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**MACHIDA et al.**(10) **Pub. No.: US 2025/0263459 A1**(43) **Pub. Date: Aug. 21, 2025**(54) **STABILIZED PACAP PEPTIDE****Publication Classification**(71) Applicant: **SENJU PHARMACEUTICAL CO., LTD.**, Osaka (JP)(51) **Int. Cl.**  
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(2013.01)(73) Assignee: **SENJU PHARMACEUTICAL CO., LTD.**, Osaka (JP)(57) **ABSTRACT**(21) Appl. No.: **17/610,821**(22) PCT Filed: **May 14, 2020**(86) PCT No.: **PCT/JP2020/019344**

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The purpose of the present invention is to provide a PACAP peptide having improved stability. The present invention is based on the finding that the stability of PACAP is significantly improved by substituting aspartic acid at positions 3 and 8 in the sequence of PACAP with at least one amino acid selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya), and oxadiazolone-substituted aspartic acid (5Oxa).

**Specification includes a Sequence Listing.**

**STABILIZED PACAP PEPTIDE****SEQUENCE LISTING**

**[0001]** A sequence listing in electronic (ASCII text file) format is filed with this application and incorporated herein by reference. The name of the ASCII text file is "2021\_2336\_8\_30\_24\_ST25.txt"; the file was created on Aug. 30, 2024; the size of the file is 36,476 bytes.

**TECHNICAL FIELD**

**[0002]** The present invention relates to a stabilized peptide having a physiological activity of PACAP, and to a neuroprotective agent, a vascular endothelial function ameliorator, a corneal neurite formation promoter, a lacrimal secretagogue, a dry-eye treatment agent, a corneal epithelial or corneal endothelial damage treatment agent, a neurotrophic keratitis treatment agent, an anti-inflammatory agent or a pharmaceutical composition, which includes the peptide.

**BACKGROUND ART**

**[0003]** Neurons are cells constituting the nervous system, which is largely divided into the central nervous system and the peripheral nervous system. Neurons are susceptible to damage by external factors, including cerebrovascular accidents such as stroke and cerebral infarction, and internal factors including accumulation of abnormal proteins, oxidative stress and inflammation, while regenerative ability is also low, and therefore when damaged they can result in notable impairment of patient QOL. Neurodegenerative diseases associated with neurodegeneration and loss of the central nervous system include neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis, and optic neurodegenerative diseases such as glaucoma or optic neuropathy, and also sensory neurodegenerative diseases such as nerve deafness.

**[0004]** Advances in neuroscience have led to the discovery of numerous neuroprotective factors, and they are expected for developing as a preventive or therapeutic agent for nervous disorder. Drugs that decrease free radicals or excitatory amino acids responsible for neurodegeneration, and drugs that are able to protect and/or repair neurons (for example, immunophilin ligands, such as neurotrophic factors, or for immunosuppressive agents), have been found to exhibit neuroprotective action. Endogenous proteins such as pituitary adenylate cyclase-activating polypeptide (PACAP), CD44 and human brain carboxypeptidase B (HBCPB) have also been shown to exhibit neuroprotective action (PTLs 1 and 2).

**[0005]** Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide originally discovered in sheep hypothalamus extract. PACAP can exhibit activity which stimulates cAMP formation in anterior pituitary cells. PACAP includes PACAP38 which consists of 38 amino acid residues, and PACAP27 which consists of 27 amino acid residues, both of which have equivalent activity (NPLs 1 and 2). PACAP belongs to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon superfamily, and the sequence of human PACAP27 has 68% identity with vasoactive intestinal polypeptide (VIP). PACAP and VIP both bind to PAC1 receptor (PAC1R), VPAC1 receptor (VPAC1R) and VPAC2 receptor (VPAC2R), but they differ in their affinities for their respective receptors. PAC1R binds to PACAP with high

selectivity. The affinity of PAC1R for PACAP is more than 1000 times higher than the affinity of PAC1R for VIP. VPAC1R and VPAC2R, on the other hand, both have equal affinity for PACAP and VIP. PACAP has a variety of physiological effects, being known to have physiological activity as a neuroprotective substance, immunosuppressive factor, vasodilating factor, exocrine secretagogue (PTL 3) and neurite formation promoting factor (PTL 4).

**[0006]** Medical drug is being developed utilizing the various physiological activities of PACAP. However, most peptides such as PACAP are unstable in aqueous solution and are associated with the problem of a short half-life due to lack of protease resistance.

**CITATION LIST****Patent Literature**

**[0007]** [PTL 1] Japanese Patent Public Inspection No. 2014-510101

**[0008]** [PTL 2] Japanese Unexamined Patent Publication No. 2012-232952

**[0009]** [PTL 3] Japanese Unexamined Patent Publication No. 2009-269818

**[0010]** [PTL 4] International Patent Publication No. WO2005/102375, pamphlet

**Non Patent Literature**

**[0011]** [NPL 1] S. Bourgault (2009) Current Medicinal Chemistry 16, 4462-4480

**[0012]** [NPL 2] Louise Dickson (2009) Pharmacology & Therapeutics, 12, 294-316

**SUMMARY OF INVENTION****Technical Problem**

**[0013]** The present invention is intended to provide a stabilized PACAP peptide with high stability in aqueous solution, as well as a neuroprotective agent, a neurite formation promoter, a lacrimal gland secretagogue, a therapeutic or prophylactic pharmaceutical composition for dry-eye, a therapeutic or prophylactic pharmaceutical composition for corneal epithelial or corneal endothelial damage, a therapeutic or prophylactic pharmaceutical composition for neuropathy, or a therapeutic or prophylactic composition for neurotrophic keratitis, which includes the peptide.

**Solution to Problem**

**[0014]** The present inventors have conducted avid research for the purpose of increasing the stability of PACAP in aqueous solution, and have completed this invention upon finding that it is possible to vastly increase its stability in aqueous solution by independently replacing each of the aspartic acids at position 3 and position 8 of PACAP peptide with a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cy) and oxadiazolone-substituted aspartic acid (5Oxa).

**[0015]** The present invention relates to the following:

**[0016]** [1] A peptide consisting of the sequence represented by H-X<sub>1</sub>-D-G-X<sub>2</sub>-F-X<sub>3</sub>-D-X<sub>4</sub>-Y-X<sub>5</sub>-R-Y-R-K-X<sub>6</sub>-X<sub>7</sub>-A-V-K-K-Y-L-A-A-V-L (SEQ ID NO: 3)

{wherein

**[0017]** X<sub>1</sub> is a neutral amino acid,

**[0018]** X<sub>2</sub> is a neutral amino acid,

**[0019]** X<sub>3</sub> is a neutral amino acid,

**[0020]** X<sub>4</sub> is alanine, serine, Ai or Cn,

**[0021]** X<sub>5</sub> is alanine, serine, Ai or Cn,

**[0022]** X<sub>6</sub> is glutamine or alanine and

**[0023]** X<sub>7</sub> is non-polar amino acid},

with the aspartic acids at position 3 and position 8 each independently replaced by a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa), or its modified sequence, wherein the peptide has affinity for PAC1R and VPAC1R, and the modified sequence is the sequence listed as SEQ ID NO: 3 having a deletion or addition of one or more amino acids.

**[0024]** [2] The peptide according to [1] above, wherein X<sub>1</sub> is serine or alanine.

**[0025]** [3] The peptide according to [1] or [2] above, wherein X<sub>2</sub> is isoleucine or alanine.

**[0026]** [4] The peptide according to any one of [1] to [3] above, wherein X<sub>3</sub> is alanine or threonine.

**[0027]** [5] The peptide according to any one of [1] to [4] above, wherein X<sub>7</sub> is methionine, leucine, norleucine or alanine.

**[0028]** [6] The peptide according to any one of [1] to [5], wherein in cases where the amino acids at position 3 and position 8 are tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz) or tetrazole-substituted homoglutamic acid (nvTz), the amino acids are the L-forms, D-forms or racemic forms.

**[0029]** [7] The peptide according to any one of [1] to [6] above, wherein the N-terminus of the peptide is modified with a protecting group.

**[0030]** [8] The peptide according to [7] above, wherein the protecting group is an acyl group.

**[0031]** [9] The peptide according to any one of [1] to [8] above, wherein the C-terminus of the peptide is modified with a protecting group.

**[0032]** [10] The peptide according to [9] above, wherein the protecting group is an amide group or ester group.

**[0033]** [11] The peptide according to any one of [1] to [10], wherein the modified sequence is the sequence with 1 to 3 amino acids deleted from the C-terminus of the sequence.

**[0034]** [12] The peptide according to any one of [1] to [10], wherein the modified sequence is the sequence with 1 to 3 amino acids added to the N-terminus of the sequence.

**[0035]** [13] The peptide according to any one of [1] to [10] or [12] above, wherein the modified sequence is the sequence with one sequence selected from the group consisting of the following:

GKRYKQRVKKNK; (SEQ ID NO: 43)

GKRYKQRVKN; (SEQ ID NO: 44)

GKRYKQRVK; (SEQ ID NO: 45)

GKRYKQRV; (SEQ ID NO: 46)

GKRYKQR; (SEQ ID NO: 47)

GKRYKQ; (SEQ ID NO: 48)

GKRYK; (SEQ ID NO: 49)

GKRY (SEQ ID NO: 50)

GKRY

GKR;

GRR;

GK;

and

GR

G;

added to the C-terminus of the peptide.

**[0036]** [14] A neuroprotective agent containing the peptide according to any one of [1] to [13] above.

**[0037]** [15] A lacrimal secretagogue containing the peptide according to any one of [1] to [13] above.

**[0038]** [16] An anti-inflammatory agent containing the peptide according to any one of [1] to [13] above.

**[0039]** [17] A vascular endothelial function ameliorator containing the peptide according to any one of [1] to [13] above.

**[0040]** [18] A corneal epithelial or corneal endothelial damage treatment agent containing the peptide according to any one of [1] to [13] above.

**[0041]** [19] A dry-eye treatment agent containing the peptide according to any one of [1] to [13] above.

**[0042]** [20] A pharmaceutical composition containing the peptide according to any one of [1] to [13] above.

**[0043]** [21] A neuroprotection method for a subject in need of neuroprotection, wherein the method includes administering an effective dose of the peptide according to any one of [1] to [13] to the subject.

**[0044]** [22] A lacrimal secretion promoting method for a subject in need of lacrimal secretion, wherein the method includes administering the peptide according to any one of [1] to [13] to the subject.

**[0045]** [23] A vascular endothelial function amelioration method for a subject in need of ameliorating vascular endothelial function, wherein the method includes administering the peptide according to any one of [1] to [13].

[0046] [24] A method of inhibiting inflammation in a subject suffering from inflammation, wherein the method includes administering the peptide according to any one of [1] to [13].

[0047] [25] A method for ameliorating corneal epithelial or corneal endothelial damage in a subject suffering from corneal epithelial or corneal endothelial damage, wherein the method includes administering the peptide according to any one of [1] to [13].

[0048] [26] A method for treatment or prevention of dry-eye in a subject suffering from dry-eye, wherein the method includes administering the peptide according to any one of [1] to [13].

[0049] [27] The peptide according to any one of [1] to [13], which is for use as a medicament.

[0050] [28] The peptide according to any one of [1] to [13], which is for use in treatment of nervous disorder.

[0051] [29] The peptide according to any one of [1] to [13], which is for use in treatment or prevention of nervous disorder by neuroprotection.

[0052] [30] The peptide according to any one of [1] to [13], which is for use in treatment or prevention of dry-eye and diseases associated with dry-eye.

[0053] [31] The peptide according to any one of [1] to [13] above, which is for use in treatment or prevention of corneal epithelial or corneal endothelial damage.

[0054] [32] The peptide according to any one of [1] to [13] above, which is for use in treatment or prevention of inflammatory disease.

[0055] [33] The peptide according to any one of [1] to [13] above, which is for use in treatment or prevention of vascular endothelial disorders.

[0056] [34] The use of the peptide according to any one of [1] to [13] for production of a drug selected from the group consisting of neuroprotective agents, lacrimal secretagogues, anti-inflammatory drugs, vascular endothelial function ameliorators, corneal epithelial or corneal endothelial damage treatment agents and dry-eye treatment agents.

#### Advantageous Effects of Invention

[0057] According to the invention it is possible to greatly stabilize PACAP, which has been unstable in aqueous solution.

#### DESCRIPTION OF EMBODIMENTS

[0058] The present invention relates to a peptide consisting of a sequence in which the aspartic acid residues at position 3 and position 8 of PACAP are each independently replaced by a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa), or its partially modified sequence, wherein the peptide has an affinity for PAC1R, VPAC1R and/or VPAC2R equivalent to that of PACAP. The "equivalent affinity" means that the EC50 value of the peptide for each receptor is up to 100 times that of PACAP. The PACAP may be PACAP38 consisting of 38 residues, or PACAP27 consisting of 27 residues. PACAP38 and PACAP27 have the following sequences:

PACAP38: (SEQ ID NO: 1)

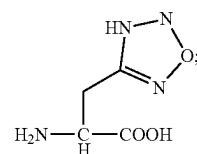
HSDGIFTDSYSRYRKQMAVKKYLAALVGKRYKQRVKNK

PACAP27: (SEQ ID NO: 2)

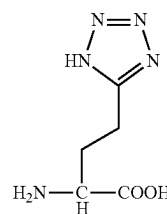
HSDGIFTDSYSRYRKQMAVKKYLAALV

[0059] According to the invention, tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz) and tetrazole-substituted homoglutamic acid (nvTz) are non-natural amino acid residues having a structure in which the carboxyl group on the side chain of aspartic acid, glutamic acid or homoglutamic acid, respectively, is replaced with the carboxyl group isostere tetrazole. These are represented by the following formulas:

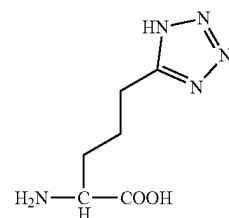
[Chemical Formula 1]



Tetrazole-substituted aspartic acid (Tz)



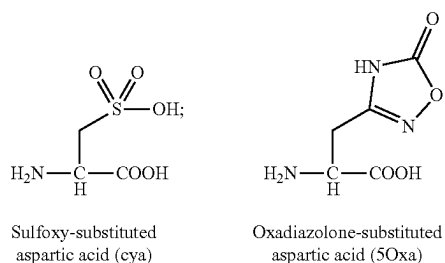
Tetrazole-substituted glutamic acid (egTz)



Tetrazole-substituted homoglutamic acid (nvTz)

[0060] According to the invention, sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa) are residues having a structure in which the carboxyl group on the side chain of aspartic acid is replaced with the carboxyl group isostere sulfoxy or 1,2,4-oxadiazol-5-one (oxadiazolone), respectively. These are represented by the following formulas:

[Chemical Formula 2]



**[0061]** Non-natural amino acid residues with aspartic acid at position 3 and position 8 replaced may be the L-forms, D-forms or racemic forms, so long as they have the equivalent level of physiological activity as PACAP. In particular, a peptide substituted with the L-form tetrazole-substituted aspartic acid and a peptide substituted with the D-form tetrazole-substituted aspartic acid have the equivalent level of affinity as PAC1R, VPAC1R and/or VPAC2R. With no intention to be limited to any particular theory, it is possible that including a D-form amino acid is less likely to be degraded by enzymes, allowing the stability to be increased. Non-natural amino acids other than protein-constituting amino acids may also be introduced into peptides together with protecting groups by methods such as the Fmoc method, similar to protein-constituting amino acids.

**[0062]** According to the invention, a “sequence which is partially modified” is a sequence in which one or more amino acids of the original sequence have been substituted, deleted or added. More preferably, a “sequence which is partially modified” is a sequence in which one or several amino acids of the original sequence have been substituted, deleted or added.

**[0063]** An amino acid substitution may be at any position so long as the affinity of the peptide consisting of the sequence which is partially modified, for PAC1R, VPAC1R and/or VPAC2R, does not change. From the viewpoint of maintaining affinity, the amino acid substitution in the peptide consisting of the partially modified sequence may be at position 2, position 5, position 7, position 9, position 11, position 16 and position 17 of PACAP27. According to a particularly preferred aspect, the amino acids at positions  $X_1$  to  $X_7$  in the following sequence:

(SEQ ID NO: 3)

H- $X_1$ -D-G- $X_2$ -F- $X_3$ -D- $X_4$ -Y- $X_5$ -R-Y-R-K- $X_6$ - $X_7$ -A-V-K-K-Y-L-A-A-V-L

may be substituted.

**[0064]** One aspect of the invention, therefore, relates to a peptide consisting of the sequence represented by the following formula:

(SEQ ID NO: 3)

H- $X_1$ -D-G- $X_2$ -F- $X_3$ -D- $X_4$ -Y- $X_5$ -R-Y-R-K- $X_6$ - $X_7$ -A-V-K-K-Y-L-A-A-V-L

{wherein

**[0065]**  $X_1$  to  $X_6$  are neutral amino acids, and

**[0066]**  $X_7$  is a non-polar amino acid},

with the aspartic acids at position 3 and position 8 each independently replaced with a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz),

tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa), or its modified sequence, wherein the peptide has affinity for PAC1R, VPAC1R and/or VPAC2R.

**[0067]** The substitution may also be conservative substitution with an amino acid having the same attributes. Examples of conservative substitutions include substitutions of amino acids within the following groups:

**[0068]** (i) Non-polar amino acids: Val, Leu, Ile, Met, Phe, Trp, Pro, Nle, Ala

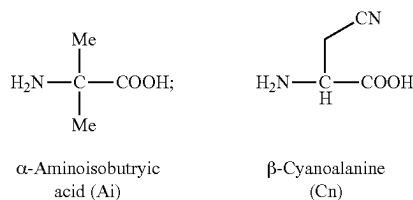
**[0069]** (ii) Neutral amino acids: Ala, Ser, Thr, Tyr, Cys, Asn, Gln, Gly

**[0070]** (iii) Basic amino acids: Lys, Arg, His

**[0071]** (iv) Acidic amino acids: Asp, Glu

**[0072]** Among neutral amino acids,  $\alpha$ -aminoisobutyric acid (Ai) having a methyl group substituted at the  $\alpha$ -position of alanine and  $\beta$ -cyanoalanine (Cn) having the hydrogen atom at the  $\beta$ -position of alanine substituted with a cyano group, may be used instead of alanine. In particular,  $\alpha$ -aminoisobutyric acid (Ai) and  $\beta$ -cyanoalanine (Cn) may be used as neutral amino acids at position 9 and position 11. The amino acids  $\alpha$ -aminoisobutyric acid (Ai) and  $\beta$ -cyanoalanine (Cn) have the following structures:

[Chemical Formula 3]



**[0073]** In the sequence represented by SEQ ID NO: 3 of the invention, the amino acids represented by  $X_1$  to  $X_7$  are preferably the following: The neutral amino acid of  $X_1$  is, in particular, alanine or serine.

**[0074]** The neutral amino acid of  $X_2$  is, in particular, isoleucine or alanine.

**[0075]** The neutral amino acid of  $X_3$  is, in particular, alanine or threonine.

**[0076]** The neutral amino acids of  $X_4$  and  $X_5$  are, in particular, alanine, serine,  $\alpha$ -aminoisobutyric acid or  $\beta$ -cyanoalanine.

**[0077]** The neutral amino acid of  $X_6$  is, in particular, glutamine or alanine.

**[0078]** The non-polar amino acid of  $X_7$  is, in particular, methionine, leucine, norleucine or alanine.

**[0079]** According to the invention, a “modified sequence” is a sequence in which one or more amino acids of the original sequence have been substituted, deleted or added. More preferably, a “sequence which is partially modified” is a sequence in which one or several amino acids of the original sequence have been substituted, deleted or added.

**[0080]** The amino acid substitution may include substitution of one or more amino acids, but from the viewpoint of maintaining an activity, any number from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, amino acids may be substituted.

A substitution of 1 to 4 amino acids is particularly preferred, a substitution of 3 amino acids is more preferred, a substitution of two amino acids is even more preferred and a substitution of one amino acid is most preferred.

**[0081]** An amino acid deletion may be at any position so long as the affinity of the peptide consisting of the sequence which is partially modified, for PAC1R, VPAC1R and/or VPAC2R, does not change. The number of amino acids deleted is selected as any number from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. From the viewpoint of not altering affinity, a deletion of one or two amino acids, and especially a deletion of one amino acid, is particularly preferred. The amino acid deletion may be a deletion at the N-terminal end or C-terminal end of the original sequence, or a deletion within the sequence. Since PACAP27 and PACAP38 have equivalent binding affinity for PAC1R, VPAC1R and/or VPAC2R, it is believed that deletion of amino acids present at the C-terminal end of PACAP38 have little effect on the affinity.

**[0082]** An amino acid addition may be at any position so long as the affinity of the peptide consisting of the sequence which is partially modified, for PAC1R, VPAC1R and/or VPAC2R, does not change. The number of amino acids added is selected as any number from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. The amino acids may be added at the N-terminal end or C-terminal end of the original sequence, or they may be added within the sequence. Since PACAP27 and PACAP38 have equivalent binding affinity for PAC1R, VPAC1R and/or VPAC2R, it is believed that addition of any amino acids at the C-terminus of PACAP27 has little effect on the affinity.

**[0083]** A modified sequence of the sequence listed as SEQ ID NO: 3 wherein the aspartic acid residues at position 3 and position 8 are each independently replaced by a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa), is preferably the amino acid sequence listed as SEQ ID NO: 3 with a deletion or addition of one or more amino acids. More preferably, one or two amino acids may be deleted from the amino acid sequence listed as SEQ ID NO: 3, or the following C-terminal added sequence may be added to the amino acid sequence listed as SEQ ID NO: 3. The amino acid sequence listed as SEQ ID NO: 3 may also have an addition of 1 to 3 amino acids (such as methionine) at the N-terminus.

**[0084]** It is not our intention to be limited to any particular theory, but since PACAP27 and PACAP38 have equivalent binding affinity for PAC1R, VPAC1R and/or VPAC2R, addition of any amino acids at the C-terminal end of PACAP27 does not effect the binding affinity for PAC1R. Therefore, the peptide with a modified sequence according to the invention may include a C-terminal added sequence at the C-terminus of the amino acid sequence listed as SEQ ID NO: 3, similar to PACAP27. A C-terminal added sequence is a sequence comprising 1 to 11 arbitrary amino acids. The C-terminal added sequence preferably corresponds to the amino acids at position 28 to position 38 of PACAP38. The following sequences may therefore be mentioned as C-terminal sequences:

GKRYKQRVKNK;	(SEQ ID NO: 43)
GKRYKQRVKN;	(SEQ ID NO: 44)
GKRYKQRVK;	(SEQ ID NO: 45)
GKRYKQRV;	(SEQ ID NO: 46)
GKRYKQR;	(SEQ ID NO: 47)
GKRYKQ;	(SEQ ID NO: 48)
GKRYK;	(SEQ ID NO: 49)
GKRY	(SEQ ID NO: 50)
GKR;	
GRR;	
GK;	
and	
GR	
G.	

**[0085]** The peptide of the invention may use either the D-form or L-form of amino acids, or the racemic forms of amino acids, so long as affinity for PAC1R, VPAC1R and/or VPAC2R is not lost. The peptide of the invention may also comprise non-natural amino acids such as 2-aminoisobutyric acid, and may include derivatives with optional modification of N-terminal amino groups, C-terminal carboxyl groups or amino acid side chain functional groups. Examples of modifications include addition of protecting groups on the amino group (for example, acylation (formylation, acetylation, propionylation, butyrylation or isobutyrylation), mesylation, ureation, carbamation, Boc-protection or Fmoc-protection), and esterification (such as ethylation) of the carboxyl group. It may also include modification that can commonly take place in the body, such as phosphorylation, amidation, methylation, esterification and acetylation, as well as modification that takes place during the synthesis process or that is used to facilitate purification, such as biotinylation. For the purpose of extending the in vivo half-life of the peptide, modification such as PEGylation may also be added. From the viewpoint of increasing the stability, in particular, the free amino group of the N-terminal amino acid may be protected with a protecting group (such as an acyl group). For example, the free amino groups of N-terminal amino acids may be acylated (for example, acetylated, propionylated, butyrylated or isobutyrylated), or mesylated. Similarly, the free carboxyl groups of C-terminal amino acids may be protected by protecting groups (such as amide or ester groups). For example, the free carboxyl groups of C-terminal amino acids may be amidated or esterified. Acetylation or mesylation of the N-terminus of a peptide of the invention further increases the stability. The C-terminus may be a carboxyl group ( $-\text{COOH}$ ), carboxylate ( $-\text{COO}-$ ), amide

(—CONH<sub>2</sub>) or ester (—COOR). The peptide of the invention may also have a glycosyl chain addition (see WO2017/027848, for example).

**[0086]** According to one specific aspect, the invention relates to the peptides having the sequences listed in Table 1 below, or their modified sequences:

TABLE 1

	N-terminus	Sequence	C-terminus	SEQ ID No.
Peptide 1	H	HST <sub>2</sub> GI FAT <sub>2</sub> AY SRYRK QMAVK KYLAA VL	NH <sub>2</sub>	4
Peptide 2	H	HST <sub>2</sub> GI FAT <sub>2</sub> SY ARYRK QMAVK KYLAA VL	NH <sub>2</sub>	5
Peptide 3	H	HST <sub>2</sub> GI FAT <sub>2</sub> AY ARYRK QMAVK KYLAA VL	NH <sub>2</sub>	6
Peptide 4	H	HST <sub>2</sub> GI FAT <sub>2</sub> AIY SRYRK QMAVK KYLAA VL	NH <sub>2</sub>	7
Peptide 5	H	HST <sub>2</sub> GI FAT <sub>2</sub> SY AIRYRK QMAVK KYLAA VL	NH <sub>2</sub>	8
Peptide 6	H	HST <sub>2</sub> GI FAT <sub>2</sub> AIY AIRYRK QMAVK KYLAA VL	NH <sub>2</sub>	9
Peptide 7	H	HST <sub>2</sub> GI FTT <sub>2</sub> AIY AIRYRK QMAVK KYLAA VL	NH <sub>2</sub>	10
Peptide 8	H	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QMAVK KYLA	NH <sub>2</sub>	11
Peptide 9	H	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QMAVK KYLAA V	NH <sub>2</sub>	12
Peptide 10	H	HScyaGI FTcyaSY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	13
Peptide 11	H	HScyaGI FTcyaSY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	14
Peptide 12	H	MHST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	15
Peptide 13	H	HST <sub>2</sub> (D)GI FTT <sub>2</sub> (D)SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	16
Peptide 14	H	HST <sub>2</sub> GI FTT <sub>2</sub> (D)SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	17
Peptide 15	H	HST <sub>2</sub> (D)GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	18
Peptide 16	H	HST <sub>2</sub> GI FTegT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	19
Peptide 17	CH <sub>2</sub> CF <sub>2</sub> CO	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	20
Peptide 18	CH <sub>2</sub> CF <sub>2</sub> CH <sub>2</sub> CO	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	21
Peptide 19	(CH <sub>3</sub> ) <sub>2</sub> CHCO	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	22
Peptide 20	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CO	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	23
Peptide 21	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CO	HST <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	24
Peptide 22	Ac	HS(50xa)GI FT(50xa)SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	25
Peptide 23	Ac	HST <sub>2</sub> GA FTT <sub>2</sub> SY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	26
Peptide 24	Ac	HST <sub>2</sub> GI FAT <sub>2</sub> SY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	27
Peptide 25	Ac	HAT <sub>2</sub> GI FAT <sub>2</sub> SY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	28
Peptide 26	Ac	HAT <sub>2</sub> GI FIT <sub>2</sub> SY SRYRK ALAVK KYLAA VL	NH <sub>2</sub>	29
Peptide 27	Ac	HST <sub>2</sub> GI FAT <sub>2</sub> AY SRYRK QLAVK KYLAA VL	NH <sub>2</sub>	30
Peptide 28	Ac	HST <sub>2</sub> GI FAT <sub>2</sub> SY SRYRK ALAVK KYLAA VL	NH <sub>2</sub>	31
Peptide 29	Ac	HST <sub>2</sub> GI FAT <sub>2</sub> SY SRYRK QAAVK KYLAA VL	NH <sub>2</sub>	32
Peptide 30	Ac	HAT <sub>2</sub> GI FAT <sub>2</sub> AY SRYRK AAVK KYLAA VL	NH <sub>2</sub>	33
Synthetic Example 1	H	HST <sub>2</sub> GI FAT <sub>2</sub> SY CnRYRK QMAVK KYLAA VL	NH <sub>2</sub>	34
Synthetic Example 2	H	HST <sub>2</sub> GI FTT <sub>2</sub> CnY CnRYRK QMAVK KYLAA VL	NH <sub>2</sub>	35
Synthetic Example 3	H	HSegT <sub>2</sub> GI FIT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	36
Synthetic Example 4	H	HSegT <sub>2</sub> GI FTegT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	37
Synthetic Example 5	H	HSnvT <sub>2</sub> GI FTT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	38
Synthetic Example 6	H	HST <sub>2</sub> GI FTnvT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	39
Synthetic Example 7	H	HSnvT <sub>2</sub> GI FTnvT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	40
Synthetic Example 8	H	HSegT <sub>2</sub> GI FTnvT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	41
Synthetic Example 9	H	HSnvT <sub>2</sub> GI FTegT <sub>2</sub> SY SRYRK QNLAVK KYLAA VL	NH <sub>2</sub>	42

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**[0087]** In the table, Tz represents tetrazole-substituted aspartic acid, egTz represents tetrazole-substituted glutamic acid, nvTz represents tetrazole-substituted homoglutamic acid, cya represents sulfoxy-substituted aspartic acid, (50xa) represents oxadiazolone-substituted aspartic acid, Ai represents  $\alpha$ -aminoisobutyric acid and Cn represents  $\beta$ -cyanoalanine. Tz(D) represents that tetrazole-substituted aspartic acid is the D-form.

**[0088]** The peptide of the invention can be produced by any production method. For example, it can be produced by solid phase synthesis or liquid phase synthesis using the Boc or Fmoc method. It can also be produced by other methods, using a method of transferring nucleic acid coding for the peptide of the invention into a gene, introducing the gene into host cells and synthesizing the peptide in the host cells. Purification after expression can be easily accomplished by

a design in which a tag peptide such as a polyhistidine tag is added at the ends of the peptide.

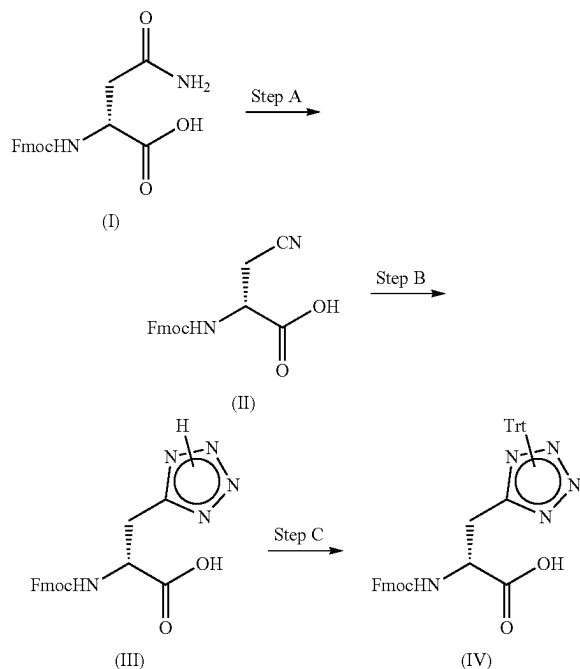
**[0089]** The peptide of the invention also includes its pharmaceutically acceptable salts. Pharmaceutically acceptable salts include salts of inorganic acids (for example, hydrochlorides, hydrobromides, sulfates and phosphates),

salts of organic acids (for example, methanesulfonates, benzenesulfonates, p-toluenesulfonates, formates, acetates, trifluoroacetates, oxalates, citrates, malonates, fumarates, maleates, tartrates, succinates and malates), or salts with bases (for example, ammonium salts, methylpyridinium salts and acetylpyridinium salts). The peptide of the invention also includes hydrates or solvates.

**[0090]** An Fmoc-N protected amino acid wherein the side chain carboxyl group of an amino acid (aspartic acid) has been replaced by the carboxyl group isostere tetrazole can be produced by the method represented by Reaction Scheme 1, or a similar method, using Fmoc-N protected asparagine as the starting substance.

Reaction Scheme 1

[Chemical Formula 4]



{wherein

[0091] Trt represents a trityl group, and

[0092] H or Trt group is substituting at N1 or N2 of the tetrazole}

[0093] In the method of Reaction Scheme 1, the compound represented by (I) (for example, asparagine with the amino group protected by Fmoc) is reacted with EDCI in the presence of a base in step A, to produce compound (II). In step A, the base is used at 1 to 50 equivalents and preferably 10 to 25 equivalents with respect to compound (I). The base used may be pyridine, 4-dimethylaminopyridine, triethylamine, N,N-diisopropylethylamine or N-methylmorpholine, with pyridine being preferred. EDCI is used at 1 to 10 equivalents and preferably 1 or 2 equivalents. The solvent is not particularly restricted so long as it does not affect the reaction, and examples include N,N-dimethylformamide, N,N-dimethyl acetamide, toluene, dichloromethane, tetrahydrofuran and acetonitrile, preferably acetonitrile. The reaction temperature will usually be -10 to 60° C. and is preferably 0 to 30° C., while the reaction time is 0.5 to 12 hours and preferably 1 to 5 hours.

[0094] In step B, compound (II) is reacted with trimethylsilyl azide and dibutyltin oxide in toluene to produce compound (III). Trimethylsilyl azide is used at 1 to 5 equivalents and preferably 2 to 4 equivalents with respect to compound (II). Dibutyltin oxide is used at 0.3 to 1 equivalent and preferably 0.7 to 0.95 equivalent with respect to compound (II). The solvent is not particularly restricted so long as it does not affect the reaction, and examples include benzene, toluene and xylene, preferably toluene. The reaction time will usually be 0.5 to 24 hours and is preferably 0.5 to 2 hours. The reaction temperature will usually be 80 to

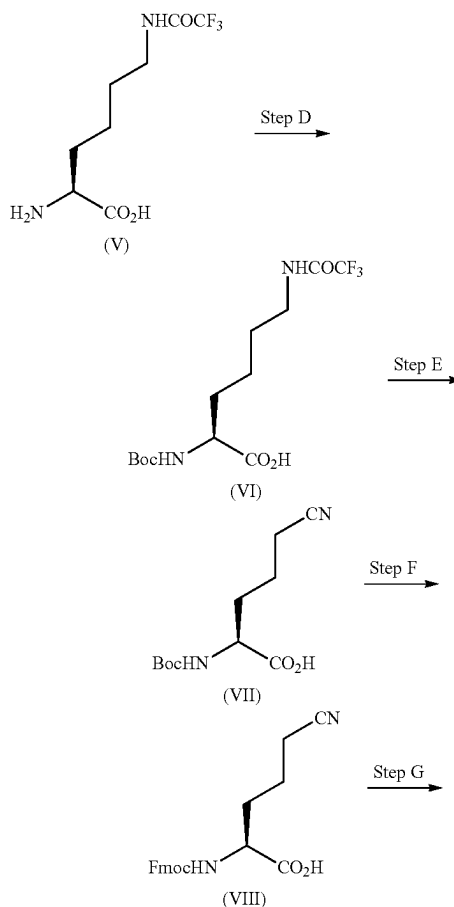
150° C. and is preferably 80 to 100° C. The heating method may be with an oil bath or microwave, with microwave heating being preferred.

[0095] In the subsequent step C, compound (IV) can be produced by reacting trityl chloride in the presence of a base. The base used in step C may be pyridine, 4-dimethylaminopyridine, triethylamine, N,N-diisopropylethylamine or N-methylmorpholine, with N-methylmorpholine being preferred. The amount of base used is 1 to 3 equivalents and preferably 1 to 1.5 equivalents with respect to compound (IX). Trityl chloride is used at 1 to 3 equivalents and preferably 1.05 to 1.1 equivalents. The solvent is not particularly restricted so long as it does not affect the reaction, and examples include N,N-dimethylformamide, N,N-dimethyl acetamide, toluene, dichloromethane, tetrahydrofuran and acetonitrile, preferably tetrahydrofuran. The reaction temperature will usually be -10 to 60° C. and is preferably 0 to 30° C. The reaction time is 0.5 to 12 hours and preferably 1 to 2 hours.

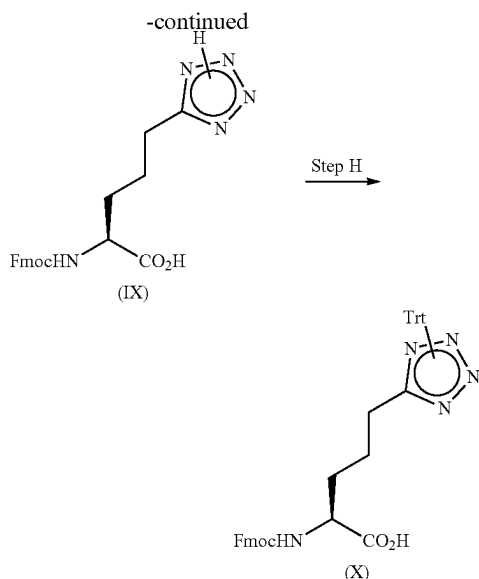
[0096] Fmoc-nvTz(Trt)-OH, having tetrazole bonded at the α-position of the amino acid via an alkyl having 3 carbon atoms, can be produced by the method represented by Reaction Scheme 2 or a similar method.

Reaction Scheme 2

[Chemical Formula 5]







**[0097]** In step D of the method of Reaction Scheme 2, a compound represented by general formula for compound (V) is reacted with  $\text{Boc}_2\text{O}$  in the presence of a base to produce compound (VI). The base is used at 1 to 10 equivalents and preferably 1 to 2 equivalents with respect to compound (V). The base used may be triethylamine, N,N-diisopropylamine, N-methylmorpholine, sodium hydroxide, potassium carbonate or sodium hydrogencarbonate, preferably sodium hydroxide. The  $\text{Boc}_2\text{O}$  is used at 1 to 10 equivalents and preferably 1 to 2 equivalents with respect to compound (V). The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF, DMF or 1,4-dioxane, and preferably 1,4-dioxane. The reaction temperature will usually be 0 to 25° C. and is preferably 10 to 25° C. The reaction time will usually be 1 to 24 hours and is preferably 18 to 24 hours. So long as the reaction is not affected, N-methylpiperazine may be added to process the excess  $\text{Boc}_2\text{O}$ .

**[0098]** In the subsequent step E, compound (VI) is reacted with nickel sulfate hexahydrate, sodium peroxodisulfate and sodium hydroxide to produce compound (VII). In step E, the sodium peroxodisulfate is used at 1 to 4 equivalents and preferably 1 to 2 equivalents with respect to compound (VI). The nickel sulfate hexahydrate is used at 0.01 to 0.1 equivalent and preferably 0.08 to 0.1 equivalent with respect to compound (VI). The base used may be sodium hydroxide, at 1 to 10 equivalents and preferably 6 to 8 equivalents in total with respect to compound (VI). The solvent used is water. The reaction temperature is 10 to 40° C. and preferably 10 to 25° C. The reaction time will usually be 1 to 24 hours and is preferably 12 to 24 hours.

**[0099]** In the subsequent step F, after reacting compound (VII) and TFA, the pH is adjusted to basicity and reaction is carried out with Fmoc-OSu to produce compound (VIII). The acid used in step F may be hydrochloric acid, sulfuric acid or trifluoroacetic acid, etc., and is preferably trifluoroacetic acid. The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF, dichloromethane or 1,4-dioxane, with dichloromethane being preferred. The reaction temperature is 0 to 25° C. and preferably 10 to 25° C. The reaction time will usually be 0.5

to 5 hours and is preferably 0.5 to 2 hours. The inorganic base used to adjust the pH after the reaction, and as the base, may be sodium hydroxide, potassium hydroxide, sodium hydrogencarbonate, sodium carbonate or potassium carbonate, with potassium carbonate being preferred. Fmoc-OSu is used at 1 to 3 equivalents and preferably 1 to 1.5 equivalents with respect to compound (VII). The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF, N,N-dimethylformamide, dichloromethane or 1,4-dioxane, with 1,4-dioxane being preferred. The reaction temperature is 0 to 25° C. and preferably 10 to 25° C. The reaction time will usually be 1 to 36 hours and is preferably 1 to 24 hours.

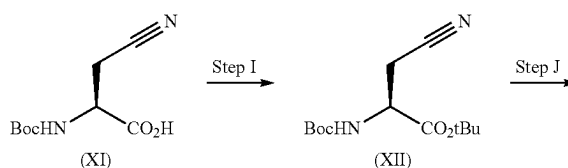
**[0100]** In the subsequent step G, compound (VIII) is reacted with trimethylsilyl azide and dibutyltin oxide in toluene to produce compound (IX). In step G, trimethylsilyl azide is used at 1 to 10 equivalents and preferably 2 to 4 equivalents with respect to compound (II). Dibutyltin oxide is used at 0.3 to 1 equivalent and preferably 0.7 to 1.0 equivalent with respect to compound (II). The solvent is not particularly restricted so long as it does not affect the reaction, and examples include benzene, toluene and xylene, with toluene being preferred. The reaction time will usually be 0.5 to 48 hours and is preferably 0.5 to 2 hours. The reaction temperature will usually be 80 to 150° C. and is preferably 80 to 100° C. The heating method may be with an oil bath or microwave, with microwave heating being preferred.

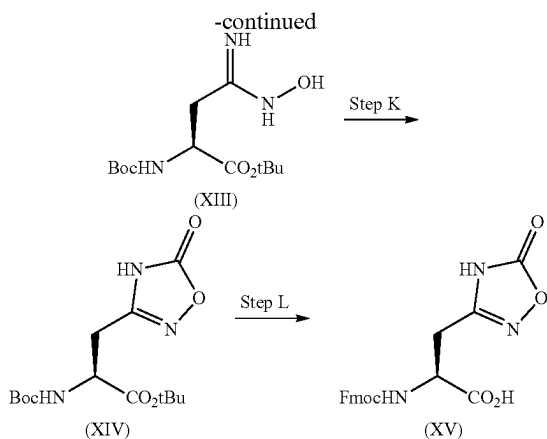
**[0101]** In the subsequent step H, compound (IX) may be reacted with trityl chloride in the presence of a base to produce compound (X). The base used for production of compound (X) may be pyridine, 4-dimethylaminopyridine, triethylamine, N,N-diisopropylethylamine or N-methylmorpholine, with N-methylmorpholine being preferred. The amount of base used is 1 to 3 equivalents and preferably 1 to 1.5 equivalents with respect to compound (IX). It may be used at 1 to 3 equivalents and preferably 1.05 to 1.1 equivalents with respect to the trityl chloride compound (IX). The solvent is not particularly restricted so long as it does not affect the reaction, and examples include N,N-dimethylformamide, N,N-dimethyl acetamide, toluene, dichloromethane, tetrahydrofuran and acetonitrile, with tetrahydrofuran being preferred. The reaction temperature will usually be -10 to 60° C. and is preferably 20 to 30° C., while the reaction time is 0.5 to 12 hours and preferably 1 to 2 hours.

**[0102]** Fmoc-(5Oxa)-OH, having the carboxyl group of the aspartic acid replaced with 1,2,4-oxadiazol-5-one, can be produced by the method represented by Reaction Scheme 3 or a similar method.

Reaction Scheme 3

[Chemical Formula 6]





[0103] In step I of the method of Reaction Scheme 3, a compound represented by the general formula of compound (XI) is reacted with 2-bromo-2-methylpropane in the presence of benzyltriethyl ammonium chloride and a base to produce compound (XII). In step I, the base is used at 10 to 30 equivalents and preferably 10 to 25 equivalents with respect to compound (XI). The base used may be potassium carbonate. Benzyltriethylammonium chloride is used at 1 to 3 equivalents and preferably 1 to 1.2 equivalents with respect to compound (XI). The 2-bromo-2-methylpropane is used at 10 to 50 equivalents and preferably 10 to 40 equivalents with respect to compound (XI). The solvent used is N,N-dimethyl acetamide. The reaction time will usually be 1 to 24 hours and is preferably 18 to 24 hours. The reaction temperature will usually be 25 to 70° C. and is preferably 25 to 55° C.

[0104] In the subsequent step J, compound (XII) is reacted with hydroxylamine hydrochloride in the presence of a base to produce compound (XIII). The base used in step J may be triethylamine or N,N-diisopropylethylamine, and is preferably triethylamine. The base used with compound (XII) is used at 1 to 3 equivalents and preferably 1 to 2 equivalents. The hydroxylamine hydrochloride is used at 1 to 2 equivalents and preferably 1 to 1.5 equivalents with respect to compound (XII). The solvent is not particularly restricted so long as it does not affect the reaction, and examples include methanol, ethanol, propanol and isopropanol, with ethanol being preferred. The reaction temperature will usually be 80 to 100° C. and is preferably 80 to 95° C. The reaction time will usually be 1 to 6 hours and is preferably 1 to 4 hours.

[0105] In the subsequent step K, compound (XIII) is reacted with carbonyldiimidazole to produce compound (XIV). In step K, the carbodiimidazole is used at 1 to 2 equivalents and preferably 1 to 1.2 equivalents with respect to compound (XIII). The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF or 1,4-dioxane, and preferably THF. The reaction temperature is 40 to 60° C. and preferably 40 to 55° C. The reaction time will usually be 1 to 6 hours and is preferably 1 to 3 hours. For continuous cyclization reaction, the solvent is not particularly restricted so long as it does not affect the reaction, and it may be toluene, xylene or benzene, or preferably toluene. The reaction temperature will usually be 100 to 130° C. and is preferably 100 to 120° C.

[0106] In the subsequent step L, the compound (XIV) is reacted with TFA in the presence of a scavenger, the pH is

adjusted to basicity and reaction is conducted with Fmoc-OSu to produce compound (XV). The acid used in step L may be hydrochloric acid, sulfuric acid or trifluoroacetic acid, and is preferably trifluoroacetic acid. The scavenger used may be triethylsilane or triisopropylsilane, and is preferably triethylsilane. The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF, dichloromethane or 1,4-dioxane, with dichloromethane being preferred. The reaction temperature is 0 to 25° C. and preferably 10 to 25° C. The reaction time will usually be 0.5 to 5 hours and is preferably 0.5 to 3 hours. The inorganic base used to adjust the pH after the reaction, and as the base, may be sodium hydroxide, potassium hydroxide, sodium hydrogencarbonate, sodium carbonate or potassium carbonate, with sodium hydrogencarbonate being preferred. Fmoc-OSu is used at 1 to 3 equivalents and preferably 1 to 1.5 equivalents with respect to compound (VII). The solvent is not particularly restricted so long as it does not affect the reaction, and it may be THF, N,N-dimethylformamide, dichloromethane or 1,4-dioxane, with 1,4-dioxane being preferred. The reaction temperature is 0 to 25° C. and preferably 10 to 25° C. The reaction time will usually be 1 to 36 hours and is preferably 1 to 24 hours.

[0107] The peptide of the invention can exhibit physiological activity similar to PACAP, by binding with PAC1R, VPAC1R and/or VPAC2R. As specific examples, the peptide of the invention can exhibit physiological activity as a neuroprotective substance, a nerve regeneration factor, a wound healing promoting factor, an inflammation suppressing factor or an exocrine secretagogue. According to another aspect, therefore, the invention may relate to a neuroprotective agent, a nerve-regenerating agent, a wound healing agent, a suppressing agent for inflammation, a corneal epithelial or corneal endothelial damage treatment agent, a dry-eye treatment agent, an exocrine gland secretagogue or a neurotrophic keratitis treatment agent, containing a peptide of the invention. According to yet another aspect, the invention relates to a pharmaceutical composition containing the peptide of the invention.

[0108] The neuroprotective agent of the invention is an agent having neuroprotective action. Therefore, a neuroprotective agent is one that can protect nerves from disorder brought on by neuronal damages, degeneration and/or cell death, and it can be a neuronal death (apoptosis and/or necrosis) inhibiting agent, a neuronal degeneration inhibiting agent, a neuronal stress reducing agent, a neurocytotoxicity resistance improving agent, a neuronal viability improving agent or an abnormal protein accumulation inhibiting agent.

[0109] Throughout the present specification, “neuroprotective action” refers to action of protecting neurons from their damage, degeneration and/or cell death, and preferably it is action of protecting them from neuronal death. More specifically, neuroprotective action may include inhibiting neuron death (apoptosis and/or necrosis), inhibiting neuronal degeneration, alleviating neuronal stress, increasing neurocytotoxicity resistance, improving neuron viability, or inhibiting accumulation of abnormal proteins. Neurons suffer damage due to physical damage, as well as exposure to neurotoxic substances and lack of oxygen or nutrients, and they can undergo cell death if a certain level of damage is exceeded. Neurons also suffer denaturation by accumulation of neurotoxic substances, eventually leading to cell death. Neurotoxic substances are largely classified as exogenous

toxic substances or endogenous toxic substances. Exogenous toxic substances include heavy metals, alcohol, and chemical substances such as botulinum toxin. Endogenous toxic substances include active oxygen species, neurotransmitters such as glutamic acid, and abnormal proteins. Neuroprotective action can be easily measured by a person skilled in the art. As an example, neurons may be cultured in culture medium containing a test substance (drug group) or culture medium lacking the test substance (control group) under conditions with various types of stress, such as low oxygen load, exposure to neurotoxic substances, nutrient depletion or ultraviolet irradiation, measuring the viable cell count and dead cell count in each culture medium and calculating the percentage of viable cells with respect to the total number of cells, and the test substance can be assessed to have neuroprotective action if the viable cell percentage in the drug group is higher than the viable cell percentage in the control group. As a more preferred method, it can be measured by comparison with a positive control group containing an added substance known to have neuroprotective action, such as IGF-1 or NGF, determining whether or not the test substance has protective action equal to or greater than the positive control group. As another example, the neuroprotective action can be measured by an *in vivo* animal experiment.

**[0110]** The peptide of the invention has the exocrine gland secretagogue action of PACAP. The exocrine gland may be a lacrimal gland or salivary gland, and the peptide may be used as an exocrine gland secretagogue such as a lacrimal secretagogue promoting agent or salivary secretagogue. Without being limited to any particular theory, presumably PACAP and the peptide of the invention promote secretion of saliva or lacrimal fluid by binding to receptors (PAC1R, VPAC1R, VPAC2R) expressed on acinar cells of exocrine glands. Lacrimal fluid covers the surface of the keratoconjunctiva to maintain its wettability, with the lacrimal fluid filling the indentations formed by microvilli on the corneal surface and allowing clear images to be obtained. Epithelial cells of the keratoconjunctiva undergo active metabolism, and when unnecessary cells or metabolites are shed and discharged from the outermost surface, lacrimal fluid flushes them out while supplying the necessary oxygen and nutrients. Lacrimal fluid also flushes out contaminants that have infiltrated the keratoconjunctiva surface, while the bacteriostatic action of lacrimal fluid plays an important role for protection from infection by viruses, bacteria and fungi infiltrating from the outside environment. It also functions as synovial fluid between the eyelids and keratoconjunctiva, for smooth blinking and eye movement. Although the lacrimal fluid constitutes only a small amount of fluid forming a minute thin-film on the keratoconjunctiva surface, it is indispensable for maintaining corneal transparency and homeostasis by a variety of elaborate mechanisms. The conditions of an abnormal keratoconjunctiva surface due to disorder in secretion of lacrimal fluid is generally referred to as “dry-eye”, and compounds have been created that promote lacrimal secretion when keratoconjunctival damage has occurred due to dry-eye, serving as prophylactic and therapeutic agents that are useful for dry-eye and conditions associated with dry-eye. The peptide of the invention can be included as a lacrimal secretagogue in an ophthalmic eye drop.

**[0111]** The peptide of the invention can also be used as a suppressing agent for inflammation, since it has the anti-

inflammatory action of PACAP. With no intention to be limited to any particular theory, PACAP and the peptide of the invention are able to suppress production of inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-12, etc.). The peptide of the invention can therefore be used as an anti-inflammatory drug. The PACAP receptors VPAC1R and VPAC2R, in particular, are widely distributed in the gastrointestinal tract and also exhibit anti-inflammatory action, and can be used for treatment of inflammatory intestinal diseases, in particular.

**[0112]** According to another aspect, the invention relates to a pharmaceutical composition containing a therapeutically effective dose of the peptide described above. The pharmaceutical composition of the invention can be used for treatment or prevention of a disease that is improved by physiological action of PACAP. Diseases that are improved by physiological action of PACAP include nervous disorder, diseases related to reduced lacrimal fluid, inflammatory diseases, and neurotrophic keratitis. The pharmaceutical composition of the invention can treat a disease that is improved by physiological action of PACAP, by administration to a patient, or it can prevent the disease by administration to a patient with the potential for developing the disease. Here, “treatment” means that after onset of a disorder or disease, worsening of the condition is prevented, or the current condition is maintained, alleviated or reversed, while “prevention” means that onset of a disorder or disease is prevented before onset.

**[0113]** Nervous disorder is a pathological condition where neuronal function is lost due to degeneration or cell death, and it includes cerebrovascular disorders and neurodegenerative disease.

**[0114]** Cerebrovascular disorders include hemorrhagic disorders, such as cerebral hemorrhage and subarachnoid hemorrhage, and cerebrovascular obstruction, such as cerebral thrombus, cerebral infarction and cerebral circulatory insufficiency. Both hemorrhagic disorders and obstructive disorders leave neurons in the brain in a state of hypoxia, leading to cell death. Therefore, the peptide, neuroprotective agent or pharmaceutical composition of the invention can be administered for the purpose of treatment or prevention of such cerebrovascular disorders.

**[0115]** Neurodegenerative diseases include, but are not limited to, degenerative diseases of the brain and central nervous system, such as dementia, Parkinson's disease, spinocerebellar degeneration, Creutzfeldt-Jakob disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, mad cow disease and epilepsy, motor neurodegenerative diseases such as spontaneous progressive muscular atrophy, amyotrophic lateral sclerosis and spinal-bulbar muscular atrophy, and sensory neurodegenerative diseases. Sensory neurodegenerative diseases include, but are not limited to, degenerative diseases of visual, auditory, tactile, gustatory and olfactory nerves, with visual degenerative diseases including glaucoma, retinitis pigmentosa, age-related macular degeneration and diabetic retinopathy, and auditory neurodegenerative diseases including hearing impairment.

**[0116]** Diseases related to reduced lacrimal fluid include, but are not limited to, dry-eye, keratoconjunctivitis sicca and reduced lacrimation.

**[0117]** Neurotrophic keratitis is a degenerative corneal disease resulting from damage to the trigeminal nerve, and they lead to damage of the corneal epithelium. The most

severe type may lead to corneal ulceration, fusion or perforation, and loss of vision by the patient.

**[0118]** A vascular endothelial function ameliorator is a drug that improves vascular endothelial function. Vascular endothelial cells are cells on the innermost layer of blood vessels, which regulate adhesion of inflammatory cells on vascular walls, vascular permeability and the clotting/fibrinolytic system, which includes governance of vascular wall contraction and relaxation (hardness/softness of blood vessels). Because the peptide of the invention has a vasodilating effect as a physiological action of PACAP, it improves vascular endothelial disorders such as arteriosclerosis.

**[0119]** Corneal epithelial or endothelial disorders are conditions in which the corneal epithelial cells and corneal endothelial cells are impaired. Corneal epithelial cells are proliferative cells, but in cases of corneal epithelial cell impairment, their proliferation potency is suppressed, with progressive epithelial shedding leading to an imbalance in epithelial homeostasis. Corneal endothelial cells do not proliferate in the body and decrease with aging. Corneal epithelial or corneal endothelial damage means corneal epithelial or endothelial injury caused by intrinsic disease factors such as corneal ulcer, corneal epithelial detachment, diabetic keratopathy, keratoconjunctivitis sicca, chronic superficial keratitis, superficial punctate keratopathy, corneal erosion and protracted corneal epithelial loss, extrinsic diseases factors such as drugs, trauma or contact lens usage, or physical or chemical injury.

**[0120]** Dry-eye is a chronic disease of lacrimal fluid and the conjunctival epithelium due to a variety of factors, and it is accompanied by eye discomfort and visual function abnormalities. Lacrimal fluid abnormalities include the quantitative abnormality of reduced lacrimal fluid flow, and the qualitative abnormality of change in lacrimal fluid quality or ability to retain lacrimal fluid. Dry-eye also includes the dry-eye that accompanies reduced lacrimation, lacrimal fluid-evaporative dry eye, Sjogren's syndrome, Stevens-Johnson syndrome, corneal epithelial erosion, blepharitis, ophthalmic pemphigus, vernal conjunctivitis, allergic conjunctivitis and vitamin A deficiency.

**[0121]** Inflammatory diseases include, but are not limited to, asthma, atopic dermatitis, hives, pollen hypersensitivity, anaphylactic shock, sinusitis (including eosinophilic sinusitis), rheumatism, multiple sclerosis, arthritis, systemic lupus erythematosus, psoriasis, ankylosing spondylitis, inflammatory intestinal disease (for example, ulcerative colitis, Crohn disease and gluten-sensitive intestinal disease), Sjogren's syndrome, chronic graft-versus-host disease (GVHD), corneal infectious disease, allergic conjunctivitis, corneal trauma, polymyositis, dermatomyositis, myasthenia gravis, chronic obstructive pulmonary disease (COPD) and dermatosclerosis.

**[0122]** The peptide of the invention, or a neuroprotective agent, nerve-regenerating agent, suppressing agent for inflammation, wound healing promoter or exocrine gland secretagogue containing the peptide, or the pharmaceutical composition, may be administered parenterally or orally, depending on the disease to be treated. Oral administration may be sublingual, intraoral or oral administration. Examples of parenteral administration include administration by intravenous, intraarterial, subcutaneous, local, intraperitoneal, intramuscular, nasal, transdermal, transmucosal, intrameningeal, perrectal, intramuscular, intracerebral, intrameningeal, subarachnoid, intradural, epidural, eye drop, ear

drop, nasal drop or intraocular routes. Intraocular routes include, more specifically, subconjunctival, sub-tenon and intravitreal routes. A pharmaceutical composition containing the peptide of the invention may be prepared in an appropriate dosage form for the route of administration, and may be an eye drop, injection, powdered drug, infusion preparation, granules, tablets or suppository, for example, but for parenteral administration it is preferably an eye drop, injection or infusion preparation, or a powdered drug for preparation prior to use. A formulation for intraocular administration may be an intravitreal injection, subconjunctival injection or sub-tenon injection, for example. Such formulations may also contain various pharmaceutically acceptable adjuvants, i.e. additives such as carriers or other auxiliary agents, which include stabilizers, antiseptic agents, soothing agents and emulsifiers. They may also be used in combination with other drugs that have neuroprotective effects, inflammation-suppressing effects or exocrine gland secretion effects.

**[0123]** All of the publications mentioned throughout the present specification are incorporated herein in their entirety by reference.

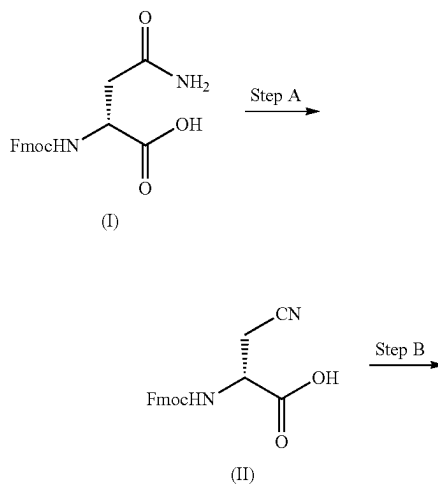
**[0124]** The examples of the invention described below are intended to serve merely as illustration and do not limit the technical scope of the invention. The technical scope of the invention is limited solely by the description in the Claims. Modifications of the invention, such as additions, deletions or substitutions to the constituent features of the invention, are possible so long as the gist of the invention is maintained.

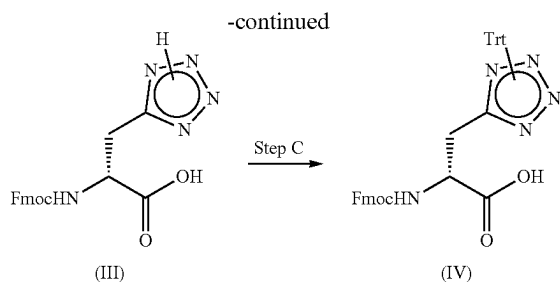
## EXAMPLES

### Example 1A: Synthesis of Fmoc-(D)-Tz(Trt)-OH (compound IV)

**[0125]** Fmoc-D-Tz(Trt)-OH (compound IV) was synthesized according to the following reaction scheme.

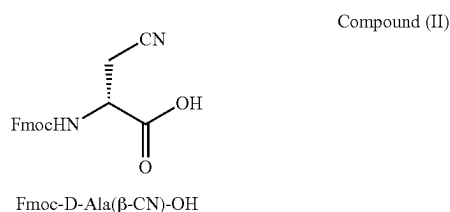
[Chemical Formula 7]





### Step A: Synthesis of Fmoc-D-Ala(β-CN)-OH

[Chemical Formula 8]

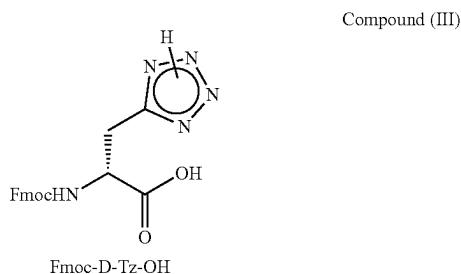


**[0126]** After adding a solution of pyridine (30 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.96 g, 21 mmol) suspended in acetonitrile (35 mL) to a solution of Fmoc-D-Asn-OH (5.04 g, 14 mmol) in acetonitrile (35 mL), at 0° C., the mixture was stirred for 4 hours at room temperature. The reaction mixture was concentrated under reduced pressure, ethyl acetate was added to the residue, the mixture was washed with 3 M hydrochloric acid and brine and dried over sodium sulfate, and the solvent was removed by distillation under reduced pressure. The obtained crude product was dissolved in acetone and hexane/diethyl ether (1/1) was added to produce a precipitate, which was then concentrated under reduced pressure and filtered to obtain the target compound (4.76 g, 99%) as a white solid.

**[0127]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.20 (1H, brs), 8.08 (1H, d, J=8.4 Hz), 7.90 (2H, d, J=7.6 Hz), 7.72 (2H, d, J=7.2 Hz), 7.42 (2H, t, J=7.2 Hz), 7.33 (2H, t, J=7.6 Hz), 4.39-4.32 (3H, m), 4.25 (1H, t, J=7.2 Hz), 2.98 (1H, dd, J=16.8, 4.8 Hz), 2.88 (1H, dd, J=16.8, 9.2 Hz).

### Step B: Synthesis of Fmoc-D-Tz-OH

[Chemical Formula 9]



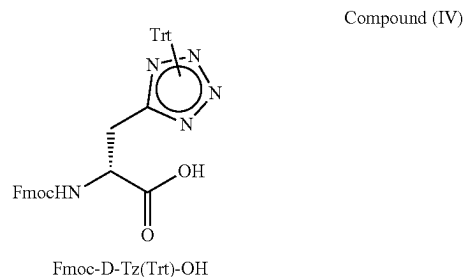
In the structural formula shown above, H is bonded to either N1 or N2 on the tetrazole ring.

**[0128]** After adding dibutyltin oxide (0.710 g, 2.9 mmol) and trimethylsilyl azide (1.8 mL, 14 mmol) to a solution of Fmoc-D-Ala(β-CN)-OH (1.01 g, 3.0 mmol) in toluene (18 mL), the mixture was heated in a microwave at 100° C. for 1.5 hours. The reaction mixture was returned to room temperature and filtered. The obtained filtered substance was washed with toluene and hexane and dissolved in ethanol/dichloromethane (3/2.25 mL), and then cooled to 4° C., after which 1 M hydrochloric acid (5 mL) was added and the mixture was stirred for 2 hours and 15 minutes at room temperature. The reaction mixture was washed with 1 M hydrochloric acid and brine, the solvent was removed by distillation under reduced pressure, and then hexane was added to form a suspension which was filtered to obtain a pale yellow solid of the target compound (1.03 g, 90%).

**[0129]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 16.00 (1H, brs), 13.07 (1H, brs), 7.89 (2H, d, H=7.6 Hz), 7.83 (1H, d, J=8.4 Hz), 7.65 (2H, t, J=8.0 Hz), 7.41 (2H, t, J=7.6 Hz), 7.31 (2H, t, J=7.6 Hz), 4.53-4.48 (1H, m), 4.27-4.18 (3H, m), 3.40 (1H, dd, J=15.2, 6.0 Hz), 3.28 (1H, dd, J=15.2, 8.8 Hz).

### Step C: Synthesis of Fmoc-D-Tz(Trt)-OH

[Chemical Formula 10]



In the structural formula shown above, a Trt group is bonded to N1 or N2 on the tetrazole ring.

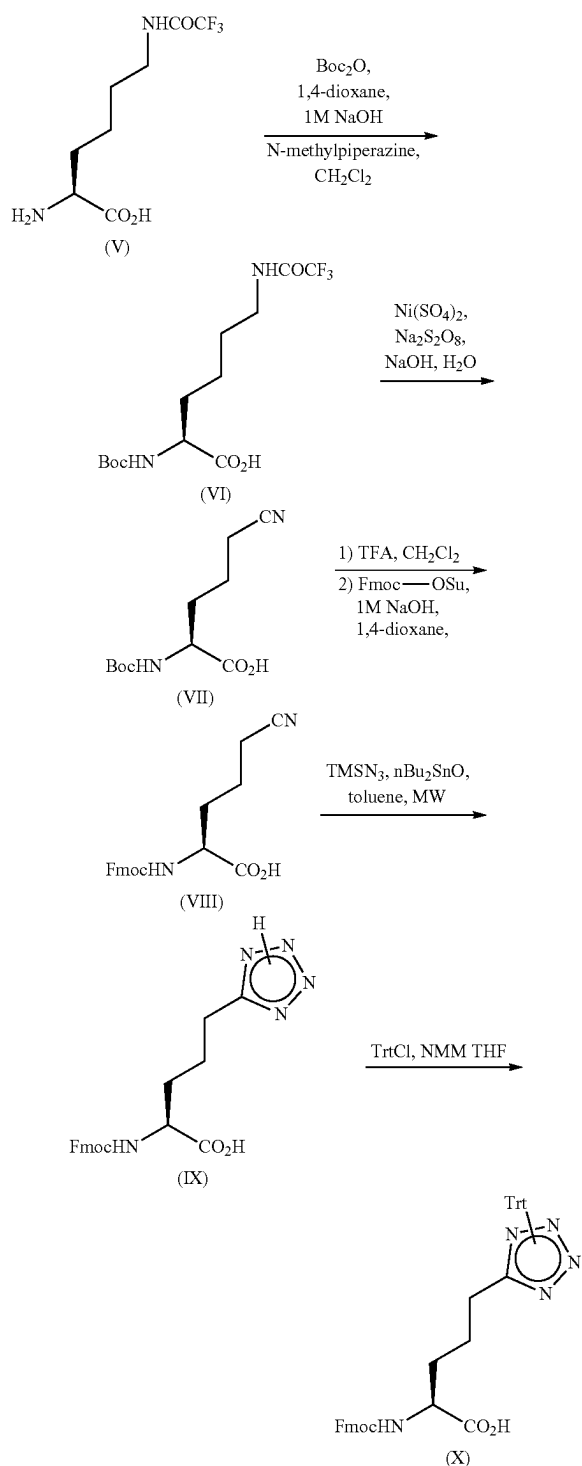
**[0130]** After adding N-methylmorpholine (0.72 mL, 7.2 mmol) and trityl chloride (1.63 g, 5.9 mmol) to a solution of Fmoc-D-Tz-OH (1.99 g, 5.3 mmol) in THE (10 mL), the mixture was stirred for 1.5 hours at room temperature. The reaction mixture was filtered and the filtered substance was washed with THE and combined with the filtrate, after which the solvent was removed by distillation under reduced pressure. The residue was purified by column chromatography (ODS, H<sub>2</sub>O/CH<sub>3</sub>CN=80/20→30/70→20/80), the fraction containing the target compound was extracted with ethyl acetate, washed with brine and dried over sodium sulfate, and then the solvent was removed by distillation under reduced pressure and vacuum drying was carried out at 60° C. to obtain the target compound (1.29 g, 40%) as a white solid.

**[0131]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.03 (1H, brs), 7.91 (2H, d, J=7.6 Hz), 7.79 (1H, d, J=8.8 Hz), 7.67 (2H, brd, J=6.8 Hz), 7.44-7.23 (13H, m), 7.01-6.99 (6H, m), 4.52-4.46 (1H, m), 4.28-4.15 (3H, m), 3.40 (1H, dd, J=15.2, 5.2 Hz), 3.27 (1H, dd, J=15.2, 9.2 Hz).

Example 1B: Synthesis of Fmoc-nvTz(Trt)-OH  
(Compound X)

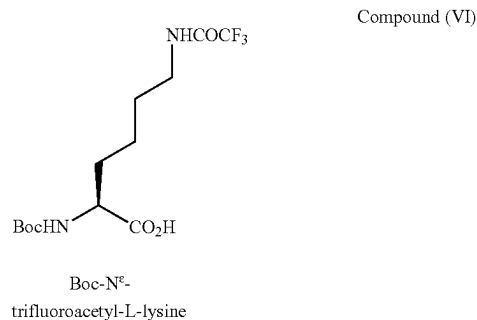
[0132] Fmoc-nvTz(Trt)-OH (compound X) was synthesized according to the following reaction scheme.

[Chemical Formula 11]



Step D: Synthesis of  
Boc-Ng-trifluoroacetyl-L-lysine

[Chemical Formula 12]

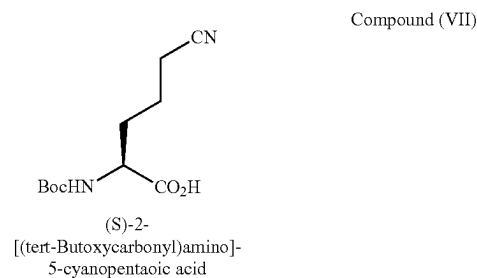


[0133] After adding a 1 M sodium hydroxide aqueous solution (42 mL, 42 mmol) and  $\text{Boc}_2\text{O}$  (30% THF solution, 49.8 g, 68 mmol) to a solution of Ng-tri fluoroacetyl-L-lysine (10.0 g, 41 mmol) in 1,4-dioxane (70 mL), the mixture was stirred for 1 day at room temperature. After then adding N-methylpiperazine (3.5 mL) to the reaction mixture and stirring for 2 hours at room temperature, 1 M hydrochloric acid was added to adjust the reaction mixture to pH 2, and it was extracted with ethyl acetate and washed with brine. The organic layer was dried over sodium sulfate and the solvent was removed by distillation under reduced pressure to obtain the title compound (15.9 g, >100%) as a white solid.

[0134]  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.69 (1H, brs), 5.12 (1H, d,  $J=6.8$  Hz), 4.30 (1H, brs), 3.38 (2H, q,  $J=6.8$  Hz), 1.94-1.86 (1H, m), 1.74-1.57 (3H, m), 1.52-1.41 (3H, m), 1.45 (9H, s).

Step E: Synthesis of (S)-2-[(tert-butoxycarbonyl)amino]-5-cyanopentanoic acid

[Chemical Formula 13]



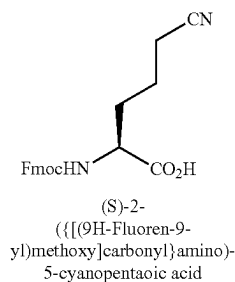
[0135] After adding Boc-Ne-trifluoroacetyl-L-lysine (1.00 g, 2.9 mmol), sodium hydroxide (276.4 mg, 6.9 mmol) and water (15 mL) to a flask, a nickel(II) sulfate hexahydrate (77.0 mg, 0.29 mmol) aqueous solution (1.0 mL), sodium peroxodisulfate (1.43 g, 6.0 mmol) and sodium hydroxide (529.5 mg, 13 mmol) were added and the mixture was stirred for 19.5 hours at room temperature. The reaction mixture was adjusted to pH 2 with 1 M hydrochloric acid and extracted with ethyl acetate, the obtained organic layer was washed with brine and dried over sodium sulfate, and the

solvent was removed by distillation under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane/methanol=100/0→95/5) to obtain the title compound (661.5 mg, 86%) as a white solid.

**[0136]**  $^1\text{H}$  NMR (400 Mz, DMSO- $d_6$ )  $\delta$  12.53 (1H, brs), 7.14 (1H, d,  $J=8.4$  Hz), 3.93-3.88 (1H, m), 2.53-2.48 (2H, m), 1.81-1.74 (1H, m), 1.69-1.56 (3H, m), 1.39 (9H, m)

Step G: Synthesis of (S)-2-([[(9H-fluoren-9-yl)methoxy]carbonyl]amino)-5-cyanopentanoic acid

[Chemical Formula 14]



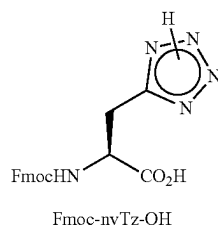
Compound (VIII)

**[0137]** A solution of (S)-2-[(tert-butoxycarbonyl)amino]-5-cyanopentanoic acid (586.3 g, 2.4 mmol) in dichloromethane (2.5 mL) was cooled to 0° C., and then trifluoroacetic acid (2.5 mL) was added and the mixture was stirred for 1 hour at room temperature. The solvent of the reaction mixture was removed by distillation under reduced pressure. After adding 1,4-dioxane (3.0 mL) to the obtained residue, a 10% potassium carbonate aqueous solution and solid potassium carbonate were added to pH 9, and then Fmoc-OSu (1.26 g, 3.7 mmol) was added and the mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with an ethyl acetate/hexane (1/1) solution and washed with 0.5 M hydrochloric acid and brine, and then the obtained organic layer was dried over sodium sulfate and the solvent was removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (ethyl acetate/hexane=10/90→31/69) to obtain the title compound (731.1 mg, 83%) as a white solid.

**[0138]**  $^1\text{H}$  NMR (4000 MHz,  $\text{CDCl}_3$ )  $\delta$  7.76 (2H, d,  $J=7.2$  Hz), 7.58 (2H, brd,  $J=6.8$  Hz), 7.40 (2H, t,  $J=7.2$  Hz), 7.31 (2H, t,  $J=6.8$  Hz), 5.38 (1H, d,  $J=7.6$  Hz), 4.64-4.36 (3H, m), 4.20 (1H,  $J=6.4$  Hz), 2.38 (1H, brt,  $J=6.4$  Hz), 2.28-2.07 (2H, m), 1.88-1.41 (3H, m)

Step H: Synthesis of Fmoc-nvTz-OH

[Chemical Formula 15]



Compound (IX)

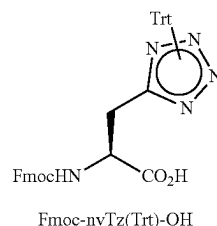
In the structural formula shown above, H is bonded to either N1 or N2 on the tetrazole ring.

**[0139]** After adding trimethylsilyl azide (1.2 mL, 9.2 mmol) to a solution of (S)-2-([[(9H-fluoren-9-yl)methoxy]carbonyl]amino)-5-cyanopentanoic acid (851 mg, 2.3 mmol) and dibutyltin oxide (581 mg, 2.3 mmol) in toluene (17 mL), the mixture was heated in a microwave at 100° C. for 1.5 hours. The reaction mixture was returned to room temperature and filtered, and the obtained solid was washed with toluene and hexane to obtain a pale orange solid. After adding ethanol/dichloromethane (3/2.50 mL) to the solid and cooling to 0° C., 1 M hydrochloric acid (5.0 mL) was added and the mixture was stirred for 2 hours at room temperature. Ethyl acetate was added to the reaction mixture and the resulting mixture was washed with 1 M hydrochloric acid and brine and dried over sodium sulfate, and the solvent was removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (dichloromethane/methanol=100/0→95/5) to obtain the title compound (367 mg, 39%).

**[0140]**  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  7.88 (2H, d,  $J=7.6$  Hz), 7.72 (2H, d,  $J=7.2$  Hz), 7.63 (1H, d,  $J=7.6$  Hz), 7.40 (2H, t,  $J=7.6$  Hz), 7.31 (2H, t,  $J=7.2$  Hz), 4.29-4.20 (3H, m), 4.01-3.96 (1H, m), 2.89 (2H, m), 1.78-1.46 (5H, m).

Step I: Synthesis of Fmoc-nvTz(Trt)-OH

[Chemical Formula 16]



Compound (X)

In the structural formula shown above, a Trt group is bonded to either N1 or N2 on the tetrazole ring.

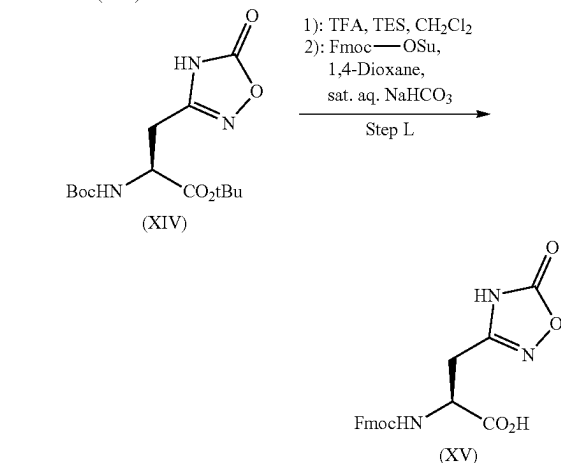
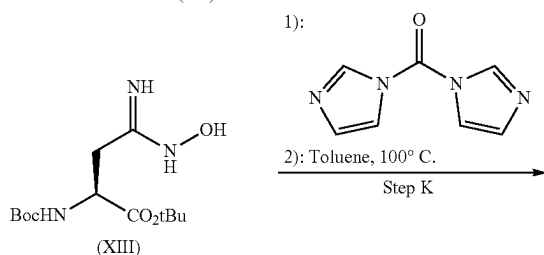
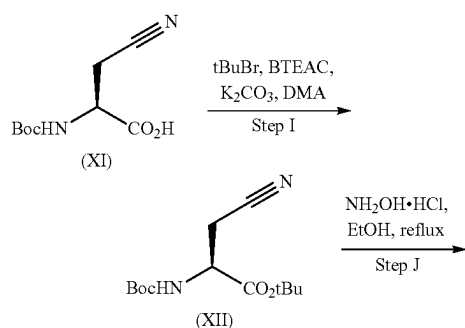
**[0141]** After adding N-methylmorpholine (110  $\mu\text{L}$ , 1.0 mmol) and trityl chloride (261 mg, 0.94 mmol) to a solution of Fmoc-nvTz-OH (365 mg, 0.89 mmol) in THE (4.0 mL), the mixture was stirred for 2 hours at room temperature. Upon completion of the reaction and filtering, the solvent of the obtained filtrate was distilled off under reduced pressure to obtain the title compound (540 mg, 93%) as a white solid.

**[0142]**  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.67 (1H, s), 7.89 (2H, d,  $J=7.2$  Hz), 7.72 (2H, m), 7.66 (1H, brs), 7.39-7.19 (13H, m), 7.02-6.98 (6H, m), 4.34-4.20 (3H, m), 3.98-3.94 (1H, m), 2.88-2.83 (2H, m), 1.77-1.62 (4H, m)

Example 1C: Synthesis of (S)-2-([[(9H-fluoren-9-yl)methoxy]carbonyl]amino)-3-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)propanoic acid (compound XV)

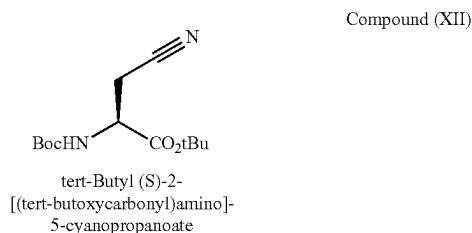
**[0143]** The compound (S)-2-([[(9H-fluoren-9-yl)methoxy]carbonyl]amino)-3-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)propanoic acid (compound XV) was synthesized according to the following reaction scheme.

[Chemical Formula 17]



Step I: Synthesis of tert-butyl (S)-2-[(tert-butoxycarbonyl)amino]-3-cyanopropanoate

[Chemical Formula 18]



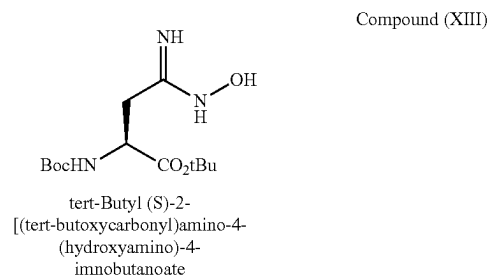
[0144] After adding 2-bromo-2-methylpropane (46 ml, 409 mmol) to a suspension of Boc-Ala(CN)—OH (2.23 g, 10.4 mmol), benzyltriethylammonium chloride (2.38 g, 10.4 mmol) and potassium carbonate (37.0 g, 268 mmol) in DMA

(60 mL), the mixture was stirred for 1 day at 55° C. The reaction mixture was returned to room temperature, water was added, extraction was performed with ethyl acetate and the obtained organic layer was washed with water and brine and dried over sodium sulfate, and then the solvent was removed by distillation under reduced pressure. The obtained residue was purified by flash silica gel column chromatography (hexane/ethyl acetate=1/0→1/4) to obtain the title compound (2.79 g, 99%) as a colorless oil.

[0145] <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.45 (1H, brd, J=4.8 Hz), 4.37 (1H, brq, J=5.2 Hz), 2.94 (1H, dd, J=16.8, 5.2 Hz), 2.88 (1H, dd, J=16.8, 4.8 Hz), 1.51 (9H, s), 1.45 (9H, s).

Step J: Synthesis of tert-butyl (S)-2-[(tert-butoxycarbonyl)amino]-4-(hydroxyamino)-4-iminobutanoate

[Chemical Formula 19]

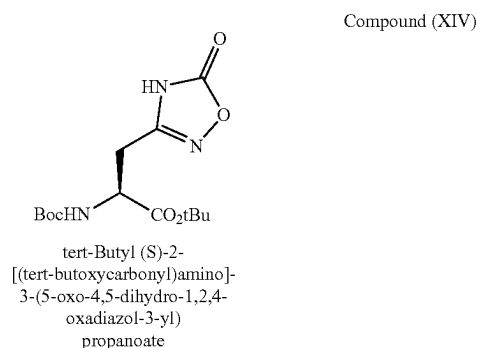


[0146] After adding triethylamine (2.8 mL, 20 mmol) and hydroxylamine hydrochloride (1.08 g, 16 mmol) to a solution of tert-butyl (S)-2-[(tert-butoxycarbonyl)amino]-3-cyanopropanoate (2.79 g, 10 mmol) in ethanol (30 mL), the mixture was stirred for 4 hours at 95° C. The reaction mixture was returned to room temperature, water was added, the mixture was extracted with ethyl acetate and dried over sodium sulfate, and the solvent was removed by distillation under reduced pressure and vacuum dried to obtain the title compound (2.85 g, 91%) as a white amorphous solid.

[0147] <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.47 (1H, brs), 5.47 (1H, brs), 4.63 (2H, brs), 4.37 (1H, brs), 2.62-2.54 (2H, m), 1.46 (9H, s), 1.44 (9H, s).

Step K: Synthesis of tert-butyl (S)-2-[(tert-butoxycarbonyl)amino]-3-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)propanoate

[Chemical Formula 20]



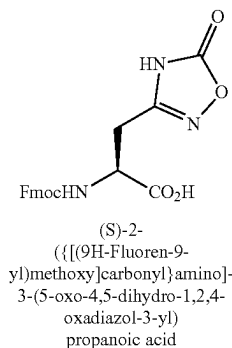


[0148] After adding 1,1-carbonyldiimidazole (627 mg, 3.9 mmol) to a solution of tert-butyl (S)-2-[(tert-butoxycarbonyl)amino]-4-(hydroxyamino)-4-iminobutanoate (1.04 g, 3.4 mmol) in THE (20 mL), the mixture was stirred for 1.5 hours at room temperature. The reaction mixture was concentrated, toluene (20 mL) was added and the mixture was stirred for 2 hours at 100° C. The reaction mixture was returned to room temperature, the solvent was removed by distillation under reduced pressure, and the obtained residue was purified by flash silica gel column chromatography (hexane/ethyl acetate=1/1) to obtain the title compound (658.9 mg, 58%) as a white amorphous solid.

[0149] <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 12.23 (1H, brs), 7.36 (1H, d, J=8.0 Hz), 4.24 (1H, q, J=8.0 Hz), 2.90 (1H, dd, J=15.2, 7.2 Hz), 2.81 (1H, dd, J=15.2, 8.4 Hz), 1.38 (9H, s), 1.37 (9H, s).

Step L: Synthesis of (S)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)propanoic acid

[Chemical Formula 21]



[0150] After adding triethylsilane (1.0 mL) and trifluoroacetic acid (5.0 mL) to a solution of tert-butyl(S)-2-[(tert-butoxycarbonyl)amino]-3-(5-oxo-4,5-dihydro-1,2,4-oxadi-

azol-3-yl)propanoate (658.9 mg, 2.0 mmol) in dichloromethane (4.0 mL) at 0° C., the mixture was stirred for 3 hours at room temperature. The solvent was removed by distillation under reduced pressure, diethyl ether and hexane were added, the solvent was again removed by distillation under reduced pressure, 1,4-dioxane (10 mL) was added, and a saturated sodium hydrogencarbonate aqueous solution was added to pH 9. Next, Fmoc-OSu (813 mg, 2.4 mmol) was added at 0° C. and the mixture was stirred overnight at room temperature. After adding a 10% citric acid aqueous solution to the reaction mixture for adjustment to pH 3, it was extracted with ethyl acetate and dried over sodium sulfate, the solvent was removed by distillation under reduced pressure, and the obtained residue was purified by silica gel column chromatography (dichloromethane/methanol=100/0→93/7) to obtain the title compound (465.3 mg, 59%) as a white amorphous solid.

[0151] <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.89 (2H, d, J=7.2 Hz), 7.70-7.63 (3H, m), 7.42 (2H, t, J=7.2 Hz), 7.32 (2H, t, J=7.2 Hz), 4.33-4.20 (4H, m), 2.96 (1H, dd, J=15.2, 5.6 Hz), 2.80 (1H, dd, 15.2, 8.8 Hz),

## Example 2: Peptide Synthesis

[0152] The peptide used in the test was synthesized using a peptide synthesizer (model: PSSM-8 by Shimadzu Corp.), using solid phase synthesis by the Fmoc method. The non-natural amino acids Fmoc-Tz-OH, Fmoc-Tz(trt)-OH and Fmoc-egTz(trt)-OH used for solid phase synthesis were purchased from Astatech Co., Fmoc-cya-OH was purchased from Anaspec Co., and compounds (IV), (X) and (XV) synthesized in Example 1 were used. F-moc-Ai-OH and Fmoc-Cn-OH were purchased from Watanabe Chemical Engineering, and peptides 1 to 28 having the following sequences were synthesized. The molecular weights of the synthetic peptides were analyzed by mass spectrometry (MALDI TOF). As shown in Table 2, all of the measured values matched theoretical values. N-terminal and C-terminal modification were carried out by methods known to those skilled in the art.

TABLE 2-1

	N-terminus	Sequence	C-terminus	Theoretical value [M + H] <sup>+</sup>	Measured Value
Peptide 1	H	HSTzGI FATzAY SRYRK QMAVK KYLA A VL (Seq ID No. 4)	NH2	3148.70	3148.2
Peptide 2	H	HSTzGI FATzSY ARYRK QMAVK KYLA A VL (Seq ID No. 5)	NH2	3148.70	3148.6
Peptide 3	H	HSTzGI FATzAY ARYRK QMAVK KYLA A VL (Seq ID No. 6)	NH2	3132.70	3132.6
Peptide 4	H	HSTzGI FATzAiY SRYRK QMAVK KYL AA VL (Seq ID No. 7)	NH2	3162.71	3162.3
Peptide 5	H	HSTzGI FATzSY AiRYRK QMAVK KYL AA VL (Seq ID No. 8)	NH2	3162.71	3162.9
Peptide 6	H	HSTzGI FATzAiY AiRYRK QMAVK KY LAA VL (Seq ID No. 9)	NH2	3160.73	3161.2
Peptide 7	H	HSTzGI FTTzAiY AiRYRK QMAVK KY LAA VL (Seq ID No. 10)	NH2	3190.74	3190.3

TABLE 2-1-continued

	N-terminus	Sequence	C-terminus	Theoretical value [M + H] <sup>+</sup>	Measured Value
Peptide 8	H	HSTzGI FTTzSY SRYRK QMAVK KYLA (Seq ID No. 11)	NH2	2911.51	2911.2
Peptide 9	H	HSTzGI FTTzSY SRYRK QMAVK KYLA A V (Seq ID No. 12)	NH2	3081.62	3081.4
Peptide 10	H	HScyaGI FTcyasY SRYRK QLAVK KY LAA VL (Seq ID No. 13)	NH2	3200.63	3200.9
Peptide 11	H	HScyaGI FTcyasY SRYRK QNIAVK K YLAA VL (Seq ID No. 14)	NH2	3200.63	3201.0
Peptide 12	H	MHSTzGI FTTzSY SRYRK QLAVK KYL AA VL (Seq ID No. 15)	NH2	3307.79	3307.3
Peptide 13	H	HSTz(D)GI FTTz(D)SY SRYRK QNIA VK KYLAA VL (Seq ID No. 16)	NH2	3176.75	3176.8
Peptide 14	H	HSTzGI FTTz(D)SY SRYRK QNIAVK KYLAA VL (Seq ID No. 17)	NH2	3176.75	3176.6
Peptide 15	H	HSTz(D)GI FTTzSY SRYRK QNIAVK KYLAA VL (Seq ID No. 18)	NH2	3176.75	3176.8
Peptide 16	H	HSTzGI FTegTzSY SRYRK QNIAVK K YLAA VL (Seq ID No. 19)	NH2	3190.76	3191.1
Peptide 17	CH <sub>3</sub> CH <sub>2</sub> CO	HSTzGI FTTzSY SRYRK QNIAVK KYL AA VL (Seq ID No. 20)	NH2	3232.77	3232.5
Peptide 18	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CO	HSTzGI FTTzSY SRYRK QNIAVK KYL AA VL (Seq ID No. 21)	NH2	3246.79	3246.3
Peptide 19	(CH <sub>3</sub> ) <sub>2</sub> CH CO	HSTzGI FTTzSY SRYRK QNIAVK KYL AA VL (Seq ID No. 22)	NH2	3246.79	3246.3
Peptide 20	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CO	HSTzGI FTTzSY SRYRK QNIAVK KYL AA VL (Seq ID No. 23)	NH2	3260.80	3260.8

TABLE 2-2

	N-terminus	Sequence	C-terminus	Theoretical value [M + H] <sup>+</sup>	Measured Value
Peptide 21	(CH <sub>3</sub> ) <sub>2</sub> CH CH <sub>2</sub> CO	HSTzGI FTTzSY SRYRK QNIAVK KYL AA VL (Seq ID No. 24)	NH2	3260.80	3260.5
Peptide 22	Ac(CH <sub>3</sub> CO)	HS(50xa)GI FT(50xa)SY SRYRK QN IAVK KYLAA VL (Seq ID No. 25)	NH2	3250.72	3250.3
Peptide 23	Ac	HSTzGA FTTzSY SRYRK QLAVK KYLA A VL (Seq ID No. 26)	NH2	3176.71	3176.2
Peptide 24	Ac	HSTzGI FATzSY SRYRK QLAVK KYLA A VL (Seq ID No. 27)	NH2	3188.75	3188.8
Peptide 25	Ac	HATzGI FATzSY SRYRK QLAVK KYLA A VL (Seq ID No. 28)	NH2	3172.75	3172.4
Peptide 26	Ac	HATzGI FITzSY SRYRK ALAVK KYLA A VL (Seq ID No. 29)	NH2	3145.74	3146.2
Peptide 27	Ac	HSTzGI FATzAY SRYRK QLAVK KYLA A VL (Seq ID No. 30)	NH2	3172.75	3172.4
Peptide 28	Ac	HSTzGI FATzSY SRYRK ALAVK KYLA A VL (Seq ID No. 31)	NH2	3131.72	3131.2

TABLE 2-2-continued

	N-terminus	Sequence	C-terminus	Theoretical value [M + H] <sup>+</sup>	Measured Value
Peptide 29	Ac	HSTzGI FATzSY SRYRK QAAVK KYLA A VL (Seq ID No. 32)	NH2	3146.70	3147.0
Peptide 30	Ac	HATzGI FATzAY SRYRK AAAVK KYLA A VL (Seq ID No. 33)	NH2	3057.69	3057.5
Synthetic Example 1	H	HSTzGI FATzSY CnRYRK QMAVK KYL AA VL (Seq ID No. 34)	NH2	3173.69	3173.3
Synthetic Example 2	H	HSTzGI FTTzCnY CnRYRK QMAVK KY LAA VL (Seq ID No. 35)	NH2	3212.70	3212.7
Synthetic Example 3	H	HSegTzGI FTTzSY SRYRK QNIAVK K YLAA VL (Seq ID No. 36)	NH2	3190.76	3190.9
Synthetic Example 4	H	HSegTzGI FTegTzSY SRYRK QNIAVK KYLAA VL (Seq ID No. 37)	NH2	3204.78	3204.8
Synthetic Example 5	H	HSnvTzGI FTTzSY SRYRK QNIAVK K YLAA VL (Seq ID No. 38)	NH2	3204.78	3204.9
Synthetic Example 6	H	HSTzGI FTnvTzSY SRYRK QNIAVK K YLAA VL (Seq ID No. 39)	NH2	3204.78	3204.8
Synthetic Example 7	H	HSnvTzGI FTnvTzSY SRYRK QNIAVK KYLAA VL (Seq ID No. 40)	NH2	3232.81	3232.8
Synthetic Example 8	H	HSegTzGI FTnvTzSY SRYRK QNIAVK KYLAA VL (Sea ID No. 41)	NH2	3218.79	3218.4
Synthetic Example 9	H	HSnvTzGI FTegTzSY SRYRK QNIAVK KYLAA VL (Seq ID No. 42)	NH2	3218.79	3218.7

TABLE 2-3

	N-terminus	Sequence	C-terminus	Theoretical value [M + H] <sup>+</sup>	Measured Value
Reference Example 1 (PACAP27)	H	HSDGIFTDSYSRYRKQMAVKKYLA AVL (Seq ID No. 2)	NH2	3146.66	3147.0
Reference Example 2	Ac	HSDGIFTDSYSRYRKQMAVKKYLA AVL (Seq ID No. 51)	NH2	3188.67	3188.4
Reference Example 3	H	HSTzGIFTTzSY SRYRKQ NIAVKKYLA AVL (Seq ID No. 52)	NH2	3176.75	3177.0
Reference Example 4	H	HSTzGIFTTzSY SRYRKQLAVKKYLA AVL (Seq ID No. 53)	NH2	3176.75	3217.9
Reference Example 5	Ac	HSTzGIFTTzSY SRYRKQ NIAVKKYLA AVL (Seq ID No. 54)	NH2	3218.76	3219.2
Reference Example 6	Ac	HSTzGIFTTzSY SRYRKQLAVKKYLA AVL (Seq ID No. 55)	NH2	3218.76	3218.2

[0153] In the table, Tz represents tetrazole-substituted aspartic acid, egTz represents tetrazole-substituted glutamic acid, nvTz represents tetrazole-substituted homoglutamic acid, cya represents sulfoxy-substituted aspartic acid, (5Oxa) represents oxadiazolone-substituted aspartic acid, Ai represents  $\alpha$ -aminoisobutyric acid and Cn represents  $\beta$ -cyanoalanine. Tz(D) indicates that tetrazole-substituted aspartic acid is the D-form.

[0154] The Tz introduced for peptides 1 to 9 was the racemic form. The amino acids used for the other peptides were L-forms unless otherwise specified.

### Example 3: Stability Test 1

#### [Measuring Sample Preparation 1]

[0155] The peptides synthesized in Example 2 (peptides 1 to 9) were weighed and dissolved in 0.1% phosphate buffer (pH 7.0) to prepare 1.0 mM peptide solutions. The 1.0 mM peptide solutions were then diluted with phosphate buffer to 100  $\mu$ M. After filtering with Chromatdisk (product of Merck Millipore, Millex-GV, 0.22  $\mu$ m), each filtrate was dispensed into an LC vial (Waters Deactivated Qsert Vial). The prepared peptide solutions were incubated for one month or 2 months in a 40° C. thermostatic bath to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

#### [Moisture Permeability]

[0156] Using the total weight of the aqueous peptide solution and storage vessel as the specimen weight, measurements were recorded as the specimen weights before storage and the specimen weights after storage under different storage conditions. The empty weight of the storage vessel was also measured, and the moisture permeability was determined by the following formula.

#### [Mathematical Formula 1]

Vessel moisture permeability (%) =

$$\frac{\text{Specimen weight before storage (g)} - \text{specimen weight after storage (g)}}{\text{Specimen weight before storage (g)} - \text{storage vessel weight (g)}} \times 100$$

[0157] After stirring the standard sample and the stored samples with a vortex mixer, each peptide solution was transferred to an HPLC vial (Deactivated Qsert vial by Waters Co.). Reverse-phase HPLC (HPLC system: Prominence by Shimadzu Corp.) was conducted under the conditions shown in Table 3 below for analysis of the peptide solutions, and chromatograms were obtained.

#### [HPLC Analysis Conditions]

[0158] Column: XSelect CSH C18, 5  $\mu$ m, 4.6 $\times$ 250 mm, by Waters Co.

[0159] Guard column: XSelect CSH C18, 5  $\mu$ m, 4.6 $\times$ 20 mm Guard Cartridge by Waters Co.

[0160] Detection wavelength: 220 nm

[0161] Mobile phase A: 0.1% formic acid aqueous solution

[0162] mobile phase B: 0.1% acetonitrile formate solution

[0163] Measuring time: 30 minutes

[0164] Measuring sample injection rate: 50  $\mu$ L

[0165] Flow rate: 1.0 mL/min

[0166] Column temperature: 40° C.

[0167] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 3 for linear concentration gradient control.

TABLE 3

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-30.0	90→75	10→25
30.0-30.5	75→0	25→100
30.5-36.5	0	100
36.5-37.0	0→90	100→10
37.0-49.5	90	10

[0168] The peak area value for the peptide in the chromatogram was calculated, and the survival rate (survival rate before correction for water) was calculated using formula (2). The survival rate after correction for water was also calculated from the survival rate before correction for water using formula (3) to take into account the permeability of the container.

#### [Mathematical Formula 2]

Survival rate before correction for water (%) =

$$\frac{\text{Peptide peak area of stored sample}}{\text{peptide peak area of standard sample}} \times 100$$

#### [Mathematical Formula 3]

Survival rate after correction for water (%) =

$$\frac{\text{Survival rate before correction for water (%)} \times (100\% - \text{vessel moisture permeability (\%)})}{100}$$

[0169] Table 4 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 4

Peptide name	40° C. 4 week	80° C. 8 week
Peptide 1	91.1%	81.5%
Peptide 2	91.9%	83.7%
Peptide 3	92.9%	85.4%
Peptide 4	95.4%	88.4%
Peptide 5	94.4%	85.4%
Peptide 6	94.0%	87.5%
Peptide 7	91.8%	83.5%
Peptide 8	95.1%	84.1%
Peptide 9	92.4%	83.3%
PACAP27	37.4%	21.3%

[0170] All of the peptides exhibited high aqueous solution stability at 4 weeks and 8 weeks at 40° C., compared to PACAP27.

## [Measuring Sample Preparation 2]

[0171] Peptide solutions prepared by the same method as [Measuring sample preparation 1] for the peptides synthesized in Example 2 (peptides 13 to 16) were incubated for 2 weeks and 8 weeks in a thermostatic bath at 40° C. to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

## [HPLC Analysis Conditions]

[0172] Measurement was carried out under the same conditions as in [Measuring sample preparation 1], changing only the measuring time and mobile phase delivery conditions as shown below.

[0173] Measuring time: 20 minutes

[0174] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 5 for linear concentration gradient control.

TABLE 5

Time after sample injection (min) <sup>②</sup>	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	85→75	15→25
20.0-20.1	75→0	25→100
20.1-24.5	0	100
24.5-25.0	0→85	100→15
25.0-40.5	85	15

② indicates text missing or illegible when filed

[0175] Table 6 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 6

Peptide name	40° C. 2 week	40° C. 8 week
Peptide 13	96.2%	84.6%
Peptide 14	96.3%	84.5%
Peptide 15	95.6%	81.3%
Peptide 16	94.4%	80.3%
PACAP27	64.3%	23.7%

Even peptides with different the stereochemistry of the amino acid residues substituted with tetrazole and different lengths between the tetrazole and the peptide main chain exhibited higher stability than PACAP27.

## [Measuring Sample Preparation 3]

[0176] Peptide solutions prepared by the same method as [Measuring sample preparation 1] for the peptides synthesized in Example 2 (peptides 17 to 21) were incubated for 4 weeks in a thermostatic bath at 40° C. to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

## [HPLC Analysis Conditions]

[0177] Measurement was carried out under the same conditions as in [Measuring sample preparation 1], changing only the measuring time and mobile phase delivery conditions as shown below.

[0178] Measuring time: 20 minutes

[0179] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 7 for linear concentration gradient control.

TABLE 7

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	85→75	15→25
20.0-20.1	75→0	25→100
20.1-24.5	0	100
24.5-25.0	0→85	100→15
25.0-40.5	85	15

[0180] Table 8 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 8

Peptide name	40° C. 4 week
Peptide 17	59.2%
Peptide 18	65.1%
Peptide 19	68.7%
Peptide 20	69.5%
Peptide 21	69.0%
PACAP27	23.9%

Peptides with different acyl groups substituting at the N-terminus exhibited higher stability than PACAP27.

## [Measuring Sample Preparation 4]

[0181] A peptide solution prepared by the same method as [Measuring sample preparation 1] for peptide 22 synthesized in Example 2 was incubated for 1 week and 4 weeks in a thermostatic bath at 40° C. to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

## [HPLC Analysis Conditions]

[0182] Measurement was carried out under the same conditions as in [Measuring sample preparation 1], changing only the measuring time and mobile phase delivery conditions as shown below.

[0183] Measuring time: 20 minutes

[0184] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 9 for linear concentration gradient control.

TABLE 9

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	85→75	15→25
20.0-20.1	75→0	25→100
20.1-24.5	0	100
24.5-25.0	0→85	100→15
25.0-40.5	85	15

[0185] Table 10 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 10

Peptide name	40° C. 1 week	40° C. 4 week
Peptide 22	89.7%	79.7%
PACAP27	74.9%	33.0%

Peptides having substitution of the tetrazole-substituted aspartic acid with oxadiazolone-substituted aspartic acid at position 3 and position 8 exhibited higher stability than PACAP27.

[Measuring Sample Preparation 5]

[0186] Peptides 23 to 30 synthesized in Example 2 were weighed and dissolved in Tris buffer (pH 7.0) to prepare 1.0 mM peptide solutions. The solution was filtered with a Chromatdisk (product of Merck Millipore, Millex-GV, 0.22  $\mu$ m). The filtrate was diluted to 100  $\mu$ M with Tris buffer (pH 7.0) and dispensed into a tube (Protein LoBind Tube by Eppendorf Co.). The prepared peptide solutions were incubated for 4 weeks or 8 weeks in a 40° C. thermostatic bath, and for one week or 2 weeks in a thermostatic bath at 60° C., to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample was stored at -30° C. and the stored samples were stored at -30° C., until sample analysis.

[HPLC Analysis Conditions 1]

[0187] Measurement was carried out under the same conditions as in [Measuring sample preparation 1], changing only the measuring time and mobile phase delivery conditions as shown below.

[0188] Measuring time: 20 minutes

[0189] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 11 for linear concentration gradient control.

TABLE 11

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	85→75	15→25
20.0-20.1	75→0	25→100
20.1-24.5	0	100

TABLE 11-continued

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
24.5-25.0	0→85	100→15
25.0-40.5	85	15

The following gradient time was used only for peptide 30.

TABLE 12

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	82.5→72.5	17.5→27.5
20.0-20.1	72.5→	27.5→100
20.1-24.5	0	100
24.5-25.0	0→82.5	100→17.5
25.0-40.0	82.5	17.5

[0190] Table 13 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 13

Peptide name	60° C. 1 week	60° C. 2 week	40° C. 4 week	40° C. 8 week
Peptide 23	96.4%	91.3%	87.7%	89.1%
Peptide 24	99.5%	91.1%	90.5%	83.5%
Peptide 25	97.3%	93.1%	91.3%	85.1%
Peptide 26	97.9%	94.4%	93.2%	89.7%
Peptide 27	97.3%	95.5%	90.9%	84.3%
Peptide 28	98.6%	90.9%	94.1%	89.8%
Peptide 29	96.3%	91.9%	86.1%	77.4%
Peptide 30	96.7%	93.7%	91.5%	83.4%
PACAP27	26.4%	14.6%	37.9%	15.6%

#### Example 4: Stability Test 2

[0191] N-terminal acylated peptides were prepared for peptides selected from the group consisting of peptides 1 to 16. Peptides without N-terminal acylated modification were prepared for peptides selected from the group consisting of peptides 17 to 30. The N-terminal modified peptides and the N-terminal non-modified peptides were each dissolved in Tris buffer (pH 7.0) to prepare 1.0 mM peptide solutions. The solution was filtered with a Chromatdisk (product of Merck Millipore, Millex-GV, 0.22  $\mu$ m). The filtrate was diluted to 100  $\mu$ M with Tris buffer (pH 7.0) and dispensed into a tube (Protein LoBind Tube by Eppendorf Co.). The prepared peptide solutions were incubated for 1 week or 2 weeks in a 60° C. thermostatic bath to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

[0192] The stored sample was analyzed under appropriate HPLC analysis conditions, and the survival rate after correction for water for the peptide in the stored sample was calculated in the same manner as Example 3. The N-terminal-modified, and especially N-terminal-acylated, and further acetylated peptides can be confirmed to have improved stability.

#### Reference Example: Stability Test 3

[0193] The peptides prepared for Reference Examples 1 to 6 were weighed and dissolved in Tris buffer (pH 7.0) to

prepare 1.0 mM peptide solutions. The solution was filtered with a Chromatdisk (product of Merck Millipore, Millex-GV, 0.22  $\mu$ m). The filtrate was diluted to 100  $\mu$ M with Tris buffer (pH 7.0) and dispensed into a tube (Protein LoBind Tube by Eppendorf Co.). The prepared peptide solutions were incubated for 1 week or 2 weeks in a 60° C. thermostatic bath to obtain stored samples. A simultaneously prepared sample among the peptide solutions that was not stored was used as a standard sample (initial sample). The standard sample and the stored samples were stored at -30° C. until sample analysis.

[0194] Each stored sample was measured under the following HPLC analysis conditions, and the survival rate after correction for water for each peptide among the stored samples was calculated in the same manner as Example 3. The results are shown in Table 15.

[HPLC Analysis Conditions]

- [0195] Column: XSelect CSH C18 by Waters Co., 5  $\mu$ m, 4.6×250 mm
- [0196] Guard column: XSelect CSH C18 by Waters Co., 5  $\mu$ m, 4.6×20 mm Guard Cartridge
- [0197] Detection wavelength: 220 nm
- [0198] Mobile phase A: 0.1% formic acid aqueous solution
- [0199] Mobile phase B: 0.1% acetonitrile formate solution
- [0200] Measuring time: 20 minutes
- [0201] Measuring sample injection rate: 50  $\mu$ L
- [0202] Flow rate: 1.0 mL/min
- [0203] Column temperature: 40° C.
- [0204] Sample cooler: 25° C.
- [0205] Mobile phase delivery: The mixing ratio of mobile phase A and mobile phase B was varied as shown in Table 14 for linear concentration gradient control.

TABLE 14

Time after sample injection (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20.0	85→75	15→25
20.0-20.1	75→0	25→100
20.1-24.5	0	100
24.5-25.0	0→85	100→15
25.0-40.5	85	15

[0206] Table 15 shows the survival rate after correction for water for each peptide among the stored samples.

TABLE 15

Peptide	60° C. 1 week preservation	60° C. 2 weeks preservation
Reference Example 1	26.6%	15.8%
Reference Example 2	28.7%	17.2%
Reference Example 3	91.3%	82.4%
Reference Example 4	91.7%	84.8%
Reference Example 5	98.1%	92.9%
Reference Example 6	98.4%	92.8%

[0207] Reference Example 1 (PACAP) had very poor stability, at 26.6% after storage for 1 week at 60° C. and 15.8% after storage for 2 weeks, while Reference Example 2, which had the N-terminus of PACAP acetylated, exhibited

only equivalent stability. Reference Examples 3 and 4, on the other hand, which had the carboxyl groups of the aspartic acids at position 3 and position 8 replaced with tetrazole (Reference Example 3 also having the methionine at position 17 replaced by norleucine (indicated as “NI” in the sequence) and Reference Example 4 further having the methionine at position 17 replaced by leucine), had drastically increased stability, at 91.3% and 91.7% after storage for 1 week at 60° C., and 82.4% and 84.8% after storage for 2 weeks. In Reference Examples 5 and 6, wherein the N-termini of Reference Examples 3 and 4 were further acetylated, stability was further improved, with 98.1% and 98.4% after storage for 1 week at 60° C., and 92.9% and 92.8% after storage for 2 weeks. An accelerated test for 1 week and 2 weeks at 60° C. corresponds respectively to approximately 1 year and 2 years at room temperature (25° C.). Therefore, Reference Examples 5 and 6 are expected to be stable (survival rate of 90% or higher) for 2 years at room temperature.

Example 5: CAMP Assay of PACAP27 and its Modified Peptide

[Cell Culturing]

[0208] CHO-K1 cells (PAC1 or VPAC1 receptor high-expressing cell line: purchased from DiscoverX Co.) that had been mitomycin-treated, and frozen cells were prepared with Cell plating reagent (product of DiscoverX) at 1.35×10<sup>4</sup> cells/100  $\mu$ L/well, and then seeded in a 96-well culture plate. The cells were cultured for 18 to 24 hours at 37° C. in a 5% CO<sub>2</sub> incubator, adhering the cells to the plate.

[Reagent Preparation]

[0209] Peptides 1 to 30 synthesized in Example 2, and powder of Reference Example 1 (PACAP) were dissolved in water to 0.1 mM, and then diluted to 20  $\mu$ M with Cell assay buffer (DiscoverX) (containing 0.5 mM IBMX and 0.001% BSA). A 5-fold dilution series was then prepared with the same Cell assay buffer and used in an assay.

[cAMP Assay]

[0210] The cAMP assay was carried out using a Hit Hunter cAMP Assay for Biologics kit (product of DiscoverX, Cat. No. 90-0075LM25), according to the included kit instructions. The cAMP antibody solution and the solutions of peptides 1 to 30 diluted to different concentrations were combined to prepare peptide-cAMP antibody liquid mixtures. Next, the culture medium was removed from the CHO-K1 cell culture plate, washing was performed with PBS, and the peptide-cAMP antibody liquid mixtures were added to the cells and incubated for 30 minutes under a 5% CO<sub>2</sub> atmosphere at 37° C. A working detection solution was then added and the culture plate was shielded from light with aluminum foil and incubated at 25° C. for 1 hour. After incubation, Solution A was added and the culture plate was shielded from light with aluminum foil and then incubated at 25° C. for 3 hours. Finally, the chemiluminescence signal was detected using a GloMax detector (Promega) under conditions with luminescence and an integration time of 1 sec. The obtained value for the Relative Luminescence Unit (RLU) was analyzed with GraphPad Prism Ver 6.05 (product of Graph Pad) and the EC50 value was calculated for each peptide.

TABLE 16

Peptide name	N-terminus	C-terminus	PAC1	VPAC1
Reference Example 1 (PACAP27)	H	NH2	0.052 nM	0.097 nM
Peptide 1	H	NH2	1.220 nM	0.097 nM
Peptide 2	H	NH2	0.610 nM	0.085 nM
Peptide 3	H	NH2	0.689 nM	0.111 nM
Peptide 4	H	NH2	2.360 nM	0.121 nM
Peptide 5	H	NH2	0.695 nM	0.078 nM
Peptide 6	H	NH2	1.170 nM	0.110 nM
Peptide 7	H	NH2	0.319 nM	0.096 nM
Peptide 8	H	NH2	0.679 nM	0.045 nM
Peptide 9	H	NH2	0.287 nM	0.079 nM
Peptide 10	H	NH2	0.447 nM	0.057 nM
Peptide 11	H	NH2	0.911 nM	0.080 nM
Peptide 12	M	NH2	4.280 nM	0.651 nM
Peptide 13	H	NH2	0.738 nM	0.082 nM
Peptide 14	H	NH2	0.857 nM	0.107 nM
Peptide 15	H	NH2	0.079 nM	0.243 nM
Peptide 16	H	NH2	1.620 nM	0.142 nM
Peptide 17	CH <sub>3</sub> CH <sub>2</sub> CO	NH2	0.398 nM	0.071 nM
Peptide 18	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CO	NH2	0.189 nM	0.069 nM
Peptide 19	(CH <sub>3</sub> ) <sub>2</sub> CHCO	NH2	0.256 nM	0.069 nM
Peptide 20	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CO	NH2	0.574 nM	0.131 nM
Peptide 21	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CO	NH2	1.130 nM	0.141 nM
Peptide 22	Ac(CH <sub>3</sub> CO)	NH2	0.376 nM	0.077 nM
Peptide 23	Ac	NH2	1.150 nM	0.047 nM
Peptide 24	Ac	NH2	1.260 nM	0.042 nM
Peptide 25	Ac	NH2	0.693 nM	0.041 nM
Peptide 26	Ac	NH2	0.209 nM	0.068 nM
Peptide 27	Ac	NH2	2.530 nM	0.111 nM
Peptide 28	Ac	NH2	2.120 nM	0.072 nM
Peptide 29	Ac	NH2	3.280 nM	0.123 nM
Peptide 30	Ac	NH2	2.290 nM	0.113 nM

[0211] To summarize the results described above, the peptides of the invention (peptides 1 to 30) have extremely improved stability in aqueous solution compared to PACAP, while also maintaining physiological activity equivalent to that of PACAP. In particular, because they have storage life exceeding 2 years at room temperature, they allow liquid formulations to be developed as products such as vials, ampules and eye drops.

#### Formulation Examples

[0212] A medicine containing a peptide of the invention as an active ingredient can be produced by the following formulation. The medicine of the present invention will now be further illustrated by formulation examples, with the understanding that the invention is not limited to the formulation examples.

#### 1. Capsules

(1) Peptide 5	40 mg
(2) Lactose	70 mg
(3) Microcrystalline cellulose	9 mg
(4) Magnesium stearate	1 mg
1 capsule:	120 mg

[0213] After mixing the total amounts of (1), (2) and (3) and ½ of (4), the mixture is granulated. The remaining amount of (4) is added and the entire mixture is encapsulated into gelatin capsules.

#### 2. Tablets

(1) Peptide 6	40 mg
(2) Lactose	58 mg
(3) Corn starch	18 mg
(4) Microcrystalline cellulose	3.5 mg
(5) Magnesium stearate	0.5 mg
1 tablet	120 mg

[0214] After mixing the total amounts of (1), (2) and (3), ⅔ of (4) and ½ of (5), the mixture is granulated. The remaining amounts of (4) and (5) are added to the granules, which are then pressure molded into tablets.

#### 3. Vitreous injection

In 1 ml:	
(1) Peptide 5	40 mg
(2) Refined sucrose	50 mg
(3) Sodium chloride	2.34 mg
(4) Polysorbate 80	q.s.
(5) Disodium hydrogenphosphate	q.s.
(6) Sodium dihydrogenphosphate	q.s.
(7) Sterilized purified water	q.s.

[0215] Components (1) to (6) are dissolved in the sterilized purified water (7) to prepare a vitreous injection.

#### 4. Ophthalmic drug

In 100 mL:	
(1) Peptide 6	100 mg
(2) Trometamol	300 mg
(3) Sodium chloride	900 mg
(4) Benzalkonium chloride	q.s.
(5) Sterilized purified water	q.s.

Components (1) to (4) were dissolved in the sterilized purified water (5) and the pH was adjusted to prepare eye drops.

#### SEQUENCE LISTING

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<212> TYPE: PRT

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<400> SEQUENCE: 1

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1           5           10           15
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Gln Arg Val Lys Asn Lys
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 2

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His Ser Asp Gly Ile Phe Thr Asp Ser Tyr Ser Arg Tyr Arg Lys Gln
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu
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<223> OTHER INFORMATION: Xaa is Gln or Ala
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<223> OTHER INFORMATION: Xaa is non-polar amino acid

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His Xaa Asp Gly Xaa Phe Xaa Asp Xaa Tyr Xaa Arg Tyr Arg Lys Xaa
1           5           10           15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu
          20          25

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<223> OTHER INFORMATION: AMIDATION

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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: AMIDATION

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1 5 10 15

Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 6

His Ser Xaa Gly Ile Phe Ala Xaa Ala Tyr Ala Arg Tyr Arg Lys Gln  
1 5 10 15

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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: AMIDATION  
  
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His Ser Xaa Gly Ile Phe Ala Xaa Xaa Tyr Ser Arg Tyr Arg Lys Gln  
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: AMIDATION  
  
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His Ser Xaa Gly Ile Phe Ala Xaa Ser Tyr Xaa Arg Tyr Arg Lys Gln  
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

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<223> OTHER INFORMATION: AMIDATION

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His Ser Xaa Gly Ile Phe Ala Xaa Xaa Tyr Xaa Arg Tyr Arg Lys Gln  
1 5 10 15

Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 10  
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<223> OTHER INFORMATION: Xaa is Aib  
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<223> OTHER INFORMATION: Xaa is Aib  
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<223> OTHER INFORMATION: AMIDATION

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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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Met Ala Val Lys Lys Tyr Leu Ala  
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val  
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<223> OTHER INFORMATION: AMIDATION

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Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: AMIDATION

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<223> OTHER INFORMATION: AMIDATION

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<210> SEQ ID NO 16

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<223> OTHER INFORMATION: AMIDATION  
  
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His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
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<223> OTHER INFORMATION: Xaa is D- amino acid wherein carboxy group of  
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<223> OTHER INFORMATION: AMIDATION  
  
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His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1                   5                   10                   15  
  
Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
                 20                   25

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<223> OTHER INFORMATION: Xaa is D-amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
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Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
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<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of glutamic acid is substituted with tetrazole  
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<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 19

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 20  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: PROPYONYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN



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<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 20

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 21  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: BUTYRYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 21

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 22  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ISOBUTYRYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN

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<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 22

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 23  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: PENTANOYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 23

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 24  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ISOPENTANOYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN

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<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 24

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 25  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with oxadiazolone  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with oxadiazolone  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 25

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 26  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN

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<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 26

His Ser Xaa Gly Ala Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 27  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 27

His Ser Xaa Gly Ile Phe Ala Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 28  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

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<400> SEQUENCE: 28

His Ala Xaa Gly Ile Phe Ala Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15  
Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 29

<211> LENGTH: 27

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: modified PACAP peptide

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: ACETYLTATION

<220> FEATURE:

<221> NAME/KEY: MUTAGEN

<222> LOCATION: (3)..(3)

<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole

<220> FEATURE:

<221> NAME/KEY: MUTAGEN

<222> LOCATION: (8)..(8)

<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (27)..(27)

<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 29

His Ala Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Ala  
1 5 10 15  
Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 30

<211> LENGTH: 27

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: modified PACAP peptide

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: ACETYLTATION

<220> FEATURE:

<221> NAME/KEY: MUTAGEN

<222> LOCATION: (3)..(3)

<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole

<220> FEATURE:

<221> NAME/KEY: MUTAGEN

<222> LOCATION: (8)..(8)

<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (27)..(27)

<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 30

His Ser Xaa Gly Ile Phe Ala Xaa Ala Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15  
Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

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<210> SEQ ID NO 31  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 31

His Ser Xaa Gly Ile Phe Ala Xaa Ser Tyr Ser Arg Tyr Arg Lys Ala  
1 5 10 15

Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 32  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 32

His Ser Xaa Gly Ile Phe Ala Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Ala Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 33  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:

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<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 33

His Ala Xaa Gly Ile Phe Ala Xaa Ala Tyr Ser Arg Tyr Arg Lys Ala  
1 5 10 15

Ala Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 34  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (11)..(11)  
<223> OTHER INFORMATION: Xaa is b-cyano alanine  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 34

His Ser Xaa Gly Ile Phe Ala Xaa Ser Tyr Xaa Arg Tyr Arg Lys Gln  
1 5 10 15

Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 35  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of

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asparatic acid is substituted with tetrazole

<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (9)..(9)  
<223> OTHER INFORMATION: Xaa is b-cyano alanine  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (11)..(11)  
<223> OTHER INFORMATION: Xaa is b-cyano alanine  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 35

His Ser Xaa Gly Ile Phe Thr Xaa Xaa Tyr Xaa Arg Tyr Arg Lys Gln  
1 5 10 15

Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 36  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
glutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 36

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 37  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
glutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
glutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)



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<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 37

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 38  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
homoglutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 38

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 39  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
homoglutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 39

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His	Ser	Xaa	Gly	Ile	Phe	Thr	Xaa	Ser	Tyr	Ser	Arg	Tyr	Arg	Lys	Gln
1				5					10					15	

Xaa	Ala	Val	Lys	Lys	Tyr	Leu	Ala	Ala	Val	Leu
			20				25			

<210> SEQ ID NO 40  
 <211> LENGTH: 27  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: modified PACAP peptide  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (3)..(3)  
 <223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
 homoglutamic acid is substituted with tetrazole  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (8)..(8)  
 <223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
 homoglutamic acid is substituted with tetrazole  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (17)..(17)  
 <223> OTHER INFORMATION: Xaa is Nle  
 <220> FEATURE:  
 <221> NAME/KEY: MOD\_RES  
 <222> LOCATION: (27)..(27)  
 <223> OTHER INFORMATION: AMIDATION  
 <400> SEQUENCE: 40

His	Ser	Xaa	Gly	Ile	Phe	Thr	Xaa	Ser	Tyr	Ser	Arg	Tyr	Arg	Lys	Gln
1				5					10					15	

Xaa	Ala	Val	Lys	Lys	Tyr	Leu	Ala	Ala	Val	Leu
			20				25			

<210> SEQ ID NO 41  
 <211> LENGTH: 27  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: modified PACAP peptide  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (3)..(3)  
 <223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
 glutamic acid is substituted with tetrazole  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (8)..(8)  
 <223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
 homoglutamic acid is substituted with tetrazole  
 <220> FEATURE:  
 <221> NAME/KEY: MUTAGEN  
 <222> LOCATION: (17)..(17)  
 <223> OTHER INFORMATION: Xaa is Nle  
 <220> FEATURE:  
 <221> NAME/KEY: MOD\_RES  
 <222> LOCATION: (27)..(27)  
 <223> OTHER INFORMATION: AMIDATION  
 <400> SEQUENCE: 41

His	Ser	Xaa	Gly	Ile	Phe	Thr	Xaa	Ser	Tyr	Ser	Arg	Tyr	Arg	Lys	Gln
1				5					10					15	

Xaa	Ala	Val	Lys	Lys	Tyr	Leu	Ala	Ala	Val	Leu
			20				25			

<210> SEQ ID NO 42

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<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
homoglutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
glutamic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (27)..(27)  
<223> OTHER INFORMATION: AMIDATION

<400> SEQUENCE: 42

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15  
Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 43  
<211> LENGTH: 11  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Gly Lys Arg Tyr Lys Gln Arg Val Lys Asn Lys  
1 5 10

<210> SEQ ID NO 44  
<211> LENGTH: 10  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Gly Lys Arg Tyr Lys Gln Arg Val Lys Asn  
1 5 10

<210> SEQ ID NO 45  
<211> LENGTH: 9  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Gly Lys Arg Tyr Lys Gln Arg Val Lys  
1 5

<210> SEQ ID NO 46  
<211> LENGTH: 8  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Gly Lys Arg Tyr Lys Gln Arg Val  
1 5

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<210> SEQ ID NO 47  
<211> LENGTH: 7  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Gly Lys Arg Tyr Lys Gln Arg  
1 5

<210> SEQ ID NO 48  
<211> LENGTH: 6  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Gly Lys Arg Tyr Lys Gln  
1 5

<210> SEQ ID NO 49  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Gly Lys Arg Tyr Lys  
1 5

<210> SEQ ID NO 50  
<211> LENGTH: 4  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Gly Lys Arg Tyr  
1

<210> SEQ ID NO 51  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLTATION

<400> SEQUENCE: 51

His Ser Asp Gly Ile Phe Thr Asp Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 52  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole

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<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 52

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 53  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole

<400> SEQUENCE: 53

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu  
20 25

<210> SEQ ID NO 54  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: modified PACAP peptide  
<220> FEATURE:  
<221> NAME/KEY: MOD\_RES  
<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: ACETYLATION  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: Xaa is amino acid wherein carboxy group of  
asparatic acid is substituted with tetrazole  
<220> FEATURE:  
<221> NAME/KEY: MUTAGEN  
<222> LOCATION: (17)..(17)  
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 54

His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln  
1 5 10 15

Xaa Ala Val Lys Lys Tyr Leu Ala Ala Val Leu

-continued

20	25
<p>&lt;210&gt; SEQ ID NO 55 &lt;211&gt; LENGTH: 27 &lt;212&gt; TYPE: PRT &lt;213&gt; ORGANISM: Artificial Sequence &lt;220&gt; FEATURE: &lt;223&gt; OTHER INFORMATION: modified PACAP peptide &lt;220&gt; FEATURE: &lt;221&gt; NAME/KEY: MOD_RES &lt;222&gt; LOCATION: (1) .. (1) &lt;223&gt; OTHER INFORMATION: ACETYLTATION &lt;220&gt; FEATURE: &lt;221&gt; NAME/KEY: MUTAGEN &lt;222&gt; LOCATION: (3) .. (3) &lt;223&gt; OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole &lt;220&gt; FEATURE: &lt;221&gt; NAME/KEY: MUTAGEN &lt;222&gt; LOCATION: (8) .. (8) &lt;223&gt; OTHER INFORMATION: Xaa is amino acid wherein carboxy group of asparatic acid is substituted with tetrazole</p>	
<p>&lt;400&gt; SEQUENCE: 55</p>	
His Ser Xaa Gly Ile Phe Thr Xaa Ser Tyr Ser Arg Tyr Arg Lys Gln	
1 5 10 15	
Leu Ala Val Lys Lys Tyr Leu Ala Ala Val Leu	
20 25	

1. A peptide consisting of the sequence represented by H-X<sub>1</sub>-D-G-X<sub>2</sub>-F-X<sub>3</sub>-D-X<sub>4</sub>-Y-X<sub>5</sub>-R-Y-R-K-X<sub>6</sub>-X<sub>7</sub>-A-V-K-K-Y-L-A-A-V-L (SEQ ID NO: 3) wherein

X<sub>1</sub> is a neutral amino acid,  
X<sub>2</sub> is a neutral amino acid,  
X<sub>3</sub> is a neutral amino acid,  
X<sub>4</sub> is alanine, serine, Ai or Cn,  
X<sub>5</sub> is alanine, serine, Ai or Cn,  
X<sub>6</sub> is glutamine or alanine and  
X<sub>7</sub> is non-polar amino acid,

with the aspartic acids at position 3 and position 8 each independently replaced by a residue selected from the group consisting of tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz), tetrazole-substituted homoglutamic acid (nvTz), sulfoxy-substituted aspartic acid (cya) and oxadiazolone-substituted aspartic acid (5Oxa), or its modified sequence, wherein the peptide has affinity for PAC1R and VPAC1R, and the modified sequence is the sequence listed as SEQ ID NO: 3 having a deletion or addition of one or more amino acids.

2. The peptide according to claim 1, wherein X<sub>1</sub> is serine or alanine.

3. The peptide according to claim 1, wherein X<sub>2</sub> is isoleucine or alanine.

4. The peptide according to claim 1, wherein X<sub>3</sub> is alanine or threonine.

5. The peptide according to claim 1, wherein X<sub>7</sub> is methionine, leucine, norleucine or alanine.

6. The peptide according to claim 1, wherein in cases where the amino acids at position 3 and position 8 are tetrazole-substituted aspartic acid (Tz), tetrazole-substituted glutamic acid (egTz) or tetrazole-substituted homoglutamic acid (nvTz), the amino acids are the L-forms, D-forms or racemic forms.

- 7. The peptide according to claim 1, wherein the N-terminus of the peptide is modified with a protecting group.
- 8. The peptide according to claim 7, wherein the protecting group is an acyl group.
- 9. The peptide according to claim 1, wherein the C-terminus of the peptide is modified with a protecting group.
- 10. The peptide according to claim 9, wherein the protecting group is an amide group or ester group.
- 11. The peptide according to claim 1, wherein the modified sequence is the sequence with 1 to 3 amino acids deleted from the C-terminus of the sequence.
- 12. The peptide according to claim 1, wherein the modified sequence is the sequence with 1 to 3 amino acids added to the N-terminus of the sequence.
- 13. The peptide according to claim 1, wherein the modified sequence is the sequence with one sequence selected from the group consisting of the following:

GKRYKQRVKKN;	(SEQ ID NO: 43)
GKRYKQRVKN;	(SEQ ID NO: 44)
GKRYKQRVK;	(SEQ ID NO: 45)
GKRYKQRV;	(SEQ ID NO: 46)
GKRYKQR;	(SEQ ID NO: 47)
GKRYKQ;	(SEQ ID NO: 48)

-continued

(SEQ ID NO: 49)

GKRYK;

(SEQ ID NO: 50)

GKRY

GKR;

GRR;

GK;

and

GR

G;

added to the C-terminus of the peptide.

14. A method of neuroprotection in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

15. A method of promoting lacrimal secretion in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

16. A method for inhibiting inflammation in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

17. A method of ameliorating vascular endothelial function in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

18. A method for ameliorating corneal epithelial or corneal endothelial damage in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

19. A method for treating or preventing dry-eye in a subject, comprising administering to a subject in need thereof a therapeutically-effective dose of a peptide according to claim 1.

20. A pharmaceutical composition containing the peptide according to claim 1.

\* \* \* \* \*