture was allowed to stand overnight at room temperature. The obtained reaction solution was concentrated under reduced pressure using Genevac EZ-2 Elite so as to give a concentration of 12.5 mM.

The obtained crude product was subjected to solid-phase extraction using a column manufactured by Gilson (column: Gilson ASPEC C18 500 mg 3 mL): (1) the column was washed with extraction solution A (0.1% TFA in 95% MeCN/H_2O, 3 mL). (2) The column was equilibrated with extraction solution B (0.1% TFA in 5% MeCN/H_2O, 3 mL). (3) 0.2 mL of the above solution was loaded onto the column. (4) The column was washed with extraction

solution B (4 mL). (5) Extraction was performed with extraction solution A (4 mL). The obtained extract solution was concentrated under reduced pressure using EZ-2 Elite.

The purity of one of the main peaks of the target product was calculated from the area ratio of the LC/MS (UV wavelength 220 nm) chromatogram under the following analytical conditions and was 60%.

Analytical conditions: retention time = 1.77 min; column: Kinetex EVO C18 1.7 μm 2.1×50 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (% B conc): 5-95% over 2.10 minutes, followed by 95-95% over 0.75 minutes; flow rate: 0.6 mL/min

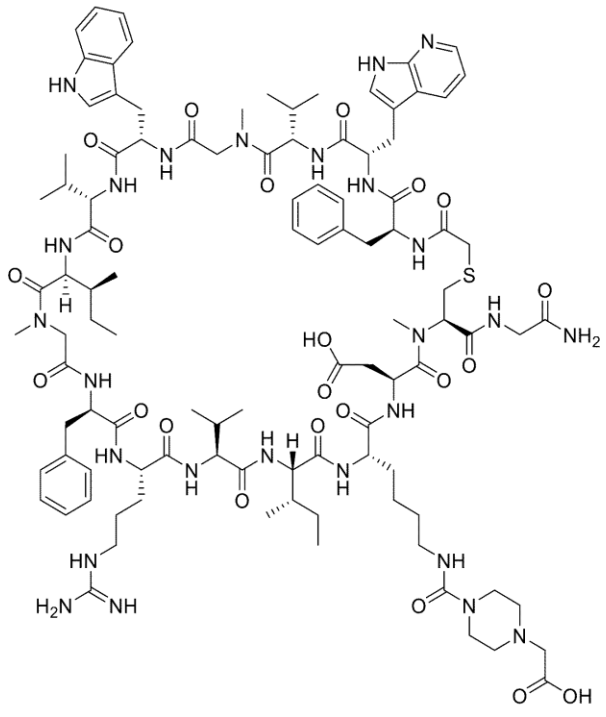
ESI-MS (+) observed value m/z = 1276 (M+4H)4+.

[Example 3]

Synthesis of monomer structure number 53-1

[0159]

[Chemical Formula 3]



[0160]

Using Sieber amide resin (Watanabe Chemical, 0.54 mmol/g), the target peptide was synthesized by the aforementioned general method starting from removal of the Fmoc group. At that time, Liberty Blue manufactured by CEM Corporation was used as a solid-phase synthesizer, and the synthesis was carried out according to the manufacturer's manual. For

introduction of each residue, Fmoc-AA/DIPCI/Oxyma pure (4.2 equivalents/8 equivalents/4 equivalents) relative to 1 equivalent of resin was used, and the reaction was carried out once for 3 minutes at 90°C in DMF. However, for the 3rd residue and 7th residue, the reaction was carried out twice for 30 minutes at 75°C. For the 10th residue, the reaction was carried out twice for 15 minutes at 50°C. For the 14th residue, the reaction was carried out twice for 10 minutes at 90°C. For the 15th residue, the reaction was carried out once for 15 minutes at 50°C. In addition, when introducing Fmoc-W7N-OH, a solution obtained by dissolving the amino acid in NMP was used.

Fmoc removal was carried out under conditions of reacting with a DMF solution of 10% pyrrolidine at 90°C for 1 minute, or under conditions in which reaction at room temperature for 1 minute was carried out twice consecutively.

Introduction of the chloroacetyl group was carried out by adding a DMF solution of ClAcOSu (10 equivalents) to the solid-phase resin and shaking at room temperature for 60 minutes.

For side-chain deprotection and cleavage from the solid-phase resin, first, the resin obtained after the chloroacetyl group introduction step was washed successively with DMF, methylene chloride, and further with diethyl ether, and then dried under reduced pressure, after which reagent cocktail-A (a mixture of TFA/H_2O/TIS/DODT at a volume ratio of 92.5:2.5:2.5:2.5) was added to a reaction vessel containing the solid-phase resin, followed by shaking at room temperature for 90 minutes. The reaction solution was collected by filtration through a frit. When this filtrate was added to an excess amount of a cooled mixed solvent of diisopropyl ether/hexane (1/1), a cloudy precipitate formed, and this mixture was centrifuged, followed by decantation of the solution. The obtained solid was washed again with cooled diethyl ether and then dried under reduced pressure. The obtained solid was used in the next cyclization reaction.

For the peptide cyclization reaction, the peptide was dissolved in MeCN/H_2O (1/1) such that the final concentration of the peptide was 5 mM based on the number of moles of the solid-phase resin, and after adding triethylamine (10 equivalents), the mixture was shaken at room temperature for 3 hours, after which acetic acid was added. The obtained reaction solution was concentrated using EZ-2 Elite. The obtained crude product was purified under the following conditions.

Column: YMC-Triart Prep C18-S 20 μm 20×250 mm;

Mobile phase: A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN; temperature: 50°C; gradient (%B conc): 33-38% over 6 CV, followed by 38% over 1 CV; flow rate: 17 mL/min.

The purity of the target product was calculated from the area ratio of the LC/MS (UV wavelength 225 nm) chromatogram under the following analytical conditions and was 93.51%.

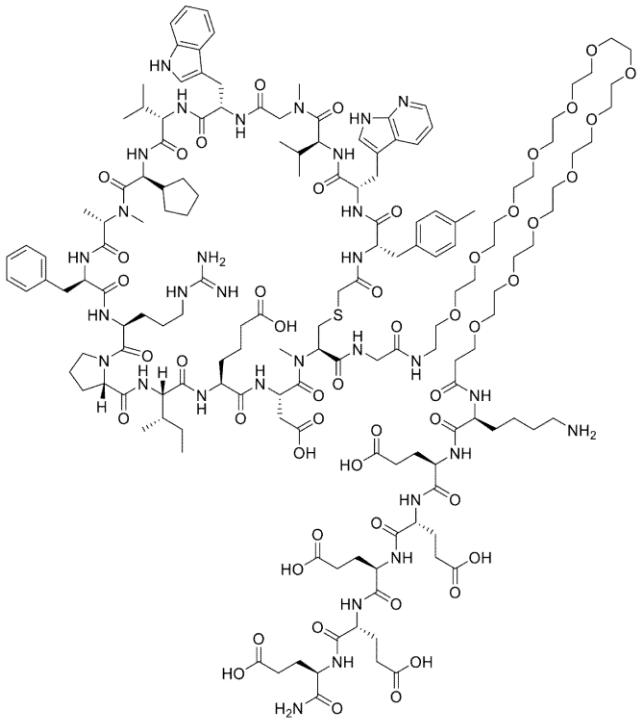
Analytical conditions: retention time = 4.02 min; column: Kinetex EVO C18 2.6 μm 2.1×150 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (%B conc): 20-60% over 7.15 minutes, followed by 60-95% over 0.30 minutes, followed by 95-95% over 1.55 minutes; flow rate: 0.5 mL/min; ESI-MS (+) observed value m/z = 1068 (M+2H)2+.

[Example 4]

[0161]

Synthesis of monomer structure number 88-7

[Chemical Formula 4]



[0162]

Using Sieber amide resin (Watanabe Chemical, 0.6 mmol/g), the target peptide was synthesized by the aforementioned general method starting from removal of the Fmoc group. At that time, Liberty Blue HT manufactured by CEM Corporation was used as a solid-phase synthesizer, and the synthesis was carried out according to the manufacturer's manual. For introduction of each residue, Fmoc-AA/DIPCI/Oxyma pure (4.2 equivalents/4 equivalents/4 equivalents) relative to 1 equivalent of resin was used, and the reaction was carried out once for 20 minutes at 50°C in DMF. However, for the 3rd residue and 7th residue, the reaction was

carried out twice for 30 minutes at 75°C. For the 10th residue, 11th residue, and 14th residue, the reaction was carried out twice for 20 minutes at 50°C. For the 19th residue and 20th residue, the reaction was carried out once for 10 minutes at 75°C. In addition, when introducing Fmoc-W7N-OH, a solution obtained by dissolving the amino acid in NMP was used.

Fmoc removal was carried out under conditions in which reaction with a DMF solution of 10% pyrrolidine at 25°C for 1 minute was carried out twice consecutively.

Introduction of the chloroacetyl group was carried out by adding a DMF solution of ClAcOSu (5 equivalents) to the solid-phase resin and shaking at room temperature for 60 minutes.

For side-chain deprotection and cleavage from the solid-phase resin, first, the resin obtained after the chloroacetyl group introduction step was washed successively with DMF, methylene chloride, and further with diethyl ether, and then dried under reduced pressure, after which reagent cocktail-A (a mixture of TFA/H_2O/TIS/DODT at a volume ratio of 92.5:2.5:2.5:2.5) was added to a reaction vessel containing the solid-phase resin, followed by shaking at room temperature for 70 minutes. The reaction solution was collected by filtration through a frit. When this filtrate was added to an excess amount of a cooled mixed solvent of diisopropyl ether/hexane (1/1), a cloudy precipitate formed, and this mixture was centrifuged, followed by decantation of the solution. The obtained solid was washed again with cooled diethyl ether and then dried under reduced pressure. The obtained solid was used in the next cyclization reaction.

For the peptide cyclization reaction, the peptide was dissolved in DMSO/H_2O (9/1) such that the final concentration of the peptide was 3.8 mM based on the number of moles of the solid-phase resin, and after adding triethylamine (20 equivalents), the mixture was shaken at room temperature for 3 hours, after which acetic acid was added. The obtained reaction solution was concentrated using Genevac HT-12. The obtained crude product was purified under the following conditions.

Column: Waters XBridge C18 5 μm 50×150 mm;

Mobile phase: A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN; temperature: 40°C; gradient (%B conc): 8% over 2 minutes, 8-33% over 1 minute, followed by 33-38% over 8 minutes, followed by 38-60% over 1 minute; flow rate: 20 mL/min over 1 minute, followed by (20 mL/min-120 mL/min) over 1 minute, followed by 120 mL/min.

The purity of the target product was calculated from the area ratio of the LC/MS (UV wavelength 225 nm) chromatogram under the following analytical conditions and was 94.21%.

Analytical conditions: retention time = 4.28 min; column: Kinetex EVO C18 2.6 μm

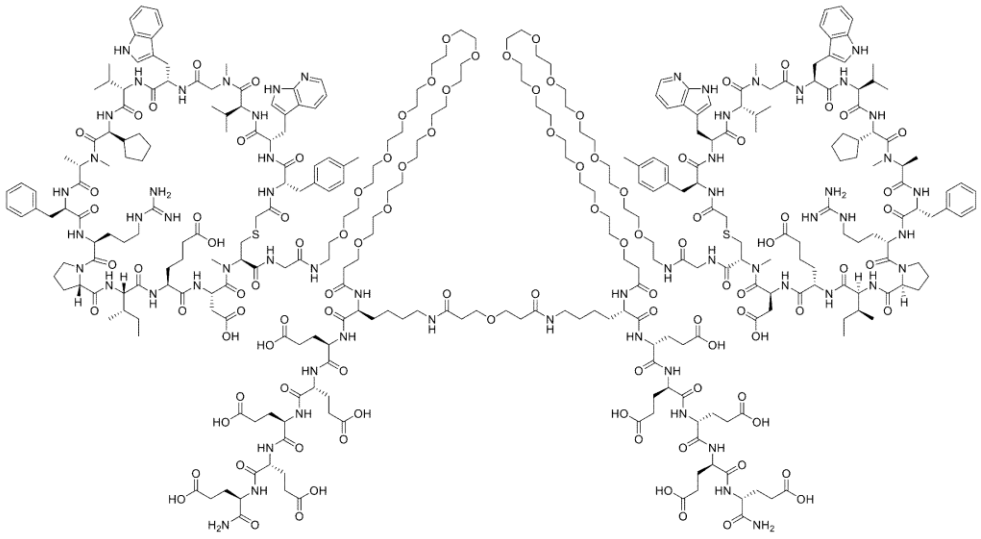
2.1×150 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (%B conc): 20-60% over 7.15 minutes, followed by 60-95% over 0.30 minutes, followed by 95-95% over 1.55 minutes; flow rate: 0.5 mL/min; ESI-MS (+) observed value m/z = 1131 (M+3H)3+.

[Example 5]

[0163]

Synthesis of peptide complex (dimer structure number 108)

[Chemical Formula 5]



[0164]

For synthesis of the peptide complex, monomer structure number 88-7 (49 mg) was dissolved in DMSO, after which NHS-cPEG1c-NHS (0.4 equivalents) and DIEA (10 equivalents) were added, and the mixture was stirred at room temperature for 1 hour, after which acetic acid was added.

The obtained crude product was purified under the following conditions.

Column: Waters XSelect CSH Prep C18 5 μm OBD 50×250 mm; mobile phase: A = 1% AcOH in H_2O, B = 1% AcOH in MeCN, C = 0.2 M TEAA in H_2O, D = MeCN;

Temperature: 50°C;

Main pump gradient (%A conc): 0.1% over 5.0 minutes, followed by 0.1-100% over 0.1 minute, and after 5.1 minutes (100%-%B); (%B conc): 0% over 5.1 minutes, followed by 0-4.2% over 1.9 minutes, followed by 4.2-60% over 20 minutes, followed by 60-90% over 4 minutes; (%C conc): 99.9% over 5.0 minutes, followed by 99.9-0% over 0.1 minute, and 0% after 5.1 minutes; (%D conc): 0%. Flow rate: 18 mL/min over 8 minutes, followed by (18

mL/min-118 mL/min) over 2 minutes, and thereafter 118 mL/min.

At-column-dilution pump: 1% AcOH in H_2O/MeCN (1/1). Flow rate: 2 mL/min.

The purity of the target product was calculated from the area ratio of the LC/MS (UV wavelength 225 nm) chromatogram under the following analytical conditions and was 96.29%.

Analytical conditions: retention time = 5.21 min; column: Kinetex EVO C18 2.6 μm 2.1×150 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (%B conc): 20-60% over 7.15 minutes, followed by 60-95% over 0.30 minutes, followed by 95-95% over 1.55 minutes; flow rate: 0.5 mL/min; ESI-MS (+) observed value m/z = 1383 (M+5H)5+.

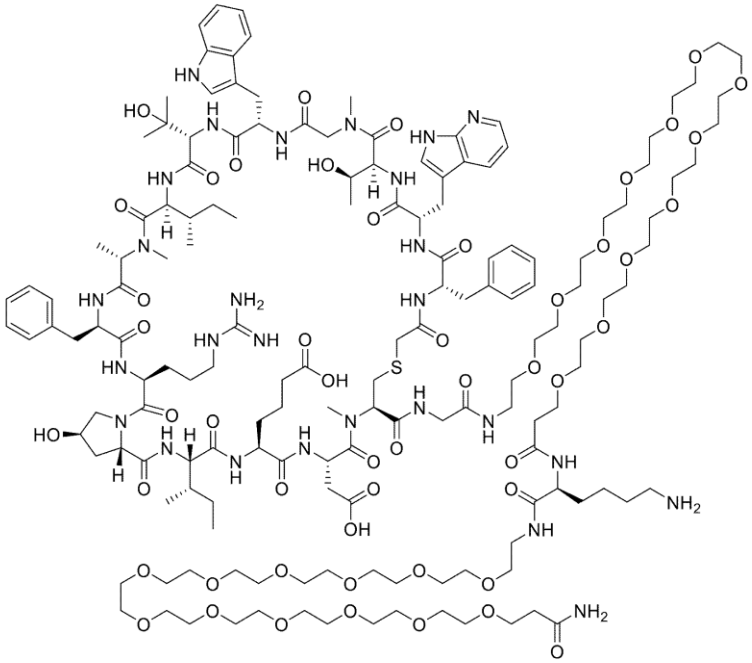
[Example 6]

[0165]

Synthesis of peptide complex (dimer structure number 103)

<6-1> Synthesis of monomer structure number 71-8

[Chemical Formula 6]



[0166]

Using Sieber amide resin (Watanabe Chemical, 0.54 mmol/g), the target peptide was synthesized by the aforementioned general method starting from removal of the Fmoc group. At that time, Liberty Blue manufactured by CEM Corporation was used as a solid-phase synthesizer, and the synthesis was carried out according to the manufacturer's manual. For introduction of each residue, Fmoc-AA/DIPCI/Oxyma pure (4.2 equivalents/8 equivalents/4

equivalents) relative to 1 equivalent of resin was used, and the reaction was carried out once for 3 minutes at 90°C in DMF. However, for the 3rd residue and 7th residue, the reaction was carried out twice for 30 minutes at 75°C. For the 5th residue and 14th residue, the reaction was carried out twice for 10 minutes at 90°C. For the 10th residue, the reaction was carried out twice for 15 minutes at 50°C. For the 15th residue, the reaction was carried out once for 15 minutes at 50°C. In addition, when introducing Fmoc-W7N-OH, a solution obtained by dissolving the amino acid in NMP was used.

Fmoc removal was carried out under conditions of reacting with a DMF solution of 10% pyrrolidine at 90°C for 1 minute, or under conditions in which reaction at room temperature for 1 minute was carried out twice consecutively.

Introduction of the chloroacetyl group was carried out by adding a DMF solution of ClAcOSu (10 equivalents) to the solid-phase resin and shaking at room temperature for 60 minutes.

For side-chain deprotection and cleavage from the solid-phase resin, first, the resin obtained after the chloroacetyl group introduction step was washed successively with DMF, methylene chloride, and further with diethyl ether, and then dried under reduced pressure, after which reagent cocktail-A (a mixture of TFA/H_2O/TIS/DODT at a volume ratio of 92.5:2.5:2.5:2.5) was added to a reaction vessel containing the solid-phase resin, followed by shaking at room temperature for 60 minutes. The reaction solution was collected by filtration through a frit. When this filtrate was added to an excess amount of a cooled mixed solvent of diethyl ether/hexane (1/1), a cloudy precipitate formed, and this mixture was centrifuged, followed by decantation of the solution. The obtained solid was washed again with cooled diethyl ether and then dried under reduced pressure. The obtained solid was used in the next cyclization reaction.

For the peptide cyclization reaction, the peptide was dissolved in DMSO/2-propanol/H_2O (90/5/5) such that the final concentration of the peptide was 2.5 mM based on the number of moles of the solid-phase resin, and after adding triethylamine (10 equivalents), the mixture was shaken at room temperature for 3 hours, after which acetic acid was added. The obtained reaction solution was concentrated using Genevac HT-12. The obtained crude product was purified under the following conditions.

Column: Waters XBridge C18 5 μm 50×150 mm;

Mobile phase: A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN; temperature: 40°C; gradient (%B conc): 6% over 2 minutes, 6-31% over 1 minute, followed by 31-36% over 8 minutes, followed by 36-60% over 1 minute; flow rate: 20 mL/min over 1 minute, followed by

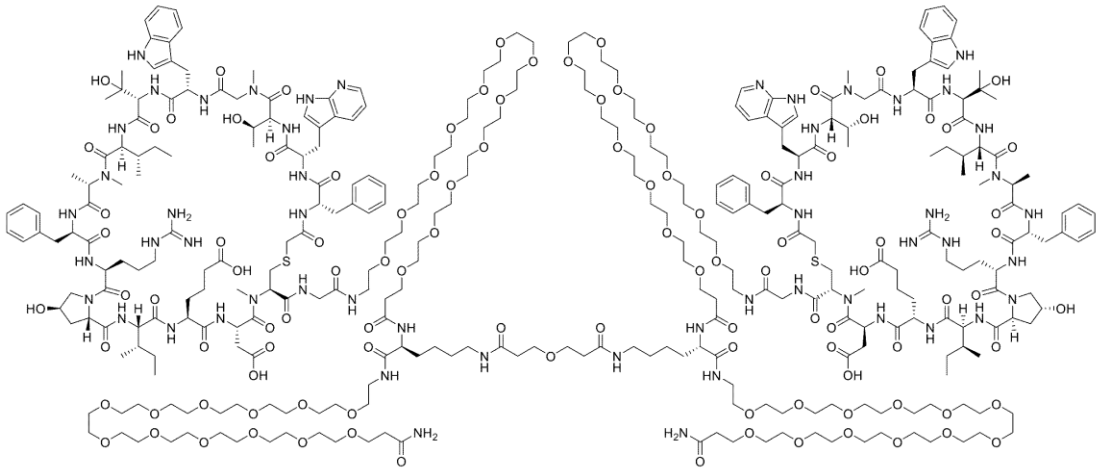
(20 mL/min-120 mL/min) over 1 minute, and thereafter 120 mL/min.

After lyophilization, monomer structure number 71-8 (15 mg) was obtained.

[0167]

<6-2> Synthesis of peptide complex (dimer structure number 103)

[Chemical Formula 7]



[0168]

The peptide conjugate was synthesized by dissolving monomer structure number 71-8 (15 mg) in DMSO, then adding NHS-cPEG1c-NHS (0.3 equivalents) and DIEA (5 equivalents), and stirring at room temperature for 30 minutes, after which acetic acid was added.

The obtained crude product was purified using the following conditions. Column: Waters XSelect C18 5 μm 30 × 150 mm; A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN; temperature: 40°C; gradient (%B conc): from 9% to 34% over 3 minutes, then from 34% to 39% over 8 minutes, then from 39% to 60% over 1 minute; flow rate: 45 mL/min.

The purity of the target compound was calculated from the LC/MS chromatogram peak area under the following analytical conditions (UV wavelength 225 nm) and was 99.21%.

Analytical conditions: retention time = 4.81 min; column: Kinetex EVO C18 2.6 μm 2.1 × 150 mm, 100 Å; mobile phase: A = 0.025% TFA in H_2O, B = 0.025% TFA in MeCN; temperature: 60°C; gradient (%B conc): 20-60% over 7.15 minutes, followed by 60-95% over 0.30 minutes, followed by 95-95% over 1.55 minutes; flow rate: 0.5 mL/min; ESI-MS (+) observed m/z = 1367 (M+5H)5+.

[Example 7]

[0169]

Synthesis of Various Peptides

In the present Example, various peptides and peptide conjugates were chemically synthesized in the same manner as in Examples 1–6. The sequences of the synthesized cyclic peptides are shown in Tables 2-1 and 2-2, the structures of the linkers are shown in Table 3, and the peptide conjugates in which cyclic peptides were dimerized via linkers are shown in Table 4.

The synthesized peptides and peptide conjugates were analyzed under any of the analytical conditions described in the above basic analytical instruments and basic conditions, and their structures were confirmed by ESI-MS (+) in mass spectrometric analysis. The obtained ESI-MS (+) observed values, retention times, valences, and concentration gradients (%) of mobile phase B used in the analysis are shown in Tables 2-1, 2-2, and 4.

In Tables 2-1, 2-2, and 4, when the "monomer structure number" is described as "A-B," A indicates the peptide sequence number and B indicates the linker structure number. In addition, in dimer structure numbers 68–117 of Table 4, it indicates that the compounds represented by the "monomer structure number" form homodimers via the linker represented by the "linker structure number." In the tables, ClAc at the N-terminus means a chloroacetyl group. However, a peptide specified by a certain sequence number may be one in which the N-terminus is not substituted with ClAc (and may furthermore be a pharmaceutically acceptable salt or solvate of the peptide). In particular, when the peptide means a cyclic peptide, the N-terminal ClAc may be removed when the ClAc binds to any group within the sequence to form the cyclic peptide. In particular, when the peptide is a cyclic peptide, it is preferable that the N-terminus not be substituted with ClAc. In the event of any discrepancy between the Sequence Listing and the following tables, the tables shall be construed as correct.

The sequence numbers in the tables mean peptide sequence numbers.

[0170]

Table 2-1 Sequences of the synthesized cyclic peptides

[Table 2-1-1-1]

SEQ ID No.	SEQ															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
2	ClAc	F	W7N	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
3	ClAc	F	W	R	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
4	ClAc	F	W	Cit	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
5	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	E	D	MeC
6	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	I	D	MeC
7	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	F	D	MeC
8	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	W	D	MeC
9	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	Y	D	MeC
10	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	R	D	MeC
11	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	Cit	D	MeC
12	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	Atp	D	MeC
13	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
14	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	H	MeC
15	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	R	MeC
16	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	S	MeC
17	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	Q	MeC
18	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	SMe	MeC
19	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	Cit	MeC
20	ClAc	A	W	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
21	ClAc	Q	W	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
22	ClAc	F	W7N	V	MeG	W	V	V	MeG	df	R	V	I	E	D	MeC
23	ClAc	F	W7N	V	MeG	W	V	V	MeG	df	R	V	I	Atp	D	MeC
24	ClAc	F	W7N	V	MeG	W	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
25	ClAc	F	W	T	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC

[Table 2-1-1-2]

SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
						Concentration Gradient B (%)
1	1-1	1	5.40	955	2	y
	1-2	2	1.67	1169	3	x
	1-3	3	1.77	1143	2	x
	1-4	4	1.78	1231	2	x
	1-5	5	1.79	880	3	x
	1-6	6	1.66	1009	3	x
	1-8	8	1.69	1080	3	x
2	2-2	2	1.55	1169	3	x
	2-5	5	1.34	880	3	x
3						
4						
5	5-5	5	1.63	894	3	x
6						
7						
8						
9						
10	10-5	5	1.53	903	3	x
11						
12	12-5	5	1.46	903	3	x
13	13-5	5	1.58	950	3	x
14						
15						
16						
17						
18						
19						
20	20-5	5	1.39	854	3	x
21	21-5	5	1.36	873	3	x
22	22-5	5	1.51	894	3	x
23	23-5	5	1.39	903	3	x
24	24-5	5	1.34	951	3	x
25	25-2	2	1.63	1170	3	x

[Table 2-1-2-1]

SEQ ID No.	SEQ														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
26	ClAc	F	W	alT	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
27	ClAc	F	W	V	MeG	W	V	I	MeG	df	R	V	I	S	D	MeC
28	ClAc	F	W	V	MeG	W	V	all	MeG	df	R	V	I	S	D	MeC
29	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	V	S	D	MeC
30	ClAc	F	W	S	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
31	ClAc	F	W	V	MeG	W	T	V	MeG	df	R	V	I	S	D	MeC
32	ClAc	F	W	V	MeG	W	alT	V	MeG	df	R	V	I	S	D	MeC
33	ClAc	F	W	V	MeG	W	V	V	MeG	df	Q	V	I	S	D	MeC
34	ClAc	F	W	V	MeG	W	V	V	MeG	df	KCOpipzaa	V	I	S	D	MeC
35	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	T	I	S	D	MeC
36	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	alT	I	S	D	MeC
37	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	Hgl	D	MeC
38	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	E	MeC
39	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	I	S	Hgl	MeC
40	ClAc	E	W7N	V	MeG	W	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
41	ClAc	Q	W7N	V	MeG	W	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
42	ClAc	F	W7N	T	MeG	W	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
43	ClAc	F	W7N	V	MeG	W1Me	V	V	MeG	df	R	V	I	KCOpipzaa	D	MeC
44	ClAc	F	W7N	V	MeG	W	V	all	MeG	df	R	V	I	KCOpipzaa	D	MeC
45	ClAc	F	W7N	V	MeG	W	V	Eva	MeG	df	R	V	I	KCOpipzaa	D	MeC
46	ClAc	F	W7N	V	MeG	W	V	V	MeA	df	R	V	I	KCOpipzaa	D	MeC
47	ClAc	F	W7N	V	MeG	W	V	V	MeG	df	R	V	I	Hgl	D	MeC
48	ClAc	F	W7N	V	MeG	W1Me	V	all	MeA	df	R	V	I	KCOpipzaa	D	MeC
49	ClAc	F	W7N	V	MeG	W1Me	V	all	MeA	df	R	V	I	Hgl	D	MeC
50	ClAc	F	W	V	MeG	W1Me	V	all	MeA	df	R	V	I	KCOpipzaa	D	MeC

[Table 2-1-2-2]

SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
						Concentration Gradient B (%)
26	26-2	2	1.62	1170	3	x
27	27-2	2	1.68	1174	3	x
28	28-2	2	1.68	1174	3	x
29	29-2	2	1.65	1165	3	x
30						
31						
32						
33						
34						
35						
36						
37						
38						
39						
40	40-5	5	1.29	945	3	x
41	41-5	5	1.29	944	3	x
42	42-5	5	1.32	951	3	x
	42-7	7	1.34	1166	3	x
43	43-5	5	1.40	955	3	x
44	44-5	5	1.38	955	3	x
45	45-5	5	1.40	960	3	x
46	46-5	5	1.38	955	3	x
47	47-5	5	1.46	899	3	x
48	48-5	5	1.51	965	3	x
49	49-5	5	1.72	913	3	x
	49-7	7	1.48	1128	3	x
50	50-5	5	1.65	964	3	x

[Table 2-1-3-1]

SEQ ID No.	SEQ														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
51	ClAc	F	W	V	MeG	W1Me	V	all	MeA	df	R	V	I	Hgl	D	MeC
52	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
53	ClAc	F	W7N	V	MeG	W	V	I	MeG	df	R	V	I	KCOpipzaa	D	MeC
54	ClAc	F	W7N	V	MeG	W	V	I	MeG	df	R	V	I	Hgl	D	MeC
55	ClAc	F	W7N	V	MeG	Nal1	V	I	MeA	df	R	V	I	Hgl	D	MeC
56	ClAc	F	W7N	V	MeG	W	V	I	Meda	df	R	V	I	Hgl	D	MeC
57	ClAc	F	W7N	V	MeG	W	V	I	MeKCOpipzaa	df	R	V	I	Hgl	D	MeC
58	ClAc	F	W7N	V	MeG	W	V	I	dp	df	R	V	I	Hgl	D	MeC
59	ClAc	F4COO	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
60	ClAc	F3Me	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
61	ClAc	F3C	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
62	ClAc	F4Me	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
63	ClAc	F4C	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
64	ClAc	F	W1Me7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
65	ClAc	F	W1aa	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
66	ClAc	F	W7N	V	MeG	W	V	I	EtG	df	R	V	I	Hgl	D	MeC
67	ClAc	F	W7N	V	MeG	W	V	I	EtA	df	R	V	I	Hgl	D	MeC
68	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
69	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	Hpr	I	Hgl	D	MeC
70	ClAc	F	W7N	T	MeG	W	V	I	MeA	df	R	Hyp	I	Hgl	D	MeC
71	ClAc	F	W7N	T	MeG	W	dMeS	I	MeA	df	R	Hyp	I	Hgl	D	MeC
72	ClAc	F4Me	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
73	ClAc	F4Me	W7N	V	MeG	W	V	I	MeA	df	R	Hpr	I	Hgl	D	MeC
74	ClAc	F4Me	W7N	V	MeG	W	V	Tbg	MeA	df	R	P	I	Hgl	D	MeC
75	ClAc	F4OMe	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC

[Table 2-1-3-2]

SEQ ID No.	Momomer Structure No.	Linker Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
						Concentration Gradient B (%)
51	51-5	5	1.60	913	3	x
52	52-2	2	1.52	1197	3	x
	52-7	7	1.34	1123	3	x
	52-5	5	1.36	908	3	x
53	53-1	1	4.02	1068	2	y
	53-2	2	1.43	1245	3	x
	53-5	5	1.38	955	3	x
54	54-2	2	1.45	1193	3	x
55	55-2	2	1.51	1201	3	x
56	56-2	2	1.48	1197	3	x
57	57-2	2	1.39	1273	3	x
58	58-2	2	1.47	1202	3	x
59						
60						
61						
62	62-7	7	1.46	1128	3	x
63						
64						
65						
66						
67						
68	68-7	7	1.44	1122	3	x
69						
70	70-8	8	1.46	1113	3	x
71	71-8	8	1.43	839	4	x
72	72-7	7	1.46	1127	3	x
73	73-7	7	1.47	1132	3	x
74	74-7	7	1.36	1127	3	x
75						

[Table 2-1-4-1]

SEQ ID No.	SEQ															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
76	ClAc	3Py	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
77	ClAc	Cha	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
78	ClAc	F4aao	W7N	V	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
79	ClAc	F	W7N	T	MeG	W	V	I	MeA	df	R	P	I	Hgl	D	MeC
80	ClAc	F	W7N	V	MeG	W	dMeS	I	MeA	df	R	P	I	Hgl	D	MeC
81	ClAc	F	W7N	V	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC
82	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	Hyp	I	Hgl	D	MeC
83	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	Mor	I	Hgl	D	MeC
84	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	P	Gthp	Hgl	D	MeC
85	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	P	G4pipaa	Hgl	D	MeC
86	ClAc	F4Me	W7N	V	MeG	W	V	I	MeA	df	R	Hyp	I	Hgl	D	MeC
87	ClAc	F4OMe	W7N	V	MeG	W	V	I	MeA	df	R	Hyp	I	Hgl	D	MeC
88	ClAc	F4Me	W7N	V	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC
89	ClAc	F4Me	W7N	V	MeG	W	V	Gcpe	MeA	df	R	Mor	I	Hgl	D	MeC
90	ClAc	F4Me	W7N	T	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC
91	ClAc	F4Me	W7N	T	MeG	W	V	Gcpe	MeA	df	R	Mor	I	Hgl	D	MeC
92	ClAc	F4aao	W7N	V	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC
93	ClAc	F4aao	W7N	V	MeG	W	V	Gcpe	MeA	df	R	Mor	I	Hgl	D	MeC
94	ClAc	F4aao	W7N	T	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC
95	ClAc	F4aao	W7N	T	MeG	W	V	Gcpe	MeA	df	R	Mor	I	Hgl	D	MeC
96	ClAc	F4OMe	W7N	V	MeG	W	V	Gcpe	MeA	df	R	P	I	Hgl	D	MeC

[Table 2-1-4-2]

SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
						Concentration Gradient B (%)
76						
77						
78	78-7	7	1.31	1147	3	x
79						
80						
81						
82						
83						
84						
85						
86						
87						
88	88-6	6	1.46	1045	3	x
	88-7	7	4.28	1131	3	y
89	89-7	7	1.36	1137	3	x
90	90-7	7	1.34	1132	3	x
91	91-7	7	1.33	1137	3	x
92	92-7	7	1.32	1151	3	x
93	93-7	7	1.33	1157	3	x
94	94-7	7	1.31	1152	3	x
95	95-7	7	1.29	1157	3	x
96	96-7	7	1.37	1137	3	x

[0171]

Table 2-2 Sequences of the synthesized cyclic peptides

[Table 2-2-1-1]

Peptide SEQ ID No.	SEQ															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
97	ClAc	F	Ahp	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
98	ClAc	F	W1Me7N	V	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
99	ClAc	F	W	I	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
100	ClAc	F	W	all	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
101	ClAc	F	W	Q	MeG	W	V	V	MeG	df	R	V	I	S	D	MeC
102	ClAc	F	W	V	MeG	W1Me	V	V	MeG	df	R	V	I	S	D	MeC
103	ClAc	F	W	V	MeG	Nal1	V	V	MeG	df	R	V	I	S	D	MeC
104	ClAc	F	W	V	MeG	Nal2	V	V	MeG	df	R	V	I	S	D	MeC
105	ClAc	F	W	V	MeG	W	V	Ahp	MeG	df	R	V	I	S	D	MeC
106	ClAc	F	W	V	MeG	W	V	Nle	MeG	df	R	V	I	S	D	MeC
107	ClAc	F	W	V	MeG	W	V	V	EtG	df	R	V	I	S	D	MeC
108	ClAc	F	W	V	MeG	W	V	V	MeA	df	R	V	I	S	D	MeC
109	ClAc	F	W	V	MeG	W	V	V	Meda	df	R	V	I	S	D	MeC
110	ClAc	F	W	V	MeG	W	V	V	dp	df	R	V	I	S	D	MeC
111	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	P	I	S	D	MeC
112	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	Hpr	I	S	D	MeC
113	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	Tic	I	S	D	MeC
114	ClAc	F	W	V	MeG	W	V	V	MeG	df	R	V	all	S	D	MeC
115	ClAc	F4aao	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
116	ClAc	F3COO	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
117	ClAc	F3aao	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
118	ClAc	4Py	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
119	ClAc	3Py	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
120	ClAc	Cha	W7N	V	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
121	ClAc	F	W7N	T	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
122	ClAc	F	W7N	TMe	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
123	ClAc	F	W7N	KCOpipzaa	MeG	W	V	I	MeA	df	R	V	I	Hgl	D	MeC
124	ClAc	F	W7N	V	MeG	W	Tbg	I	MeA	df	R	V	I	Hgl	D	MeC
125	ClAc	F	W7N	V	MeG	W	TMe	I	MeA	df	R	V	I	Hgl	D	MeC

[Table 2-2-1-2]

Peptide SEQ ID No.	Monomer Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
					Concentration Gradient B (%)
97	97-2	1.69	1150	3	x
98	98-2	1.59	1174	3	x
99	99-2	1.68	1174	3	x
100	100-2	1.69	1174	3	x
101	101-2	1.60	1179	3	x
102	102-2	1.70	1174	3	x
103	103-2	1.72	1173	3	x
104	104-2	1.72	1173	3	x
105	105-2	1.71	1179	3	x
106	106-2	1.69	1174	3	x
107	107-2	1.68	1174	3	x
108	108-2	1.69	1174	3	x
109	109-2	1.70	1174	3	x
110	110-2	1.70	1178	3	x
111	111-2	1.65	1169	3	x
112	112-2	1.66	1173	3	x
113	113-2	1.69	1189	3	x
114	114-2	1.66	1169	3	x
115	115-2	1.38	1222	3	x
116	116-2	1.50	1212	3	x
117	117-2	1.44	1222	3	x
118	118-2	1.35	1198	3	x
119	119-2	1.36	1198	3	x
120	120-2	1.50	1199	3	x
121	121-2	1.43	1198	3	x
122	122-2	1.45	1203	3	x
123	123-2	1.38	1264	3	x
124	124-2	1.48	1202	3	x
125	125-2	1.47	1203	3	x

[Table 2-2-2-1]

Peptide SEQ ID No.	SEQ															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
126	ClAc	F	W7N	V	MeG	W	alTMe	I	MeA	df	R	V	I	Hgl	D	MeC
127	ClAc	F	W7N	V	MeG	W	dMeS	I	MeA	df	R	V	I	Hgl	D	MeC
128	ClAc	F	W7N	V	MeG	W	Gthp	I	MeA	df	R	V	I	Hgl	D	MeC
129	ClAc	F	W7N	V	MeG	W	G4pipaa	I	MeA	df	R	V	I	Hgl	D	MeC
130	ClAc	F	W7N	V	MeG	W	Cit	I	MeA	df	R	V	I	Hgl	D	MeC
131	ClAc	F	W7N	V	MeG	W	V	L	MeA	df	R	V	I	Hgl	D	MeC
132	ClAc	F	W7N	V	MeG	W	V	Tbg	MeA	df	R	V	I	Hgl	D	MeC
133	ClAc	F	W7N	V	MeG	W	V	IMe	MeA	df	R	V	I	Hgl	D	MeC
134	ClAc	F	W7N	V	MeG	W	V	Cbg	MeA	df	R	V	I	Hgl	D	MeC
135	ClAc	F	W7N	V	MeG	W	V	Gcpe	MeA	df	R	V	I	Hgl	D	MeC
136	ClAc	F	W7N	V	MeG	W	V	Chg	MeA	df	R	V	I	Hgl	D	MeC
137	ClAc	F	W7N	V	MeG	W	V	alTMe	MeA	df	R	V	I	Hgl	D	MeC
138	ClAc	F	W7N	V	MeG	W	V	I	MeQ	df	R	V	I	Hgl	D	MeC
139	ClAc	F	W7N	V	MeG	W	V	I	Medq	df	R	V	I	Hgl	D	MeC
140	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	TMe	I	Hgl	D	MeC
141	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	alTMe	I	Hgl	D	MeC
142	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	V	alTMe	Hgl	D	MeC
143	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	V	dMeS	Hgl	D	MeC
144	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	V	Gthp	Hgl	D	MeC
145	ClAc	F	W7N	V	MeG	W	V	I	MeA	df	R	V	G4pipaa	Hgl	D	MeC

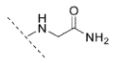
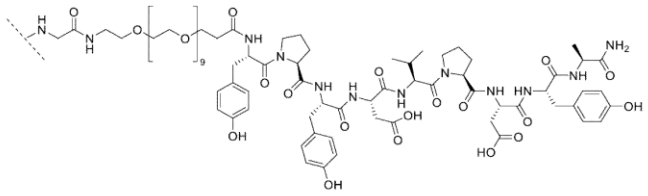
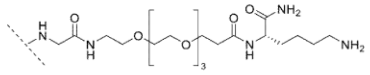
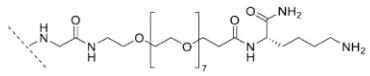
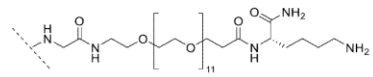
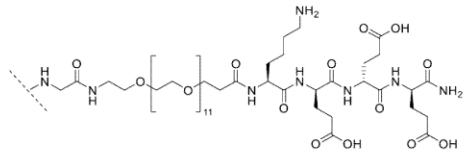
[Table 2-2-2-2]

Peptide SEQ ID No.	Monomer Structure No.	Ret. Time	ESI (m/z)	[M+XH]X+	QC Condition
					Concentration Gradient B (%)
126	126-2	1.46	1203	3	x
127	127-2	1.44	1203	3	x
128	128-2	1.44	1212	3	x
129	129-2	1.38	1231	3	x
130	130-2	1.42	1217	3	x
131	131-2	1.47	1197	3	x
132	132-2	1.46	1197	3	x
133	133-2	1.47	1202	3	x
134	134-2	1.45	1197	3	x
135	135-2	1.47	1201	3	x
136	136-2	1.48	1206	3	x
137	137-2	1.44	1198	3	x
138	138-2	1.47	1216	3	x
139	139-2	1.44	1217	3	x
140	140-2	1.46	1203	3	x
141	141-2	1.46	1203	3	x
142	142-2	1.45	1198	3	x
143	143-2	1.44	1198	3	x
144	144-2	1.45	1207	3	x
145	145-2	1.41	1226	3	x

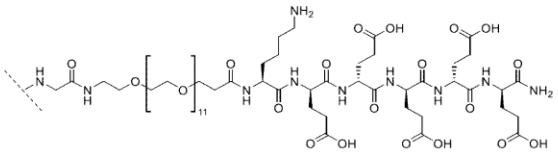
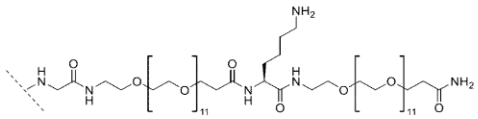
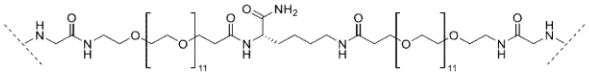
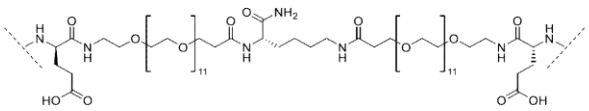
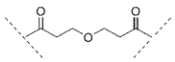
[0172]

Table 3 Structure of linkers

[Table 3-1]

Linker Structure No.	Name	Structure
1	NH2	
2	tag	
3	P04	
4	P08	
5	P12	
6	P12de3	

[Table 3-2]

Linker Structure No.	Name	Structure
7	P12de5	
8	P12P12	
9	P12K_d	
10	deP12K_d	
11	KP01K_d	

[0173]

Table 4 Peptide complexes

[Table 4-1]

Dimer Structure No.	Peptide SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret time	ESI (m/z)	[M+XH]X+	QC Condition
							Concentration Gradient B (%)
1	1		9	1.84	1283	4	x
2	2		9	1.60	1283	4	x
3	3		9	1.53	1311	4	x
4	4		9	1.67	1312	4	x
5	5		9	1.74	1304	4	x
6	6		9	1.81	1296	4	x
7	7		9	1.82	1313	4	x
8	8		9	1.80	1332	4	x
9	9		9	1.77	1321	4	x
10	10		9	1.60	1317	4	x
11	11		9	1.75	1318	4	x
12	12		9	1.78	1317	4	x
13	13		9	1.68	1388	4	x
14	14		9	1.59	1294	4	x
15	15		9	1.59	1303	4	x
16	16		9	1.75	1269	4	x
17	17		9	1.74	1289	4	x
18	18		9	1.77	1276	4	x
19	19		9	1.74	1304	4	x
20	25		9	1.71	1284	4	x
21	26		9	1.71	1284	4	x
22	27		9	1.78	1290	4	x
23	28		9	1.78	1290	4	x
24	29		9	1.75	1276	4	x
25	30		9	1.70	1277	4	x
26	31		9	1.73	1284	4	x
27	32		9	1.72	1284	4	x
28	33		9	2.00	1269	4	x
29	34		9	1.87	1354	4	x
30	35		9	1.72	1284	4	x

[Table 4-2]

Dimer Structure No.	Peptide SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret time	ESI (m/z)	[M+XH]X+	QC Condition
							Concentration Gradient B (%)
31	36		9	1.73	1284	4	x
32	37		9	1.76	1311	4	x
33	38		9	1.76	1290	4	x
34	39		9	1.76	1297	4	x
35	52		9	1.53	1325	4	x
36	53		9	1.49	1396	4	x
37	54		9	1.51	1319	4	x
38	55		9	1.60	1331	4	x
39	56		9	1.56	1325	4	x
40	57		9	1.44	1439	4	x
41	58		9	1.54	1332	4	x
42	59		9	1.49	1348	4	x
43	60		9	1.55	1332	4	x
44	61		9	1.56	1343	4	x
45	62		9	1.55	1332	4	x
46	63		9	1.56	1342	4	x
47	64		9	1.59	1332	4	x
48	65		9	1.65	1354	4	x
49	66		9	1.53	1325	4	x
50	67		9	1.55	1332	4	x
51	68		9	1.53	1325	4	x
52	69		9	1.54	1331	4	x
53	68		10	1.57	1088	5	x
54	72		9	1.62	1065	5	x
55	75		9	1.58	1071	5	x
56	76		9	1.43	1324	4	x
57	77		9	1.63	1062	5	x
58	78		9	1.54	1361	4	x
59	79		9	1.54	1060	5	x
60	80		9	1.56	1066	5	x

[Table 4-3]

Dimer Structure No.	Peptide SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret time	ESI (m/z)	[M+XH]X+	QC Condition
							Concentration Gradient B (%)
61	81		9	1.59	1064	5	x
62	82		9	1.55	1066	5	x
63	83		9	1.56	1066	5	x
64	84		9	1.57	1071	5	x
65	85		9	1.52	911	6	x
66	86		9	1.57	1071	5	x
67	87		9	1.55	1078	5	x
68	1	1-3	11	6.02	1175	4	y
69	1	1-4	11	6.09	1263	4	y
70	1	1-5	11	6.15	1351	4	y
71	1	1-6	11	6.19	1236	5	y
72	1	1-8	11	6.42	1320	5	y
73	2	2-5	11	5.24	1351	4	y
74	5	5-5	11	6.34	1372	4	y
75	10	10-5	11	5.42	1385	4	y
76	12	12-5	11	2.71	1385	4	z
77	13	13-5	11	5.73	1165	5	y
78	20	20-5	11	5.74	1312	4	y
79	21	21-5	11	5.48	1341	4	y
80	22	22-5	11	5.24	1372	4	y
81	23	23-5	11	5.10	1386	4	y
82	24	24-5	11	4.94	1166	5	y
83	40	40-5	11	4.52	1448	4	y
84	41	41-5	11	4.40	1447	4	y
85	42	42-5	11	4.20	1167	5	y
86	42	42-7	11	4.14	1424	5	y
87	43	43-5	11	5.41	1171	5	y
88	44	44-5	11	4.76	1171	5	y
89	45	45-5	11	4.97	1177	5	y
90	46	46-5	11	4.70	1171	5	y

[Table 4-4]

Dimer Structure No.	Peptide SEQ ID No.	Monomer Structure No.	Linker Structure No.	Ret time	ESI (m/z)	[M+XH]X+	QC Condition
							Concentration Gradient B (%)
91	47	47-5	11	5.16	1379	4	y
92	48	48-5	11	5.19	1183	5	y
93	49	49-5	11	5.76	1400	4	y
94	49	49-7	11	5.46	1378	5	y
95	50	50-5	11	6.33	1182	5	y
96	51	51-5	11	3.42	1399	4	z
97	52	52-7	11	5.17	1373	5	y
98	52	52-5	11	5.26	1393	4	y
99	53	53-5	11	5.32	1171	5	y
100	62	62-7	11	5.11	1379	5	y
101	68	68-7	11	5.15	1372	5	y
102	70	70-8	11	4.92	1361	5	y
103	71	71-8	11	4.81	1367	5	y
104	72	72-7	11	5.17	1378	5	y
105	73	73-7	11	5.20	1383	5	y
106	74	74-7	11	5.08	1378	5	y
107	78	78-7	11	5.18	1402	5	y
108	88	88-7	11	5.21	1383	5	y
109	88	88-6	11	5.57	1279	5	y
110	89	89-7	11	5.07	1389	5	y
111	90	90-7	11	4.91	1383	5	y
112	91	91-7	11	4.72	1390	5	y
113	92	92-7	11	5.26	1407	5	y
114	93	93-7	11	4.84	1413	5	y
115	94	94-7	11	5.00	1407	5	y
116	95	95-7	11	4.85	1414	5	y
117	96	96-7	11	5.64	1389	5	y

[Example 8]

[0174]

Evaluation of Binding Activity to TPOR by ELISA

An ELISA-based binding activity assay was performed in order to evaluate the binding activity of the peptides of the present invention to TPOR. The specific test method is described below.

His-tagged fusion TPOR (R&D Systems; 4444-TR) was added at 3 pmol per well to an anti-his-tag antibody-coated 96-well plate (GenScript; L00440C), and TPOR was immobilized on the plate by allowing it to stand for 30 minutes. After washing three times with PBS-T, HA-tagged fusion peptide diluted to 5 nM was added and allowed to stand for 1 hour. After washing three times with PBS-T, Anti-HA-tag mAb-HRP-Direct (MBL; M180-7), diluted 5000-fold with PBS-T containing 1% BSA, was added and allowed to stand for 30 minutes. After washing three times with PBS-T, the detection reagent SureBlue® TMB 1-Component

Microwell Peroxidase Substrate (SeraCare; 5120-0077) was added and allowed to stand for 10 minutes, after which the reaction was stopped by adding TMB Stop Solution (SeraCare; 5150-0021) in an amount equal to that of the TMB. Absorbance at 450 nm was measured using EnSpire (PerkinElmer). The binding signal for each peptide was calculated by subtracting the absorbance of the peptide-free well from the absorbance of the well to which each peptide had been added.

For monomer structure numbers 1-2, 2-2, 25-2 to 29-2, and 52-2 to 58-2, the calculated binding signals are shown in Table 5-1.

For monomer structure numbers 97-2 to 145-2, the calculated binding signals are shown in Table 5-2.

In Tables 5-1 and 5-2, when the "monomer structure number" is described as "A-B," A indicates the peptide sequence number and B indicates the linker structure number.

As shown in Tables 5-1 and 5-2, these peptides were shown to bind to TPOR.

[0175]

Table 5-1 Evaluation of Binding Activity to TPOR

[Table 5-1]

Peptide SEQ ID No.	Monomer Structure No.	ELISA signal/SPR	
		ELISA	SPR (KD)
1	1-2	1.4	
2	2-2	0.2	
25	25-2	1.3	
26	26-2	0.5	
27	27-2	1.2	
28	28-2	1.5	
29	29-2	0.9	
52	52-2	2.0	
53	53-2	1.2	
54	54-2	1.3	
55	55-2	1.3	
56	56-2	0.8	
57	57-2	0.9	
58	58-2	0.5	
1	1-1		1.E-09
53	53-1		2.E-09
88	88-7		3.E-08

[0176]

Table 5-2 Evaluation of Binding Activity to TPOR

[Table 5-2-1]

Peptide SEQ ID No.	Monomer Structure No.	ELISA signal
97	97-2	0.3
98	98-2	0.3
99	99-2	1.1
100	100-2	0.6
101	101-2	0.4
102	102-2	1.5
103	103-2	1.0
104	104-2	0.5
105	105-2	0.4
106	106-2	0.2
107	107-2	1.0
108	108-2	1.8
109	109-2	1.4
110	110-2	1.2
111	111-2	0.2
112	112-2	0.4
113	113-2	0.2
114	114-2	0.4
115	115-2	1.1
116	116-2	0.4
117	117-2	1.1
118	118-2	0.9
119	119-2	1.7
120	120-2	1.7
121	121-2	1.6

[Table 5-2-2]

Peptide SEQ ID No.	Monomer Structure No.	ELISA signal
122	122-2	0.3
123	123-2	0.6
124	124-2	0.6
125	125-2	0.5
126	126-2	1.2
127	127-2	1.8
128	128-2	1.1
129	129-2	0.3
130	130-2	0.7
131	131-2	1.5
132	132-2	1.8
133	133-2	2.0
134	134-2	1.1
135	135-2	1.9
136	136-2	0.4
137	137-2	1.0
138	138-2	0.8
139	139-2	1.3
140	140-2	1.7
141	141-2	2.2
142	142-2	1.4
143	143-2	0.9
144	144-2	1.6
145	145-2	1.5

[Example 9]

[0177]

Evaluation of Intermolecular Interaction Between TPOR and Peptides by Surface Plasmon Resonance (SPR)

In order to evaluate the binding activity of the peptides of the present invention to

TPOR, an intermolecular interaction evaluation by surface plasmon resonance (SPR) was carried out. The specific test method is described below.

[SPR Measurement]

An NTA sensor chip (Cytiva) was inserted into a Biacore T200 (Cytiva), and priming operation was performed three times using running buffer: HBS-P+, pH 7.4 (Cytiva), followed by equilibration at a flow rate of 30 μL/min.

Ligand immobilization operation was carried out at a flow rate of 10 μL/min. A 350 mM EDTA solution (Nacalai Tesque) was flowed for 30 seconds to wash the NTA sensor chip. Thereafter, a 500 μM nickel chloride solution (Hampton Research) was flowed for 30 seconds to coordinate nickel ions on the carboxymethyl dextran chains. A washing operation was then performed using a 50 mM EDTA solution (Nacalai Tesque). After mixing 50 μL each of a 60 mM EDC solution (Cytiva) and a 650 mM NHS solution (Cytiva), the mixture was reacted for 420 seconds at a flow rate of 10 μL/min. A 100 nM TPOR solution was reacted at a flow rate of 10 μL/min for 420 seconds, and immobilization was carried out so as to achieve approximately 2000 RU by amine coupling through crosslinking of NTA with nickel ions. A 1.0 M aqueous ethanolamine solution (Cytiva) was reacted for 420 seconds at a flow rate of 10 μL/min to perform capping treatment.

A peptide solution prepared at 10 mM in DMSO solution was diluted with running buffer so that the final concentration became a 10 μM peptide solution, and peptide solutions of 100 nM, 50 nM, 25 nM, 10 nM, and 5 nM were prepared. The above peptide samples were reacted at a flow rate of 30 μL/min for 120 seconds, followed by dissociation for 600 seconds, and the kinetics of the peptides toward TPOR were obtained by SPR measurement.

The kinetics evaluation model was Single Cycle Kinetics, and curve fitting was performed using Biacore T200 Evaluation Software Version 3.0 (Cytiva). Curve fitting by the least-squares method was performed on the obtained sensorgrams, and binding of the peptides to TPOR was evaluated by determining the KD values.

For monomer structure numbers 1-1, 53-1, and 88-7, the calculated KD values are shown in Table 5-1.

As shown in Table 5-1, these peptides were shown to bind to TPOR.

[Example 10]

[0178]

Evaluation of ERK Activation by AlphaLISA

In order to evaluate the ERK activation ability of the peptide conjugates of the present

invention, phosphorylation of ERK was evaluated using AlphaLISA. The specific test method is described below.

TPO Reporter HEK293 Cells (InvivoGen; hkb-tpo) were cultured in DMEM (Thermo Fisher Scientific) containing 10% FBS, 100 μg/mL Nomocin (InvivoGen), 50 μg/mL Gentamicin (Nacalai Tesque), and 1× HEK-Blue selection (Invivogen). After detaching the cells using PBS, the cells were seeded in a Poly-D-lysine-coated 96-well plate for adherent cells so that each well contained 30,000 cells, and cultured for 24 hours. For starvation, the medium was replaced with DMEM containing 0.1% BSA (Sigma) and 50 μg/mL Gentamicin (Nacalai Tesque), followed by culture for 20 hours. Thereafter, Recombinant Human Thrombopoietin (R&D Systems) or peptide was added, and stimulation was performed at 37°C for 20 minutes in a CO_2 incubator. Thereafter, the cells were lysed using the Lysis Buffer attached to the AlphaLISA SureFire Ultra p-Erk1/2 (Thr202/Tyr204) Assay kit (PerkinElmer). The assay was carried out according to the kit protocol, and signal detection was performed using a SpectraMax Paradigm multimode microplate reader (Molecular Devices). The obtained signals were analyzed with GraphPad Prism, and % activation was calculated by setting the maximum value of the signal induced by TPO as 100% and the unstimulated condition as 0%.

[0179]

The addition concentration of TPO was tested in the range of 0.002 nM to 10 nM, and the maximum induced signal value (Emax) was calculated.

For dimer structure numbers 1–34, peptide addition concentrations of 10, 100, and 1000 nM were tested, and those showing % activation of 50% or more upon addition at 10 nM were designated as A-1, those showing % activation of 50% or more upon addition at 100 nM were designated as A-2, those showing % activation of 50% or more upon addition at 1000 nM were designated as A-3, and those showing % activation of 5% or more and less than 50% upon addition at 1000 nM were designated as A-4, as shown in Table 6.

For dimer structure numbers 35–67, peptide addition concentrations of 1, 10, and 100 nM were tested, and those showing % activation of 50% or more upon addition at 1 nM were designated as B-1, those showing % activation of 50% or more upon addition at 10 nM were designated as B-2, those showing % activation of 50% or more upon addition at 100 nM were designated as B-3, and those showing % activation of 5% or more and less than 50% upon addition at 100 nM were designated as B-4, as shown in Table 6.

There were no compounds corresponding to B-1.

For dimer structure numbers 68–107 and 110–117, peptide addition concentrations of 1, 10, and 100 nM were tested, and those showing % activation of 50% or more upon addition

at 1 nM were designated as C-1, those showing % activation of 50% or more upon addition at 10 nM were designated as C-2, those showing % activation of 50% or more upon addition at 100 nM were designated as C-3, and those showing % activation of 5% or more and less than 50% upon addition at 100 nM were designated as C-4, as shown in Table 6.

[0180]

For dimer structure numbers 108 and 109, peptide addition concentrations of 0.039, 0.156, 0.625, and 2.5 nM were tested, and those showing % activation of 50% or more upon addition at 0.039 nM were designated as D-1, those showing % activation of 50% or more upon addition at 0.156 nM were designated as D-2, those showing % activation of 50% or more upon addition at 0.625 nM were designated as D-3, those showing % activation of 50% or more upon addition at 2.5 nM were designated as D-4, and those showing % activation of 5% or more and less than 50% upon addition at 2.5 nM were designated as D-5, as shown in Table 6.

There were no compounds corresponding to D-1, D-3, D-4, or D-5.

As shown in Table 6, these peptides were shown to have the ability to activate ERK signaling in TPO Reporter HEK293 Cells.

[0181]

Table 6 Evaluation of ERK Activation and Evaluation of JAK-STAT Pathway Activation

[Table 6-1]

Dimer Structure No.	pERK assay Results	Reporter assay Results
1	A-2	NT
2	A-2	NT
3	A-2	NT
4	A-4	NT
5	A-2	NT
6	A-4	NT
7	A-2	NT
8	A-4	NT
9	A-3	NT
10	A-2	NT
11	A-2	NT
12	A-2	NT
13	A-2	NT
14	A-4	NT
15	A-2	NT
16	A-4	NT
17	A-3	NT
18	A-4	NT
19	A-4	NT
20	A-4	NT
21	A-4	NT
25	A-4	NT
26	A-4	NT
27	A-4	NT
22	A-2	NT
23	A-2	NT
28	A-3	NT
29	A-4	NT
30	A-4	NT
31	A-3	NT

[Table 6-2]

Dimer Structure No.	pERK assay Results	Reporter assay Results
24	A-2	NT
32	A-2	NT
33	A-4	NT
34	A-4	NT
36	B-4	NT
37	B-3	NT
35	B-3	NT
42	B-4	NT
43	B-3	NT
44	B-3	NT
45	B-3	NT
46	B-3	NT
47	B-3	NT
48	B-4	NT
38	B-3	NT
40	B-4	NT
49	B-4	NT
50	B-4	NT
39	B-3	NT
41	B-4	NT
52	B-2	NT
54	B-3	NT
55	B-3	NT
56	B-3	NT
57	B-3	NT
58	B-3	NT
51	B-3	NT
59	B-3	NT
60	B-2	NT
61	B-2	NT

[Table 6-3]

Dimer Structure No.	pERK assay Results	Reporter assay Results
62	B-3	NT
63	B-3	NT
64	B-3	NT
65	B-3	NT
53	B-3	NT
66	B-3	NT
67	B-3	NT
68	C-2	E-1
69	C-2	E-1
70	C-2	F-4
71	C-3	NT
72	C-3	NT
78	C-4	NT
79	C-4	NT
73	C-2	NT
74	C-2	NT
75	C-2	NT
76	C-3	NT
77	C-2	NT
80	C-3	NT
81	C-3	NT
82	C-2	NT
83	C-4	NT
84	C-4	NT
85	C-2	NT
87	C-1	NT
99	C-1	NT
88	C-1	F-2
89	C-1	NT
90	C-1	NT

[Table 6-4]

Dimer Structure No.	pERK assay Results	Reporter assay Results
91	C-1	NT
92	C-1	F-3
93	C-1	F-2
95	C-2	NT
96	C-2	NT
86	C-2	NT
94	C-2	NT
102	C-2	NT
103	C-3	NT
100	C-2	NT
101	C-2	NT
104	C-1	NT
105	C-2	NT
106	C-2	NT
97	C-2	NT
98	C-2	NT
108	D-2	F-2
110	C-1	NT
111	C-1	NT
112	C-1	NT
107	C-1	NT
113	C-1	NT
114	C-1	NT
115	C-2	NT
116	C-3	NT
117	C-1	NT
109	D-2	F-1

[Example 11]

[0182]

Evaluation of STAT5-Dependent Transcriptional Activation by STAT5-Inducible Secreted Embryonic Alkaline Phosphatase (SEAP) Reporter Assay

In order to evaluate the STAT5-dependent transcriptional activation ability of the

peptide conjugates of the present invention, transcriptional activity was evaluated using a STAT5-inducible SEAP reporter. The specific test method is described below.

TPO Reporter HEK293 Cells used in the AlphaLISA assay were cultured in DMEM (Thermo Fisher Scientific) containing 10% FBS, 100 μg/mL Nomocin (InvivoGen), 50 μg/mL Gentamicin (Nacalai Tesque), and 1× HEK-Blue selection (InvivoGen). After detaching the cells using PBS, the cells were suspended in DMEM containing 0.1% BSA (Sigma) and 50 μg/mL Gentamicin (Nacalai Tesque), and seeded into an adherent-cell 96-well plate at 54,000 cells per well. Immediately after seeding, TPO or peptide was added, and the cells were cultured at 37°C for 24 hours in a CO_2 incubator. Then, 20 μL of the culture supernatant was transferred to a new 96-well plate for adherent culture (Thermo Fisher Scientific), and 180 μL of QUANTI-BLUE (InvivoGen) was added. The plate was incubated at 37°C for 2 hours in a CO_2 incubator, and absorbance at 620 nm was measured. The obtained values were analyzed using GraphPad Prism, and % activation was calculated by setting the maximum signal induced by TPO as 100% and the unstimulated condition as 0%.

[0183]

The TPO addition concentration was tested in the range of 0.002 nM to 10 nM, and the maximum value (Emax) of the induced signal was calculated.

For dimer structure numbers 68 and 69, tests were conducted with peptide addition concentrations of 1, 10, and 100 nM, and those in which % activation was 50% or more upon addition at 1 nM were designated as E-1, those in which % activation was 50% or more upon addition at 10 nM were designated as E-2, those in which % activation was 50% or more upon addition at 100 nM were designated as E-3, and those in which % activation was 5% or more and less than 50% upon addition at 100 nM were designated as E-4, as shown in Table 6.

There were no cases corresponding to E-2, E-3, or E-4.

For dimer structure numbers 70, 88, 92, 93, 108, and 109, tests were conducted with peptide addition concentrations of 0.0156, 0.0625, 0.25, and 1 nM, and those in which % activation was 50% or more upon addition at 0.0156 nM were designated as F-1, those in which % activation was 50% or more upon addition at 0.0625 nM were designated as F-2, those in which % activation was 50% or more upon addition at 0.25 nM were designated as F-3, those in which % activation was 50% or more upon addition at 1 nM were designated as F-4, and those in which % activation was 5% or more and less than 50% upon addition at 1 nM were designated as F-5, as shown in Table 6.

There were no cases corresponding to F-5. In addition, NT indicates that the test was not conducted.

As shown in Table 6, these peptides were shown to have the ability to activate transcription of STAT5-dependent genes in TPO Reporter HEK293 Cells.

Industrial Applicability

[0184]

This invention can be utilized in fields such as the pharmaceutical industry.

CLAIMS

[Claim 1]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising:

an amino acid sequence represented by Formula A1; or

an amino acid sequence derived from the amino acid sequence represented by Formula A1, wherein a plurality of amino acid residues are substituted, deleted, added, or inserted;

A1: X^1-X^2-X^3-MeG-X^4-X^5-X^6-X^7-df-X^8-X^9-X^{10}-X^{11}-X^{12}-MeC

wherein,

X^1 is any amino acid residue,

X^2 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

X^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members which may be substituted,

X^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^{11} is any amino acid residue, and

X^{12} is any amino acid residue,

wherein, in Formula A1, the amino acid sequence is described from an N-terminus to a C-terminus.

[Claim 2]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 1,

wherein, in Formula A1,

X^1 is F, A, Q, E, F4COO, F3Me, F3C, F4Me, F4C, F4OMe, 3Py, Cha, or F4aao,

X^2 is W, W7N, W1Me7N, or W1aa,

X^3 is V, R, Cit, T, alT, or S,

X^4 is W, W1Me, or Nal1,

X^5 is V, T, alT, or dMeS,

X^6 is V, I, alI, Eva, Tbg, or Gcpe,

X^7 is MeG, MeA, Meda, MeKCOpipzaa, dp, EtG, or EtA,

X^8 is R, Q, or KCOpipzaa,

X^9 is V, T, alT, P, Hpr, Hyp, or Mor,

X^{10} is I, V, Gthp, or G4pipaa,

X^{11} is S, E, I, F, W, Y, R, Cit, Atp, KCOpipzaa, or Hgl, and

X^{12} is D, H, R, S, Q, SMe, Cit, E, or Hgl.

[Claim 3]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising: an amino acid sequence represented by Formula A2; or an amino acid sequence derived from the amino acid sequence represented by Formula A2, wherein 1 to 12 amino acid residues at one or more of positions 1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, and 14 are substituted, deleted, added or inserted.

A2: F-W-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 1).

[Claim 4]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 1,

wherein, in Formula A1,

X^1 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^2 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group

which may be substituted,

X^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

X^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

X^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

X^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group, or a cyclic secondary amino acid residue having 4 to 6 members,

X^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group,

X^{11} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, and

X^{12} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted.

[Claim 5]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 4,

wherein, in Formula A1,

X^1 is F or F4Me,

X^2 is W or W7N,

X^3 is V, T, or alT,

X^4 is W or Nal1,

X^5 is V,

X^6 is V, I, alI, or Gcpe,

X^7 is MeG, MeA, Meda, MeKCOpipzaa, or dp,

X^8 is R,

X^9 is V or P,

X^{10} is I or V,

X^{11} is S, KCOpipzaa, or Hgl, and

X^{12} is D.

[Claim 6]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 1 to 5, wherein the peptide is a cyclic peptide.

[Claim 7]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 1 to 5, wherein the peptide has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in Formula A1 or Formula A2 contained in the peptide, are bound.

[Claim 8]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 1 to 5, further comprising an additional amino acid residue.

[Claim 9]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 4 or 5, wherein the peptide has TPO receptor binding ability.

[Claim 10]

A peptide, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, the peptide comprising:

an amino acid sequence represented by Formula B1; or

an amino acid sequence derived from the amino acid sequence represented by Formula B1, wherein a plurality of amino acid residues are substituted, deleted, added, or inserted,

B1: Y^1-Y^2-Y^3-MeG-Y^4-Y^5-Y^6-Y^7-df-R-Y^8-Y^9-Y^{10}-D-MeC

wherein,

Y^1 is any amino acid residue,

Y^2 is any amino acid residue,

Y^3 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^4 is an amino acid residue having, in a side chain, an aryl group which may be substituted,

Y^5 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^6 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^7 is any N-alkylated amino acid residue, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^8 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted, or a cyclic secondary amino acid residue having 4 to 6 members,

Y^9 is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

Y^{10} is an amino acid residue having, in a side chain, an aliphatic hydrocarbon group which may be substituted,

wherein, in Formula B1, the amino acid sequence is described from the N-terminus to the C-terminus.

[Claim 11]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 10,

wherein, in Formula B1,

Y^1 is F, F4aao, F3COO, F3aao, 4Py, 3Py, or Cha,

Y^2 is Ahp, W1Me7N, W, or W7N,

Y^3 is V, I, alI, Q, T, TMe, or KCOpipzaa,

Y^4 is W, W1Me, Nal1, or Nal2,

Y^5 is V, Tbg, TMe, alTMe, dMeS, Gthp, G4pipaa, or Cit,

Y^6 is V, Ahp, Nle, I, L, Tbg, IMe, Cbg, Gcpe, Chg, or alTMe,

Y^7 is MeG, EtG, MeA, Meda, dp, MeQ, or Medq,

Y^8 is V, P, Hpr, Tic, TMe, or alTMe,

Y^9 is I, alI, alTMe, dMeS, Gthp, or G4pipaa, and

Y^{10} is S or Hgl.

[Claim 12]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically

acceptable solvate thereof according to claim 10, wherein the peptide comprises: an amino acid sequence represented by Formula B2; or an amino acid sequence derived from the amino acid sequence represented by Formula B2, wherein 1 to 10 amino acid residues at one or more of positions 1, 2, 3, 5, 6, 7, 8, 11, 12, and 13 are substituted, deleted, added or inserted,

B2: F-Ahp-V-MeG-W-V-V-MeG-df-R-V-I-S-D-MeC (SEQ ID NO: 97).

[Claim 13]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 10 to 12, wherein the peptide is a cyclic peptide.

[Claim 14]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 10 to 12, wherein the peptide has a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC, which is the 15th amino acid residue in Formula B1 or Formula B2 contained in the peptide, are bound.

[Claim 15]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 10 to 12, further comprising an additional amino acid residue.

[Claim 16]

The peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 10 to 12, wherein the peptide has TPO receptor binding ability.

[Claim 17]

A peptide complex, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, comprising a first peptide and a second peptide,

wherein the first peptide and the second peptide are identical to or different from each other, and are each the peptide according to claim 1 or claim 10.

[Claim 18]

The peptide complex, a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof according to claim 17, wherein the peptide complex comprises the first peptide, the second peptide, and a linker connecting the first peptide and the second peptide, and wherein the second peptide is identical to or different from the first peptide and has an amino acid sequence represented by Formula A1 or an amino acid sequence consisting of a sequence resulting from substitution, deletion, addition, or insertion of a plurality of amino acid residues in the amino acid sequence represented by Formula A1.

[Claim 19]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the first peptide and the second peptide have a sequence identity of 90% or more and 100% or less.

[Claim 20]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the first peptide and the second peptide are substantially the same peptide.

[Claim 21]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the first peptide and the second peptide are each a cyclic peptide.

[Claim 22]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the first peptide and the second peptide each have a cyclic structure in which an amino acid residue derived from a chloroacetylated amino acid and MeC contained in the first peptide or the second peptide are bound.

[Claim 23]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the first peptide

and the second peptide further comprise additional amino acid residues.

[Claim 24]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the C-terminus of the first peptide and the C-terminus of the second peptide are linked via the linker.

[Claim 25]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the linker is a PEG linker or a linker consisting of PEG and an amino acid residue.

[Claim 26]

The peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18, wherein the peptide complex has intracellular signaling activation ability.

[Claim 27]

A pharmaceutical composition comprising the peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 1 to 5 and 10 to 12.

[Claim 28]

A pharmaceutical composition comprising the peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18.

[Claim 29]

A cell culture composition used for cell culture, comprising the peptide complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18.

[Claim 30]

A composition for medical, diagnostic, or research use, comprising the peptide

complex, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to claim 18.

[Claim 31]

A composition for medical, diagnostic, or research use, comprising the peptide, the pharmaceutically acceptable salt thereof, or the pharmaceutically acceptable solvate thereof according to any one of claims 1 to 5 and 10 to 12.