

(12) PATENT APPLICATION
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 200177371 A1

(54) Title
**Peptides promoting the activation of latent TGF-b and method for screening
TGF-b activity regulators**

(51)⁷ International Patent Classification(s)
C12Q 001/00 C07K 014/495
A61K 038/18

(21) Application No: **200177371**

(22) Application Date: **2001.10.03**

(43) Publication Date : **2001.11.29**

(43) Publication Journal Date : **2001.11.29**

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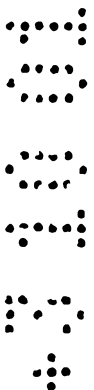
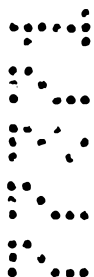
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Peptides Promoting the Activation of Latent TGF- β and Method for Screening TGF- β Activity Regulators

Abstract

The present invention provides a method of screening a compound to be used for
5 treatment or prevention of TGF- β -related diseases, which comprises: measuring the
amount of latent TGF- β bound to animal cells after addition of latent TGF- β to said cells;
measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF-
 β and a compound to be evaluated to said cells; and evaluating the inhibiting activity or
promoting activity of said compound on the binding of latent TGF- β to animal cells from
10 the change in the amount of latent TGF- β bound to animal cells caused by the addition of
said compound.



AUSTRALIA

PATENTS ACT 1990

COMPLETE SPECIFICATION

FOR A STANDARD PATENT

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Invention Title:

Peptides Promoting the Activation of Latent TGF- β and
Method for Screening TGF- β Activity Regulators

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

SPECIFICATION

PEPTIDES PROMOTING THE ACTIVATION OF LATENT TGF- β AND METHOD FOR
SCREENING TGF- β ACTIVITY REGULATORS5 Technical Field

The present invention relates to novel peptides which promote conversion of latent TGF- β (TGF- β type ordinarily secreted) into active transforming growth factor- β (hereinafter occasionally abbreviated as active TGF- β or merely as TGF- β)
 10 having a variety of physiological activities such as inhibition of cell growth, promotion of cell differentiation, immunosuppression, and stimulation of chemotaxis of fibroblasts and which are useful as therapeutic agents for diseases pointed out to be related to the lack of TGF- β activity and diseases
 15 against which administration of exogenous TGF- β is considered to be effective, such as cancer, bone fracture, myocardial infarction, myocardial disorder after ischemia reperfusion, cerebral infarction and retinal detachment.

The present invention also relates to methods of screening
 20 compounds which regulate the binding of latent transforming growth factor- β (hereinafter occasionally abbreviated as LTGF- β) to cells or compounds which regulate the release of active TGF- β from latent TGF- β , and to compounds obtainable by the above methods which are useful for the treatment or prevention
 25 of TGF- β -related diseases.

Background Art

In mammals including humans exist some types of TGF- β such as TGF- β 1, β 2 and β 3, and all of them are secreted as inactive
 30 LTGF- β [Robert, A.B. & Sporn, M.B., Peptide Growth Factors and Their Receptors, Handbook of Experimental Pharmacology, Part 1, SPRINGER-VERLAG, Berlin, p. 419-472 (1990)] and need to be activated after the secretion to exhibit their activities. LTGF- β is divided into two types: small molecular weight latent TGF- β
 35 β (hereinafter abbreviated as SLTGF- β) wherein a latency associated peptide (hereinafter occasionally abbreviated as

LAP) is non-covalently bound to TGF- β and large molecular weight latent TGF- β (hereinafter occasionally abbreviated as LLTGF- β) wherein latent TGF- β binding protein (hereinafter occasionally abbreviated as LTBP) is bound to SLTGF- β by SS bond with LAP. LTGF- β is secreted mostly in the form of LLTGF- β [EMBO Journal, 10, 1091 (1991)]. TGF- β and LAP are biosynthesized as the same protein molecule (TGF- β precursor) having a signal peptide and the amino acid sequence thereof is known [Nature, 316, 701 (1985)].

Some protease enzymes have been pointed out to participate in the activation of latent TGF- β , and plasmin has been analyzed most closely among these enzymes. That is, non-covalently bound TGF- β is released by the limited degradation of LAP by plasmin [Journal of Cell Biology, 110, 1361 (1990)]. The analysis of the activation of latent TGF- β by plasmin at the cell level has revealed the following: the activation by plasmin is carried out on the surface of the cell membrane [Journal of Cell Biology, 109, 309 (1989)], binding of latent TGF- β to the cell membrane is necessary for the activation [Journal of Cell Biology, 121, 439 (1993), *ibid.*, 120, 995 (1993), *ibid.*, 123, 1249 (1993)], and latent TGF- β is bound to the cell membrane via LAP [Journal of Cell Biology, 123, 1249 (1993), Tohoku Journal of Experimental Medicine, 179, 23 (1996)]. However, it is not clear how the regulation of the binding of latent TGF- β to a cell membrane is associated with the regulation of TGF- β activity.

It is recognized that latent TGF- β is bound to vascular smooth muscle cells, but not to vascular endothelial cells [Journal of Cell Biology, 123, 1249 (1993)].

TGF- β has a variety of physiological activities such as inhibition of cell growth, promotion of cell differentiation, immunosuppression, and stimulation of chemotaxis of fibroblasts. TGF- β is considered to be associated with various diseases. For example, it has been reported that lack of TGF- β activity is related to diabetic retinopathy [Journal of Cell Biology, 109, 309 (1989), Archives of Ophthalmology, 66, 366 (1961)] and initial lesion of atherosclerosis [Nature Medicine,

1, 1067 (1995)]. TGF- β itself is expected to have a therapeutic effect on bone fracture, myocardial infarction, myocardial disorder after ischemia reperfusion, cerebral infarction and retinal detachment [Journal of Cell Biology, 119, 1017 (1992)].

5 Further, TGF- β is known to inhibit the growth of various cancer cells [Endocrinology, 128, 1981 (1991), Journal of Clinical Investigation, 87, 277 (1991), Cell Growth & Differentiation, 1, 549 (1990)] and is expected as an anti-tumor agent [Proceedings of the National Academy of Science U.S.A., 92, 4254

10 (1995)].

Only a part of latent TGF- β produced and secreted in vivo is activated and exhibits its activity, and accordingly, it is considered that the activity of TGF- β can be enhanced by increasing the activation efficiency of latent TGF- β in vivo.

15 Therefore, a compound which promotes the activation of latent TGF- β is expected to be effective as a therapeutic agent for diseases pointed out to be related to the lack of TGF- β activity and diseases against which administration of exogenous TGF- β is considered to be effective, for example, cancer, diabetic

20 retinopathy, atherosclerosis, bone fracture, myocardial infarction, myocardial disorder after ischemia reperfusion, cerebral infarction and retinal detachment.

On the other hand, there have been known various diseases basically accompanied by development of extracellular matrix which are caused by advance of TGF- β activation. A substance

25 which inhibits the TGF- β activation is expected to be effective as a therapeutic agent for diseases such as glomerulonephritis, diabetic nephropathy, renal graft rejection, HIV nephropathy, sudden pulmonary fibrosis, autoimmune pulmonary fibrosis,

30 hepatic cirrhosis, venous constrictive hepatopathy (often occurring after treatments of cancer), systemic sclerosis, keloid, eosinophilia-muscle ache syndrome, re-stricture after angioplasty, intraocular fibrosis, rheumatic arthritis and fibrosis such as nasal polyp [Border W.A. & Noble N.A.,

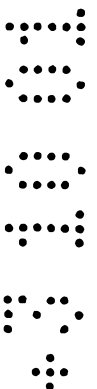
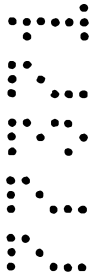
35 Transforming growth factor- β in tissue fibrosis, New Engl. J. Med., 331, 1286 (1994) and Border W.A. & Rouslahti E.,

Transforming growth factor- β in disease: The dark side of tissue repair, J. Clin. Invest., 90, 1, (1992)].

Disclosure of the Invention

According to this invention there is provided a method of screening a compound to
 5 be used for treatment or prevention of TGF- β -related diseases, which comprises:
 measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β
 to said cells; measuring the amount of latent TGF- β bound to animal cells after addition
 of latent TGF- β and a compound to be evaluated to said cells; and evaluating the
 inhibiting activity or promoting activity of said compound on the binding of latent TGF- β
 10 to animal cells from the change in the amount of latent TGF- β bound to animal cells
 caused by the addition of said compound.

In another embodiment, the present invention provides a peptide having an activity
 to promote the release of active TGF- β from latent TGF- β or an activity to promote the
 binding of latent TGF- β or an activity to promote the binding of latent TGF- β to a cell
 15 membrane which is represented by general



formula (I), wherein A is an amino acid sequence selected from partial sequences of an amino acid sequence selected from the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence and the sequences of TGF- β precursors other than human TGF- β 1 corresponding to the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence when aligned with the human TGF- β 1 sequence, and 1 to 5 amino acid residues in said partial sequence may be deleted, substituted or added, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a peptide having an activity to promote the release of active TGF- β from latent TGF- β or an activity to promote the binding of latent TGF- β to a cell membrane which is represented by general formula (I), wherein A is any one of the amino acid sequences of SEQ ID NOS: 1-16 in which 1 to 5 amino acid residues may be deleted, substituted or added, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a method of screening a compound to be used for the treatment or prevention of TGF- β -related diseases which comprises measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β to said cells, measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β and a compound to be evaluated to said cells, and evaluating the inhibiting activity or promoting activity of said compound on the binding of latent TGF- β to animal cells from the change in the amount of latent TGF- β bound to animal cells caused by the addition of said compound.

In another embodiment, the present invention provides a method of screening a compound to be used for the treatment or prevention of TGF- β -related diseases which comprises measuring the amount of TGF- β after addition of a peptide represented by general formula (I) or a pharmaceutically acceptable salt thereof to animal cells, measuring the amount of TGF- β after addition of a compound to be evaluated and a peptide represented

by general formula (I) or a pharmaceutically acceptable salt thereof to animal cells, and evaluating the inhibiting activity or promoting activity of said compound on the conversion of latent TGF- β into TGF- β from the change in the amount of TGF- β caused by the addition of said compound.

5 In another embodiment of the present invention, a compound having inhibiting activity or promoting activity on the binding of latent TGF- β to cells or on the conversion of latent TGF- β into TGF- β is obtainable according to either of the above two methods, and a compound to be used for the treatment or prevention of TGF- β -related diseases or a pharmaceutically acceptable salt thereof is provided.

10 The peptides represented by general formula (I) are hereinafter referred to as Compounds (I).

In the definitions of the groups in general formula (I), the alkanoyl includes alkanoyl groups having 1 to 20 carbon atoms, such as formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, heptanoyl, lauroyl and icosanoyl.

15 Examples of the aryl moiety of the aroyl and the aryloxycarbonyl are phenyl and naphthyl.

Examples of the heteroaryl moiety of the heteroarylcarbonyl and the heteroaryloxycarbonyl are furyl, thienyl, pyridyl, pyrrolyl, pyrazolyl, pyrimidinyl, pyrazinyl, indolyl, quinolyl, isoquinolyl and quinazolinyl.

20 The alkyl moiety of the alkoxycarbonyl and the alkoxy includes alkyl groups having 1 to 20 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, heptyl, decyl, dodecyl and icosyl.

The substituted alkanoyl, the substituted alkoxycarbonyl and the substituted alkoxy each has 1 to 3 substituents which are the same or different. Examples of the substituents
25 are hydroxy, carboxyl, alicyclic alkyl groups having 3 to 8 carbon atoms (e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl), substituted or unsubstituted phenyl, and fluorenyl. The substituted phenyl has 1 to 3

substituents which are the same or different. Examples of the substituents are alkyl, alkoxy, hydroxy, nitro, sulfo, cyano and halogen. The halogen includes fluorine, chlorine, bromine and iodine. The alkyl moiety of the alkyl and the alkoxy as the substituents of the substituted phenyl has the same significance as the above-mentioned alkyl moiety of the alkoxycarbonyl and the alkoxy.

The substituted aroyl, the substituted aryloxycarbonyl, the substituted heteroarylcarbonyl and the substituted heteroaryloxycarbonyl each has 1 to 3 substituents which are the same or different. The substituents are the same as the substituents of the above substituted phenyl.

The substituted amino has 1 to 2 substituents which are the same or different, and examples of the substituents are substituted or unsubstituted alkyl and substituted or unsubstituted aryl. The alkyl has the same significance as the above-mentioned alkyl moiety of the alkoxy, etc., including the substituents thereof. The aryl group has the same significance as the above-mentioned aryl moiety of the aroyl and the aryloxycarbonyl, including the substituents thereof.

As the TGF- β precursor sequence, any kind of TGF- β sequence derived from any animal may be employed. Suitable examples are human TGF- β 1 (J05114) [Nature, 316, 701 (1985)] (SEQ ID NO: 17), human TGF- β 2 (Y00083) [EMBO. J., 6, 3673 (1987)] (SEQ ID NO: 18), human TGF- β 3 (J03241) [Proc. Natl. Acad. Sci., USA, 85, 4715 (1988)] (SEQ ID NO: 19), murine TGF- β 1 (M13177) [J. Biol. Chem., 261, 4377 (1986)] (SEQ ID NO: 20), murine TGF- β 2 (X57413) [Mol. Endocrinol., 3, 1108 (1989)] (SEQ ID NO: 21), murine TGF- β 3 (M32745) [Mol. Endocrinol., 3, 1926 (1989)] (SEQ ID NO: 22), rat TGF- β 1 (X52498) [Nucleic Acids Res., 18, 3059 (1990)] (SEQ ID NO: 23), rat TGF- β 3 (U03491) [J. Biol. Chem., 270, 2722 (1995)] (SEQ ID NO: 24), bovine TGF- β 1 (M36271) [Mol. Endocrinol., 1, 693 (1987)] (SEQ ID NO: 25), porcine TGF- β 1 (Y00111) [Nucleic Acids Res., 15, 3187 (1987)] (SEQ ID NO: 26), porcine TGF- β 3 (X14150) [EMBO J., 7, 3737 (1988)] (SEQ ID NO: 27), canine TGF- β 1 (L34956) [Gene, 155, 307 (1995)] (SEQ ID NO: 28).

28), ovine TGF- β 1 (X76916) [Gene, **150**, 371 (1994)] (SEQ ID NO:29), chicken TGF- β 2 (X58071) [Mol. Endocrinol., **7**, 175 (1991)] (SEQ ID NO:30), chicken TGF- β 3 (M31154) [Mol. Endocrinol., **2**, 747 (1988)] (SEQ ID NO:31), chicken TGF- β 4 (M31160) [Mol. Endocrinol., **6**, 989 (1992)] (SEQ ID NO: 32), Simian (African green monkey) TGF- β (M16658) [DNA, **6**, 239 (1987)] (SEQ ID NO:33) and frog (*Xenopus laevis*) TGF- β 5 (J05180) [J. Biol. Chem., **265**, 1089 (1990)] (SEQ ID NO:34). The numbers in parentheses following the names of TGF- β precursor sequences indicate the accession numbers of GenBank.

There is no restriction in employing A as long as A is an amino acid sequence which is selected from partial sequences of a TGF- β precursor sequence in which 1 to 5 amino acid residues may be deleted, substituted or added. It is preferable that A is an amino acid sequence which is a partial sequence of a sequence selected from the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence and the sequences of TGF- β precursors other than human TGF- β 1 corresponding to the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence when aligned with the human TGF- β 1 sequence, and in which 1 to 5 amino acid residues may be deleted, substituted or added.

It is particularly preferably that A is a partial sequence of a sequence selected from the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence and the sequences of TGF- β precursors other than human TGF- β 1 corresponding to the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence when aligned with the human TGF- β 1 sequence.

The above animal-derived TGF- β precursors which were aligned with the human TGF- β 1 precursor sequence are shown in Tables 1-1 to 1-8. The figures before and after each sequence indicate the position numbers of amino acids, and "-" in the amino acid sequences indicates gap positions.

Table 1-1

Origin	Sequence
Human TGF- β 1	1:MPPSGRLRLPLLLPLLWLLVLTGPPAAGLSTCKTIDMELVKKRIEAI RGQILSKLRLA 60
Human TGF- β 2	1:-----M-HYCVLSAFLILHLVTVALSLSTCSTLDMQFMKKRIEAI RGQILSKLKL T 51
Human TGF- β 3	1:-----MK-MHLQRALVVLALLNFATVSLSLSTCTTLD FGHIKKKRVEAI RGQILSKLR L T 54
Murine TGF- β 1	1:MPPSGRLRLPLLLPLPWLVLTPGRPAAGLSTCKTIDMELVKKRIEAI RGQILSKLRLA 60
Murine TGF- β 2	1:-----M-HYCVLSTFLLHLVPVALSLSTCSTLDMQFMKKRIEAI RGQILSKLKL T 51
Murine TGF- β 3	1:-----MHLQRALVVLALLNLATISLSLSTCTTLD FGHIKKKRVEAI RGQILSKLR L T 52
Rat TGF- β 1	1:MPPSGRLRLPLLLPLPWLVLTPGRPAAGLSTCKTIDMELVKKRIEAI RGQILSKLRLA 60
Rat TGF- β 3	1:-----MK-MHLQRALVVLALLNLATVSLSLSTCTTLD FGHIKKKRVEAI RGQILSKLR L T 54
Bovine TGF- β 1	1:-----
Porcine TGF- β 1	1:MPPSGRLRLPLLLPLLWLLVLTGPRPAAGLSTCKTIDMELVKKRIEAI RGQILSKLRLA 60
Porcine TGF- β 3	1:-----MHLQRALVVLALLNFATVSLSMSTCTTLD FGHIKKKRVEAI RGQILSKLR L T 52
Canine TGF- β 1	1:MPPSGRLRLPLLLPLLRLVLTPGRPAAGLSTCKTIDMELVKKRIEAI RGQILSKLR L S 60
Ovine TGF- β 1	1:MPPSGRLRLPLLLPLLWLLMLTPGRPVAGLSTCKTIDMELVKKRGIEAI RGQILSKLRLA 60
Chicken TGF- β 2	1:-----M-HCYLLSVFLTDLAAVALSLSTCSTLDMQFMKKRIEAI RGQILSKLKL T 51
Chicken TGF- β 3	1:-----MK-MYAQRALVLLSLSFATVSLALSSCTTLDLEHIKKKRVEAI RGQILSKLR L T 54
Chicken TGF- β 4	1:-----ALSTCQRDL EAAKKKRIEAVRGQILSKLR L T 32
Simian TGF- β	1:MPPSGRLRLPLLLPLLWLLVLTPSRPAAGLSTCKTIDMELVKKRIETI RGQILSKLRLA 60
Frog TGF- β 5	1:-----MEVLWMLLVLLVLHLSSLAMSLSTCKAVDMEEVRKKRIEAI RGQILSKL K L D 52

Table 1-2

Origin	Sequence
Human GF- β 1	61:SPPSQGEVPPGPLPEAVLALYNST---RDRVAG-ESAEPEP-EPEADYYAKEVTRVLMVE 115
Human GF- β 2	52:SPP-EDYPEPEEVPPEVISTYNSTRDLLQEKASRRAAACERERSDEEYYAKEVYKIDMPP 110
Human GF- β 3	55:SPP--EPTVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENTESEYYAKEIHKFDMIQ 112
Murine GF- β 1	61:SPPSQGEVPPGPLPEAVLALYNST---RDRVAG-ESADPEP-EPEADYYAKEVTRVLMVD 115
Murine GF- β 2	52:SPP-EDYPEPDEVPEVISTYNSTRDLLQEKASRRAAACERERSEQEYYAKEVYKIDMPS 110
Murine GF- β 3	53:SPP--EPSVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQETSESEYYAKEIHKFDMIQ 110
Rat TGF- β 1	61:SPPSQGEVPPGPLPEAVLALYNST---RDRVAG-ESADPEP-EPEADYYAKEVTRVLMVD 115
Rat TGF- β 3	55:SPP--EPSVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQETSESEYYAKEIHKFDMIQ 112
Bovine TGF- β 1	1:-----AILALYNST---RDRVAG-ESAETEP-EPEADYYAKEVTRVLMVE 40
Porcine TGF- β 1	61:SPPSQGDVPPGPLPEAVLALYNST---RDRVAG-ESVEPEP-EPEADYYAKEVTRVLMLE 115
Porcine TGF- β 3	53:SPP--DPSMLANIPTQVLDLYNSTRELLEEVHGERGDDCTQENTESEYYAKEIYKFDMIQ 110
Canine TGF- β 1	61:SPPSQGEVPPVPLPEAVLALYNST---RDRVAG-ESAEPEP-EPEADYYAKEVTRVLMVE 115
Ovine TGF- β 1	61:SPPSQGDVPPGPLPEAILALYNST---RDRVAG-ESAETEP-EPEADYYAKEVTRVLMVE 115
Chicken TGF- β 2	52:SPP-DEYPEPEEVPPEVISTYNSTRDLLQEKANHRAATCERERSDEEYYAKEVYKIDMQP 110
Chicken TGF- β 3	55:SPP--ESVGAHVYPYQILALYNSTRELLEEMEEEEKEESCSQENTESEYYAKEIHKFDMIQ 112
Chicken TGF- β 4	33:APPPASETPPRPLPDDVRALYNST---QELLKQARLRPPP-DGPDEYWAKELRRIPMET 88
Simian TGF- β	61:SPPSQGEVPPGPLPEAVLALYNST---RDRVAG-ESAEPEP-EPEADYYAKEVTRVLMVE 115
Frog TGF- β 5	53:KTP-DVDSEKMTVPSEAIFLYNSTLEVIREKATREEEHVGHQNIQDYAKQ-----V-- 104

Table 1-3

Origin	Sequence
Human TGF- β 1	116:THNEIYDKFKQSTHSIYMFNTSELREAVPEPVLLSRA--ELRL--RR---LKLKVEQHV 169
Human TGF- β 2	111:FFPSENAIPPTFYRPFYFRIVRFDVSAMEKNASNLVKAEFRVFRQLQNPKEARVPEQRIELYQ 170
Human TGF- β 3	113:GLAEHNELAVCPKGITSKVFRFNVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQ 172
Murine TGF- β 1	116:RNNAIYEKTKDISHSIYMFNTSDIREAVPEPPLLSRA--ELR-L-QR---LKSSVEQHV 168
Murine TGF- β 2	111:HLPSENAIPPTFYRPFYFRIVRFDVSTMEKNASNLVKAEFRVFRQLQNPKEARVPEQRIELYQ 170
Murine TGF- β 3	113:GLAEHNELAVCPKGITSKVFRFNVSSVEKNGTNLFRAEFRVLRVPNPSSKRTEQRIELFQ 172
Rat TGF- β 1	111:GLAEHNELAVCPKGITSKVFRFNVSSVEKNGTNLFRAEFRVLRVPNPSSKRTEQRIELFQ 170
Rat TGF- β 3	116:RNNAIYDKTKDITHSIYMFNTSDIREAVPEPPLLSRA--ELR-L-QR---FKSTVEQHV 168
Bovine TGF- β 1	41:YGNKIYDKMKSSSHSIYMFNTSELREAVPEPVLLSRA--DVRLL--R---LKLKVEQHV 93
Porcine TGF- β 1	116:SGNQIYDKFKGTPHSLYMLFNTSELREAVPEPVLLSRA--ELRL--R---LKLKVEQHV 168
Porcine TGF- β 3	111:GLEEHNDLAVCPKGITSKIFRFNVSSVEKNETNLFRAEFRVLRMPNPSSKRSEQRIELFQ 170
Canine TGF- β 1	116:NTNKIYEKVKKSPHSIYMLFNTSELREAVPEPVLLSRA--ELRL--R---LKLKAEQHV 168
Ovine TGF- β 1	116:YGNKIYDKMKSSSHSIYMFNTSELREAVPEPVLLSRA--DVRLL--R---LKLKVEQHV 168
Chicken TGF- β 2	111:FYP-ENAIPPSYSLYFRIVRFDVSAMEKNASNLVKAEFRVFRQLQNSKARVSEQRIELYQ 169
Chicken TGF- β 3	113:GLPEHNELGICPKGVTSNVFRFNVSSAEKNSTNLFRAEFRVLRVPNPSSKRSEQRIELFQ 172
Chicken TGF- β 4	89:TWDGAMEHWQPQSHSIFVFVNSRARRGGR-PTLLHRA--ELRMLRQKAAADSAGTEQRL 145
Simian TGF- β	116:THNEIYDKFKQSTHSIYMFNTSELREAVPEPVLLSRA--ELRL--R---LKLKVEQHV 168
Frog TGF- β 5	105:-YRFESITELEDHEFKFKFNASHVRENVGMNSLLHHAELRMYK--KQTDKNMDQRMELFW 161

Table 1-4

Origin	Sequence
Human TGF- β 1	170:ELVQKYSNNSW-RYLSNRL LAPSDSPEWLSFDVTGVVRQWLSRGGEIEGFRLSAHCSC-- 226
Human TGF- β 2	171:ILKSKDLTSPQRYIDSKVVKTRAEGEWLSFDVTDVHVEWLHHKDRNLGFKISLHPCCT 230
Human TGF- β 3	173:ILRPDE-HIAKQRYIGGKNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHPCHT 231
Murine TGF- β 1	169:ELVQKYSNNSW-RYLGNRLLTPTDTPEWLSFDVTGVVRQWLNQGDGIQGFRLSAHCSC-- 225
Murine TGF- β 2	171:ILKSKDLTSPQRYIDSKVVKTRAEGEWLSFDVTDVQEWLHHKDRNLGFKISLHPCCT 230
Murine TGF- β 3	171:ILRPDE-HIAKQRYIGGKNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHPCHT 229
Rat TGF- β 1	169:ELVQKYSNNSW-RYLGNRLLTPTDTPEWLSFDVTGVVRQWLNQGDGIQGFRLSAHCSC-- 225
Rat TGF- β 3	173:ILRPDE-HIAKQRYIGGKNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHPCHT 231
Bovine TGF- β 1	94:ELVQKYSNNSW-RYLSNRL LAPSDSPEWLSFDVTGVVRQWLTRREEIEGFRLSAHCSC-- 150
Porcine TGF- β 1	169:ELVQKYSNDSW-RYLSNRL LAPSDSPEWLSFDVTGVVRQWLTRREAIEGFRLSAHCSC-- 225
Porcine TGF- β 3	171:ILQPDE-HIAKQRYIDGKNLPTRGAAEWLSFDVTDTVREWLLRRESNLGLEISIHPCHT 229
Canine TGF- β 1	169:ELVQKYSNDSW-RYLSNRL LAPSDTPEWLSFDVTGVVRQWLSHGGEVEGFRLSAHCSC-- 225
Ovine TGF- β 1	169:ELVQKYSNNSW-RYLSNRL LAPSDSPEWLSFDVTGVVRQWLTHREEIEGFRLSAHCSC-- 225
Chicken TGF- β 2	170:VLKSKELSSPGQRYIDSKVVKTRAEGEWLSFDVTEAVHEWLHHRDRNLGFKISLHPCCT 229
Chicken TGF- β 3	173:ILRPDE-HIAKQRYLSGRNVQTRGSPEWLSFDVTDTVREWLLHRESNLGLEISIHPCHT 231
Chicken TGF- β 4	146:ELYQGYGNASW-RYLHGRSVRATADDEWLSFDVTDVHQLSGSELLGVFKLSVHCPC-- 202
Simian TGF- β	169:ELVQKYSNNSW-RYLSNRL LAPSNSPEWLSFDVTGVVRQWLSRGGEIEGFRLSAHCSC-- 225
Frog TGF- β 5	162:K-YQENGTHS-RYLESKYITPVTDDEWMSFDVTKTVNEWLKRAEENEQGLQPACKCPT 219

Table 1-5

Origin	Sequence
Human TGF- β 1	227:-----D---SRDNTLQVDI-N--GFTTGRRGDLATIHGMN-----R-PFLLLMATPLER 268
Human TGF- β 2	231:FVPSNNYIIPNKSEELEAFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLMLLPSYR 290
Human TGF- β 3	232:FQPNGD-ILENIHEVMEIKFKGVDNEDDHGRGD--LGR-LKKQKDHHNPHLILMMIPPHR 287
Murine TGF- β 1	226:-----D---SKDNKLHVEI-N--GISPKRRGDLGTIHD MN-----R-PFLLLMATPLER 267
Murine TGF- β 2	231:FVPSNNYIIPNKSEELEAFAGIDGTSTYASGDQKTIKSTRKKTSKTPHLLMLLPSYR 290
Murine TGF- β 3	230:FQPNGD-ILENVHEVMEIKFKGVDNEDDHGRGD--LGR-LKKQKDHHNPHLILMMIPPHR 285
Rat TGF- β 1	226:-----D---SKDNVLHVEI-N--GISPKRRGDLGTIHD MN-----R-PFLLLMATPLER 267
Rat TGF- β 3	232:FQPNGD-ILENVHEVMEIKFKGVDNEDDHGRGD--LGR-LKKQKDHHNPHLILMMIPPHR 287
Bovine TGF- β 1	151:-----D---SKDNTLQVDI-N--GFSSGRRGDLATIHGMN-----R-PFLLLMATPLER 192
Porcine TGF- β 1	226:-----D---SKDNTLHVEI-N--GFNSGRRGDLATIHGMN-----R-PFLLLMATPLER 267
Porcine TGF- β 3	230:FQPNGD-ILENIQEVMEIKFKGVDSEDDPGRGD--LGR-LKKKKE-HSPHLILMMIPPDR 284
Canine TGF- β 1	226:-----D---SKDNTLQVDI-N--GFSSSRRGDLATIHGMN-----R-PFLLLMATPLER 267
Ovine TGF- β 1	226:-----D---SKDNTLQVDI-N--GFSSGRRGDLATIHGMN-----R-PFLLLMATPLER 267
Chicken TGF- β 2	230:FVPSNNYIIPNKSEEPEARFAGIDD-YTYSSGDVKALKSNRKKYSKTPHLLMLLPSYR 288
Chicken TGF- β 3	232:FQPNGD-ILENLHEVLEIKFKGIDSEDDYGRGD--LGR-LKKQKDLENPHLILMMLPPHR 287
Chicken TGF- β 4	203:-----EM--GPGHAEEMRI-SI-EGFEQQRGDMQSIKKHR---RVPYVLAMALPAER 248
Simian TGF- β	226:-----D---SKDNTLQVDI-N--GFTTGRRGDLATIHGMN-----R-PFLLLMATPLER 267
Frog TGF- β 5	220:--P-----QAQDIDIE-GFPALRGD-LASLSSKENTKPYL-MITSM--PAER 259

Table 1-6

Origin	Sequence
Human TGF- β 1	269:--AQHLQSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 324
Human TGF- β 2	291:L-ESQQTNRKKRALDAAYCF--RNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYNANFCA 347
Human TGF- β 3	288:LDNPGQGGQKKRALDTNYCF--RNLEENCCVRPLYIDFRQDLGWKWWHEPKGYANFCS 345
Murine TGF- β 1	268:--AQHLHSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Murine TGF- β 2	291:L-ESQSSRRKKRALDAAYCF--RNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYNANFCA 347
Murine TGF- β 3	286:LDSPGQGSQKKRALDTNYCF--RNLEENCCVRPLYIDFRQDLGWKWWHEPKGYANFCS 343
Rat TGF- β 1	268:--AQHLHSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Rat TGF- β 3	288:LDSPGQGGQKKRALDTNYCF--RNLEENCCVRPLYIDFRQDLGWKWWHEPKGYANFCS 345
Bovine TGF- β 1	193:--AQHLHSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 248
Porcine TGF- β 1	268:--AQHLHSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Porcine TGF- β 3	285:LDNPLGAQRKKRALDTNYCF--RNLEENCCVRPLYIDFRQDLGWKWWHEPKGYANFCS 342
Canine TGF- β 1	268:--AQHLHSSRQRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Ovine TGF- β 1	268:--AQHLHSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Chicken TGF- β 2	289:L-ESQPSRRKKRALDAAYCF--RNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYHANFCA 345
Chicken TGF- β 3	288:LESPTLGGQKKRALDTNYCF--RNLEENCCVRPLYIDFRQDLGWKWWHEPKGYANFCS 345
Chicken TGF- β 4	249:--ANELHSARRRRDLTDYCFGPGTDEKNCCVRPLYIDFRKDLQWKWIHEPKGYMANFCM 306
Simian TGF- β	268:--AQHLQSSRHRRALDTNYCF--SST-EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCL 323
Frog TGF- β 5	260:I--DTVTSSRKKRGVGOEYCFG--NNGPNCCVKPLYINFRKDLGWKWIHEPKGYEANYCL 315

Table 1-7

Origin	Sequence
Human TGF- β 1	325:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 384
Human TGF- β 2	348:GACPYLWSSDTQHSRVLSTYNTINPEASAPCCVSDLEPLTILYVIGTKPKIEQLSNMI 407
Human TGF- β 3	346:GPCPYLRSADTTHTSVLGLYNTLNPEASAPCCVPQDLEPLTILYVIGRTPKVEQLSNMV 405
Murine TGF- β 1	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Murine TGF- β 2	348:GACPYLWSSDTQHTKVLSTYNTINPEASAPCCVSDLEPLTILYVIGNTPKIEQLSNMI 407
Murine TGF- β 3	344:GPCPYLRSADTTHTSVLGLYNTLNPEASAPCCVPQDLEPLTILYVIGRTPKVEQLSNMV 403
Rat TGF- β 1	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Rat TGF- β 3	346:GPCPYLRSSDTTHTSVLGLYNTLNPEASAPCCVPQDLEPLTILYVIGRTPKVEQLSNMV 405
Bovine TGF- β 1	249:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 308
Porcine TGF- β 1	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Porcine TGF- β 3	343:GPCPYLRSADTTHTSVLGLYNTLNPEASAPCCVPQDLEPLTILYVIGRTAKVEQLSNMV 402
Canine TGF- β 1	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Ovine TGF- β 1	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Chicken TGF- β 2	346:GACPYLWSSDTQHSRVLSTYNTINPEASAPCCVSDLEPLTILYVIGTKPKIEQLSNMI 405
Chicken TGF- β 3	346:GPCPYLRSADTTHTSVLGLYNTLNPEASAPCCVPQDLEPLTILYVIGRTPKVEQLSNMV 405
Chicken TGF- β 4	307:GPCPYIWSADTQYTKVLALYNQHNPGASAAPCCVPQTLDPPIIYVGRNVRVEQLSNMV 366
Simian TGF- β	324:GPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMI 383
Frog TGF- β 5	316:GNCPYIWSMDTQYSKVLSTYNNQNPASISPCVPDVLEPLPIIYVIGRTAKVEQLSNMV 375

Table 1-8

Origin	Sequence	
Human TGF- β 1	385:VRSCCKCS	391
Human TGF- β 2	408:VKSCCKCS	414
Human TGF- β 3	406:VKSCCKCS	412
Murine TGF- β 1	384:VRSCCKCS	390
Murine TGF- β 2	408:VKSCCKCS	414
Murine TGF- β 3	404:VKSCCKCS	410
Rat TGF- β 1	384:VRSCCKCS	390
Rat TGF- β 3	406:VKSCCKCS	412
Bovine TGF- β 1	309:VRSCCKCS	315
Porcine TGF- β 1	384:VRSCCKCS	390
Porcine TGF- β 3	403:VKSCCKCS	409
Canine TGF- β 1	384:VRSCCKCS	390
Ovine TGF- β 1	384:VRSCCKCS	390
Chicken TGF- β 2	406:VKSCCKCS	412
Chicken TGF- β 3	406:VKSCCKCS	412
Chicken TGF- β 4	367:VRACKCS	373
Simian TGF- β	384:VRSCCKCS	390
Frog TGF- β 5	376:VRSCNCS	382

The parts corresponding to the sequences of amino acids 30 to 60, 142 to 186, and 269 to 297 in the human TGF- β 1 precursor sequence (the underlined amino acid sequences in the human TGF- β 1 precursor sequence in Table 1) are, for example, the sequences
 5 of amino acids 21 to 51, 137 to 188, and 291 to 320, respectively, in the human TGF- β 2 precursor sequence, and the sequences of amino acids 24 to 54, 139 to 190, and 288 to 318, respectively, in the human TGF- β 3 precursor sequence. This kind of alignment can be carried out by the method of Barton & Sternberg [Journal
 10 of Molecular Biology, 198, 327 (1987)].

Preferred Compounds (I) are peptides wherein A is an amino acid sequence selected from the sequences of SEQ ID NOS: 1 to 16 in which 1 to 5 amino acid residues may be deleted, substituted or added, and pharmaceutically acceptable salts thereof.
 15 Particularly preferred are peptides wherein A is an amino acid sequence selected from the sequences of SEQ ID NOS: 1 to 16.

The expression "1 to 5 amino acid residues may be deleted, substituted or added in the sequence" herein means that the sequence may contain deletion, substitution or addition of a
 20 single or plural amino acid residues at a single or plural arbitrarily selected positions therein, and the total number of such residues deleted, substituted or added is 1 to 5, which deletion, substitution and addition may be simultaneously contained in the sequence. It does not matter whether or not
 25 the substituted or added amino acid is a natural one.

Examples of the addition are addition of amino acids having a thiol group (e.g. cysteine and homocysteine) or organic groups at both ends of the sequence. Examples of the substitution are substitution of a cysteine residue existing in the sequence to
 30 a serine or alanine residue, and substitution of a serine or alanine residue to a cysteine residue. A disulfide bond may be formed between two thiol groups contained in the sequence for cyclization. An amide bond represented by CO-NH or a reversed amide bond represented by NH-CO may be formed between the
 35 N-terminal amino group or a side-chain amino group and the

C-terminal carboxyl group or a side-chain carboxyl group for cyclization.

Examples of the natural amino acids are glycine, L-alanine, L-threonine, L-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, L-valine, L-leucine, L-serine, L-methionine, L-isoleucine, L-phenylalanine, L-tyrosine, L-lysine, L-arginine, L-histidine, L-proline, L-cysteine and L-tryptophan.

The term latent TGF- β includes small molecular weight latent TGF- β (SLTGF- β) comprising TGF- β and LAP which inhibits the activity thereof, and large molecular weight latent TGF- β (LLTGF- β) comprising TGF- β , LAP which inhibits the activity thereof, and LTBP.

The pharmaceutically acceptable salts of the compounds obtainable by the method of the present invention and Compounds (I) include acid addition salts, metal salts, organic base addition salts, etc. Examples of the pharmaceutically acceptable acid addition salts are inorganic acid addition salt such as hydrochloride, sulfate and phosphate, and organic acid addition salts such as acetate, maleate, fumarate, tartrate and citrate. Examples of the pharmaceutically acceptable metal salts are alkali metal salts such as sodium salt and potassium salt, alkaline earth metal salts such as magnesium salt and calcium salt, aluminum salt and zinc salt. Examples of the pharmaceutically acceptable organic base addition salts are salts with primary amines, e.g. methylamine, ethylamine and aniline, secondary amines, e.g. dimethylamine, diethylamine, pyrrolidine, piperidine, morpholine and piperazine, and tertiary amines, e.g. trimethylamine, triethylamine, N,N-dimethylaniline and pyridine, and ammonium salt.

The abbreviations for amino acids and their protecting groups used herein are described below.

The abbreviations for amino acids and their protecting groups follow the recommendations by IUPAC-IUB Joint Commission on Biochemical Nomenclature [European Journal of Biochemistry, 138, 9 (1984)].

The abbreviations for amino acids and their protecting groups are as follows, unless otherwise specified.

	Gly or G; Glycine
	Ala or A; L-Alanine
5	Thr or T; L-Threonine
	Asp or D; L-Aspartic acid
	Asn or N; L-Asparagine
	Asx; L-Aspartic acid or L-asparagine
	Glu or E; L-Glutamic acid
10	Gln or Q; L-Glutamine
	Glx; L-Glutamic acid or L-glutamine
	Val or V; L-Valine
	Leu or L; L-Leucine
	Ser or S; L-Serine
15	Met or M; L-Methionine
	Ile or I; L-Isoleucine
	Phe or F; L-Phenylalanine
	Tyr or Y; L-Tyrosine
	Lys or K; L-Lysine
20	Arg or R; L-Arginine
	His or H; L-Histidine
	Pro or P; L-Proline
	Cys or C; L-Cysteine
	Trp or W; L-Tryptophan
25	Fmoc; 9-Fluorenylmethyloxycarbonyl
	t-Bu; t-Butyl
	Trt; Trityl
	Pmc; 2,2,5,7,8-Pentamethylchroman-6-sulfonyl
	Boc; t-Butyloxycarbonyl

The abbreviations for side-chain-protected amino acids are as follows.

	Fmoc-Asp(Ot-Bu)-OH; N ^α -9-Fluorenylmethyloxycarbonyl-L-aspartic acid β-t-butyl ester
35	Fmoc-Glu(Ot-Bu)-OH; N ^α -9-Fluorenylmethyloxycarbonyl-L-glutamic acid γ-t-butyl ester

	Fmoc-Thr (t-Bu) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-O-t-butyl-L-threonine
	Fmoc-Ser (t-Bu) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-O-t-butyl-L-serine
5	Fmoc-Tyr (t-Bu) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-O-t-butyl-L-tyrosine
	Fmoc-Lys (Boc) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ^ε -t-butyloxycarbonyl-L-lysine
10	Fmoc-Asn (Trt) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ^γ -trityl-L-asparagine
	Fmoc-Gln (Trt) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ^δ -trityl-L-glutamine
15	Fmoc-Arg (Pmc) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ⁹ -2,2,5,7,8-pentamethylchroman-6-sulfonyl-L-arginine
	Fmoc-His (Trt) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ^{im} -trityl-L-glutamine
	Fmoc-Cys (Trt) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-S-trityl-L-cysteine
20	Fmoc-Trp (Boc) -OH;	N ^α -9-Fluorenylmethyloxycarbonyl-N ^{ind} -t-butyloxycarbonyl-L-tryptophan

The abbreviations for reaction solvents, reaction reagents, etc. are as follows.

25	PyBOP;	Benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate
	HOBt;	N-Hydroxybenzotriazole
	DCC;	Dicyclohexylcarbodiimide
	NMM;	N-Methylmorpholine
30	DMF;	N,N-Dimethylformamide
	NMP;	N-Methylpyrrolidone
	TFA;	Trifluoroacetic acid
	DTT;	Dithiothreitol
35	HBTU;	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
	DIPC;	N,N'-Diisopropylcarbodiimide

DIEA; N,N-Diisopropylethylamine

DCM; Dichloromethane

5 The processes for producing Compounds (I) are described below.

Compounds (I) can be synthesized by general liquid phase or solid phase peptide synthetic methods [Fundamentals and Experiments of Peptide Synthesis, Nobuo Izumiya, et al., Maruzen (1985)], or appropriate combinations thereof. Compounds (I) can
10 also be synthesized by using an automatic peptide synthesizer. That is, the peptide synthesis can be carried out by the use of commercially available peptide synthesizers from Shimadzu Corporation, Applied Biosystems Inc., U.S.A. (ABI), Advanced ChemTech Inc., U.S.A. (ACT), etc. using an appropriately
15 side-chain-protected N α -9-fluorenylmethyloxycarbonyl amino acid or N α -t-butyloxycarbonyl amino acid according to respective synthesis programs.

The protected amino acids which are starting materials for the synthesis of Compounds (I) and carrier resins are available
20 from ABI, Shimadzu Corporation, Kokusan Chemical Works Co., Ltd., Nova Biochem Co., Watanabe Chemical Industries, Ltd., ACT, and Peptide Institute Inc.

Cyclization may be carried out after all the constituent amino acid residues and organic groups are bonded by a liquid
25 phase method, a solid phase method or a combination thereof, or in the course of elongation of the peptide chain. In the latter case, the obtained cyclization product is subjected to further condensation with amino acid residues or organic groups to prepare Compound (I). The cyclic structure may be formed by
30 forming, at the final step of the process, a disulfide bond, an amide bond or a reversed amide bond which forms a cyclic structure in general formula (I), or by forming an amide bond in an ordinary sequence, after the above bonds are formed, between an amino acid residue and the adjacent amino acid residue which are to be
35 constituents of the cyclic structure. The cyclization process is described in detail below.

1. Cyclization by disulfide bond formation

First a peptide which has, at two positions in the sequence, amino acid residues having appropriately protected thiol groups is prepared by a solid phase method, a liquid phase method or a combination thereof. Then, protecting groups other than the thiol-protecting groups are removed, followed by removal of the thiol-protecting groups. The thus obtained precursor peptide is subjected to oxidation reaction and the product is purified by general purification steps in organic chemical reactions to give the desired peptide having a cyclic structure with a disulfide bond.

1-1 Cyclization by disulfide bond formation according to a liquid phase method

The peptide having a cyclic structure with a disulfide bond can be prepared by subjecting the above precursor peptide to air oxidation or reaction with an oxidizing agent in an inert solvent. The reaction is carried out at a peptide concentration of 0.5-5000 $\mu\text{mol/l}$, preferably 50-500 $\mu\text{mol/l}$. As the solvent, buffers such as 50 mM - 1 M tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCl) buffer adjusted to pH 4-9, preferably pH 6-8, 5-50% aqueous acid, water, and organic solvents such as DMF, DMSO, acetonitrile, tetrahydrofuran, methanol and ethanol can be used alone or in combination.

Examples of the oxidizing agents are potassium ferricyanide and iodine, which are used respectively in the amounts of 0.1-1 time and 0.5-5 times (preferably one time) that of the precursor peptide (weight:weight). DMSO can also be used as the oxidizing agent at a concentration of 10-50%. The reaction is usually carried out at 0-40°C for one hour to one week. In some cases, the yield of oxidation product can be increased by addition of glutathione, and the reaction may be carried out in the presence of oxidized glutathione in an amount of 0.5-5 times that of the precursor peptide (weight:weight) and reduced glutathione in an amount of one-half the weight of oxidized glutathione [Journal of American Chemical Society, 103, 5867 (1981); Development of

Medicines, second series, vol. 14, Peptide Synthesis, p. 239, compiled under the supervision of Haruaki Yajima, Hirokawa Shoten (1991)]. When iodine is used as the oxidizing agent, zinc powder is added after the completion of reaction until the color of iodine disappears from the reaction mixture, and the mixture is purified as such, or after concentration under reduced pressure, by means of various kinds of chromatography. When potassium ferricyanide is used as the oxidizing agent, the reaction mixture is made weakly acidic by addition of acetic acid and then is purified as such, or after concentration under reduced pressure, by means of various kinds of chromatography. Alternatively, an anion exchange resin such as Dowex 1X2 (AcO-) (Dow Chemical Co.) may be added to the reaction mixture to remove excess potassium ferricyanide (ferricyan ion and ferrocyan ion) by adsorption, and then the mixture is purified as such, or after concentration under reduced pressure, by means of various kinds of chromatography.

It is also possible to pyridylsulfenylate or 2-nitropyridylsulfenylate one of the thiol groups and then force the cyclization reaction to proceed to completion simultaneously with the selective removal of the other thiol protecting group [International Journal of Peptide and Protein Research, 29, 162 (1987)]. The solvent, reaction temperature, reaction time, etc. for the cyclization reaction are substantially the same as described above. Further, after the protection groups of the two thiol groups are removed, an equivalent amount of a reagent for pyridylsulfenylation or 2-nitropyridylsulfenylation may be introduced. Pyridylsulfenylation can be carried out by adding 1-3 equivalents of a reagent such as 2,2'-dithiodipyridine to a solvent containing the peptide, followed by stirring. 2-Nitropyridylsulfenylation can be carried out in a similar manner. The solvent, reaction temperature, reaction time, etc. for the reaction are substantially the same as described above [Peptide Chemistry, 1991, 125 (1992)].

1-2 Cyclization by disulfide bond formation according to a solid phase method

A peptide which has, at two positions in the sequence, amino acid residues having appropriately protected thiol groups is elongated by a solid phase method. Before cleavage of the peptide from the resin, the thiol-protecting groups are selectively removed and the peptide is subjected to oxidation reaction to prepare a peptide moiety having a cyclic structure. Then, the peptide is cleaved from the resin and the remaining protecting groups are removed, whereby the desired peptide having a cyclic structure is obtained.

Examples of the thiol-protecting groups include acetamidomethyl (Acm) group and trityl (Trt) group. By reaction of the protected peptide on the resin with iodine in an appropriate solvent such as DMF or DCM, Acm group and Trt group are removed and an intramolecular disulfide bond is formed. The reaction is carried out using 0.5-2 ml of a solvent for 50 mg of the resin, and iodine in an amount of 0.5-5 times, preferably one time the calculated weight of the peptide on the resin. The reaction is usually carried out at 0-40°C for one hour to one week. After the completion of reaction, the resin is subjected to a usual treatment in a solid phase method, that is, washing with a small amount of a solvent such as DMF or DCM, and then subjected to the subsequent reaction.

The pyridylsulfenylation or 2-nitropyridylsulfenylation of one of the thiol groups as described in 1-1 above can be applied to a solid phase method. The solvent, reaction temperature, reaction time, etc. for the cyclization reaction are substantially the same as described above. Further, similarly to the above-described liquid phase method, an equivalent amount of a reagent for pyridylsulfenylation or 2-nitropyridylsulfenylation may be introduced after the protecting groups of the two thiol groups are removed. Pyridylsulfenylation can be carried out by adding 1-3 equivalents of a reagent such as 2,2'-dithiodipyridine to the resin swollen with a solvent, followed by stirring. 2-

Nitropyridylsulfenylation can be carried out in a similar manner. The solvent, reaction temperature, reaction time, etc. for the reaction are substantially the same as in the above cyclization reaction in the solid phase method.

5

2. Cyclization by amide bond or reversed amide bond formation

By a solid phase method, a liquid phase method, or a combination thereof, a peptide is prepared which has, at two positions in the sequence, an amino acid residue having an appropriately protected amino group and an amino acid residue having an appropriately protected carboxyl group and in which the side chains, N-terminus and C-terminus are protected. After the amino- and carboxyl-protecting groups are selectively removed, the peptide is subjected to intramolecular condensation, followed by general purification steps in organic chemical reactions to give a peptide which has a cyclic structure and in which the side chains, N-terminus and C-terminus are protected. Then, the remaining protecting groups are removed, whereby the desired peptide is obtained. The desired peptide can also be prepared by first preparing a peptide moiety having a cyclic structure and then elongating it.

2-1 Cyclization by amide bond or reversed amide bond formation according to a liquid phase method

By a solid phase method, a peptide is prepared which has, at two positions in the sequence, an amino acid residue having an appropriately protected amino group and an amino acid residue having an appropriately protected carboxyl group. Before cleavage of the peptide from the resin, the amino- and carboxyl-protecting groups are selectively removed, and the obtained peptide having free amino group and free carboxyl group is subjected to condensation reaction to give a peptide moiety having a cyclic structure. Then, the peptide is cleaved from the resin and the remaining side-chain-protecting groups are removed, whereby the desired peptide having a cyclic structure is obtained.

When 4-methyltrityl group is used as the amino-protecting group, it can be removed by reaction using acetic acid/trifluoroethanol/DCM (1/2/7). The reaction is usually carried out at 0-40°C for 0.5-6 hours. After the completion of reaction, the peptide is precipitated by addition of diethyl ether, etc., followed by removal of the solvent, if necessary under reduced pressure. General purification steps in organic chemical reactions including such step are applicable as may be required.

When allyloxycarbonyl group is used as the amino-protecting group and allyl ester group is used as the carboxyl-protecting group, these protecting groups can be simultaneously removed by reaction with a reducing agent in the presence of a palladium catalyst. It is also possible to use only allyl ester group as the carboxyl-protecting group. Any zerovalent palladium catalysts for homogeneous system can be used in the reaction. Suitable catalysts include tetrakis(triphenylphosphine)palladium(0) and palladium(II) acetate-triphenylphosphine. The catalyst is used in an amount of 0.01-1 equivalent, preferably 0.1-0.5 equivalent, based on the above protecting groups. Further, additives such as formic acid, formic acid-triethylammonium, tributyltin hydride, triphenyltin hydride, trimethylhydrosilane, sodium borohydride, acetic acid, and acetic acid-NMM are added in an amount of one equivalent to excess based on the above protecting groups. As a solvent, ether, tetrahydrofuran, acetonitrile, DMF, chloroform, etc. are used alone or in combination. For 1 mM of allyloxycarbonyl group and allyl ester group are added the above reagents and 3-10 ml of the solvent. The reaction is carried out at -20 to 80°C, preferably 0 to 30°C for 10 minutes to 6 hours. After the completion of reaction, general purification steps in organic chemical reactions can be applied.

The obtained peptide is then subjected to reaction for forming an intermolecular amide bond between the free amino group and the free carboxyl group. Typical amide bond formation reactions for cyclization are described below. Common reaction

conditions are as follows. As a solvent, DMF, NMP, methylene chloride, chloroform, acetonitrile, tetrahydrofuran, etc. are used alone or in combination. The peptide is used at a concentration of 0.5-5000 $\mu\text{mol/l}$, preferably 50-500 $\mu\text{mol/l}$.

- 5 The reaction is carried out usually at 0-40°C, preferably 4-25°C, with stirring for 3 hours to one week. After the completion of reaction, general purification steps in organic chemical reactions can be applied.

- Amide bond formation reaction can be carried out by using
10 carbodiimide such as dicyclohexylcarbodiimide (DCC) or water-soluble carbodiimide (WSC) in an amount of 1-10 equivalents based on the carboxyl group. NMM, DIEA or sodium hydrogencarbonate is added in an amount of 1.5-2 equivalents based on carbodiimide. If necessary, HOBt or HONSu may be added
15 in an equimolar amount based on carbodiimide.

- Amide bond formation reaction can also be carried out by using diphenylphosphoryl azide (DPPA) or diethyl
phosphorocyanidate (DEPC) in an amount of 1-10 equivalents based
on the carboxyl group. NMM, DIEA or sodium hydrogencarbonate is
20 added in an amount of 1.5-2 equivalents based on carbodiimide.

- Further, amide bond formation reaction can be carried out
by using PyBOP or HBTU in an amount of 1-10 equivalents,
preferably 2-5 equivalents based on the carboxyl group, and HOBt
in an equimolar amount based on PyBOP or HBTU. NMM, DIEA or sodium
25 hydrogencarbonate is added in an amount of 1.5-2 equivalents based on PyBOP or HBTU.

- It is also possible to convert the carboxyl group into an active ester, selectively remove the amino-protecting group, and then form an amide bond. Examples of the active esters are
30 p-nitrophenyl ester, pentafluorophenyl ester, and N-oxy succinimide ester. The active esters can be formed by various methods. For example, DCC is added in an amount of 1-10 equivalents based on the carboxyl group, together with an equimolar amount of p-nitrophenol, pentafluorophenol or HONSu,
35 and the mixture is stirred at 0-5°C for one hour to one day, followed by removal of the formed dicyclohexylurea (DCUrea) by

filtration. Then, purification can be carried out by general purification steps in organic chemical reactions. The solvent, reaction temperature, reaction time, etc. for the formation of active esters are substantially the same as in the above amide bond formation reactions.

2-2 Cyclization by amide bond or reversed amide bond formation according to a solid phase method

By a solid phase method, a peptide is prepared which has, at two positions in the sequence, an amino acid residue or an organic group having an appropriately protected amino group and an amino acid residue or an organic group having an appropriately protected carboxyl group. Before cleavage of the peptide from the resin, the amino- and carboxyl-protecting groups are selectively removed, and the obtained peptide having free amino group and free carboxyl group is subjected to condensation reaction to give a peptide moiety having a cyclic structure. Then, the peptide is cleaved from the resin and the remaining side-chain-protecting groups are removed, whereby the desired peptide having a cyclic structure is obtained.

When 4-methyltrityl group is used as the amino-protecting group, it can be removed by reaction using 0.5-2 ml of acetic acid/trifluoroethanol/DCM (1/2/7) for 50 mg of the resin. The reaction is usually carried out at 0-40°C for 0.5-6 hours. After the completion of reaction, the resin is subjected to a usual treatment in a solid phase method, that is, washing with a small amount of a solvent such as DMF, and then subjected to the subsequent reaction.

When allyloxycarbonyl group is used as the amino-protecting group and allyl ester group is used as the carboxyl-protecting group, these protecting groups can be removed by reaction using, for example, a chloroform solution containing 0.1-0.2 M tetrakis (triphenylphosphine)palladium(0), 5% acetic acid and 2.5% NMM. For 1 mM of allyloxycarbonyl group and allyl ester group is added 3-10 ml of the above chloroform solution. The reaction is usually

carried out at 0-40°C for 0.5-6 hours. After the completion of reaction, the resin is subjected to a usual treatment in a solid phase method, that is, washing with a small amount of a solvent such as DMF, and then subjected to the subsequent reaction.

5 The subsequent reaction is carried out by using, for 50 mg of the resin, 1 ml of an organic solvent such as DMF, DCM or NMP containing PyBOP or HBTU in an amount of 1-10 equivalents, preferably 2-5 equivalents based on the calculated quantity of the carboxyl group on the resin, HOBT in an equimolar amount based
10 on PyBOP or HBTU, and NMM or DIEA in an amount of 1.5-2 equivalents based on PyBOP or HBTU. The reaction is carried out usually at 0-40°C, preferably 4-25°C, with stirring for 3 hours to one week. After the completion of reaction, the resin is subjected to a usual treatment in a solid phase method, that is, washing with
15 a small amount of a solvent such as DMF, and then subjected to the subsequent reaction.

Compounds (I) obtained by the above processes can be purified by high performance liquid chromatography (hereinafter referred to as HPLC) using reversed-phase silica gel columns
20 such as C-4, C-8 and C-18, or column chromatography or thin layer chromatography such as gel filtration using partition resins, adsorption resins, ion-exchange resins, silica gel, chemically-modified silica gel, reversed-phase silica gel,
25 alumina, diatomaceous earth or magnesium silicate.

The pharmaceutically acceptable salts of Compound (I) are obtained according to an ordinary method. That is, the acid addition salts and organic base addition salts of Compound (I) are obtained by dissolving Compound (I) in an aqueous solution
30 of the corresponding acid or organic base, followed by freeze-drying. The metal salts of Compound (I) are obtained by dissolving Compound (I) in an aqueous solution containing the corresponding metal ion, followed by purification by gel filtration or HPLC.

35 Specific examples of Compounds (I) are shown in Table 2.

Table 2

Compound	Sequence
1	H-Leu-Gln-Ser-Ser-Arg-His-Arg-Arg-Ala-Leu-Asp-Thr-Asn-Tyr-Ser-Phe-Ser-Ser-Thr-Glu-Lys-Asn-Cys-OH
2	H-Pro-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Cys-OH
3	H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys-OH
4	H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH
5	H-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH
6	H-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH
7	H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-OH
8	H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-OH
9	H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-OH
1 0	H-cyclo(Cys-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys)-OH
1 1	H-Leu-Ser-Thr-cyclo(Cys-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys)-OH
1 2	H-cyclo(Cys-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Cys)-OH
1 3	Biotinyl-cyclo(Cys-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Cys)-OH
1 4	H-Leu-Ser-Thr-Cys-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-OH
1 5	H-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Glu-Leu-Tyr-Gln-Lys-Tyr-Ser-Asn-Asn-Ser-Trp-Arg-OH
1 6	Biotinyl-Gly-Arg-Arg-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Glu-Leu-Tyr-Gln-Lys-Tyr-Ser-Asn-Asn-Ser-Trp-Arg-OH

The method of screening a compound to be used for the treatment or prevention of TGF- β -related diseases is described below which comprises measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β to said cells, measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β and a compound to be evaluated to said cells, and evaluating the inhibiting activity or promoting activity of said compound on the binding of latent TGF- β to animal cells from the change in the amount of latent TGF- β bound to animal cells caused by the addition of said compound.

Either a synthetic compound or a natural substance can be subjected to screening according to this method without any specific restriction. For example, a natural or synthetic peptide, a peptide obtained by hydrolyzing a natural protein with an enzyme, etc. can be evaluated.

The latent TGF- β may be those extracted and purified from animal cells, for example, by the method of Okada, et al. [Journal of Biochemistry, 106, 304 (1989)] or those produced by recombinant DNA techniques [Journal of Biological Chemistry, 271, 29891 (1996)]. Animal cells suitable for use in this method are those to which latent TGF- β can be bound. Examples of such cells are platelets, vascular smooth muscle cells, capillary endothelial cells, aortic endothelial cells, fibroblasts, epithelial cells and macrophages. The cells may be those isolated and purified from animals such as a human, a cow, a pig and a rat, or cultured cells derived from such cells. The cells can be isolated and purified by the method of Okada, et al. [Journal of Biochemistry, 106, 304 (1989)], or the like. The binding of latent TGF- β to cells can be carried out, for example, by first culturing cells in a medium and adding latent TGF- β thereto, followed by incubation, and then washing said cells and measuring the amount of latent TGF- β bound to the cells.

It is preferred to use latent TGF- β which has been ^{125}I -labeled according to the chloramine T method [Molecular & Cellular Biology, 2, 599 (1982)], or the like. The amount of

latent TGF- β bound to cells can be determined by measuring the radioactivity.

The amount of latent TGF- β bound to cells can also be determined by measuring the amount of active TGF- β because latent TGF- β is activated by being bound to the cells. The determination of active TGF- β can be carried out by any method. For example, the determination can be carried out by methods such as enzyme immunoassay directly using an anti-TGF- β antibody [Methods in Enzymology, 198, 303 (1991)] and the luciferase assay system of Abe, et al. [Analytical Biochemistry, 216, 276 (1994)], or by measuring the degree of migration of vascular endothelial cells [Journal of Cell Biology, 123, 1249 (1993)], growth of vascular smooth muscle cells [Tohoku Journal of Experimental Medicine, 179, 23 (1996)], growth inhibition of various cancer cells [Journal of Clinical Investigation, 87, 277 (1991), Endocrinology, 128, 1981 (1991)] and growth inhibition of mink lung epithelial cells [Methods in Enzymology, 198, 317 (1991)].

The judgment as to whether or not a compound to be evaluated is useful for the treatment or prevention of TGF- β -related diseases is made from the difference between the amount of latent TGF- β bound to the cells or active TGF- β in the absence of said compound and that in the presence of said compound. The desired compound can be preferably obtainable by screening a compound, for example, which increases or decreases the amount of active TGF- β by 10% or more when added at a concentration of 1 mM compared with that measured without addition of the compound.

The method of screening a compound to be used for the treatment or prevention of TGF- β -related diseases is described below which comprises measuring the amount of TGF- β after addition of a peptide shown by Compound (I) or a pharmaceutically acceptable salt thereof to animal cells, measuring the amount of TGF- β after addition of a compound to be evaluated and a peptide shown by Compound (I) or a pharmaceutically acceptable salt thereof to animal cells, and evaluating the inhibiting activity or promoting activity of said compound on the conversion

of latent TGF- β into TGF- β from the change in the amount of TGF- β caused by the addition of said compound.

5 Either a synthetic compound or a natural substance can be subjected to screening according to this method without any specific restriction. For example, a natural or synthetic peptide, a peptide obtained by hydrolyzing a natural protein with an enzyme, etc. can be evaluated.

10 Cells suitable for use in this method are those which secrete latent TGF- β themselves. Such cell lines are preferred because the desired compound can be selected without addition of latent TGF- β to the test system. Examples of the cells which secrete latent TGF- β are vascular endothelial cells, vascular smooth muscle cells and macrophages, and cultured cells derived therefrom.

15 The amount of active TGF- β can be determined by any method, for example, the methods mentioned above.

20 The compounds selected according to the present invention include not only the above-defined peptides but also all compounds which have an activity to promote the release of active TGF- β from latent TGF- β or an activity to promote the binding of latent TGF- β to a cell membrane.

25 The compounds obtainable according to the screening methods of the present invention, Compounds (I) and pharmaceutically acceptable salts thereof can be used for treating or preventing diseases such as cancer, diabetic retinopathy, atherosclerosis, bone fracture, myocardial infarction, myocardial disorder after ischemia reperfusion, cerebral infarction, retinal detachment, glomerulonephritis, diabetic nephropathy, renal graft rejection, HIV nephropathy, sudden pulmonary fibrosis, autoimmune pulmonary fibrosis, hepatic cirrhosis, venous constrictive hepatopathy (often occurring after treatments of cancer), systemic sclerosis, keloid, eosinophilia-muscle ache syndrome, re-stricture after angioplasty, intraocular fibrosis, rheumatic arthritis and
35 nasal polyp. Specifically, they can be preferably used as anti-fibrosis agents, anti-tumor agents and anti-

employed as such or in various administration forms. For example, a pharmaceutical composition which is appropriate as an injection can be prepared by dissolving a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof in physiological saline, or an aqueous solution of glucose, lactose or mannitol. A powdery composition for injection can be prepared by freeze-drying a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof and adding sodium chloride thereto. These pharmaceutical compositions may contain as may be appropriate an additive known in the pharmaceutical field, for example, a pharmaceutically acceptable salt.

A pharmaceutical composition for oral administration such as a tablet, granule, powder or syrup can be prepared by mixing a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof with an appropriate excipient, disintegrating agent, binder, lubricant, or the like. Further, a suppository for rectal administration can be prepared by mixing a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof with a conventional carrier.

The effective dose will vary depending upon the mode of administration, the kind of a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof, the age and symptoms of a patient, etc. The mode of administration can also be changed according to the symptoms and the dose. For example, a compound obtained according to the screening method of the present invention, Compound (I) or a pharmaceutically acceptable salt thereof can be administered in a daily dose of 0.00001-100 mg/kg, preferably 0.01-10 mg/kg.

Brief Description of the Drawings

Fig. 1 shows the degree of binding of active TGF- β to porcine vascular smooth muscle cells (PSMC) as determined by measuring the radioactivity. "Vehicle" lane shows the

5 radioactivity when a solution without a test compound was added, and the other lanes show the radioactivity when the respective test compound was added at a concentration of 100 μ g/ml.

Fig. 2 shows the degree of binding of active TGF- β to bovine vascular smooth muscle cells (BSMC) as determined by measuring
10 the radioactivity. "Vehicle" lane shows the radioactivity when a solution without a test compound was added, "cell free" lane shows the radioactivity when ^{125} I-LLTGF- β alone was added to a plate without a cell, and the other lanes show the radioactivity when the respective test compound was added at a concentration
15 of 100 μ g/ml.

Fig. 3 shows the migration inhibitory activity of active TGF- β on bovine capillary endothelial cells (BCEC) as determined by counting the number of the cells which migrated into a field of a microscope. "Vehicle" lane shows the number
20 of the cells when a solution without a test compound was added, and the other lanes show the number of the cells when the respective test compound was added at a concentration of 50 μ g/ml.

Fig. 4 shows the migration inhibitory activity of active
25 TGF- β on bovine capillary endothelial cells (BCEC) as determined by counting the number of the cells which migrated into a field of a microscope. "Vehicle" lane shows the number of the cells when a solution without a test compound was added, and the other lanes show the number of the cells when the
30 respective test compound was added at a concentration of 100 μ g/ml.

Fig. 5 shows the migration inhibitory activity of active
TGF- β on bovine capillary endothelial cells (BCEC) as determined by counting the number of the cells which migrated
35 into a field of a microscope. "Vehicle" lane shows the number of the cells when a solution without a test compound was added,

and the other lanes show the number of the cells when the respective test compound was added at the concentration indicated.

Fig. 6 shows the luminescence intensity as measured by the luciferase assay system for the determination of the amount of active TGF- β . "STD" lane shows the luminescence intensity at stationary state, "vehicle" lane shows the luminescence intensity when a solution without a test compound was added, and the other lanes show the luminescence intensity when the respective compound was added at the concentration indicated.

Fig. 7 shows the amount of active TGF- β converted from the luminescence intensity shown in Fig. 6. Each lane has the same significance as that in Fig. 6.

15 Best Modes for Carrying Out the Invention

The physicochemical properties of the compounds in Examples below were determined according to the following methods. Mass spectrometric analysis was carried out according to the FAB method using JEOL JMS-SX102A. Amino acid analysis was carried out according to the method of Bidlingmeyer, B.A., et al. [Journal of Chromatography, 336, 93 (1984)]. Hydrolysis was carried out in hydrochloric acid vapor at 110°C for 22 hours. The amino acid compositions of the resulting hydrolyzates were analyzed with Pico Tag amino acid analyzer (Waters Associates).

25 Example 1 Synthesis of Compound 1 (SEQ ID NO: 1) (H-Leu-Gln-Ser-Ser-Arg-His-Arg-Arg-Ala-Leu-Asp-Thr-Asn-Tyr-Ser-Phe-Ser-Ser-Thr-Glu-Lys-Asn-Cys-OH)

A carrier resin (Wang resin, 123 mg) combined with 62.5 μ mol of Fmoc-Cys(Trt) was put in a reactor of an automatic synthesizer (ABI, model 430A) and the following treatments were carried out by the Fmoc method according to the synthesis program developed by ABI.

35 (a) To the carrier resin was added a 20% piperidine-NMP solution, and the mixture was stirred for 20 minutes, followed by discharge of said solution.

- (b) The carrier resin was washed with NMP for 5 minutes, and the rinsings were discharged. The carrier resin combined with Cys(Trt) without Fmoc group was thus obtained.
- (c) A solution previously prepared by stirring 250 μ mol (4 equivalents based on the amino acid on the resin) of Fmoc-Asn(Trt)-OH, DCC and HOBt in NMP for 50 minutes was added to the resin, and the resulting mixture was stirred for 60 minutes, followed by discharge of said solution.
- (d) The carrier resin was washed with NMP for 3 minutes. Fmoc-Asn(Trt)-Cys(Trt) was thus synthesized on the carrier resin.

Subsequently, deprotection and washing steps (a) and (b) were carried out, and condensation reaction was carried out using Fmoc-Lys(Boc)-OH in step (c), followed by washing step (d) to synthesize Fmoc-Lys(Boc)-Asn(Trt)-Cys(Trt) on the carrier resin. Then, steps (a)-(d) were repeated to obtain the carrier resin to which a protected peptide was bound. In step (c) in the repeated procedures, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Phe-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Tyr(t-Bu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-His(Trt)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Gln(Trt)-OH and Fmoc-Leu-OH were used in turn. After deprotection and washing steps (a) and (b) were carried out, the carrier resin was washed successively with methanol and butyl ether, followed by drying under reduced pressure for 12 hours to obtain 310.3 mg of the carrier resin to which a side-chain-protected peptide was bound. To the obtained carrier resin was added 10 ml of a mixture of TFA (82.5%), thioanisole (5%), water (5%), ethyl methyl sulfide (3%), 1,2-ethanedithiol (2.5%) and thiophenol (2%), and the resulting mixture was allowed to stand at room temperature for 8 hours to remove the side-chain-protecting groups and to cleave the peptide from the resin. After the resin was separated by filtration, the filtrate was concentrated to about 2 ml under reduced pressure, and about 10

ml of ether was added thereto. The deposited precipitate was collected by centrifugation and decantation to obtain 145 mg of the crude peptide. A part of the obtained crude peptide (70 mg) was dissolved in 2 M acetic acid and then purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 25 mm). Elution was carried out with a linear concentration gradient by adding 90% aqueous acetonitrile containing 0.1% TFA to 0.1% aqueous TFA, followed by detection at 220 nm to give a fraction containing Compound 1. The obtained fraction was lyophilized to give 25.5 mg of Compound 1.

Mass spectrum [FABMS]: $m/z=2701.1$ ($M+H^+$)

Amino acid analysis: Asx 3.1 (3), Glx 2.1 (2), Ser 5.0 (5), His 1.0 (1), Arg 2.9 (3), Thr 2.0 (2), Ala 1.0 (1), Tyr 1.0 (1), Leu 1.9 (2), Phe 1.0 (1), Lys 1.1 (1), Cys 1.1 (1)

Example 2 Synthesis of Compound 2 (SEQ ID NO: 2) (H-Pro-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Cys-OH)

Condensation was carried out in the same manner as in Example 1 using 181.8 mg of a carrier resin (Wang resin) combined with 100 μ mol of Fmoc-Cys(Trt) as a starting material, and using Fmoc-Val-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Val-OH and Fmoc-Pro-OH in turn. The condensation product was washed and dried to obtain 568.4 mg of the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 320 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co.,

Ltd., CAPCELL PAK C18 30 mm I. D. x 250 mm) to give 26.4 mg of Compound 2.

Mass spectrum [FABMS]: $m/z=2870.4$ ($M+H^+$)

Amino acid analysis: Glx 3.0 (3), Ser 1.1 (1), His 1.0 (1), Arg 3.8 (4), Ala 1.1 (1), Pro 0.9 (1), Val 2.6 (3), Leu 7.3 (7), Lys 2.1 (2), Cys 1.2 (1)

Example 3 Synthesis of Compound 3 (SEQ ID NO: 3) (H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys-OH)

A carrier resin (Wang resin, 60 mg) combined with 33 μ mol of Fmoc-Cys(Trt) was put in a reactor of an automatic synthesizer (Shimadzu Corporation, PSSM-8), and the following treatments were carried out according to the synthesis program.

- 15 (a) The carrier resin was washed with 500 μ l of DMF for 3 minutes, and the rinsings were discharged.
- (b) To the carrier resin was added 500 μ l of a 30% piperidine-DMF solution, and the mixture was stirred for 4 minutes, followed by discharge of said solution. The same treatment was repeated.
- 20 (c) The carrier resin was washed with 500 μ l of DMF for one minute, and the rinsings were discharged. The same treatment was repeated 5 times. The carrier resin combined with Cys(Trt) without Fmoc was thus obtained.
- 25 (d) DMF (1155 μ l) containing 330 μ mol of Fmoc-Gly-OH, 330 μ mol of PyBOP, 330 μ mol of HOBt monohydrate and 495 μ mol of NMM was stirred for 3 minutes. The resulting solution was added to the carrier resin and the mixture was stirred for 30 minutes, followed by discharge of the solution.
- 30 (e) The carrier resin was washed with 500 μ l of DMF for one minute. The same treatment was repeated 5 times. Fmoc-Gly-Cys(Trt) was thus synthesized on the carrier resin.

Subsequently, washing and deprotection steps (a)-(c) were carried out, and condensation reaction was carried out using Fmoc-Arg(Pmc)-OH in step (d), followed by washing step (e) to

synthesize Fmoc-Arg(Pmc)-Gly-Cys(Trt) on the carrier resin. Then, steps (a)-(e) were repeated to obtain the carrier resin to which a protected peptide was bound. In step (d) in the repeated procedures, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and Fmoc-Leu-OH were used in turn. Subsequently, washing and deprotection steps (a)-(c) were carried out, and the carrier resin was washed with 500 μ l of DMF for one minute. The same treatment was repeated 5 times, and the carrier resin was washed successively with methanol and butyl ether, followed by drying under reduced pressure for 12 hours to obtain the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 103.2 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack ODS-AM 30 mm I.D. x 250 mm) to give 25.4 mg of Compound 3.

Mass spectrum [FABMS]: $m/z=2648.1$ ($M+H^+$)

Amino acid analysis: Asx 1.1 (1), Glx 2.1 (2), Ser 1.9 (2), Gly 1.1 (1), Arg 2.5 (3), Thr 1.8 (2), Ala 1.1 (1), Val 0.9 (1), Met 1.0 (1), Ile 3.0 (3), Leu 2.1 (2), Lys 3.1 (3), Cys 1.2 (1)

Example 4 Synthesis of Compound 4 (SEQ ID NO: 4) (H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH)

Condensation was carried out in the same manner as in Example 1 using 60 mg of a carrier resin (Wang resin) combined with 33 μ mol of Fmoc-Gly as a starting material, and using Fmoc-Arg(Pmc)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-

Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 115.3 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack ODS-AM 30 mm I.D. x 250 mm) to give 63.3 mg of Compound 4.

Mass spectrum [FABMS]: $m/z=2545.1$ ($M+H^+$)

Amino acid analysis: Asx 1.1 (1), Glx 2.1 (2), Ser 1.9 (2), Gly 1.1 (1), Arg 2.6 (3), Thr 1.9 (2), Ala 1.1 (1), Val 0.9 (1), Met 0.9 (1), Ile 3.0 (3), Leu 2.1 (2), Lys 3.0 (3)

Example 5 Synthesis of Compound 5 (SEQ ID NO: 5) (H-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH)

The following treatments were carried out using 51.0 mg of a carrier resin (Wang resin) combined with 25 μ mol of Fmoc-Gly as a starting material by the use of a peptide synthesizer (ACT, ACT357).

- (a) The carrier resin was washed with 1 ml of DMF for 30 seconds, and the rinsings were discharged.
- (b) To the carrier resin was added 1 ml of a 25% piperidine-DMF solution and the mixture was stirred for 2 minutes, followed by discharge of said solution. To the carrier resin was added again 1 ml of a 25% piperidine-DMF solution and the mixture was stirred for 10 minutes, followed by discharge of said solution.
- (c) The carrier resin was washed with DMF for 12 seconds, followed by discharge of the rinsings. The same treatment

was repeated 7 times. The carrier resin combined with Gly without Fmoc was thus obtained.

- (d) To the carrier resin were added 125 μ l of DMF, 500 μ l of NMP solution containing 0.25 M Fmoc-Arg(Pmc)-OH and 0.25 M HOBT monohydrate, 500 μ l of DMF solution containing 0.25 M PyBOP and 125 μ l of DMF solution containing 2.0 M NMM. After stirring for 60 minutes, the solutions were discharged.
- (e) The reactor was washed with 500 μ l of DMF, and then with 1 ml of DMF for 30 seconds with stirring, followed by further washing with 500 μ l of DMF. Fmoc-Arg(Pmc)-Gly was thus synthesized on the carrier resin.

- Subsequently, washing and deprotection steps (a)-(c) were carried out, and condensation reaction was carried out using a solution containing Fmoc-Ile-OH in place of Fmoc-Arg(Pmc)-OH in step (d), followed by washing step (e) to synthesize Fmoc-Ile-Arg(Pmc)-Gly on the carrier resin. Then, condensation was carried out using, in step (d), Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH and Fmoc-Thr(t-Bu)-OH in turn. The condensation product was washed and dried to obtain 132.5 mg of the carrier resin (Wang resin) to which a side-chain-protected peptide was bound.
- Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 58.3 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack ODS-AM 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 22.6 mg of Compound 5.

Mass spectrum [FABMS]: $m/z=2029.2$ ($M+H^+$)

Amino acid analysis: Asx 1.0 (1), Glx 2.0 (2), Gly 1.0 (1), Arg 3.0 (3), Thr 1.0 (1), Ala 1.1 (1), Val 0.9 (1), Met 1.1 (1), Ile 2.9 (3), Leu 1.1 (1), Lys 2.0 (2)

Example 6 Synthesis of Compound 6 (SEQ ID NO: 6) (H-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-OH)

Condensation was carried out in the same manner as in Example 5 using 51.0 mg of a carrier resin (Wang resin) combined with 25.0 μ mol of Fmoc-Gly as a starting material, and using Fmoc-Arg(Pmc)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain 112.2 mg of the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 48.7 mg of the crude peptide. The obtained crude peptide was dissolved in 1 ml of TFA, and the resulting solution was added dropwise into 50 ml of ether. The deposited precipitate was separated by filtration and then dried to give 35.5 mg of Compound 6.

Mass spectrum [FABMS]: $m/z=1439.0$ ($M+H^+$)

Amino acid analysis: Glx 1.1 (1), Gly 1.0 (1), Arg 3.0 (3), Ala 1.1 (1), Val 0.8 (1), Ile 1.9 (2), Leu 1.0 (1), Lys 2.0 (2)

Example 7 Synthesis of Compound 7 (SEQ ID NO: 7) (H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-OH)

Condensation was carried out in the same manner as in Example 5 using 56.8 mg of a carrier resin (Wang resin) combined with 25.0 μ mol of Fmoc-Ile as a starting material, and using Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain 280.9 mg of the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide

from the resin were carried out in the same manner as in Example 1 to obtain 123.1mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack ODS-AM 30mm I.D. x 250mm) in the same manner as in Example 1 to give 26.7mg of Compound 7.

5 Mass spectrum [FABMS]: $m/z=2019.2$ ($M+H^+$)

Amino acid analysis: Asx 1.1 (1), Glx 1.1 (1), Ser 1.7 (2), Arg 2.2 (2), Thr 1.7 (2), Val 1.0 (1), Met 1.0 (1), Ile 2.1 (2), Leu 2.1 (2), Lys 3.1 (3)

Example 8 Synthesis of Compound 8 (SEQ ID NO:8) (H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-OH)

10 Condensation was carried out in the same manner as in Example 5 using 48.1mg of a carrier resin (Wang resin) combined with 25.0 μ mol of Fmoc-Arg (Pmc) as a starting material, and using Fmoc-Lys (Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu (Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and
15 Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain 79.3mg of the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 18.3mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack
20 ODS-AM 30mm I.D. x 250mm) in the same manner as in Example 1 to give 4.7mg of Compound 8.

Mass spectrum [FABMS]: $m/z=1621.0$ ($M+H^+$)

Amino acid analysis: Asx 1.0 (1), Glx 1.1 (1), Ser 1.8 (2), Arg 1.1 (1), Thr 1.9 (2), Val 0.9 (1), Met 1.0 (1), Ile 1.0 (1), Leu 2.1 (2), Lys 2.0 (2)

25 **Example 9** Synthesis of Compound 9 (SEQ ID NO:9) (H-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-OH)

Condensation was carried out in the same manner as in Example 5 using 48.1 mg of a carrier resin (Wang resin) combined with 25.0 μ mol of Fmoc-Val as a starting material, and using Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain 120.3 mg of the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1, except that a mixture of 90% TFA, 5% 1,2-ethanedithiol and 5% thioanisole was used and the standing time was 2 hours, to obtain 49.0 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (YMC, YMC-Pack ODS-AM 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 20.2 mg of Compound 9.

Mass spectrum [FABMS]: $m/z=1336.7$ ($M+H^+$)

Amino acid analysis: Asx 1.1 (1), Glx 1.1 (1), Ser 1.8 (2), Thr 1.8 (2), Val 1.0 (1), Met 1.0 (1), Ile 1.0 (1), Leu 2.1 (2), Lys 1.0 (1)

Example 10 Synthesis of Compound 10 (SEQ ID NO: 10) [H-cyclo(Cys-Leu-Ser-Thr-Ser-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys)-OH]

Condensation was carried out in the same manner as in Example 3 using 30 mg of a carrier resin (Wang resin) combined with 16.5 μ mol of Fmoc-Cys(Trt) as a starting material, and using Fmoc-Gly, Fmoc-Arg(Pmc)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Leu-OH and Fmoc-Cys(Trt)-OH in turn. The condensation product

was washed and dried to obtain the carrier resin to which a side-chain-protected peptide was bound. The reaction time for condensation with each amino acid was 60 minutes. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 54.5 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 14.0 mg of an uncyclized form of Compound 10.

Mass spectrum [FABMS]: $m/z=2751.7$ ($M+H^+$)

The obtained compound was dissolved in 10 ml of DMSO, and aqueous ammonia was added thereto to adjust the pH of the solution to 7.1, followed by stirring at room temperature for 29 hours. The resulting solution was lyophilized and then dissolved in a 2 M aqueous solution of acetic acid, followed by freeze-drying to give 13.5 mg of Compound 10.

Mass spectrum [FABMS]: $m/z=2749.5$ ($M+H^+$)

Amino acid analysis: Asx 1.2 (1), Glx 2.2 (2), Ser 2.0 (2), Gly 1.1 (1), Arg 3.0 (3), Thr 2.0 (2), Ala 1.1 (1), Val 1.0 (1), Met 1.1 (1), Ile 3.1 (3), Leu 2.2 (2), Lys 3.1 (3), Cys 1.8 (2)

Example 11 Synthesis of Compound 11 (SEQ ID NO: 11) [H-Leu-Ser-Thr-cyclo(Cys-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-Ile-Glu-Ala-Ile-Arg-Gly-Cys)-OH]

Condensation was carried out in the same manner as in Example 3 using 30 mg of a carrier resin (Wang resin) combined with 16.5 μ mol of Fmoc-Cys(Trt) as a starting material, and using Fmoc-Gly, Fmoc-Arg(Pmc)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Met-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-

Cys(Trt)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(t-Bu)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain the carrier resin to which a side-chain-protected peptide was bound. The reaction time for condensation with each amino acid was 60 minutes. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 55.2 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 9.7 mg of an uncyclized form of Compound 11.

Mass spectrum [FABMS]: $m/z=2664.5$ ($M+H^+$)

The obtained compound was dissolved in 10 ml of DMSO, and aqueous ammonia was added thereto to adjust the pH of the solution to 7.3, followed by stirring at room temperature for 20 hours. The resulting solution was lyophilized and then dissolved in a 2 M aqueous solution of acetic acid, followed by freeze-drying to give 9.9 mg of Compound 11.

Mass spectrum [FABMS]: $m/z=2662.5$ ($M+H^+$)

Amino acid analysis: Asx 0.9 (1), Glx 2.0 (2), Ser 0.9 (1), Gly 1.1 (1), Arg 3.0 (3), Thr 1.9 (2), Ala 1.1 (1), Val 0.9 (1), Met 1.0 (1), Ile 3.2 (3), Leu 2.1 (2), Lys 2.9 (3), Cys 1.5 (2)

Example 12 Synthesis of Compound 12 (SEQ ID NO: 12) [H-cyclo(Cys-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Cys)-OH]

The following treatments were carried out using 250 mg of a carrier resin (Cl-Trt resin) combined with 140 μ mol of H-Cys(Trt) as a starting material by the use of a peptide synthesizer (ACT, ACT357).

(a) The carrier resin was washed with 2.5 ml of DMF for 3 minutes, and the rinsings were discharged. The same treatment was repeated.

- (b) To the carrier resin were added 250 μ l of DMF, 1.4 ml of NMP solution containing 0.5 M Fmoc-Lys(Boc)OH and 0.5 M HOBT monohydrate and 1.4 ml of NMP solution containing 0.5 M DIPIC. After stirring for 40 minutes, the solutions were discharged.
- (c) The carrier resin was washed with 2.5 ml of DMF for one minute, and the rinsings were discharged. The same treatment was repeated twice.
- (d) To the carrier resin were added 250 μ l of DMF, 1.4 ml of NMP solution containing 0.5 M Fmoc-Lys(Boc)-OH and 0.5 M HOBT monohydrate, 1.4 ml of DMF solution containing 0.5 M HBTU, and 0.7 ml of NMP solution containing 2.0 M DIEA. After stirring for 20 minutes, the solutions were discharged.
- (e) The same treatment as in (c) was carried out. Fmoc-Lys(Boc)-Cys(Trt) was thus synthesized on the carrier resin.
- (f) To the carrier resin was added 2.5 ml of DMF containing 25% piperidine, and the resulting mixture was stirred for two minutes, followed by discharge of said solution. To the carrier resin was added again 2.5 ml of the same solution, and the resulting mixture was stirred for 10 minutes, followed by discharge of said solution.
- (g) The carrier resin was washed with 2.5 ml of DMF for one minute, and the rinsings were discharged. The same treatment was repeated 7 times. Thus, the carrier resin combined with Lys(Boc)-Cys(Trt) without Fmoc was obtained.
- Subsequently, condensation reaction was carried out using a solution containing Fmoc-Leu-OH in place of Fmoc-Lys(Boc)-OH in steps (a)-(e), followed by deprotection and washing steps (f) and (g) to synthesize Leu-Lys(Boc)-Cys(Trt) on the carrier resin. Then, steps (a)-(e) were repeated using Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Val-OH and Fmoc-Cys(Trt)-OH in turn. The condensation product was washed and

dried to obtain the carrier resin to which a side-chain-protected peptide was bound. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 using 4/5 the amount of the
 5 obtained resin to give 137.2 mg of the crude peptide. The obtained crude peptide and 100 mg of DTT were dissolved in 2 ml of DMF, and the solution was allowed to stand at 50°C for one hour. The resulting solution was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30
 10 mm I.D. X 250 mm) in the same manner as in Example 1 to give 12.2 mg of a peptide having two free SH groups.

Mass spectrum [FABMS]: $m/z=2284.3$ ($M+H^+$)

The obtained peptide was dissolved in 5 ml of a 2 M aqueous
 15 solution of acetic acid. After the resulting solution was diluted with water to 50 ml, dilute aqueous ammonia was added thereto to adjust the pH of the solution to 5.7. To the resulting mixture was added 0.5 ml of a 0.1 M aqueous solution of $K_3Fe(CN)_6$, followed by stirring at room temperature for 2.5 hours. After
 20 addition of 1 ml of acetic acid, the reaction mixture was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 7.3 mg of Compound 12.

Mass spectrum [FABMS]: $m/z=2282.5$ ($M+H^+$)

25 Amino acid analysis: Glx 1.0 (1), Ser 1.0 (1), Arg 3.9 (4), Ala 1.1 (1), Val 0.7 (1), Leu 7.2 (7), Lys 2.1 (2), Cys 2.7 (2)

Example 13 Synthesis of Compound 13 (SEQ ID NO: 13)
 30 [Biotinyl-cyclo(Cys-Val-Leu-Leu-Ser-Arg-Ala-Glu-Leu-Arg-Leu-Leu-Arg-Arg-Leu-Lys-Leu-Lys-Cys)-OH]

After 1/5 the amount of the carrier resin with the side-chain-protected peptide obtained in Example 12 was washed with 1 ml of DMF, 440 μ l of DMF suspension containing 68.4 mg
 35 of D-biotin (Nakalai Tesque, Inc.) and NMP solution containing 0.5 M DIPIC were added thereto, followed by stirring at room

temperature for 2 days. After the solutions were discharged, the carrier resin was washed and dried to obtain the resin combined with a side-chain-protected peptide having the biotinylated N-terminus. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 47.3 mg of the crude peptide. The obtained crude peptide and 50 mg of DTT were dissolved in 1 ml of DMF, and the solution was allowed to stand at 50°C for one hour. The resulting solution was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 11.8 mg of a peptide having two free SH groups.

Mass spectrum [FAB-MS]: $m/z=2510.8$ ($M+H^+$)

The obtained peptide was dissolved in 5 ml of a 2 M aqueous solution of acetic acid. After the resulting solution was diluted with water to 50 ml, dilute aqueous ammonia was added thereto to adjust the pH of the solution to 5.5. To the resulting mixture was added 0.5 ml of a 0.1 M aqueous solution of $K_3Fe(CN)_6$, followed by stirring at room temperature for 3 hours. After addition of 1 ml of acetic acid, the reaction mixture was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 3.8 mg of Compound 13.

Mass spectrum [FABMS]: $m/z=2508.8$ ($M+H^+$)

Amino acid analysis: Glx 1.1 (1), Ser 1.0 (1), Arg 3.8 (4), Ala 1.1 (1), Val 0.8 (1), Leu 6.8 (7), Lys 2.0 (2), Cys 2.1 (2)

Example 14 Synthesis of Compound 14 (SEQ ID NO: 14) (H-Leu-Ser-Thr-Cys-Lys-Thr-Ile-Asp-Met-Glu-Leu-Val-Lys-Arg-Lys-Arg-OH)

Condensation was carried out in the same manner as in Example 3 using 50 mg of a carrier resin (Wang resin) combined with 23 μ mol of Fmoc-Arg(Pmc) as a starting material, and using Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-

Val-OH, Fmoc-Leu-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Met-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Ser(tBu)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain the carrier resin to which a side-chain-protected peptide was bound. The reaction time for condensation with each amino acid was 60 minutes. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1 to obtain 59.8 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 7.7 mg of Compound 14.

Mass spectrum [FABMS]: $m/z=1921.7$ ($M+H^+$)

Amino acid analysis: Asx 1.0 (1), Glx 1.1 (1), Ser 0.9 (1), Arg 2.2 (2), Thr 1.6 (2), Val 0.9 (1), Met 1.1 (1), Ile 1.0 (1), Leu 2.1 (2), Lys 3.1 (3), Cys 1.3 (1)

Example 15 Synthesis of Compound 15 (SEQ ID NO: 15) (H-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Glu-Leu-Tyr-Gln-Lys-Tyr-Ser-Asn-Asn-Ser-Trp-Arg-OH)

Condensation was carried out in the same manner as in Example 3 using 50 mg of a carrier resin (Wang resin) combined with 23 μ mol of Fmoc-Arg(Pmc) as a starting material, and using Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH and Fmoc-Leu-OH in turn. The condensation product was washed and dried to obtain the carrier resin to which a side-chain-protected peptide was bound. The reaction time for condensation with each amino acid was 60 minutes. Removal of the side-chain-protecting groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1, except that a mixture of the same

composition as in Example 1 additionally containing 5 mg/ml 2-methylindole was used and the standing time was 6 hours, to obtain 20.9 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. x 250 mm) in the same manner as in Example 1 to give 7.8 mg of Compound 15.

Mass spectrum [FABMS]: $m/z=2663.5$ ($M+H^+$)

Amino acid analysis: Asx 2.0 (2), Glx 3.9 (4), Ser 2.0 (2), His 1.1 (1), Arg 1.1 (1), Tyr 2.2 (2), Val 1.8 (2), Leu 3.0 (3), Lys 3.0 (3)

Example 16 Synthesis of Compound 16 (SEQ ID NO: 16)

(Biotinyl-Gly-Arg-Arg-Leu-Lys-Leu-Lys-Val-Glu-Gln-His-Val-Glu-Leu-Tyr-Gln-Lys-Tyr-Ser-Asn-Asn-Ser-Trp-Arg-OH)

Condensation was carried out in the same manner as in Example 3 using 30 mg of a carrier resin (Wang resin) combined with 13.8 μ mol of Fmoc-Arg(Pmc) as a starting material, and using Fmoc-Trp(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Leu-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH and Fmoc-Gly-OH in turn.

To the condensation product was added a solution which had been previously prepared by adding 562 μ l of DMF containing 0.5 M HBTU and 0.5 M HOBt monohydrate and 562 μ l of DMF containing 1 M DIEA to 61.9 mg of D-biotin (Nakalai Tesque, Inc.), and by stirring the mixture at room temperature for 5 minutes, and the resulting mixture was stirred for 4 hours. After the solution was discharged, the product was washed and dried to obtain the carrier resin to which a side-chain protected peptide having the biotinylated N-terminus was bound. In the condensation of an amino acid in step (d), HBTU was used in place of PyBOP and DIEA was used in place of NMM. Removal of the side-chain-protecting

groups and cleavage of the peptide from the resin were carried out in the same manner as in Example 1, except that a mixture of the same composition as in Example 1 additionally containing 5 mg/ml 2-methylindole was used and the standing time was 6 hours, to obtain 44.2 mg of the crude peptide. The obtained crude peptide was purified by HPLC using a reversed-phase column (Shiseido Co., Ltd., CAPCELL PAK C18 30 mm I.D. 250 mm) in the same manner as in Example 1 to give 10.3 mg of Compound 16.

Mass spectrum [FABMS]: $m/z=3260.0$ ($M+H^+$)

Amino acid analysis: Asx 2.1 (2), Glx 4.1 (4), Ser 2.0 (2), Gly 1.0 (1), His 1.0 (1), Arg 2.8 (3), Tyr 2.1 (2), Val 1.9 (2), Leu 3.0 (3), Lys 2.9 (3)

Example 17 Conversion of Compound 10 into acetate

Compound 10 obtained according to the process of Example 10 (22.1 mg) was dissolved in 5 ml of a 1% aqueous solution of acetic acid, and the solution was passed through an anion exchange column (Asahi Chemical Industry Co., Ltd., Asahi Pack ES-502N 21.5 mm I.D. x 100 mm) to remove TFA and convert the compound into acetate. Elution of the peptide was carried out with a 1% aqueous solution of acetic acid, followed by detection at 220 nm. The eluate was lyophilized to give 20.0 mg of acetate of Compound 10.

Example 18 Conversion of Compound 12 into acetate

Compound 12 obtained according to the process of Example 12 (17.5 mg) was dissolved in 5 ml of a 1% aqueous solution of acetic acid, and the solution was passed through an anion exchange column (Asahi Chemical Industry Co., Ltd., Asahi Pack ES-502N 21.5 mm I.D. x 100 mm) to remove TFA and convert the compound into acetate. Elution of the peptide was carried out with a 1% aqueous solution of acetic acid, followed by detection at 220 nm. The eluate was lyophilized to give 13.0 mg of acetate of Compound 12.

The biological activities of Compounds (I) and the screening methods are described by the following Examples.

Example 19 Activity to release active TGF- β from latent TGF- β examined by measuring the degree of binding of TGF- β to bovine vascular smooth muscle cells, porcine vascular smooth muscle cells, bovine capillary endothelial cells or bovine aortic endothelial cells

10 (1-1) ^{125}I -Labeling of LLTGF- β

LLTGF- β isolated and purified from human platelets by the method of Okada, et al. [Journal of Biochemistry, 106, 304 (1989)] was ^{125}I -labeled according to the chloramine T method [Molecular & Cellular Biology, 2, 599 (1982)] in the following manner.

15 To 2 μg of LLTGF- β were added 25 μl of 1 M K-phosphate buffer (pH 7.5), 37 MBq of Na- ^{125}I (DuPont NEN) and 10 μl of chloramine-T (0.5 mg/100 μl in 0.05 M K-phos. buffer, Wako Pure Chemical Industries, Ltd.), followed by stirring at room temperature for 40 seconds. After 15 μl of $\text{Na}_2\text{S}_2\text{O}_5$ (1 mg/100
20 μl in 0.05 M K-phos. buffer) was added, the mixture was stirred at room temperature for 5 seconds, followed by addition of 100 μl of an aqueous solution of tyrosine (1 mg/ml). The reaction mixture was applied to a Sephadex G25 column and the eluate was taken in 500 μl fractions. Each fraction was subjected to
25 measurement of radioactivity using a γ -counter (ARC-2000, Aloka Co., Ltd.). The fractions containing labeled protein were selected for use in the experiment.

(1-2) Preparation of cells

30 The cells to be used in the experiment were obtained according to the method of Sato, et al. [Journal of Cell Biology, 123, 1249 (1993)]. Bovine vascular smooth muscle cells (BSMC) and porcine vascular smooth muscle cells (PSMC) were isolated from bovine aorta and porcine aorta, respectively, and cultured
35 by the explant method [Journal of Cell Biology, 50, 172 (1971)]. Bovine capillary endothelial cells (BCEC) were isolated from

bovine adrenal capillary and cultured. Bovine aortic endothelial cells (BAEC) were isolated from bovine aortic and cultured.

5 (1-3) Measurement of the degree of TGF- β binding

The following procedure was carried out according to the method of Sato, et al. [Journal of Cell Biology, 123, 1249 (1993)].

10 BSMC, PSMC and BCEC, isolated and cultured in the above-described manner were respectively put into 35 mm dishes in an amount of 4×10^4 cells/dish. On the next day, the medium was replaced by Dulbecco's Modified Eagle's Medium (DMEM, Nissui Pharmaceutical Co.) containing 0.1% bovine serum albumin (BSA), followed by incubation for 5 hours. After the cells were washed
15 with 2 ml of phosphate-buffered saline containing 0.1 g/l each of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and CaCl_2 [hereinafter sometimes referred to as PBS(+); phosphate-buffered saline containing neither $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ nor CaCl_2 is referred to as PBS(-)], the medium was replaced by 1 ml of ice-cold DMEM containing 0.1% BSA, 2 ng/ml ^{125}I -LLTGF- β and a test compound, followed by incubation at 4°C for 3 hours. The test compound was added as a solution in dimethyl sulfoxide (DMSO) to give a final DMSO concentration in the system of 0.1%. After being washed three times with 2 ml of ice-cold PBS(+), the cells were lysed with 900 μl of 0.5% Triton X-100 (room
20 temperature, 0.5-1 hour), and 800 μl of the lysate was subjected to measurement of radioactivity using a γ -counter.
25

The results are shown in Figs. 1 and 2 and Tables 3 to 6.

Table 3

Test group	Radioactivity (cpm), n=4
Vehicle	163.1 $\pm$ 28.3
Cell free	70.6 $\pm$ 10.2
Compound 12	2739.9 $\pm$ 182.8

30

Table 3 shows the degree of binding of active TGF- β to bovine capillary endothelial cells (BCEC) as determined by measuring

the radioactivity. "Vehicle" lane shows the radioactivity when a solution without a test compound was added, "cell free" lane shows the radioactivity when ^{125}I -LLTGF- β alone was added to a plate without a cell, and the other lane shows the radioactivity when the test compound was added at a concentration of 30 $\mu\text{g}/\text{ml}$.

5

Table 4

Test group	Radioactivity (cpm), n=4
Vehicle	416.8 $\pm$ 89.6
Cell free	70.6 $\pm$ 10.2
Compound 14	3369.9 $\pm$ 166.8
Compound 15	5621.1 $\pm$ 889.2

Table 4 shows the degree of binding of active TGF- β to bovine capillary endothelial cells (BCEC) as determined by measuring the radioactivity. "Vehicle" lane shows the radioactivity when a solution without a test compound was added, "cell free" lane shows the radioactivity when ^{125}I -LLTGF- β alone was added to a plate without a cell, and the other lanes show the radioactivity when the respective test compound was added at a concentration of 100 $\mu\text{g}/\text{ml}$.

Table 5

Test group	Radioactivity (cpm), n=4
Vehicle	1076.5 $\pm$ 12.0
Compound 13	4704.0 $\pm$ 939.9

Table 5 shows the degree of binding of active TGF- β to bovine aortic endothelial cells (BAEC) as determined by measuring the radioactivity. "Vehicle" lane shows the radioactivity when a solution without a test compound was added, and the other lane shows

the radioactivity when the test compound was added at a concentration of 100 μ g/ml.

Table 6

Test group	Radioactivity (cpm), n=4)
Vehicle	792.0 $\pm$ 107.0
Compound 16	10495.0 $\pm$ 803.0

Table 6 shows the degree of binding of active TGF- β to bovine aortic endothelial cells (BAEC) as determined by measuring the radioactivity. "Vehicle" lane shows the radioactivity when a solution without a test compound was added, and the other lane shows the radioactivity when the test compound was added at a concentration of 100 μ g/ml.

As shown in Figs. 1 and 2 and Tables 3 to 6, the degree of binding of TGF- β to cells was increased and the release of active TGF- β from latent TGF- β was promoted by Compounds 1 to 3, 5 to 9 and 13 to 16 at a concentration of 100 μ g/ml and Compound 12 at a concentration of 30 μ g/ml.

Compounds promoting the release of active TGF- β from latent TGF- β and increasing the degree of binding of TGF- β to cells can be obtainable by the method of the present invention.

Example 20 Activity to release active TGF- β from latent TGF- β examined by measuring the degree of migration of bovine vascular endothelial cells.

The following procedure was carried out according to the method of Sato *et al.* [Journal of Cell Biology, 1249 (1993)].

BCEC were cultured in a 35mm dish to make a confluent layer and then the medium was replaced by DMEM containing 0.1% BSA, followed by incubation for 5 hours. After a part of the cells were scraped with a razor, the remaining cells were washed with PBS(-), followed by incubation in DMEM containing 0.1% BSA and a test compound. After 24 hours, the number of the cells which migrated in four fields of a microscope (IX70, Olympus) was counted for each dish. The test compound was added in the same manner as in Example 19. The results are shown in Figs. 3 to 5. The migration of cells was inhibited and the release of active TGF- β from latent TGF- β was promoted by Compounds 3 and 5 to 9 at a concentration of 50 μ g/ml. The migration of cells was also inhibited by Compound 4 at a concentration of 100 μ g/ml, and Compound 10 showed the migration inhibitory effect even at 10 μ g/ml.

Compounds promoting the release of active TGF- β from latent TGF- β and increasing the degree of binding of TGF- β to cells can be screened by the method of the present invention.

15 Example 21 Determination of active TGF- β by luciferase assay

The amount of active TGF- β was determined by measuring the luminescence of mink lung epithelial cells (MLEC) carrying the luciferase gene introduced downstream of the PAI-I promoter by luciferase assay system (Promega) according to the method of Abe, *et al.* [Analytical Biochemistry, 216, 276 (1994)] as described below.

20 BCEC were cultured in a 24-well plate to make a confluent layer and then the medium was replaced by DMEM containing 0.1% BSA. After 6 hours, the cells were scraped reticulately with a comb and the remaining cells were washed with PBS(-), followed by incubation in DMEM containing 0.1% BSA and a test compound for 24 hours. The resulting culture supernatant was taken as a sample. Six hours before the sampling of this culture supernatant, the abovementioned MLEC were put into wells of a 96-well plate in an amount of 2.8×10^4 cells/well. After the cells were cultured in DMEM containing 10% fetal calf serum (FCS) for 6 hours, the medium was replaced by the above culture supernatant sample, followed by incubation for 16 hours. The cells were washed twice with PBS(-) and then subjected to

measurement of active TGF- β concentration by luciferase assay system. The results are shown in Fig. 6. Active TGF- β was released by the addition of Compounds 3 and 10. Particularly, Compound 10 showed a remarkable effect even at a concentration of 10 μ g/ml.

Compounds promoting the release of active TGF- β from latent TGF- β and increasing the degree of binding of TGF- β to cells can be screened by the method of the present invention.

10 Industrial Applicability

The present invention provides novel peptides having an activity to promote the activation of latent TGF- β by enhancing the binding of latent TGF- β to a cell membrane, and pharmaceutically acceptable salts thereof.

According to the methods of screening compounds of the present invention, compounds having an activity to promote the release of active TGF- β from latent TGF- β or an activity to promote the binding of latent TGF- β to a cell membrane can be selected.

The compounds obtained by the screening methods of the present invention, Compounds (I) and pharmaceutically acceptable salts thereof have inhibiting activity or promoting activity on the binding of latent TGF- β to cells or on the conversion of latent TGF- β into TGF- β . Thus, they are useful for the treatment or prevention of diseases such as cancer, diabetic retinopathy, atherosclerosis, bone fracture, myocardial infarction, myocardial disorder after ischemia reperfusion, cerebral infarction, retinal detachment, glomerulonephritis, diabetic nephropathy, renal graft rejection, HIV nephropathy, sudden pulmonary fibrosis, autoimmune pulmonary fibrosis, hepatic cirrhosis, venous constrictive hepatopathy (often occurring after treatments of cancer), systemic sclerosis, keloid, eosinophilia-muscle ache syndrome, re-stricture after angioplasty, intraocular fibrosis, rheumatic arthritis and nasal polyp.

Page(s) 96 are claims pages
they appear after the sequence listing

(1) GENERAL INFORMATION

APPLICANT

KYOWA HAKKO KOGYO CO. LTD.

TITLE OF INVENTION

PEPTIDES WHICH PROMOTE ACTIVATION OF LATENT TGF- β AND METHOD OF SCREENING TGF- β
ACTIVITY-REGULATING COMPOUNDS.

FILE REFERENCE

1060

CURRENT FILING DATE

1998. 5. 12

PRIOR APPLICATION NUMBER

JP 120683/97

PRIOR APPLICATION FILING DATE

1997. 5. 12

NUMBER OF SEQUENCES

34

(2) INFORMATION FOR SEQUENCES

INFORMATION FOR SEQ ID NO:1:

LENGTH: 23

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Gln Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Ser Phe

1 5 10 15

Ser Ser Thr Glu Lys Asn Cys

20

INFORMATION FOR SEQ ID NO:2:

LENGTH: 24

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Pro Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Arg Leu Lys

1 5 10 15

Leu Lys Val Glu Gln His Val Cys

20 25

INFORMATION FOR SEQ ID NO:3:

LENGTH: 23

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg

1 5 10 15

Ile Glu Ala Ile Arg Gly Cys

20

INFORMATION FOR SEQ ID NO:4:

LENGTH: 23

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg

1

5

10

15

Ile Glu Ala Ile Arg Gly

20

INFORMATION FOR SEQ ID NO:5:

LENGTH: 17

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Ala Ile Arg

1

5

10

15

Gly

INFORMATION FOR SEQ ID NO:6:

LENGTH: 12

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Val Lys Arg Lys Arg Ile Glu Ala Ile Arg Gly

1

5

10

INFORMATION FOR SEQ ID NO:7:

LENGTH: 17

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg

1 5 10 15
Ile

INFORMATION FOR SEQ ID NO:8:

LENGTH: 14

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val Lys Arg

1 5 10 15

INFORMATION FOR SEQ ID NO:9:

LENGTH: 12

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val

1 5 10

INFORMATION FOR SEQ ID NO:10:

LENGTH: 24

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: disulfide-bonds

LOCATION: 1 and 24

SEQUENCE DESCRIPTION:

Cys Leu Ser Thr Ser Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys

1 5 10 15

Arg Ile Glu Ala Ile Arg Gly Cys

20 25

INFORMATION FOR SEQ ID NO:11:

LENGTH: 23

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: disulfide-bonds

LOCATION: 4 and 23

SEQUENCE DESCRIPTION:

Leu Ser Thr Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg

1 5 10 15

Ile Glu Ala Ile Arg Gly Cys

20

INFORMATION FOR SEQ ID NO:12

LENGTH: 19

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: disulfide-bond

LOCATION: 1 and 19

SEQUENCE DESCRIPTION:

Cys Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Arg Leu Lys

1 5 10 15

Leu Lys Cys

20

INFORMATION FOR SEQ ID NO:13:

LENGTH: 19

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: disulfide-bond

LOCATION: 1 and 19

NAME/KEY: modified site

LOCATION: 1

OTHER INFORMATION: Xaa represents N^α-biotinyl-L-systeine

SEQUENCE DESCRIPTION:

Xaa Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Arg Leu Lys

1 5 10 15

Leu Lys Cys

20

INFORMATION FOR SEQ ID NO:14:

LENGTH: 16

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Ser Thr Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg

1 5 10 15

INFORMATION FOR SEQ ID NO:15:

LENGTH: 21

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Leu Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser

1 5 10 15

Asn Asn Ser Trp Arg

20

INFORMATION FOR SEQ ID NO:16:

LENGTH: 24

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: modified site

LOCATION: 1

OTHER INFORMATION: Xaa represents N^α-biotinyl-glycine

SEQUENCE DESCRIPTION:

Xaa Arg Arg Leu Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln

1 5 10 15

Lys Tyr Ser Asn Asn Ser Trp Arg

20

25

INFORMATION FOR SEQ ID NO:17:

LENGTH: 391

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Pro Leu Leu

1 5 10 15

Trp Leu Leu Val Leu Thr Pro Gly Pro Pro Ala Ala Gly Leu Ser Thr

20

25

30

Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Ala

35

40

45

Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser

50	55	60
Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala Leu		
65	70	75
Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu Pro Glu		
85	90	95
Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu		
100	105	110
Met Val Glu Thr His Asn Glu Ile Tyr Asp Lys Phe Lys Gln Ser Thr		
115	120	125
His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu Leu Arg Glu Ala Val		
130	135	140
Pro Glu Pro Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Arg		
145	150	155
Leu Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser		
165	170	175
Asn Asn Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp		
180	185	190
Ser Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp		
195	200	205
Leu Ser Arg Gly Gly Glu Ile Glu Gly Phe Arg Leu Ser Ala His Cys		
210	215	220
Ser Cys Asp Ser Arg Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe		
225	230	235
Thr Thr Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg		
245	250	255
Pro Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu		
260	265	270
Gln Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser		
275	280	285
Ser Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg		
290	295	300
Lys Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala		
305	310	315
Asn Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln		
325	330	335
Tyr Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser		

340 345 350
 Ala Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val
 355 360 365
 Tyr Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile
 370 375 380
 Val Arg Ser Cys Lys Cys Ser
 385 390

INFORMATION FOR SEQ ID NO:18:

LENGTH: 414

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met His Tyr Cys Val Leu Ser Ala Phe Leu Ile Leu His Leu Val Thr
 1 5 10 15
 Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met Asp Gln Phe
 20 25 30
 Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu
 35 40 45
 Lys Leu Thr Ser Pro Pro Glu Asp Tyr Pro Glu Pro Glu Glu Val Pro
 50 55 60
 Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr Arg Asp Leu Leu Gln Glu
 65 70 75 80
 Lys Ala Ser Arg Arg Ala Ala Ala Cys Glu Arg Glu Arg Ser Asp Glu
 85 90 95
 Glu Tyr Tyr Ala Lys Glu Val Tyr Lys Ile Asp Met Pro Pro Phe Phe
 100 105 110
 Pro Ser Glu Asn Ala Ile Pro Pro Thr Phe Tyr Arg Pro Tyr Phe Arg
 115 120 125
 Ile Val Arg Phe Asp Val Ser Ala Met Glu Lys Asn Ala Ser Asn Leu
 130 135 140
 Val Lys Ala Glu Phe Arg Val Phe Arg Leu Gln Asn Pro Lys Ala Arg
 145 150 155 160
 Val Pro Glu Gln Arg Ile Glu Leu Tyr Gln Ile Leu Lys Ser Lys Asp
 165 170 175

Leu Thr Ser Pro Thr Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr
 180 185 190
 Arg Ala Glu Gly Glu Trp Leu Ser Phe Asp Val Thr Asp Ala Val His
 195 200 205
 Glu Trp Leu His His Lys Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu
 210 215 220
 His Cys Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro
 225 230 235 240
 Asn Lys Ser Glu Glu Leu Glu Ala Arg Phe Ala Gly Ile Asp Gly Thr
 245 250 255
 Ser Thr Tyr Thr Ser Gly Asp Gln Lys Thr Ile Lys Ser Thr Arg Lys
 260 265 270
 Lys Asn Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu Leu Pro Ser
 275 280 285
 Tyr Arg Leu Glu Ser Gln Gln Thr Asn Arg Arg Lys Lys Arg Ala Leu
 290 295 300
 Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn Cys Cys Leu Arg
 305 310 315 320
 Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp Lys Trp Ile His
 325 330 335
 Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly Ala Cys Pro Tyr
 340 345 350
 Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu Ser Leu Tyr Asn
 355 360 365
 Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Ser Gln Asp
 370 375 380
 Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys Thr Pro Lys Ile
 385 390 395 400
 Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys Cys Ser
 405 410 415

INFORMATION FOR SEQ ID NO:19:

LENGTH: 412

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Lys Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn
 1 5 10 15
 Phe Ala Thr Val Ser Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe
 20 25 30
 Gly His Ile Lys Lys Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu
 35 40 45
 Ser Lys Leu Arg Leu Thr Ser Pro Pro Glu Pro Thr Val Met Thr His
 50 55 60
 Val Pro Tyr Gln Val Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu
 65 70 75 80
 Glu Glu Met His Gly Glu Arg Glu Glu Gly Cys Thr Gln Glu Asn Thr
 85 90 95
 Glu Ser Glu Tyr Tyr Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln
 100 105 110
 Gly Leu Ala Glu His Asn Glu Leu Ala Val Cys Pro Lys Gly Ile Thr
 115 120 125
 Ser Lys Val Phe Arg Phe Asn Val Ser Ser Val Glu Lys Asn Arg Thr
 130 135 140
 Asn Leu Phe Arg Ala Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser
 145 150 155 160
 Ser Lys Arg Asn Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro
 165 170 175
 Asp Glu His Ile Ala Lys Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro
 180 185 190
 Thr Arg Gly Thr Ala Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val
 195 200 205
 Arg Glu Trp Leu Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser
 210 215 220
 Ile His Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu
 225 230 235 240
 Asn Ile His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu
 245 250 255
 Asp Asp His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp
 260 265 270
 His His Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg Leu

275	280	285
Asp Asn Pro Gly Gln Gly	Gly Gln Arg Lys Lys	Arg Ala Leu Asp Thr
290	295	300
Asn Tyr Cys Phe Arg Asn	Leu Glu Glu Asn Cys Cys	Val Arg Pro Leu
305	310	315
Tyr Ile Asp Phe Arg Gln	Asp Leu Gly Trp Lys Trp	Val His Glu Pro
325	330	335
Lys Gly Tyr Tyr Ala Asn	Phe Cys Ser Gly Pro Cys	Pro Tyr Leu Arg
340	345	350
Ser Ala Asp Thr Thr His	Ser Thr Val Leu Gly Leu	Tyr Asn Thr Leu
355	360	365
Asn Pro Glu Ala Ser Ala	Ser Pro Cys Cys Val Pro	Gln Asp Leu Glu
370	375	380
Pro Leu Thr Ile Leu Tyr	Tyr Val Gly Arg Thr Pro	Lys Val Glu Gln
385	390	395
Leu Ser Asn Met Val Val	Lys Ser Cys Lys Cys Ser	
405	410	

INFORMATION FOR SEQ ID NO:20:

LENGTH: 390

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu	Arg Leu Leu Pro Leu Leu	Pro Leu Pro
1	5	10
Trp Leu Leu Val Leu Thr	Pro Gly Arg Pro Ala Ala	Gly Leu Ser Thr
20	25	30
Cys Lys Thr Ile Asp Met	Glu Leu Val Lys Arg Lys	Arg Ile Glu Ala
35	40	45
Ile Arg Gly Gln Ile Leu	Ser Lys Leu Arg Leu Ala	Ser Pro Pro Ser
50	55	60
Gln Gly Glu Val Pro Pro	Gly Pro Leu Pro Glu Ala	Val Leu Ala Leu
65	70	75
Tyr Asn Ser Thr Arg Asp	Arg Val Ala Gly Glu Ser	Ala Asp Pro Glu
85	90	95

Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu
 100 105 110
 Met Val Asp Arg Asn Asn Ala Ile Tyr Glu Lys Thr Lys Asp Ile Ser
 115 120 125
 His Ser Ile Tyr Met Phe Phe Asn Thr Ser Asp Ile Arg Glu Ala Val
 130 135 140
 Pro Glu Pro Pro Leu Leu Ser Arg Ala Glu Leu Arg Leu Gln Arg Leu
 145 150 155 160
 Lys Ser Ser Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn
 165 170 175
 Asn Ser Trp Arg Tyr Leu Gly Asn Arg Leu Leu Thr Pro Thr Asp Thr
 180 185 190
 Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu
 195 200 205
 Asn Gln Gly Asp Gly Ile Gln Gly Phe Arg Phe Ser Ala His Cys Ser
 210 215 220
 Cys Asp Ser Lys Asp Asn Lys Leu His Val Glu Ile Asn Gly Ile Ser
 225 230 235 240
 Pro Lys Arg Arg Gly Asp Leu Gly Thr Ile His Asp Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu His
 260 265 270
 Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr
 325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ser Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380

Arg Ser Cys Lys Cys Ser

385 390

INFORMATION FOR SEQ ID NO:21:

LENGTH: 414

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met His Tyr Cys Val Leu Ser Thr Phe Leu Leu Leu His Leu Val Pro

1 5 10 15

Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met Asp Gln Phe

20 25 30

Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu

35 40 45

Lys Leu Thr Ser Pro Pro Glu Asp Tyr Pro Glu Pro Asp Glu Val Pro

50 55 60

Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr Arg Asp Leu Leu Gln Glu

65 70 75 80

Lys Ala Ser Arg Arg Ala Ala Ala Cys Glu Arg Glu Arg Ser Glu Gln

85 90 95

Glu Tyr Tyr Ala Lys Glu Val Tyr Lys Ile Asp Met Pro Ser His Leu

100 105 110

Pro Ser Glu Asn Ala Ile Pro Pro Thr Phe Tyr Arg Pro Tyr Phe Arg

115 120 125

Ile Val Arg Phe Asp Val Ser Thr Met Glu Lys Asn Ala Ser Asn Leu

130 135 140

Val Lys Ala Glu Phe Arg Val Phe Arg Leu Gln Asn Pro Lys Ala Arg

145 150 155 160

Val Ala Glu Gln Arg Ile Glu Leu Tyr Gln Ile Leu Lys Ser Lys Asp

165 170 175

Leu Thr Ser Pro Thr Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr

180 185 190

Arg Ala Glu Gly Glu Trp Leu Ser Phe Asp Val Thr Asp Ala Val Gln

195 200 205

Glu Trp Leu His His Lys Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu

210	215	220	
His Cys Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro			
225	230	235	240
Asn Lys Ser Glu Glu Leu Glu Ala Arg Phe Ala Gly Ile Asp Gly Thr			
245	250	255	
Ser Thr Tyr Ala Ser Gly Asp Gln Lys Thr Ile Lys Ser Thr Arg Lys			
260	265	270	
Lys Thr Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu Leu Pro Ser			
275	280	285	
Tyr Arg Leu Glu Ser Gln Gln Ser Ser Arg Arg Lys Lys Arg Ala Leu			
290	295	300	
Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn Cys Cys Leu Arg			
305	310	315	320
Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp Lys Trp Ile His			
325	330	335	
Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly Ala Cys Pro Tyr			
340	345	350	
Leu Trp Ser Ser Asp Thr Gln His Thr Lys Val Leu Ser Leu Tyr Asn			
355	360	365	
Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Ser Gln Asp			
370	375	380	
Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Asn Thr Pro Lys Ile			
385	390	395	400
Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys Cys Ser			
405	410	415	

INFORMATION FOR SEQ ID NO:22:

LENGTH: 410

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn Leu Ala

1

5

10

15

Thr Ile Ser Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe Gly His

20

25

30

Ile Lys Lys Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys
 35 40 45
 Leu Arg Leu Thr Ser Pro Pro Glu Pro Ser Val Met Thr His Val Pro
 50 55 60
 Tyr Gln Val Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu Glu Glu
 65 70 75 80
 Met His Gly Glu Arg Glu Glu Gly Cys Thr Gln Glu Thr Ser Glu Ser
 85 90 95
 Glu Tyr Tyr Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln Gly Leu
 100 105 110
 Ala Glu His Asn Glu Leu Ala Val Cys Pro Lys Gly Ile Thr Ser Lys
 115 120 125
 Val Phe Arg Phe Asn Val Ser Ser Val Glu Lys Asn Gly Thr Asn Leu
 130 135 140
 Phe Arg Ala Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser Ser Lys
 145 150 155 160
 Arg Thr Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro Asp Glu
 165 170 175
 His Ile Ala Lys Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro Thr Arg
 180 185 190
 Gly Thr Ala Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val Arg Glu
 195 200 205
 Trp Leu Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser Ile His
 210 215 220
 Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu Asn Val
 225 230 235 240
 His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu Asp Asp
 245 250 255
 His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp His His
 260 265 270
 Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg Leu Asp Ser
 275 280 285
 Pro Gly Gln Gly Ser Gln Arg Lys Lys Arg Ala Leu Asp Thr Asn Tyr
 290 295 300
 Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys Val Arg Pro Leu Tyr Ile
 305 310 315 320

Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp Val His Glu Pro Lys Gly
 325 330 335
 Tyr Tyr Ala Asn Phe Cys Ser Gly Pro Cys Pro Tyr Leu Arg Ser Ala
 340 345 350
 Asp Thr Thr His Ser Thr Val Leu Gly Leu Tyr Asn Thr Leu Asn Pro
 355 360 365
 Glu Ala Ser Ala Ser Pro Cys Cys Val Pro Gln Asp Leu Glu Pro Leu
 370 375 380
 Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro Lys Val Glu Gln Leu Ser
 385 390 395 400
 Asn Met Val Val Lys Ser Cys Lys Cys Ser
 405 410

INFORMATION FOR SEQ ID NO:23:

LENGTH: 390.

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Pro
 1 5 10 15
 Trp Leu Leu Val Leu Thr Pro Gly Arg Pro Ala Ala Gly Leu Ser Thr
 20 25 30
 Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Ala
 35 40 45
 Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser
 50 55 60
 Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala Leu
 65 70 75 80
 Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Asp Pro Glu
 85 90 95
 Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu
 100 105 110
 Met Val Asp Arg Asn Asn Ala Ile Tyr Asp Lys Thr Lys Asp Ile Thr
 115 120 125
 His Ser Ile Tyr Met Phe Phe Asn Thr Ser Asp Ile Arg Glu Ala Val

130 135 140
 Pro Glu Pro Pro Leu Leu Ser Arg Ala Glu Leu Arg Leu Gln Arg Phe
 145 150 155 160
 Lys Ser Thr Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn
 165 170 175
 Asn Ser Trp Arg Tyr Leu Gly Asn Arg Leu Leu Thr Pro Thr Asp Thr
 180 185 190
 Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu
 195 200 205
 Asn Gln Gly Asp Gly Ile Gln Gly Phe Arg Phe Ser Ala His Cys Ser
 210 215 220
 Cys Asp Ser Lys Asp Asn Val Leu His Val Glu Ile Asn Gly Ile Ser
 225 230 235 240
 Pro Lys Arg Arg Gly Asp Leu Gly Thr Ile His Asp Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu His
 260 265 270
 Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr
 325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ser Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380
 Arg Ser Cys Lys Cys Ser
 385 390

INFORMATION FOR SEQ ID NO:24:

LENGTH: 412

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Lys Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn

1 5 10 15

Leu Ala Thr Val Ser Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe

20 25 30

Gly His Ile Lys Lys Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu

35 40 45

Ser Lys Leu Arg Leu Thr Ser Pro Pro Glu Pro Ser Val Met Thr His

50 55 60

Val Pro Tyr Gln Val Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu

65 70 75 80

Glu Glu Met His Gly Glu Arg Glu Glu Gly Cys Thr Gln Glu Thr Ser

85 90 95

Glu Ser Glu Tyr Tyr Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln

100 105 110

Gly Leu Ala Glu His Asn Glu Leu Ala Val Cys Pro Lys Gly Ile Thr

115 120 125

Ser Lys Val Phe Arg Phe Asn Val Ser Ser Val Glu Lys Asn Gly Thr

130 135 140

Asn Leu Phe Arg Ala Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser

145 150 155 160

Ser Lys Arg Thr Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro

165 170 175

Asp Glu His Ile Ala Lys Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro

180 185 190

Thr Arg Gly Thr Ala Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val

195 200 205

Arg Glu Trp Leu Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser

210 215 220

Ile His Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu

225 230 235 240

Asn Val His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu

245 250 255

Asp Asp His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp
260 265 270

His His Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg Leu
275 280 285

Asp Ser Pro Gly Gln Gly Gly Gln Arg Lys Lys Arg Ala Leu Asp Thr
290 295 300

Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys Val Arg Pro Leu
305 310 315 320

Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp Val His Glu Pro
325 330 335

Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly Pro Cys Pro Tyr Leu Arg
340 345 350

Ser Ser Asp Thr Thr His Ser Thr Val Leu Gly Leu Tyr Asn Thr Leu
355 360 365

Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Pro Gln Asp Leu Glu
370 375 380

Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro Lys Val Glu Gln
385 390 395 400

Leu Ser Asn Met Val Val Lys Ser Cys Lys Cys Ser
405 410

INFORMATION FOR SEQ ID NO:25:

LENGTH: 315

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Ala Ile Leu Ala Leu Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu
1 5 10 15

Ser Ala Glu Thr Glu Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu
20 25 30

Val Thr Arg Val Leu Met Val Glu Tyr Gly Asn Lys Ile Tyr Asp Lys
35 40 45

Met Lys Ser Ser Ser His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu
50 55 60

Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu Ser Arg Ala Asp Val

65	70	75	80
Arg Leu Leu Arg Leu Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr			
85	90	95	
Gln Lys Tyr Ser Asn Asn Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu			
100	105	110	
Ala Pro Ser Asp Ser Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val			
115	120	125	
Val Arg Gln Trp Leu Thr Arg Arg Glu Glu Ile Glu Gly Phe Arg Leu			
130	135	140	
Ser Ala His Cys Ser Cys Asp Ser Lys Asp Asn Thr Leu Gln Val Asp			
145	150	155	160
Ile Asn Gly Phe Ser Ser Gly Arg Arg Gly Asp Leu Ala Thr Ile His			
165	170	175	
Gly Met Asn Arg Pro Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg			
180	185	190	
Ala Gln His Leu His Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn			
195	200	205	
Tyr Cys Phe Ser Ser Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr			
210	215	220	
Ile Asp Phe Arg Lys Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys			
225	230	235	240
Gly Tyr His Ala Asn Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser			
245	250	255	
Leu Asp Thr Gln Tyr Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn			
260	265	270	
Pro Gly Ala Ser Ala Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro			
275	280	285	
Leu Pro Ile Val Tyr Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu			
290	295	300	
Ser Asn Met Ile Val Arg Ser Cys Lys Cys Ser			
305	310	315	

INFORMATION FOR SEQ ID NO:26:

LENGTH: 390

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Leu
 1 5 10 15
 Trp Leu Leu Val Leu Thr Pro Gly Arg Pro Ala Ala Gly Leu Ser Thr
 20 25 30
 Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Ala
 35 40 45
 Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser
 50 55 60
 Gln Gly Asp Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala Leu
 65 70 75 80
 Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Val Glu Pro Glu
 85 90 95
 Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu
 100 105 110
 Met Leu Glu Ser Gly Asn Gln Ile Tyr Asp Lys Phe Lys Gly Thr Pro
 115 120 125
 His Ser Leu Tyr Met Leu Phe Asn Thr Ser Glu Leu Arg Glu Ala Val
 130 135 140
 Pro Glu Pro Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Leu
 145 150 155 160
 Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn
 165 170 175
 Asp Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp Ser
 180 185 190
 Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu
 195 200 205
 Thr Arg Arg Glu Ala Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser
 210 215 220
 Cys Asp Ser Lys Asp Asn Thr Leu His Val Glu Ile Asn Gly Phe Asn
 225 230 235 240
 Ser Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu His
 260 265 270

Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr
 325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380

Arg Ser Cys Lys Cys Ser
 385 390

INFORMATION FOR SEQ ID NO:27:

LENGTH: 409

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn Phe Ala
 1 5 10 15
 Thr Val Ser Leu Ser Met Ser Thr Cys Thr Thr Leu Asp Phe Asp His
 20 25 30
 Ile Lys Arg Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys
 35 40 45
 Leu Arg Leu Thr Ser Pro Pro Asp Pro Ser Met Leu Ala Asn Ile Pro
 50 55 60
 Thr Gln Val Leu Asp Leu Tyr Asn Ser Thr Arg Glu Leu Leu Glu Glu
 65 70 75 80
 Val His Gly Glu Arg Gly Asp Asp Cys Thr Gln Glu Asn Thr Glu Ser
 85 90 95
 Glu Tyr Tyr Ala Lys Glu Ile Tyr Lys Phe Asp Met Ile Gln Gly Leu

100	105	110
Glu Glu His Asn Asp Leu Ala Val Cys Pro Lys Gly Ile Thr Ser Lys		
115	120	125
Ile Phe Arg Phe Asn Val Ser Ser Val Glu Lys Asn Glu Thr Asn Leu		
130	135	140
Phe Arg Ala Glu Phe Arg Val Leu Arg Met Pro Asn Pro Ser Ser Lys		
145	150	155
Arg Ser Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Gln Pro Asp Glu		
165	170	175
His Ile Ala Lys Gln Arg Tyr Ile Asp Gly Lys Asn Leu Pro Thr Arg		
180	185	190
Gly Ala Ala Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val Arg Glu		
195	200	205
Trp Leu Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser Ile His		
210	215	220
Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu Asn Ile		
225	230	235
Gln Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Ser Glu Asp Asp		
245	250	255
Pro Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Lys Lys Glu His Ser		
260	265	270
Pro His Leu Ile Leu Met Met Ile Pro Pro Asp Arg Leu Asp Asn Pro		
275	280	285
Gly Leu Gly Ala Gln Arg Lys Lys Arg Ala Leu Asp Thr Asn Tyr Cys		
290	295	300
Phe Arg Asn Leu Glu Glu Asn Cys Cys Val Arg Pro Leu Tyr Ile Asp		
305	310	315
Phe Arg Gln Asp Leu Gly Trp Lys Trp Val His Glu Pro Lys Gly Tyr		
325	330	335
Tyr Ala Asn Phe Cys Ser Gly Pro Cys Pro Tyr Leu Arg Ser Ala Asp		
340	345	350
Thr Thr His Ser Ser Val Leu Gly Leu Tyr Asn Thr Leu Asn Pro Glu		
355	360	365
Ala Ser Ala Ser Pro Cys Cys Val Pro Gln Asp Leu Glu Pro Leu Thr		
370	375	380
Ile Leu Tyr Tyr Val Gly Arg Thr Ala Lys Val Glu Gln Leu Ser Asn		

385 390 395 400
 Met Val Val Lys Ser Cys Lys Cys Ser

405 410

INFORMATION FOR SEQ ID NO:28:

LENGTH: 390

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Leu

1 5 10 15

Arg Leu Leu Val Leu Thr Pro Gly Arg Pro Ala Ala Gly Leu Ser Thr

20 25 30

Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Ala

35 40 45

Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ser Ser Pro Pro Ser

50 55 60

Gln Gly Glu Val Pro Pro Val Pro Leu Pro Glu Ala Val Leu Ala Leu

65 70 75 80

Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu Pro Glu

85 90 95

Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu

100 105 110

Met Val Glu Asn Thr Asn Lys Ile Tyr Glu Lys Val Lys Lys Ser Pro

115 120 125

His Ser Ile Tyr Met Leu Phe Asn Thr Ser Glu Leu Arg Glu Ala Val

130 135 140

Pro Glu Pro Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Leu

145 150 155 160

Lys Leu Lys Ala Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn

165 170 175

Asp Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp Thr

180 185 190

Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu

195 200 205

Ser His Gly Gly Glu Val Glu Gly Phe Arg Leu Ser Ala His Cys Ser
 210 215 220
 Cys Asp Ser Lys Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Ser
 225 230 235 240
 Ser Ser Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu His
 260 265 270
 Ser Ser Arg Gln Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr
 325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380
 Arg Ser Cys Lys Cys Ser
 385 390

INFORMATION FOR SEQ ID NO:29:

LENGTH: 390

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Leu
 1 5 10 15
 Trp Leu Leu Met Leu Thr Pro Gly Arg Pro Val Ala Gly Leu Ser Thr
 20 25 30
 Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Gly Ile Glu Ala

35 40 45
 Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser
 50 55 60
 Gln Gly Asp Val Pro Pro Gly Pro Leu Pro Glu Ala Ile Leu Ala Leu
 65 70 75 80
 Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu Thr Glu
 85 90 95
 Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu
 100 105 110
 Met Val Glu Tyr Gly Asn Lys Ile Tyr Asp Lys Met Lys Ser Ser Ser
 115 120 125
 His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu Leu Arg Glu Ala Val
 130 135 140
 Pro Glu Pro Val Leu Leu Ser Arg Ala Asp Val Arg Leu Leu Arg Leu
 145 150 155 160
 Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn
 165 170 175
 Asn Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp Ser
 180 185 190
 Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu
 195 200 205
 Thr His Arg Glu Glu Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser
 210 215 220
 Cys Asp Ser Lys Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Ser
 225 230 235 240
 Ser Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu His
 260 265 270
 Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr

325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380

Arg Ser Cys Lys Cys Ser

385 390

INFORMATION FOR SEQ ID NO:30:

LENGTH: 412

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met His Cys Tyr Leu Leu Ser Val Phe Leu Thr Leu Asp Leu Ala Ala

1 5 10 15

Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met Asp Gln Phe

20 25 30

Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu

35 40 45

Lys Leu Thr Ser Pro Pro Asp Glu Tyr Pro Glu Pro Glu Glu Val Pro

50 55 60

Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr Arg Asp Leu Leu Gln Glu

65 70 75 80

Lys Ala Asn His Arg Ala Ala Thr Cys Glu Arg Glu Arg Ser Asp Glu

85 90 95

Glu Tyr Tyr Ala Lys Glu Val Tyr Lys Ile Asp Met Gln Pro Phe Tyr

100 105 110

Pro Glu Asn Ala Ile Pro Pro Ser Tyr Tyr Ser Leu Tyr Phe Arg Ile

115 120 125

Val Arg Phe Asp Val Ser Ala Met Glu Lys Asn Ala Ser Asn Leu Val

130 135 140

Lys Ala Glu Phe Arg Val Phe Arg Leu Gln Asn Ser Lys Ala Arg Val

145 150 155 160

Ser Glu Gln Arg Ile Glu Leu Tyr Gln Val Leu Lys Ser Lys Glu Leu
165 170 175

Ser Ser Pro Gly Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr Arg
180 185 190

Ala Glu Gly Glu Trp Leu Ser Phe Asp Val Thr Glu Ala Val His Glu
195 200 205

Trp Leu His His Arg Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu His
210 215 220

Cys Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro Asn
225 230 235 240

Lys Ser Glu Glu Pro Glu Ala Arg Phe Ala Gly Ile Asp Asp Tyr Thr
245 250 255

Tyr Ser Ser Gly Asp Val Lys Ala Leu Lys Ser Asn Arg Lys Lys Tyr
260 265 270

Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu Leu Pro Ser Tyr Arg
275 280 285

Leu Glu Ser Gln Gln Pro Ser Arg Arg Lys Lys Arg Ala Leu Asp Ala
290 295 300

Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn Cys Cys Leu Arg Pro Leu
305 310 315 320

Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp Lys Trp Ile His Glu Pro
325 330 335

Lys Gly Tyr His Ala Asn Phe Cys Ala Gly Ala Cys Pro Tyr Leu Trp
340 345 350

Ser Ser Asp Thr Gln His Ser Arg Val Leu Ser Leu Tyr Asn Thr Ile
355 360 365

Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Ser Gln Asp Leu Glu
370 375 380

Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys Thr Pro Lys Ile Glu Gln
385 390 395 400

Leu Ser Asn Met Ile Val Lys Ser Cys Lys Cys Ser
405 410

INFORMATION FOR SEQ ID NO:31:

LENGTH: 412

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Lys Met Tyr Ala Gln Arg Ala Leu Val Leu Leu Ser Leu Leu Ser
 1 5 10 15
 Phe Ala Thr Val Ser Leu Ala Leu Ser Ser Cys Thr Thr Leu Asp Leu
 20 25 30
 Glu His Ile Lys Lys Lys Arg Val Glu Ala Ile Arg Gly Gln Ile Leu
 35 40 45
 Ser Lys Leu Arg Leu Thr Ser Pro Pro Glu Ser Val Gly Pro Ala His
 50 55 60
 Val Pro Tyr Gln Ile Leu Ala Leu Tyr Asn Ser Thr Arg Glu Leu Leu
 65 70 75 80
 Glu Glu Met Glu Glu Glu Lys Glu Glu Ser Cys Ser Gln Glu Asn Thr
 85 90 95
 Glu Ser Glu Tyr Tyr Ala Lys Glu Ile His Lys Phe Asp Met Ile Gln
 100 105 110
 Gly Leu Pro Glu His Asn Glu Leu Gly Ile Cys Pro Lys Gly Val Thr
 115 120 125
 Ser Asn Val Phe Arg Phe Asn Val Ser Ser Ala Glu Lys Asn Ser Thr
 130 135 140
 Asn Leu Phe Arg Ala Glu Phe Arg Val Leu Arg Val Pro Asn Pro Ser
 145 150 155 160
 Ser Lys Arg Ser Glu Gln Arg Ile Glu Leu Phe Gln Ile Leu Arg Pro
 165 170 175
 Asp Glu His Ile Ala Lys Gln Arg Tyr Leu Ser Gly Arg Asn Val Gln
 180 185 190
 Thr Arg Gly Ser Pro Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val
 195 200 205
 Arg Glu Trp Leu Leu His Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser
 210 215 220
 Ile His Cys Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu
 225 230 235 240
 Asn Leu His Glu Val Leu Glu Ile Lys Phe Lys Gly Ile Asp Ser Glu
 245 250 255
 Asp Asp Tyr Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp

260	265	270
Leu His Asn Pro His Leu Ile Leu Met Met Leu Pro Pro His Arg Leu		
275	280	285
Glu Ser Pro Thr Leu Gly Gly Gln Arg Lys Lys Arg Ala Leu Asp Thr		
290	295	300
Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys Val Arg Pro Leu		
305	310	315
Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp Val His Glu Pro		
325	330	335
Lys Gly Tyr Phe Ala Asn Phe Cys Ser Gly Pro Cys Pro Tyr Leu Arg		
340	345	350
Ser Ala Asp Thr Thr His Ser Thr Val Leu Gly Leu Tyr Asn Thr Leu		
355	360	365
Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Pro Gln Asp Leu Glu		
370	375	380
Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro Lys Val Glu Gln		
385	390	395
Leu Ser Asn Met Val Val Lys Ser Cys Lys Cys Ser		
405	410	

INFORMATION FOR SEQ ID NO:32:

LENGTH: 373

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Ala Leu Ser Thr Cys Gln Arg Leu Asp Leu Glu Ala Ala Lys Lys Lys			
1	5	10	15
Arg Ile Glu Ala Val Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Thr			
20	25	30	
Ala Pro Pro Pro Ala Ser Glu Thr Pro Pro Arg Pro Leu Pro Asp Asp			
35	40	45	
Val Arg Ala Leu Tyr Asn Ser Thr Gln Glu Leu Leu Lys Gln Arg Ala			
50	55	60	
Arg Leu Arg Pro Pro Pro Asp Gly Pro Asp Glu Tyr Trp Ala Lys Glu			
65	70	75	80

Leu Arg Arg Ile Pro Met Glu Thr Thr Trp Asp Gly Ala Met Glu His
 85 90 95
 Trp Gln Pro Gln Ser His Ser Ile Phe Phe Val Phe Asn Val Ser Arg
 100 105 110
 Ala Arg Arg Gly Gly Arg Pro Thr Leu Leu His Arg Ala Glu Leu Arg
 115 120 125
 Met Leu Arg Gln Lys Ala Ala Ala Asp Ser Ala Gly Thr Glu Gln Arg
 130 135 140
 Leu Glu Leu Tyr Gln Gly Tyr Gly Asn Ala Ser Trp Arg Tyr Leu His
 145 150 155 160
 Gly Arg Ser Val Arg Ala Thr Ala Asp Asp Glu Trp Leu Ser Phe Asp
 165 170 175
 Val Thr Asp Ala Val His Gln Trp Leu Ser Gly Ser Glu Leu Leu Gly
 180 185 190
 Val Phe Lys Leu Ser Val His Cys Pro Cys Glu Met Gly Pro Gly His
 195 200 205
 Ala Glu Glu Met Arg Ile Ser Ile Glu Gly Phe Glu Gln Gln Arg Gly
 210 215 220
 Asp Met Gln Ser Ile Ala Lys Lys His Arg Arg Val Pro Tyr Val Leu
 225 230 235 240
 Ala Met Ala Leu Pro Ala Glu Arg Ala Asn Glu Leu His Ser Ala Arg
 245 250 255
 Arg Arg Arg Asp Leu Asp Thr Asp Tyr Cys Phe Gly Pro Gly Thr Asp
 260 265 270
 Glu Lys Asn Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Lys Asp
 275 280 285
 Leu Gln Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Met Ala Asn Phe
 290 295 300
 Cys Met Gly Pro Cys Pro Tyr Ile Trp Ser Ala Asp Thr Gln Tyr Thr
 305 310 315 320
 Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala
 325 330 335
 Pro Cys Cys Val Pro Gln Thr Leu Asp Pro Leu Pro Ile Ile Tyr Tyr
 340 345 350
 Val Gly Arg Asn Val Arg Val Glu Gln Leu Ser Asn Met Val Val Arg
 355 360 365

Ala Cys Lys Cys Ser

370

INFORMATION FOR SEQ ID NO:33:

LENGTH: 390

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu Leu

1 5 10 15

Trp Leu Leu Val Leu Thr Pro Ser Arg Pro Ala Ala Gly Leu Ser Thr

20 25 30

Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Thr

35 40 45

Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser

50 55 60

Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala Leu

65 70 75 80

Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu Pro Glu

85 90 95

Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr Arg Val Leu

100 105 110

Met Val Glu Thr His Asn Glu Ile Tyr Asp Lys Phe Lys Gln Ser Thr

115 120 125

His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu Leu Arg Glu Ala Val

130 135 140

Pro Glu Pro Val Leu Leu Ser Arg Ala Glu Leu Arg Leu Leu Arg Leu

145 150 155 160

Lys Leu Lys Val Glu Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn

165 170 175

Asn Ser Trp Arg Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asn Ser

180 185 190

Pro Glu Trp Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu

195 200 205

Ser Arg Gly Gly Glu Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser

210 215 220
 Cys Asp Ser Lys Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Thr
 225 230 235 240
 Thr Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg Pro
 245 250 255
 Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His Leu Gln
 260 265 270
 Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser
 275 280 285
 Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys
 290 295 300
 Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn
 305 310 315 320
 Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr
 325 330 335
 Ser Lys Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala
 340 345 350
 Ala Pro Cys Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr
 355 360 365
 Tyr Val Gly Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val
 370 375 380
 Arg Ser Cys Lys Cys Ser
 385 390

INFORMATION FOR SEQ ID NO:34:

LENGTH: 382

TYPE: amino acid

STRANDEDNES: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION:

Met Glu Val Leu Trp Met Leu Leu Val Leu Leu Val Leu His Leu Ser
 1 5 10 15
 Ser Leu Ala Met Ser Leu Ser Thr Cys Lys Ala Val Asp Met Glu Glu
 20 25 30
 Val Arg Lys Arg Arg Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys
 35 40 45

Leu Lys Leu Asp Lys Thr Pro Asp Val Asp Ser Glu Lys Met Thr Val
 50 55 60
 Pro Ser Glu Ala Ile Phe Leu Tyr Asn Ser Thr Leu Glu Val Ile Arg
 65 70 75 80
 Glu Lys Ala Thr Arg Glu Glu Glu His Val Gly His Asp Gln Asn Ile
 85 90 95
 Gln Asp Tyr Tyr Ala Lys Gln Val Tyr Arg Phe Glu Ser Ile Thr Glu
 100 105 110
 Leu Glu Asp His Glu Phe Lys Phe Lys Phe Asn Ala Ser His Val Arg
 115 120 125
 Glu Asn Val Gly Met Asn Ser Leu Leu His His Ala Glu Leu Arg Met
 130 135 140
 Tyr Lys Lys Gln Thr Asp Lys Asn Met Asp Gln Arg Met Glu Leu Phe
 145 150 155 160
 Trp Lys Tyr Gln Glu Asn Gly Thr Thr His Ser Arg Tyr Leu Glu Ser
 165 170 175
 Lys Tyr Ile Thr Pro Val Thr Asp Asp Glu Trp Met Ser Phe Asp Val
 180 185 190
 Thr Lys Thr Val Asn Glu Trp Leu Lys Arg Ala Glu Glu Asn Glu Gln
 195 200 205
 Phe Gly Leu Gln Pro Ala Cys Lys Cys Pro Thr Pro Gln Ala Lys Asp
 210 215 220
 Ile Asp Ile Glu Gly Phe Pro Ala Leu Arg Gly Asp Leu Ala Ser Leu
 225 230 235 240
 Ser Ser Lys Glu Asn Thr Lys Pro Tyr Leu Met Ile Thr Ser Met Pro
 245 250 255
 Ala Glu Arg Ile Asp Thr Val Thr Ser Ser Arg Lys Lys Arg Gly Val
 260 265 270
 Gly Gln Glu Tyr Cys Phe Gly Asn Asn Gly Pro Asn Cys Cys Val Lys
 275 280 285
 Pro Leu Tyr Ile Asn Phe Arg Lys Asp Leu Gly Trp Lys Trp Ile His
 290 295 300
 Glu Pro Lys Gly Tyr Glu Ala Asn Tyr Cys Leu Gly Asn Cys Pro Tyr
 305 310 315 320
 Ile Trp Ser Met Asp Thr Gln Tyr Ser Lys Val Leu Ser Leu Tyr Asn
 325 330 335

Gln Asn Asn Pro Gly Ala Ser Ile Ser Pro Cys Cys Val Pro Asp Val

340

345

350

Leu Glu Pro Leu Pro Ile Ile Tyr Tyr Val Gly Arg Thr Ala Lys Val

355

360

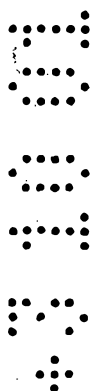
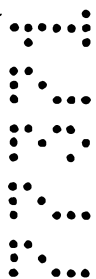
365

Glu Gln Leu Ser Asn Met Val Val Arg Ser Cys Asn Cys Ser

370

375

380



The claims defining the invention are as follows:

1. A method of screening a compound to be used for treatment or prevention of TGF- β -related diseases, which comprises:

measuring the amount of latent TGF- β bound to animal cells after addition of latent
5 TGF- β to said cells;

measuring the amount of latent TGF- β bound to animal cells after addition of latent TGF- β and a compound to be evaluated to said cells; and

evaluating the inhibiting activity or promoting activity of said compound on the binding of latent TGF- β to animal cells from the change in the amount of latent TGF- β
10 bound to animal cells caused by the addition of said compound.

2. The method according to claim 1, wherein said animal cells are vascular endothelial cells.

3. The method according to claim 1 or claim 2, wherein the promoting activity of said compound on the binding of latent TGF- β to animal cells is evaluated.

4. The method of screening a compound to be used for the treatment or prevention of TGF- β -related diseases, said method substantially as hereinbefore described with reference to Example 20 or Example 21.

5. A compound obtained by the method according to any one of claims 1 to 4.

Dated 27 September, 2001

Kyowa Hakko Kogyo Co., Ltd

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

Fig 1

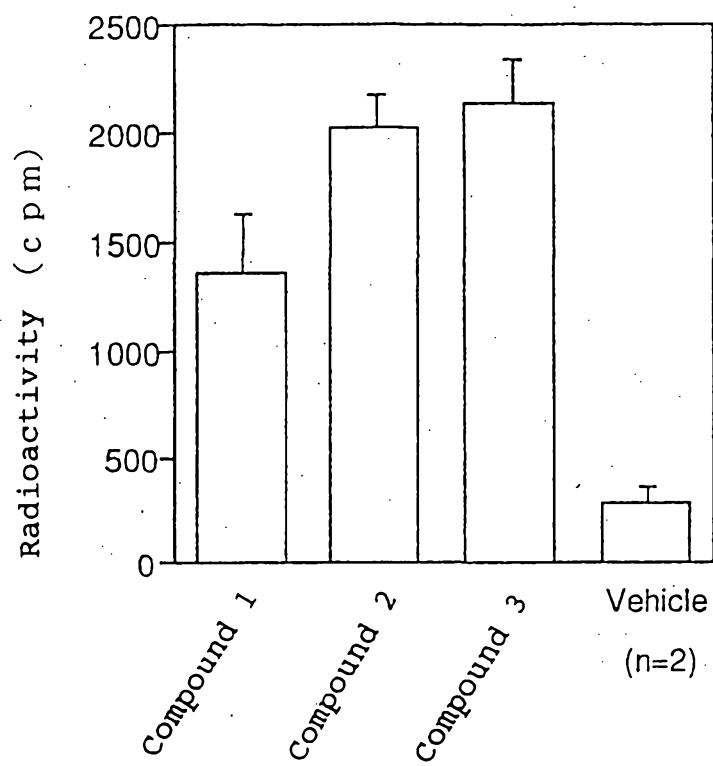


Fig 2

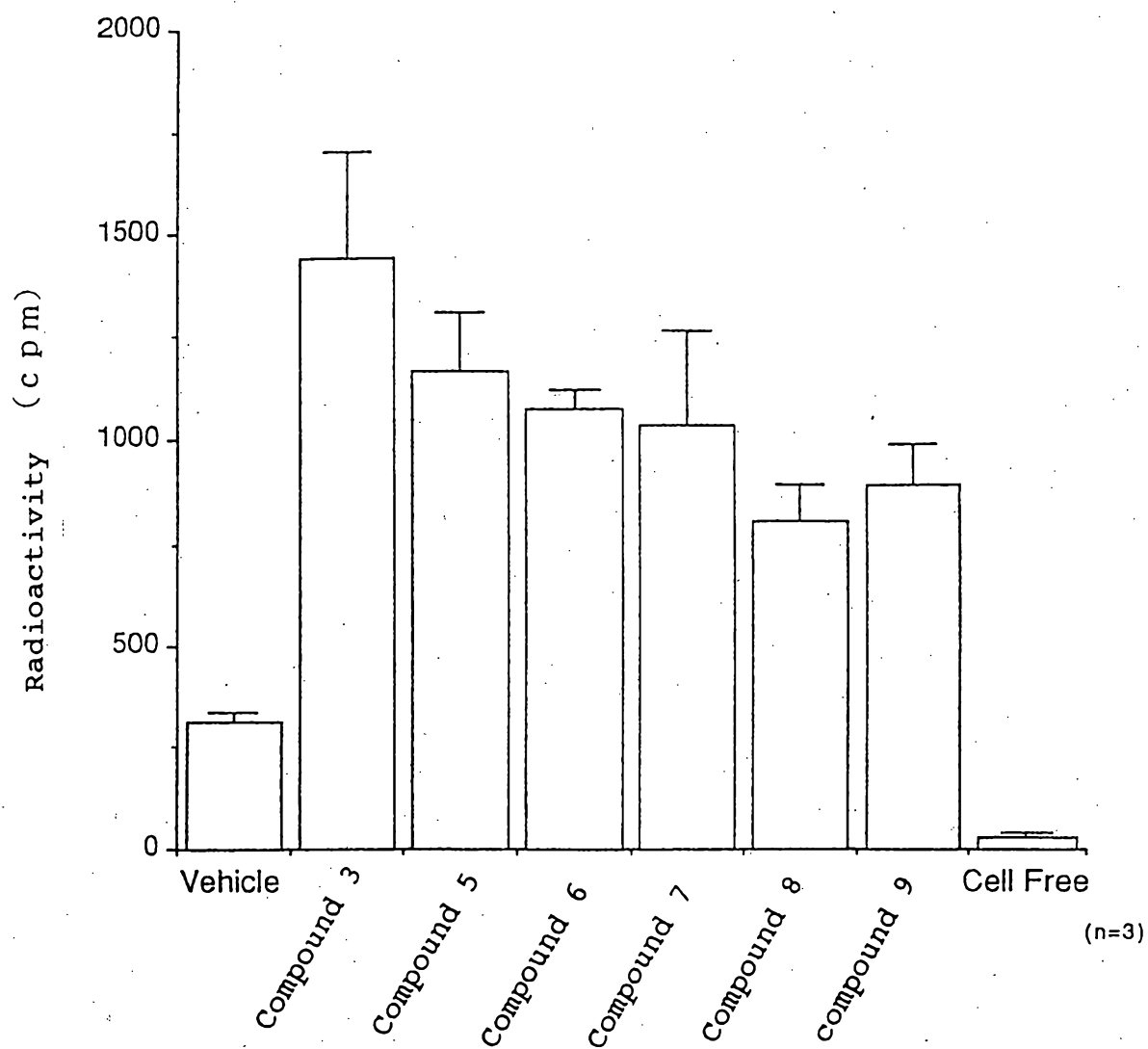


Fig 3

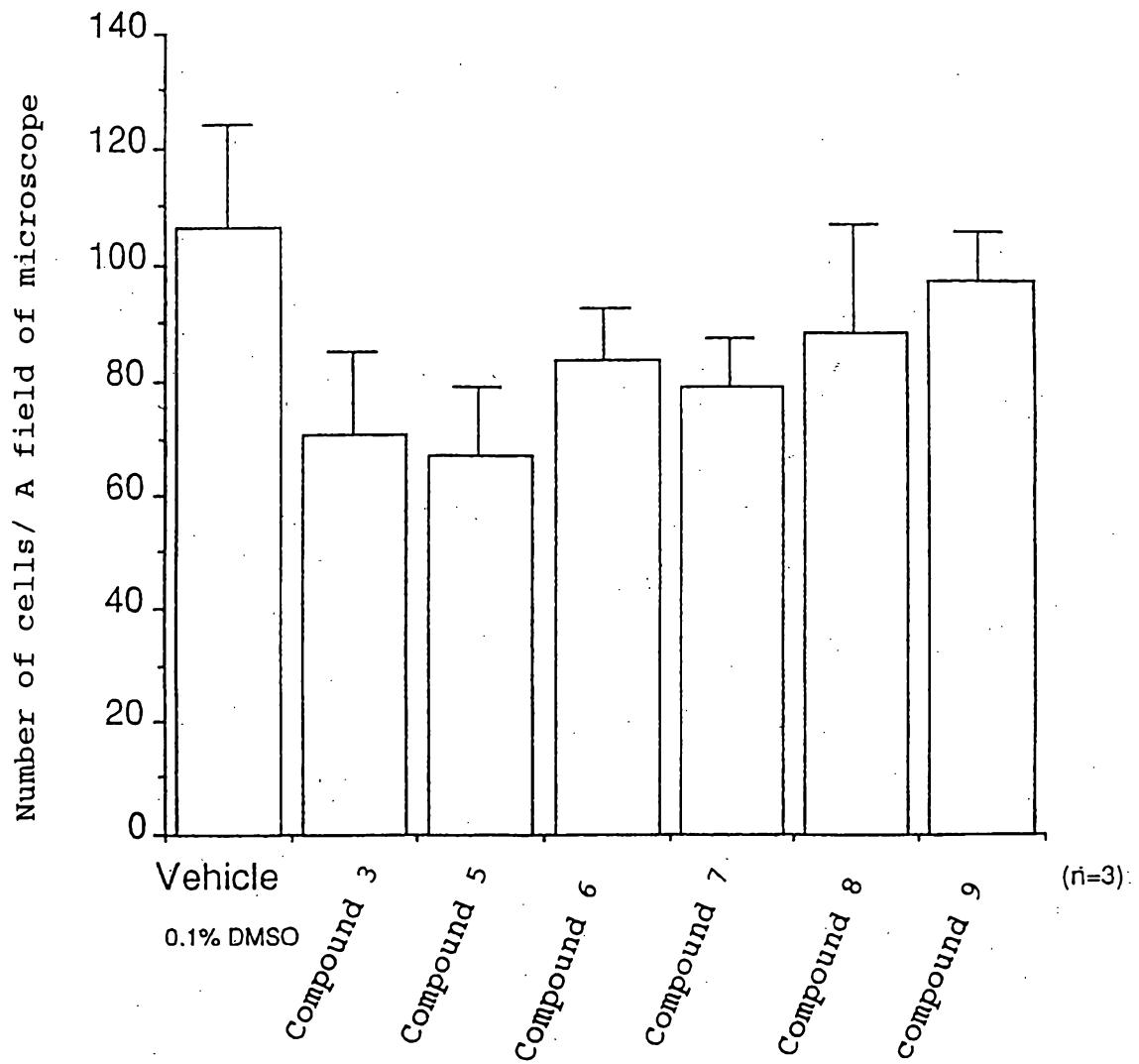
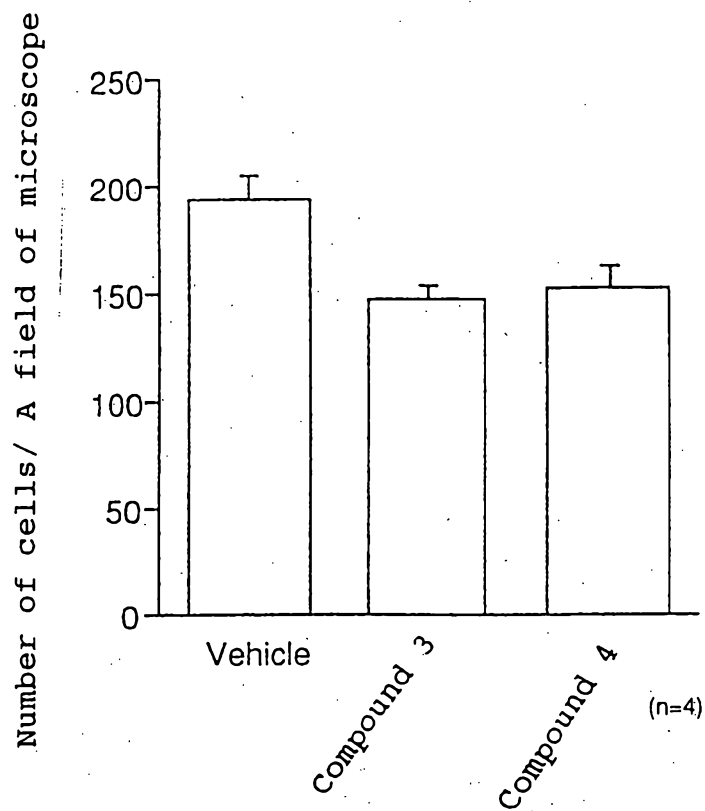


Fig 4



3 10 01 7 7 7 1

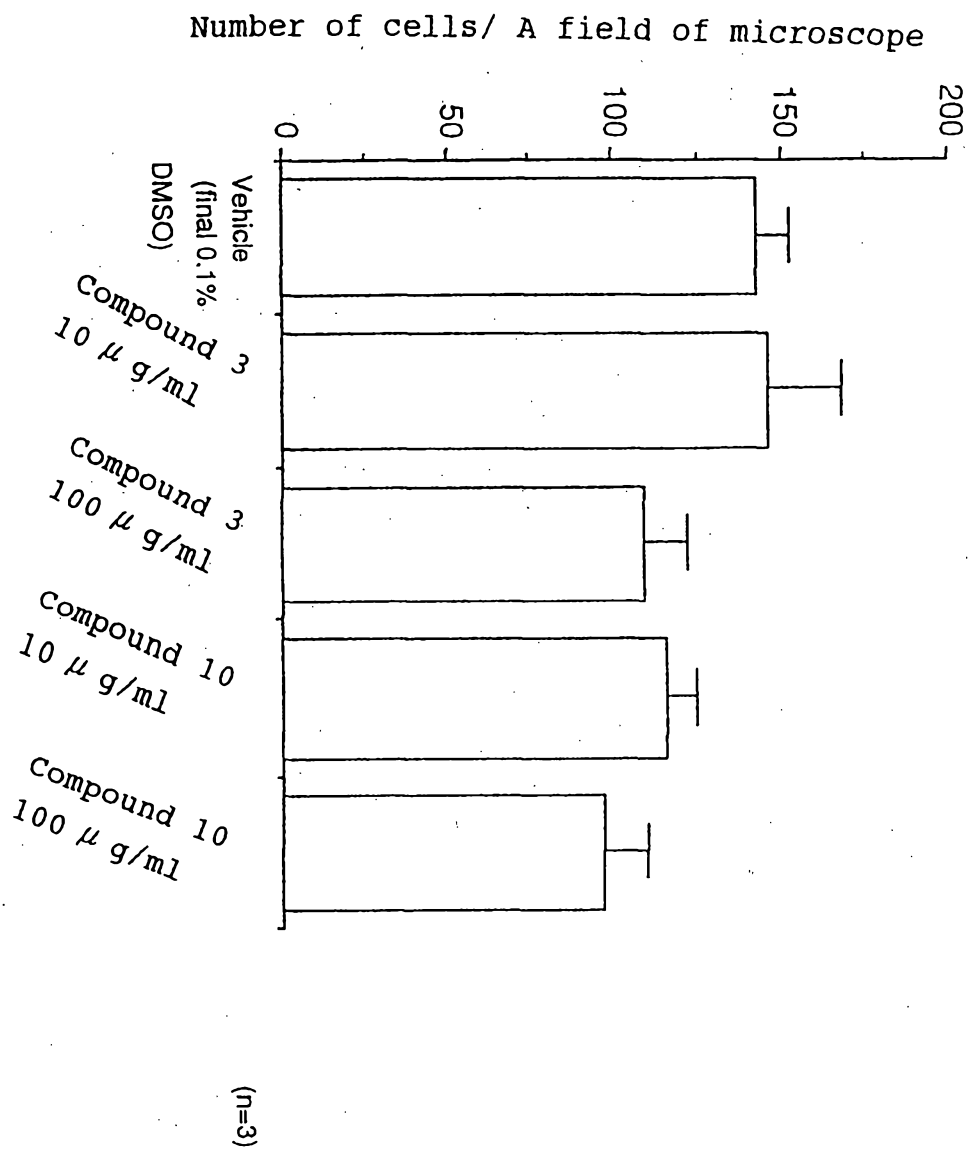


Fig 5

Fig 6

Luminescence intensity

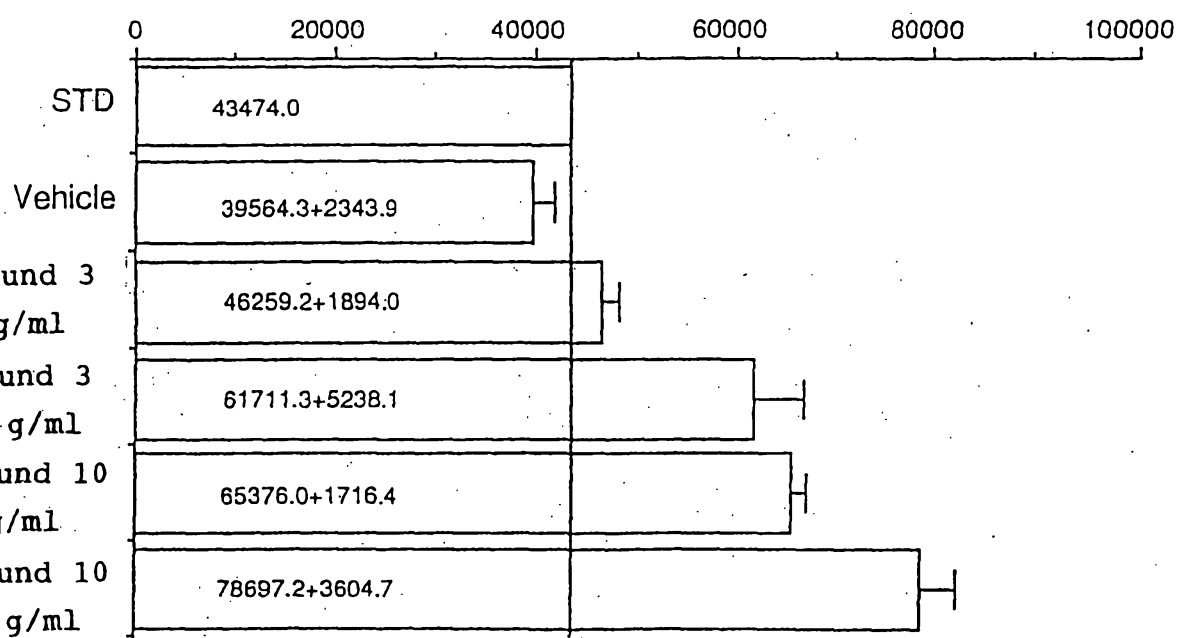


Fig 7

TGF- β (pg/ml)

(n=6)

