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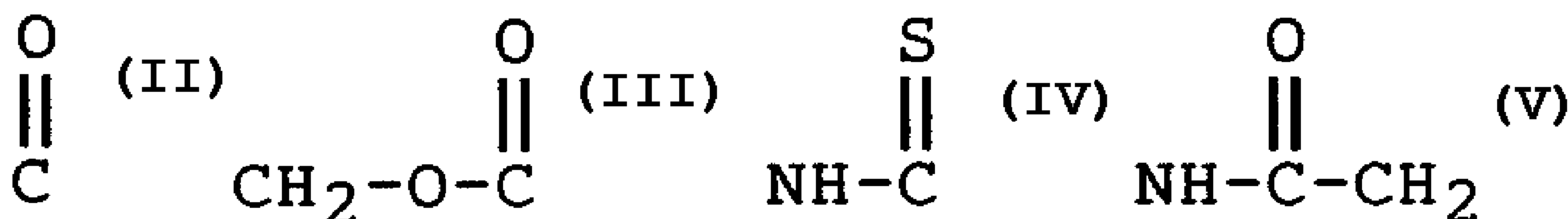
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(54) Title: MARKER FOR GROWTH HORMONE-RELEASING FACTOR RECEPTORS



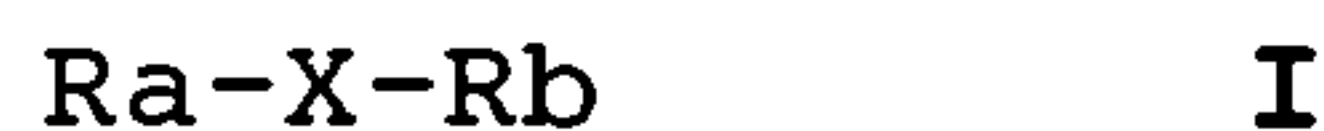
(57) Abrégé/Abstract:

The present invention relates to a compound having the formula: Ra-X-Rb I or a pharmaceutically acceptable salt thereof, wherein, X is selected from the group consisting of (see formula II), (see formula III), (see formula IV), (see formula V) and CH₂; Ra is a fluorophore selected from the group consisting of fluorescein, rhodamine, Texas red, BODIPY™, Cascade blue, coumarin, phycoerythryn, eosin, rosamine and the fluorenyl moiety; and Rb is a polypeptide moiety for binding to GRF receptors which allows for receptor physiological studies in vivo or in vitro and for distinguishing cell surface from intracellular receptor components. The present invention also relates to a method for the labeling of GRF receptors on cell surface.



ABSTRACT OF THE INVENTION

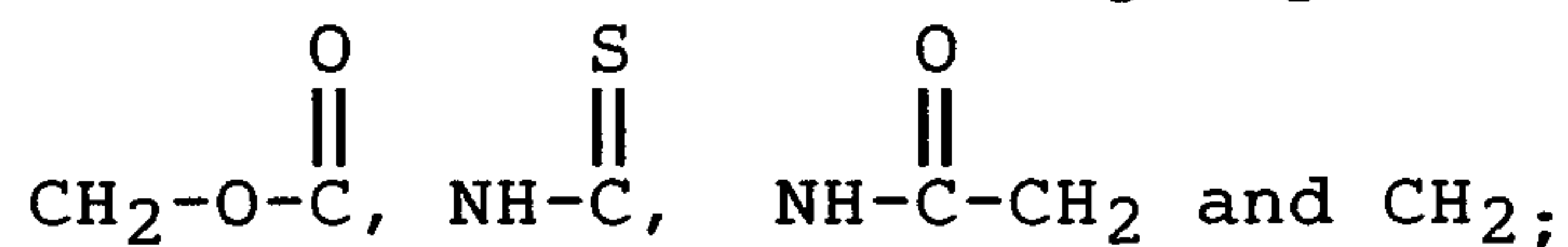
The present invention relates to a compound having the formula:



or a pharmaceutically acceptable salt thereof,

wherein,

X is selected from the group consisting of $\overset{\text{O}}{\parallel}\text{C}$,



Ra is a fluorophore selected from the group consisting of fluorescein, rhodamine, Texas red, BODIPYTM, Cascade blue, coumarin, phycoerithryn, eosin, rosamine and the fluorenyl moiety; and Rb is a polypeptide moiety for binding to GRF receptors which allows for receptor physiological studies *in vivo* or *in vitro* and for distinguishing cell surface from intracellular receptor components. The present invention also relates to a method for the labeling of GRF receptors on cell surface.

BACKGROUND OF THE INVENTION

5 The invention relates to selective marker peptides and marker polyclonal antibodies for growth hormone-releasing factor receptors, and to means for using these peptides and antibodies to characterize and visualize *in vitro* these receptors in normal and
10. tumoral tissues.

Growth hormone-releasing factor (GRF) is a peptide of 44 amino acids (human structure:

isolated first from pancreatic tumors and subsequently from hypothalami of various mammals.

25 In addition to the arcuate nucleus of the hypo-
thalamus, GRF is present in other hypothalamus nuclei
such as the suprachiasmatic nucleus and in other
regions of the brain such as the limbic system. GRF-
like immunoreactivity and/or GRF messenger ribonucleic
30 acid (mRNA) has also been found in the placenta, gas-
trointestinal tract, ovary, testis, thymus and spleen.
GRF fulfills a dual function as an hormone in these
target tissues and as a neuromodulator in the central
nervous system. Both modes of actions imply as a
35 first step the selective association of the neuropep-
tide with a specific receptor located on the plasma
membrane of target cells.

GRF binding sites have been localized and characterized in various tissues preparations and cell

cultures from normal and tumoral pituitary, and from normal hypothalamus, thymus, spleen and ovary. Pharmacological studies have demonstrated the existence of two populations of GRF binding sites in the pituitary and ovary: a low affinity and high capacity binding site and a high affinity and low capacity site corresponding to the physiologically relevant form of the receptor. Alterations of the rat pituitary GRF binding sites parameters occur in the course of aging, leading to a loss of the high affinity binding site. It has been reported by several authors that GRF(1-29)NH₂, the 29 amino acid N-terminus fragment of GRF(1-44)NH₂, exhibits the full bioactivity of GRF(1-44)NH₂. The GRF receptor binding pharmacophores have been identified in the rat pituitary (Lefrançois L. and Gaudreau P., *Neuroendocrinology*, 1994, 59:363-370). The mouse, rat, pig and human GRF receptors have been cloned and sequenced (Mayo K., *Mol. Endocrinol.*, 1992, 6:1734-1744). Some of the biochemical events mediating GRF signal transduction have also been characterized.

Moreover, degradation patterns of GRF has been elucidated in serum and plasma, liver and target tissues such as the pituitary and hypothalamus. The vulnerable peptide bonds of GRF identified so far are R²-R³, R¹⁰-R¹¹, R¹¹-R¹², R¹⁴-R¹⁵, R¹⁸-R¹⁹, R²⁰-R²¹, R²¹-R²². Modifications at these amino acid residues to prevent or decrease proteolysis will result in a longer duration of action of GRF and its analogues.

Over the past years, fluoroprobes have been developed for localizing drugs, neurotransmitters, peptides and proteins at the cellular level in tissues and cell cultures (Hazum E. et al., *Proc. of Natl. Acad. Sciences USA*, 1979, 77:3038-3041). This concept, which involves labeling purified molecules covalently with fluorochromes such as fluorescein, has permitted the

characterization of the kinetics, the distribution and the ultimate fate of a number of ligands in living cells (Taylor D.L. & Wang Yu-Li, Nature, 1980, 284:405-410).

5 It would be highly desirable to be provided with non-toxic highly sensitive tools for biochemical, pharmacological and anatomical studies of the GRF receptor in both normal and tumoral brain and peripheral tissues.

10 The few marker peptides for GRF receptors existing to date are of radioactive nature and hence have a limited half-life. No antibodies for GRF receptors have been developed so far. In addition, radioactive probes for GRF receptors are costly and
15 provide only static information on underlying biological processes.

 Further, it would be highly desirable to be provided with markers for GRF receptors which would allow for the isolation of GRF-receptor expressing
20 cells, which would permit the detection, characterization and sorting out of specific populations of GRF-receptor expressing cells, such as the somatotroph cells of the anterior pituitary.

 It would be also highly desirable to be provided with markers for GRF receptors which allows for
25 receptor physiological studies *in vivo* and *in vitro* in tissue slices and in cell cultures and for distinguishing cell surface from intracellular receptor components.

30 It would be also highly desirable to be provided with high affinity GRF receptor markers, that are superagonists and/or exhibit a greater resistance to proteolysis *in vitro* and *in vivo*.

 Finally it would be highly desirable to be
35 provided with photoactivable high affinity GRF recep-

tor markers that are agonists and selectively make covalent binding with GRF receptors, upon UV activation.

5 **SUMMARY OF THE INVENTION**

One aim of the present invention is to provide for non-toxic highly sensitive and selective marker peptides and marker polyclonal antibodies of the GRF receptors for biochemical, pharmacological and anatomical studies of the said receptors in both normal and tumoral brain and peripheral tissues.

Another aim of the present invention is to provide for marker peptides and marker polyclonal antibodies of GRF receptors which would allow for the isolation of GRF-receptor expressing cells.

Another aim of the present invention is to provide for marker peptides and marker polyclonal antibodies of GRF receptors which allows for receptor physiological studies *in vivo* or *in vitro* and for distinguishing cell surface from intracellular receptor components.

Another aim of the present invention is to provide for markers of GRF receptors that are superagonists and exhibit a greater resistance to proteolysis *in vitro* and *in vivo*.

Another aim of the present invention is to provide for high affinity GRF receptor markers that are photoactivable agonists that selectively make covalent binding with GRF receptors, upon UV activation.

30 In accordance with the present invention there is provided a compound represented by the following general formula:



or a pharmaceutically acceptable salt thereof,

X is selected from the group consisting of C, $\overset{\text{O}}{\parallel}$

5 $\text{CH}_2\text{-O-C}, \text{NH-C}, \text{NH-C-CH}_2$ and CH_2 ;

Ra is a fluorophore selected from the group consisting of fluorescein, rhodamine, Texas red, BODIPYTM, Cascade blue, coumarin, phycoerithryn, eosin
10 rosamine and the fluorenyl moiety;

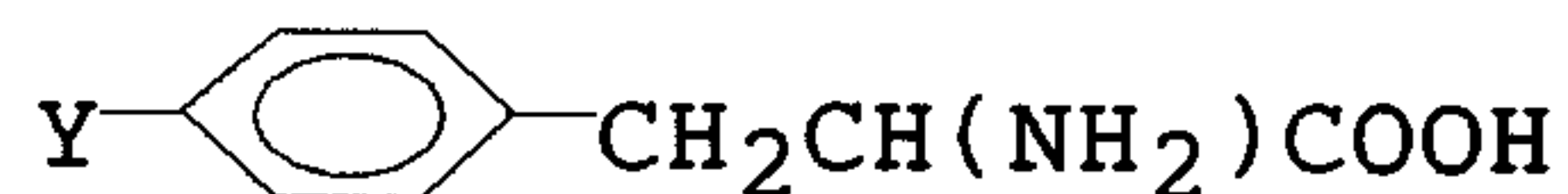
Rb is a polypeptide moiety comprising an amino acid sequence:

A-R²-R³-R⁴-R⁵-Phe-R⁷-R⁸-R⁹-R¹⁰-R¹¹-R¹²-R¹³-R¹⁴-
R¹⁵-R¹⁶-Leu-R¹⁸-R¹⁹-R²⁰-R²¹-R²²-Leu-R²⁴-B

15 A is L- or L-(aa);

wherein L is an hydrogen, a lower acyl or a thiocarbamyl;

aa is an amino acid residue derived from an amino acid selected from the group consisting
20 of histidine, 5-hydroxyindole acetic acid, 3-(4-hydroxyphenyl) propionic acid, and



wherein Y is an hydroxy, nitro, amino, azido, aryl ketone;

25 B is a carboxamide, an ester or an amino acid sequence of (aa)₁₋₁₅;

wherein aa₁₋₁₅ comprising a sufficient number of amino acids of the native human sequence (Gln³⁰-Gln³¹-Gly³²-Glu³³-Ser³⁴-Asn³⁵-Gln³⁶-
30 Glu³⁷-Arg³⁸-Gly³⁹-Ala⁴⁰-Arg⁴¹-Ala⁴²-Arg⁴³-Leu⁴⁴-NH₂ (SEQ ID NO:2), or functional derivative thereof wherein one or more amino acid may be substituted by lysine, ornithine, citrulline, norleucine, norvaline, β-alanine, cysteine, any of the other natural amino acids,
35 any of the enantiomorphic form thereof or an

aliphatic chain of $-(CH_2)_n$ (n ranges from 3 to 8);

R^2 is hydrogen, alanine or its enantiomorphous form, glycine, serine, glutamine, aminoisobutyric acid, asparagine, leucine, phenylalanine, threonine, valine or isoleucine;

R^3 is aspartic acid or its enantiomorphous form;

R^4 is alanine or an amino acid residue derived from an amino acid selected from the group of



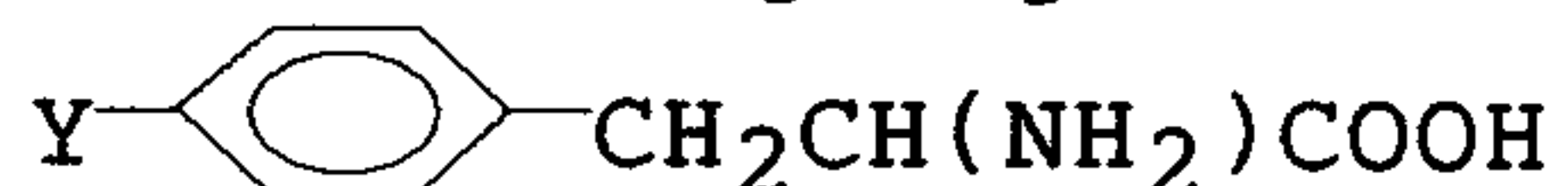
wherein Y is an amino, nitro, azido or aryl ketone;

R^5 is isoleucine, leucine, norleucine;

R^7 is threonine or serine;

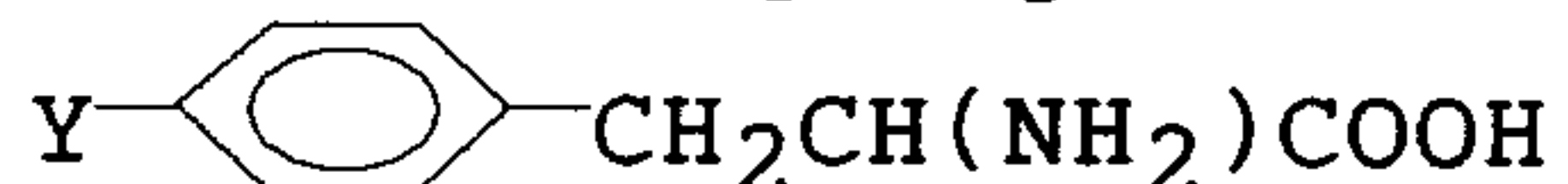
15 R^8 is asparagine or its enantiomorphous form, aminoisobutyric acid, alanine, serine, threonine, glutamine or aspartic acid wherein its β -carboxylic function is cyclized to the N- ϵ amino function of lysine¹² to form a lactam bridge;

20 R^9 is serine, alanine, aminoisobutyric acid or an amino acid residue derived from an amino acid selected from the group of



25 wherein Y is an hydroxy, amino, nitro, azido or aryl ketone;

R^{10} is tyrosine or its enantiomorphous form, or an amino acid residue derived from an amino acid selected from the group of



30 wherein Y is an amino, nitro, azido or aryl ketone;

R^{11} is arginine or its enantiomorphous form;

R^{12} is lysine or arginine or their enantiomorphous forms;

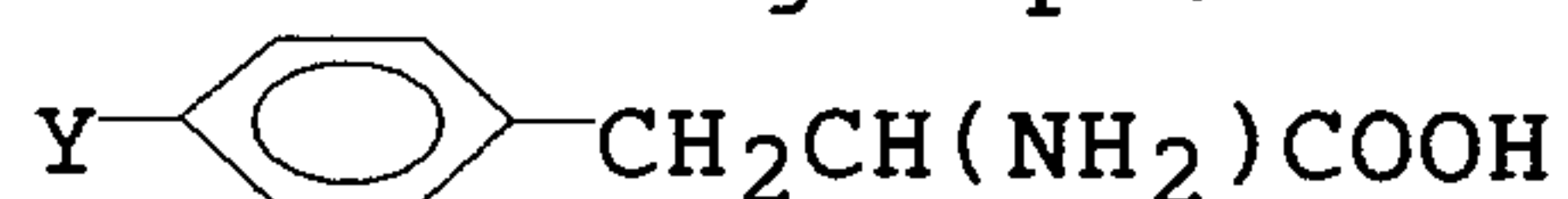
R¹³ is isoleucine or leucine;

R¹⁴ is leucine or its enantiomorphous form;

R¹⁵ is glycine, alanine, leucine or its enantiomorphous form, α-aminobutyric acid or glutamine;

5 R¹⁶ is glutamine, alanine or aminoisobutyric acid;

R¹⁸ is serine, alanine or their enantiomorphous forms, or aminoisobutyric acid, leucine or tyrosine or an amino acid residue derived from an amino acid
10 selected from the group of



wherein Y is an amino, nitro, azido or aryl ketone;

15 R¹⁹ is alanine, valine, leucine, serine or isoleucine;

R²⁰ is arginine or its enantiomorphous form;

R²¹ is lysine or arginine or their enantiomorphous forms;

20 R²² is alanine, leucine, lysine or their enantiomorphous forms, or aminoisobutyric acid;

R²⁴ is X-R²⁵-R²⁶-R²⁷-R²⁸-R²⁹;

wherein X is absent, glutamine, alanine or their enantiomorphous forms, aminoisobutyric acid, or histidine;

25 R²⁵ is absent, aspartic acid, glutamic acid, alanine or their enantiomorphous forms, aminoisobutyric acid or aspartic acid where its β-carboxylic function is cyclized to the N-ε amino function of lysine²¹ to form a lactam bridge;

30 R²⁶ is absent, isoleucine, leucine, alanine or aminoisobutyric acid;

R²⁷ is absent, methionine, leucine, isoleucine, norleucine, alanine or their enantiomorphous forms;

35 R²⁸ is absent, alanine, aspartic acid, glutamine, aminoisobutyric acid or asparagine;

R²⁹ is absent, arginine, the enantiomorphous form of arginine, 4-guanidino-butylamine (agmatine) or alanine; with the proviso that only R²⁷ and R²⁸ can be absent simultaneously.

5 Further, the amino acid sequence of the polypeptide moiety in accordance with the present invention may be lengthened further at the N- or C-terminus as long as the GRF-like biological activity is preserved.

10 In accordance with the present invention, the expression GRF biological activity is intended to mean that the polypeptide induces biological effects similar to those of GRF and/or binds with high affinity and selectivity to GRF receptors.

15 In accordance with the present invention, other fluorophores may be used where GRF-like biological activity is preserved.

 In accordance with the present invention, are provided specific polyclonal antibodies against the
20 N-terminal extracellular segment (aa₂₉₋₄₀), the third intracellular cytoplasmic segment (aa₃₁₃₋₃₂₂) and the intracellular C-terminal segment (aa₃₉₂₋₄₀₄) of the pituitary GRF receptor.

 In accordance with the present invention,
25 other GRF receptor antibodies may be used where specific labeling of the GRF receptors is preserved.

 Other applications of some of the peptides of the present invention in mammals, especially humans, may be for the treatment of hypothalamic pituitary
30 dwarfism, burns, osteoporosis, renal failure, non-union bone-fracture and other surgeries, acute/chronic debilitating illness or infection by promoting growth, wound healing, reduction of the incidence of post-surgical problems, for treatment of lactation failure,
35 for treatment of infertility in women, for prevention

or reduction of cachexia in cancer patients, for promotion of anabolism and/or for prevention of anabolic and/or catabolic problems in humans. Some of the peptides of the present invention may also be used for
5 improving serum lipid patterns in humans by decreasing in serum the amount of cholesterol and low density lipoproteins and increasing in serum the amount of high density lipoproteins. Some of the peptides of the present invention may also be used for the treatment of T-cell immunodeficiencies. Some of these
10 peptides may also be used to reverse some of the bodily changes associated with aging and to improve memory in normal subjects or in neurodegenerative conditions such as Alzheimer's disease. Some of these peptides may also be used for the treatment of GRF receptor-dependent tumors. Some of these peptides may also
15 be used for increasing muscle in animals and/or decreasing body fat, for enhancing milk production in cows and goats or increasing wool and/or fur production.
20 tion.

In accordance with the present invention, there is provided a method for *in vitro* labeling of GRF receptors on pituitary tissue sections, which comprises the steps of: a) incubating pituitary tissue
25 sections with an anti-GRF receptor antibody of the present invention for a time sufficient for the antibody to bind to GRF receptor present in the tissue sections; and b) visualizing the bound antibody of step a) using a second antibody for binding to the bound antibody of step a) directly or indirectly
30 labeled. The directly or indirectly labeled may consist in a horseradish peroxidase coupled to the second antibody, gold particles and optical or electron microscopy.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows adenopituitary immunoreactive complexes detected with the GRF receptor antibodies anti29-40 and anti392-404, after SDS-PAGE and transfer;
5

Fig. 1B shows immunoreactive complexes from wild-type and GRF receptor-transfected BHK cell membrane preparations detected with the GRF receptor antibodies anti29-40 and anti392-404, after SDA-PAGE and transfer;
10

Fig. 2A shows a light micrograph of the human pituitary immunostained for the GRF receptor with an anti-GRF-receptor segment 29-40 antiserum; and

Fig. 2B shows a light micrograph of the rat pituitary immunostained for the GRF receptor with an anti-GRF-receptor segment 29-40 antiserum.
15

DETAILED DESCRIPTION OF THE INVENTION

In general, the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (Biochemistry, 1972, 11:1726-1732).
20

For instance, Tyr, Lys, Orn, Nle, Nva, Ala, D-Ala, D-Ser, Ser, Thr, Glu, Gln, Asp, Asn, Leu, Phe, Val, Ile, D-Arg, D-Lys, D-Leu, D-Asp and D-Glu, β -Ala and Gly each represent the "residue" of L-tyrosine, L-lysine, L-ornithine, L-norleucine, L-norvaline, L-alanine, D-alanine, D-serine, L-serine, L-threonine, L-glutamic acid, L-glutamine, L-aspartic acid, L-asparagine, L-leucine, L-phenylalanine, L-valine, L-isoleucine, D-arginine, D-lysine, D-leucine, D-aspartic acid, D-glutamic acid, β -alanine and glycine.
25
30

The term "residue", when used with reference to an amino acid, means a radical derived from the corre-
35

sponding amino acid by eliminating the hydroxyl of the carboxyl group and one hydrogen of the amino group.

The term "natural amino acid" means an amino acid which occurs in nature or which is incorporated
5 as an amino acid residue in a naturally occurring peptide, exclusive of the amino acid cystine. Such amino acids are described, for example, in general textbooks of peptide chemistry (Kipple, K.D., "Peptides and Amino
10 Acids", W.A. Benjamin, Inc., New York, 1966; "The Peptides", Ed. Gross E. and Meienhofer J., Vol. 1, Academic Press, New York, 1979), and include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxylysine, hydroxyproline, isoleucine, leucine, lysine,
15 methionine, phenylalanine, proline, pyroglutamic acid, sarcosine, serine, threonine, tryptophan, tyrosine and valine.

The term "lower acyl" means an alkanoyl group containing one to eight carbon atoms and includes
20 formyl, acetyl, 1-oxopropyl, 8-aminooctanoyl, hexanoyl, etc.

The term "receptor" is intended to mean any plasma membrane protein which bind to GRF with high affinity and selectivity.

25 The peptides of the present formula are prepared by a suitable method such as by exclusively solid-phase techniques, by partial solid-phase techniques and/or by fragment condensation, or by classical solution coupling. For example, the techniques of
30 exclusively solid-phase synthesis, using t-Boc or Fmoc strategies, are described by Atherton E. and Sheppard R.C. ("Solid-phase peptide synthesis: a practical approach", IRL Press, Oxford University Press, Oxford, England, 1989, p.1-203). The fragment condensation method is exemplified
35 by the disclosure of Canadian Patent No. 1,178,950,

issued on December 4, 1984. Other available synthesis are exemplified by U.S. Patent No. 3,842,067, issued on October 15, 1974, and U.S. Patent No. 3,862,925, issued on January 28, 1975.

5 Common to such syntheses is the protection of the labile side chain groups of the various amino acid residues with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually
10 also common is the protection of an α -amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the α -amino protecting group to allow subsequent reaction to take place at that location.
15 Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with side-chain protecting groups linked to the appropriate
20 residues.

Thus, the aforementioned intermediate compounds are included within the scope of the invention.

Still another method for preparing the peptides within the scope of the invention employs recently
25 developed recombinant DNA techniques. Recombinant DNA techniques suitable for the preparation of the peptides of this invention having amino acid residues of the natural amino acids are well known (Villa-Komaroff L. et al., 1978, Proc. Natl. Acad. Sci. USA, 75:3727).

30 The terminal amino acylated derivatives of the peptides of the present formula are obtained from the corresponding free terminal amino peptides by treatment with a suitable acylating agent; for instance, the appropriate acid chloride or acid anhydride in the
35 presence of a strong organic base, e.g. triethylamine.

One of the preferred compounds in accordance with the present invention is Nε-5-carboxyfluoresceinyl-[Lys³¹]hGRF(1-44)NH₂ (Nε-CF-[Lys³¹]hGRF), defined as compound No. 4 in

5 Table 1.

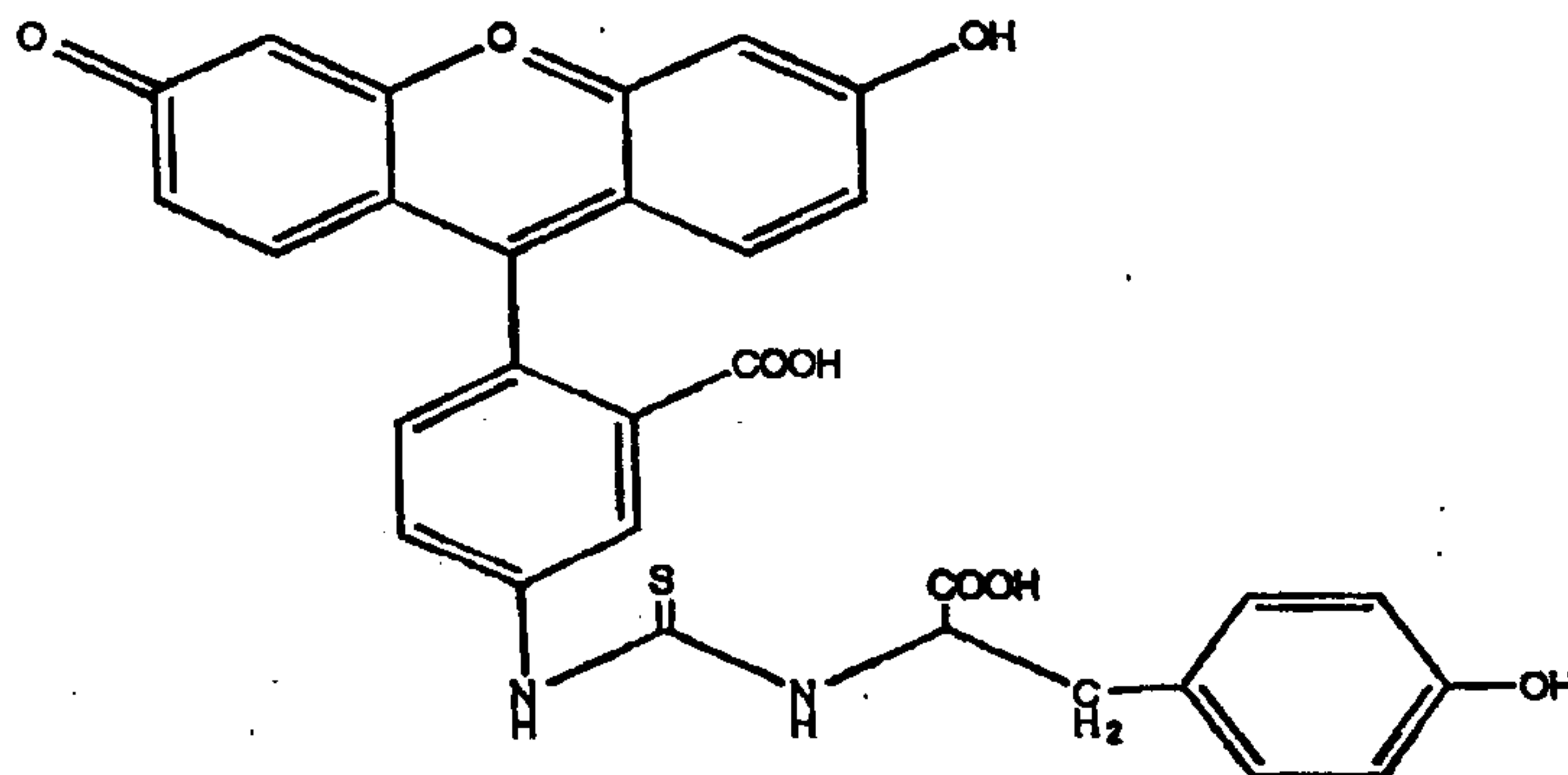
- 14 -

Table 1

Amino acid composition of compounds

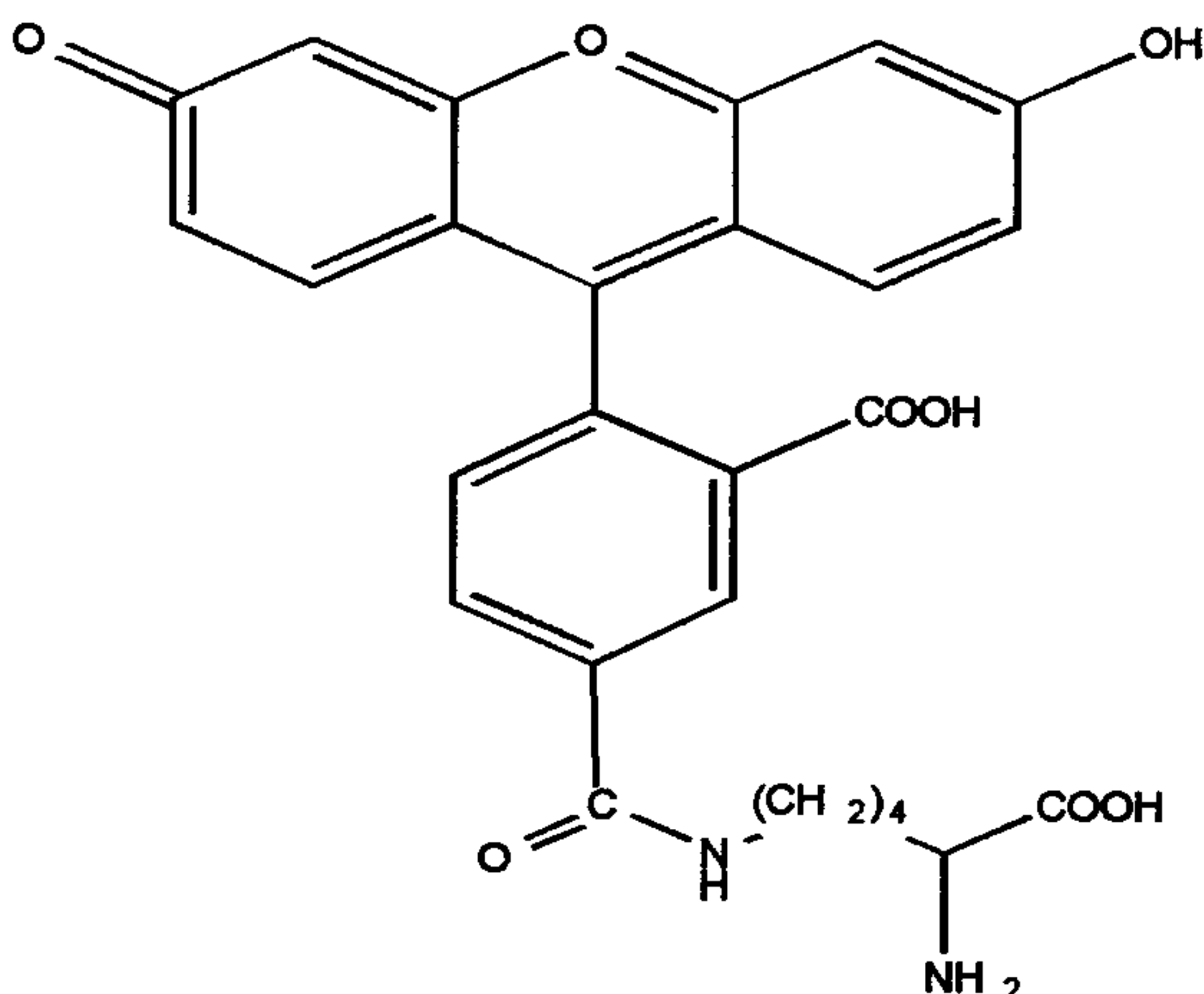
5	Number	Compound structure
	1	
		Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
10		Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH ₂ 20 25 (SEQ ID NO:3)
	2	
		Na-FTC-Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
15		Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH ₂ 20 25 (SEQ ID NO:12)
	3	
		Na-FTC-Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
20		Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly 20 25 30
		Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu-NH ₂ 35 40 (SEQ ID NO:4)
	4	
		Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
30		Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Na-CF-Lys Gly 20 25 30
		Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu-NH ₂ 35 40 (SEQ ID NO:5)

Na-FTC-Tyr is Na-fluorescein-5-thiocarbamyl-L-tyrosine



40

Nε-CF-Lys is Nε-5-carboxy-fluorescein-L-lysine:



Although there have been previous attempts at
 5 conjugating peptides with fluorescein derivatives, (Nε-
 -CF-[Lys³¹]hGRF) is the first example of a successful
 conjugation of fluorescein with GRF. The salient fea-
 tures of one compound of the present invention are:

- 10 (1) the selective attachment of fluorescein to
 the ε-amino function of Lys³¹;
- (2) the purification of the conjugated compound
 to approximately 99% purity allowing for opti-
 mal detection sensitivity;
- 15 (3) the similarity of its biological properties
 with those of the native peptide; and
- (4) the fact that it is 100% non-toxic and has
 a demonstrated shelf life of at least one year.

The fluorescent peptide compounds of the pre-
 sent invention offer a new, inexpensive and highly
 20 sensitive tool for biochemical, pharmacological,
 physiological and anatomical studies of GRF receptors
 in both brain and peripheral tissues. The present
 fluorescent probes offer several advantages over the
 use of radioactive compounds.

25 The compounds of the present invention do not
 have any of the common drawbacks of radioactive mole-
 cules such as short half-life, high cost, slow detec-

tion yield (which may imply weeks of photographic exposure) and great biohazard. Further, they compensate for a major shortcoming of current GRF radioactive probes, the fact that they essentially provide
5 static information (i.e. information that is not applicable to studying living processes in real time).

In addition to providing a non-radioactive approach to the characterization of GRF receptors, the fluorescent compounds of the present invention may be
10 used for a number of additional applications unsuited to radioactive probes. These include the following:

(1) these fluorescent compounds may be readily applied to the isolation of GRF-receptor expressing cells, using flow cytometric cell-sorting methods.
15 Similarly, receptor binding studies may be carried out on whole cells by flow cytometry.

(2) The fluorescent compounds of the present invention may be used for real time visualization of physiological processes (receptor aggregation, capping
20 and internalization) using confocal laser microscopy on brain slices or in cell culture preparation. The same technique may be used for distinguishing cell surface with respect to intracellular components.

(3) Confocal microscopic visualization of the
25 bound fluorescent compounds may be combined with that of other cell markers to study cations fluxes, such as Ca^{2+} entry in the somatotroph cell with fluorescent Ca^{2+} indicators such as Indo-1, Fura-2 or Fluo-3, after stimulation with (N ϵ -CF-[Lys³¹]hGRF). It may
30 also be conjugated to the immunocytochemical characterization of the cells and/or compartments harboring the labeled receptors, using appropriate fluorescent-tagged antibodies.

In accordance with one embodiment of the present invention (Nε-CF-[Lys³¹]hGRF) is prepared according to the following procedure.

5 **1- Fluorescent labeling of growth hormone-releasing factor**

Nε-CF-[Lys³¹]hGRF was synthesized by solid phase technique using a scheme based on Nα-tert-butyl-oxycarbonyl (t-Boc) chemistry/acid labile amino acid
10 protecting groups, using p-methylbenzhydrylamine or benzhydrylamine resin. Coupling of Boc amino acid derivatives (3 equiv) except for Boc-Asn and Boc-Gln were achieved with (benzotriazol-1-yl-oxy)-tris(dimethylamino) phosphonium hexafluorophosphate
15 (BOP) (3 equiv), using *in situ* neutralization (6 equiv diisopropylethylamine (DIEA)). Boc-Asn and Boc-Gln (3 equiv) were coupled with dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT) (3 equiv); 4-methyl-morpholine (0.5 equiv) was added when a sec-
20 ond coupling was needed. After coupling Nα-Boc,Nε-Fmoc-Lys³¹ to the growing peptide, the Nε-Fmoc group was removed by treating the peptide-resin with a solution of piperidine/methylene chloride (CH₂Cl₂) (50/50,v/v) for 20 min. The N-hydroxysuccinimide
25 ester of 5-carboxyfluorescein (3 equiv) was then coupled to the Nε-amino group of Lys³¹, in anhydrous dimethylformamide, in presence of 4-methyl-morpholine (6 equiv), for 5h at room temperature. The fluorescent derivative, Nα-Boc,Nε-5-carboxy-fluoresceinyl-Lys³¹, may also be directly coupled to the growing
30 peptide resin. The acyl-peptide-resin intermediate was then extensively washed with DMF and the synthesis pursued to completion of the peptide. Completion of the coupling was ascertain by a ninhydrin colorimetric
35 test or by amino acid analysis after hydrolysis of the

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acyl-peptide-resin intermediate. Boc-protecting groups were removed with trifluoroacetic acid (TFA)/CH₂Cl₂ (40/60, v/v) containing 1% D,L-methionine (w/v) when Boc-Met was incorporated in the growing peptide. This was followed by a neutralization with DIEA/CH₂Cl₂ (5/95, v/v) when the DCC/HOBT method was used.

After completion of the synthesis and removal of the last Boc protecting group, the peptide resin was dried in vacuo.

Deprotection of the amino acid side chains and cleavage of the peptide from the resin were performed with anhydrous hydrogen fluoride (HF)/anisole (9/1, v/v; 10 ml/g peptide-resin intermediate) at -15°C for 30 min and then at 0°C for 30 additional min. When Met was present in the peptide chain, 0.5% D,L methionine (w/v) was added to the reaction mixture. HF removal was done in vacuo followed by precipitation of the crude peptide with peroxide-free anhydrous ether and solubilization with 20% aqueous N₂-purged HOAc. Solutions were lyophilized to yield amorphous powders.

It was then purified by preparative high pressure liquid chromatography (HPLC) on a Partisil 10 ODS-3 WhatmanTM column (10-um particle size; 2.2 cm x 50 cm), using a binary solvent system consisting of 0.01% aqueous TFA, pH 2.9 and acetonitrile (CH₃CN)-0.01% TFA and an appropriate gradient. Elution of the peptide was monitored at 214 nm. Collected fractions were readily screened by analytical HPLC using both UV (214 or 280 nm) and fluorescence detection.

The purified Nε-CF-[Lys³¹]hGRF was analyzed for homogeneity by analytical HPLC on a μBondapakTM C₁₈ (10 μm particles) column (0.39 cm X 15 cm) using appropriate linear gradients of 0.01% aqueous TFA, pH 2.9 and

0.01% TFA/CH₃CN and 0.1M NaClO₄, pH 2.5 and CH₃CN. Its amino acid composition was assessed by quantitative amino acid analysis after acidic hydrolysis *in vacuo* (6 HCl, 110°C, 18h).

5 The structure of the fluorescent peptide was confirmed by mass spectral analysis (theoretical and experimental molecular mass: 5412). The degree of homogeneity was determined by U.V. and fluorescence detection to 99%. The modification of semi-protected
10 GRF with 5-carboxyfluorescein yielded a selective incorporation of one mole Nε-5-carboxyfluoresceinyl-[Lys³¹]/mole unprotected peptide. Nε-CF-[Lys³¹]GRF was evaluated to be pure as indicated by a single elution peak from reverse-phase HPLC allowing for optimal
15 detection sensitivity (Table 2). Its amino acid composition was in agreement with the theoretical values listed in Table 3. The compound is freely soluble in distilled water or aqueous buffer, and is stable if protected from light and maintained at 4°C. Finally,
20 Nε-CF-[Lys³¹]GRF is 100% environmentally safe.

 [Nα-fluorescein-5-thiocarbamyl]hGRF(1-44)NH₂ and [Nα-fluorescein-5-thiocarbamyl]hGRF(1-29)NH₂ were synthesized according to the general methods of solid-phase peptide synthesis described above. However,
25 after the deprotection of the last Nα amino group, acylation was performed by fluorescein-5-isothiocyanate (FITC, isomer 1, 6-fold excess) in anhydrous DMF containing 5% DIEA for 2-4 h at room temperature with stirring. Completion of the coupling
30 was ascertain by a ninhydrin colorimetric test. The acyl-peptide-resin intermediates were then extensively washed with DMF and dried *in vacuo*. They were submitted to HF cleavage to deprotect amino acid side chains and to cleave the fluorescein-5-thiocarbamyl (FTC)
35 peptides from the resin. The FTC-peptides were then

- 20 -

solubilized in TFA, subjected to rotary evaporation in vacuo, and further dried on a freeze drier. Their purification and characterization was accomplished according to the methods described previously.

5

Table 2

Physicochemical data of fluoresceinyl analogues of hGRF(1-29)NH₂ and hGRF(1-44)NH₂

10

No.	MW	% overall yield	HPLC	
			t _R ^a , min	% homogeneity (214 nm/280 nm)
1	3358	24	24.0 ^b	99/100 ^b
2	3774	4	24.8 ^b	95/97 ^b
3	5430	2	21.8 ^b	99/99 ^b
4	5412	2	25.0 ^b	99/99 ^b

Identification of compounds corresponds to that of Table 1.

15

a μ BondapakTM C18 (10- μ m particles) column (0.39-cm x 15-cm).

t_R retention time.

20

b Linear gradient: solvent A consisted of 0.01% aqueous TFA (pH 2.9) and solvent B consisted of CH₃CN/0.01% TFA; 0.67% B/min for 45 min, initial condition 20% B, flow rate 1.5 ml/min, 23°C.

25

Compound no. 1 is hGRF(1-29)NH₂.

Table 3

Quantitative amino acid analysis of fluoresceinyl analogues of hGRF(1-29)NH₂ and hGRF(1-44)NH₂

No.	Asx	Glx	Ser	Gly	Arg	Thr	Ala	Tyr	Val	Met	Ile	Leu	Phe	Lys	P.C. (%)
1	2.89(3)	2.11(2)	2.59(3)	1.05(1)	3.36(3)	0.87(1)	3.10(3)	2.02(2)	1.02(1)	0.90(1)	2.10(2)	4.07(4)	0.98(1)	1.94(2)	83
2	2.88(3)	2.13(2)	2.49(3)	1.06(1)	3.26(3)	0.79(1)	3.10(3)	2.00(2)	1.05(1)	0.85(1)	2.20(2)	4.02(4)	0.99(1)	1.99(2)	78
3	3.55(4)	5.06(6)	3.02(4)	3.01(3)	6.33(6)	1.02(1)	5.22(5)	2.10(2)	1.07(1)	0.88(1)	2.18(2)	4.88(5)	1.11(1)	2.02(2)	76
4	3.50(4)	5.05(5)	3.00(4)	3.08(3)	6.30(6)	1.36(1)	5.20(5)	2.13(2)	1.32(1)	0.86(1)	2.20(2)	4.91(5)	1.23(1)	3.05(3)	69

Identification of compounds corresponds to that of Table 1.

Amino acid composition was determined after acidic hydrolysis (6N HCl, 110°C, 18h) and PITC derivatization by reverse-phase HPLC (Waters PICO-TAG™ column, 0.39 X 15cm, 38°C) using a solvent system consisting of 140 mM sodium acetate (pH 6.4) and 60% aqueous CH₃CN.

Detection at 254 nm.

2-Competitive binding assay

The binding of [^{125}I -Tyr ^{10}]hGRF(1-44)NH $_2$ was performed on anterior pituitary homogenates as previously described (Gaudreau P. et al., J. Biol. Chem., 1992, 35:1864-1869). Briefly, anterior pituitaries (pit.) were dissected out, rinsed and homogenized for 8 sec with a Micro Ultrasonic Cell DisrupterTM, in ice-cold 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM MgCl $_2$ and 5 mM EDTA (1 pit./0.5 ml). These homogenates were used immediately. Competition studies were performed using 35-50 pM [^{125}I -Tyr ^{10}]hGRF(1-44)NH $_2$ as radioligand, 50 μl of homogenate (70 to 75 μg of protein), and increasing concentrations of N ϵ -CF-[Lys ^{31}]hGRF or other GRF analogues (0-1000 nM) or with 2.4 μM rGRF(1-29)NH $_2$ for determination of non-specific binding, in a total volume of 300 μl of Tris-HCl buffer, pH 7.4, containing 5 mM EDTA, 5mM MgCl $_2$ and 0.42% BSA. Incubations were carried out at 23°C for 60 min and stopped by centrifugation (12,000 g, 5 min, at 4°C). The radioactivity content in the pellet was measured by gamma counting. The affinity of hGRF(1-29) NH $_2$ was tested in each experiment to assess the validity of the assay and determine the relative affinity of the analogues. The stability of the C-terminus carboxamide form of GRF and its analogues has previously been demonstrated in this assay; 75 to 97% of their initial concentration are recovered at the end of the binding assay (Gaudreau P. et al., J. Biol. Chem., 1992, 35:1864-1869). The Ligand computerized program was used to analyze competition curves of all the GRF analogues reported in Tables 4, 7 and 11, and to determine their IC $_{50}$ and Hill coefficient. The Hill values indicate that all these peptides bind to the high and low affinity GRF binding sites found in the anterior pituitary.

3-Adenylate cyclase assay

The adenylate cyclase activity Nε-CF-[Lys³¹]hGRF was performed on anterior pituitary homogenates as previously described (Lefrançois L. and Gaudreau P., J. Chromatogr., 1993, 619:116-120). Briefly, anterior pituitaries (pit.) were dissected out, rinsed and homogenized for 8 sec with a Micro Ultrasonic Cell Disrupter™, in ice-cold 20 mM Tris-HCl buffer (pH 7.5) containing 2 mM MgCl₂ and 250 mM sucrose (1 pit./ 0.5 ml).

Samples of anterior pituitary homogenates (30-40 µg protein) were incubated in 1.5 ml Eppendorf™ tubes with 30 mM Tris-HCl, 5 mM MgCl₂, 0.5 mM EGTA, containing 0.5 mM ATP, 1 mM IBMX, 10 µM GTP and an ATP regenerating system consisting of 2 mM creatine phosphate, 0.1 mg/ml creatine kinase and, 0.1 mg/ml myokinase in a final volume of 120 µl. Incubations were initiated by adding 20 µl of the homogenate to the reaction mixture which had previously been equilibrated at 37°C for 2 min. The reactions were carried out for 8 min at 37°C and stopped by heating the samples in boiling water for 4 min. Samples were centrifuged (12000 g, 5 min, 4°C) and supernatants were filtered through 0.45 µm Millex HV₄™ filters and transferred to autosampler vials for HPLC quantification of cAMP. The Sigma Plot computerized program was used to analyze concentration-response curves of all the GRF analogues reported in Tables 5 and 12, and to determine their EC₅₀.

The peptide segments of the pituitary GRF receptor are as follows:

N-acetyl-Asp-Phe-Ile-Thr-Gln-Leu-Arg-Asp-Asp-Glu-Leu-Ala SEQ ID NO:37
29 34 40

N-acetyl-Pro-Ala-Gln-Gly-Gly-Leu-His-Thr-Arg-Ala
313 317 322

N-acetyl-Tyr-Gly-His-Asp-Pro-Glu-Leu-Leu-Pro-Ala-Arg-Arg-Thr
392 397 404

20 [N-acetyl-Asp-Phe-Ile-Thr-Gln-Leu-Arg-Asp-Asp-Glu-Leu-Ala] (n=16) -
29 34 40

25 [N-acetyl-Pro-Ala-Gln-Gly-Gly-Leu-His-Thr-Arg-Ala] (n=16) -
313 317 322

30 N-acetyl-Tyr-Gly-His-Asp-Pro-Glu-Leu-Leu-Pro-Ala-Arg-Arg-
392 397

The peptide segments of the pituitary GRF receptor and their Multiple (hexadecameric) Antigenic Peptide Systems (MAPS) were synthesized by standard solid phase methodology using a TFA/HF compatible scheme. The degree of substitution of the first amino acid coupled to the Merrifield resin was 0.36mmol/g for the linear peptides, while the degree of substitution of first amino acid coupled (BAla) to the Merrifield resin was 0.11mmol/g for the MAPS (Tam J. P.,

1988, Proc. Natl. Acad. Sci. USA, 85, 5409-5413). The peptides was purified by reverse-phase HPLC whereas the MAPS were purified by membrane dialysis with a cut-off point of 12 kD. Their homogeneity was assessed
5 by analytical HPLC using appropriate binary solvent systems. The peptides and the MAPS yielded the predicted amino acid composition with respective peptide contents of 78-87% and 72-85%. The haptenic contents represented 91-94% of the total mass of the MAPS.

10

5-Antisera production

Each MAPS was injected intramuscularly and intradermally in 3 to 4 New Zealand white male rabbits, initially at a dose of 0.5 mg/kg in complete
15 Freund adjuvant. The rabbits were subsequently boosted once every four weeks at a peptide dose of 0.165mg/kg in incomplete Freund adjuvant. Blood was obtained before the immunization and subsequently two week after each immunization.

20

6-Western blotting

The proteins from rat pituitary homogenates were separated by electrophoresis using 12% polyacrylamide gels and then transferred to nitrocellulose
25 membranes. These membranes were then incubated with the anti-GRF-receptor antisera of interest at a dilution of 1/200 to 1/5000 for 48 h at 4°C. Subsequently the membrane were incubated with a secondary antibody linked to alkaline phosphatase for 24h at 4°C and
30 labeling revealed according to the manufacturer's specifications.

7-Immunocytochemistry

For optical immunocytochemistry, adult male
35 Sprague-Dawley rats were perfused with 4% 2 in 100 mM

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phosphate buffer, pH 7.4. Their pituitaries were removed and immersed in the same fixative for 24 h. Once fixed, the tissues were dehydrated and embedded in paraffin. For ultrastructural immunocytochemical studies, the anterior pituitaries were removed, cut into 1mm³ pieces, and fixed by immersion in 4% buffered paraformaldehyde. Ultrathin frozen sections (100 nm) were cut at -120°C on a UltracutTM microtome equipped with a FO4D cryosectioning system. Additional pituitaries were embedded in LowicrylTMK4M resin. Dehydration through graded ethanol and polymerization were performed at -20°C. The tissue sections were incubated with the anti-GRF-receptor antisera of interest at a dilution of 1/50 to 1/10000, in 100 mM phosphate buffer saline for 1 h at room temperature.

Sections were then incubated with a secondary antibody coupled to horseradish peroxidase or gold particles and labeling revealed according to the manufacturer's specifications.

The antisera labeled major bands at 45kD and 65kD in rat pituitary homogenates while no signal was detected with pre-immune sera (Fig. 1A). Lanes 1-7, 50µg adenopituitary homogenate; lanes 1-3, anti29-40 at 16, 1.6 and 0.16 µg IgG; lanes 4-7, anti392-404 at 16, 1.6, 0.16 and 0.016 µg IgG. They also labeled a 45kD band membrane preparations of baby kidney hamster (BHK) cells transfected with the human GRF receptor but not in wild type cells (Fig. 1B). Lanes 1 and 5, 20 µg of wild-type membrane preparations; lanes 3-5 and 6-9, 20 µg of GRF-transfected membrane preparations; lanes 1-2, anti29-40 at 16 µg IgG; lanes 3-4, anti29-40 at 1.6 and 0.16 µg IgG; lanes 5-6, anti392-404 at 16 µg IgG; lanes 7-9, anti392-404 at 1.6, 0.16 and 0.016 µg IgG. At the optical level, GRF receptor immunoreactivity appeared as a brown deposit. Immun-

oreactivity is mainly localized at the level of cytoplasm as shown in Fig. 2B. The human (Fig. 2A) and rat (Fig. 1B) pituitary sections immunostained for the GRF receptor showed numerous immunoreactive cells (40-50%). The reaction was localized in the cytoplasm and in the nucleus of ~30% of positive cells. This immunostaining was specific since no signal was observed with corresponding preimmune sera or when the primary sera were omitted. In rat pituitary ultrathin cryosections, the immunocytochemical labeling obtained with these antisera was selective for the somatotrophs. The ultrastructural distribution of gold particles correlated with the reported distribution of ^{125}I -GRF. Highest densities were associated to the plasma membrane and secretory granules, moderate densities were found in the cytoplasmic matrix and nucleus.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

Example I

***In vitro* binding affinity of fluoresceinyl analogues of hGRF(1-29)NH₂ and hGRF(1-44)NH₂ for [^{125}I -Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary and adenylate cyclase activity of [Nε-5-carboxy-fluoresceinyl-Lys³¹]hGRF(1-44)NH₂ compared to hGRF(1-29)NH₂ in rat adenopituitary**

As shown in Tables 4 and 5, the biological activity of [Nε-5-carboxy-fluoresceinyl-Lys³¹]hGRF(1-44)NH₂ adenopituitary was similar to that of native GRF, indicating that this compound has a high affinity for GRF receptors and is a full GRF receptor agonist.

[Nα-fluoresceinthiocarbamyl]hGRF(1-44)NH₂ and [Nα-fluoresceinthiocarbamyl]hGRF(1-29)NH₂ still exhib-

ited a nanomolar affinity for the adenopituitary GRF receptor indicating that additional modifications to these compounds, such as those reported in example III, would result in additional fluorescent GRF receptor markers exhibiting a biological activity similar to that of native GRF.

Table 4

10 **Binding affinity of fluoresceinyl analogues of hGRF(1-29)NH₂ and hGRF(1-44)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary compared to hGRF(1-29)NH₂**

Number	Compounds	IC ₅₀ (nM)	Relative affinity (%)	Hill coef.
1	hGRF(1-29)NH ₂	2.67±0.12	100±4	0.50±0.03
2	[Nα-Fluoresceinylthiocarbamyl]hGRF(1-29)NH ₂	14±8	19±11	0.61±0.05
3	[Nα-Fluoresceinylthiocarbamyl]hGRF(1-44)NH ₂	14±4	19±5	0.63±0.07
4	[Nε-5-carboxyfluoresceinyl-Lys ³¹]hGRF(1-44)NH ₂	3.65±1.34	73±27	0.55±0.03

15

Values represent the mean±SD of 2 experiments performed in triplicate for the analogues and the mean ± SE of 29 experiments performed in triplicate for hGRF(1-29)NH₂. IC₅₀ is the concentration of peptide inhibiting 50% of ¹²⁵I-GRF-specific binding as determined by the LIGAND program for analysis of competition studies. The relative affinity was obtained by taking the ratio IC₅₀ of hGRF(1-29)NH₂/IC₅₀ analogue.

25

Table 5

Adenylate cyclase activity of [Nε-5-carboxy-fluo-Lys³¹]hGRF(1-44)NH₂ compared to hGRF(1-29)NH₂ in rat adenopituitary

5

No.	Compounds	IC ₅₀ (nM)	Relative activity (%)
1	hGRF(1-29)NH ₂	36.7±5.5	100±15
2	[Nε-5-carboxy-fluo-Lys ³¹] hGRF(1-44)NH ₂	67±24	55±36

Values represent the mean ± SD of 2 experiments performed in triplicate for the analogue and the mean ± SE of 10 experiments performed in triplicate for hGRF(1-29)NH₂. EC₅₀ is the concentration of peptide inducing 50% of maximal cAMP accumulation induced by 1μM hGRF(1-29)NH₂. The relative activity was obtained by taking the ratio EC₅₀ of hGRF(1-29)NH₂/EC₅₀ analogue. The maximal activity of the analogues was not significantly different from that of hGRF(1-29)NH₂.

Example II

In vitro binding affinity of photoreactive analogues of hGRF(1-29)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary

The chemical integrity of the GRF analogs presented in Tables 6 and 7 is reported in Tables 8 and 9. As shown in Table 7, a monosubstitution in position 9 or 10 of GRF with 4'-nitro-phenylalanine, the precursor of the photoreactive 4'-azido-phenylalanine, allowed to preserve the biological activity of the native peptide indicating that the introduction of the photoreactive 4'-azido-phenylalanine in these position would also allowed to preserve the biological activity of the native peptide. A monosubstitution in position 10 of GRF with bpa allowed also to preserve the

- 30 -

biological activity of native GRF. Moreover, an addition to the N α -terminus of GRF with npa or bpa, or a monosubstitution in position 4 or 5 or 6 or 7 or 8 with npa, or a monosubstitution in position 4 or 9 together with additional modifications, such as those reported in Example III, would result in photoreactive GRF receptor markers exhibiting a biological activity similar to that of native GRF and would give powerful markers to perform mapping of the GRF receptor binding site.

[4'-nitro-phenylalanine⁹]hGRF(1-29)NH₂, [4'-nitro-phenylalanine¹⁰]hGRF(1-29)NH₂ and 4'-benzoyl-phenylalanine¹⁰]hGRF(1-29)NH₂ exhibited a high affinity for adenopituitary GRF receptors. Their IC₅₀ were all in the low nanomolar range.

Table 6
Amino acid composition of compounds

Number	Compound structure
1	Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
2	Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH ₂ 20 25 (SEQ ID NO:3)
3	npa Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
4	Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH ₂ 20 25 (SEQ ID NO:13)
5	npa Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
6	Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH ₂ 20 25 (SEQ ID NO:15)
7	Tyr npa Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15

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Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 16)

5

5 Tyr Ala npa Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 17)

6

10 Tyr Ala Asp npa Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 18)

15 7

Tyr Ala Asp Ala npa Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 19)

20 8

Tyr Ala Asp Ala Ile npa Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

25 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 20)

9

Tyr Ala Asp Ala Ile Phe npa Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

30 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 21)

10

35 Tyr Ala Asp Ala Ile Phe Thr npa Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 22)

11

40 Tyr Ala Asp Ala Ile Phe Thr Asn npa Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 23)

12

45 Tyr Ala Asp Ala Ile Phe Thr Asn Ser npa Arg Lys Val Leu Gly Gln
 1 5 10 15

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Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 24)

13

5 bpa Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 14)

14

10 Tyr Ala Asp bpa Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 25)

15 15

Tyr Ala Asp Ala Ile Phe Thr Asn bpa Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

20 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 26)

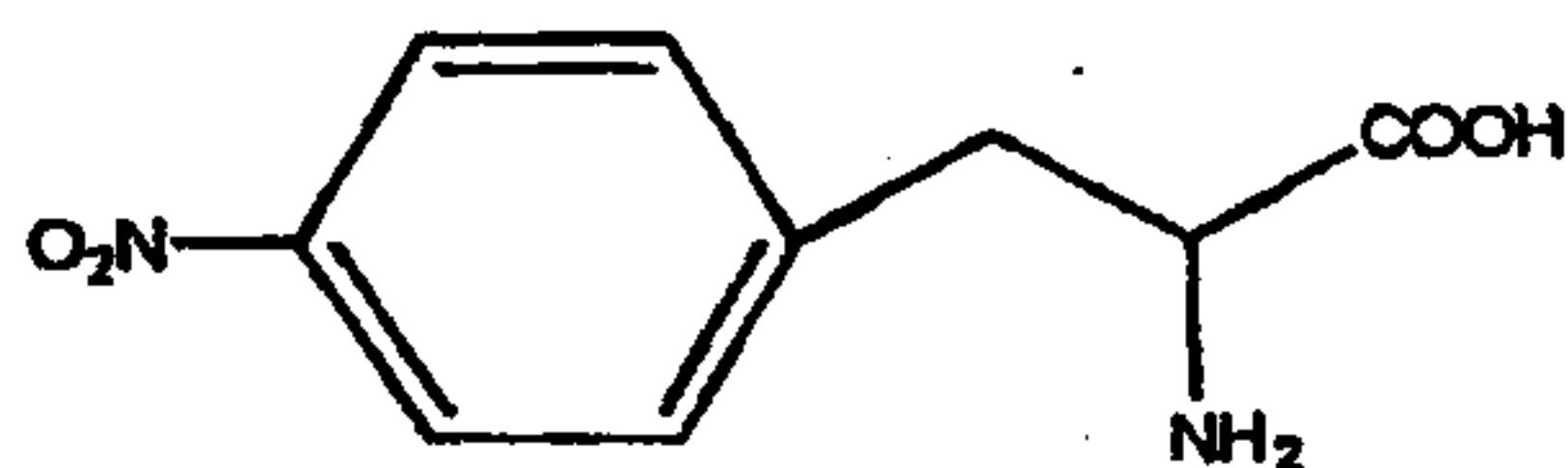
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Tyr Ala Asp Ala Ile Phe Thr Asn Ser bpa Arg Lys Val Leu Gly Gln
 1 5 10 15

25 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO: 27)

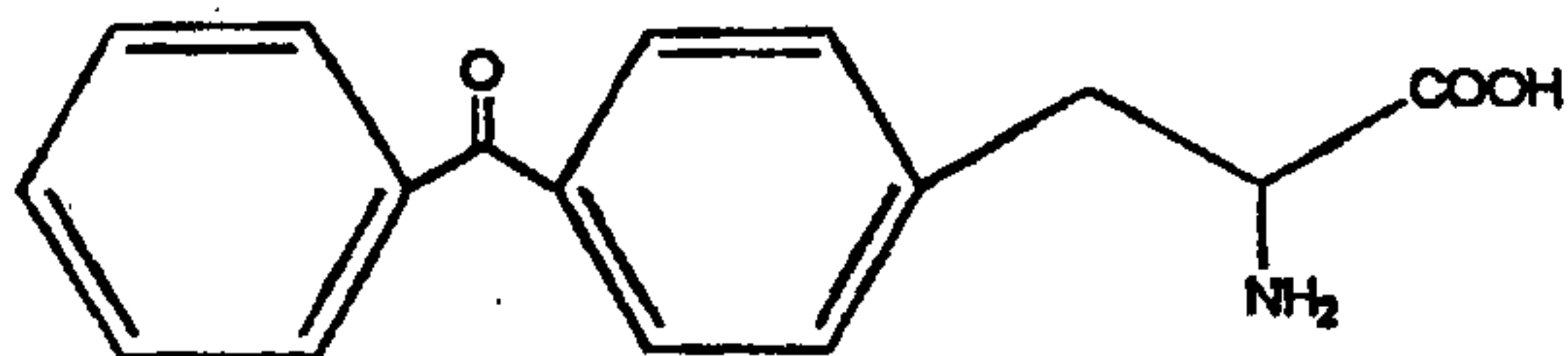
30 Detailed chemical structure of npa and bpa are:

npa is 4'-nitro-L-phenylalanine:



35

bpa is 4'-benzoyl-L-phenylalanine:



- 33 -

Table 7

5 Binding affinity of 4'-nitro-phenylalanyl (npa) and 4'-benzoylphenylalanyl (bpa) monosubstituted analogues of hGRF(1-29)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary.

Number	Substitutions	IC ₅₀ (nM)	Relative affinity (%)	Hill coef.	SEQ ID NO
1	none	2.67±0.12	100±4	0.50±0.03	3
2	npa ⁰	18.3±5.9	14±5	0.63±0.09	13
3	npa ¹	376±107	7±2	0.63±0.04	15
4	npa ²	751±97	0.30±0.04	0.68±0.03	16
5	npa ³	197±21	1.3±0.1	0.72±0.05	17
6	npa ⁴	8.65±1.63	31±6	0.66±0.06	18
7	npa ⁵	27.7±8.5	10±3	0.81±0.03	19
8	npa ⁶	30.9±1.2	8.6±0.3	0.53±0.06	20
9	npa ⁷	33.7±0.6	7.9±0.1	0.79±0.08	21
10	npa ⁸	54.1±11.5	5±1	0.37±0.06	22
11	npa ⁹	9.97±2.45	27±7	0.57±0.22	23
12	npa ¹⁰	4.43±0.26	60±3	0.43±0.03	24
13	bpa ⁰	34.5±7.0	8±2	0.63±0.07	14
14	bpa ⁴	64.7±13.6	4±1	0.72±0.08	25
15	bpa ⁹	28.4±9.8	9±3	0.61±0.06	26
16	bpa ¹⁰	5.77±0.04	46.0±0.1	0.79±0.08	27

Values represent the mean ± SD of 2 experiments or the mean ± SE of 3-6 experiments performed in triplicate for the analogues and the mean ± SE of 29 experiments performed in triplicate for hGRF(1-29)NH₂. IC₅₀ is the concentration of peptide inhibiting 50% of ¹²⁵I-GRF-specific binding as determined by the LIGAND program for analysis of competition studies. The relative affinity was obtained by taking the ratio IC₅₀ of hGRF(1-29)NH₂/IC₅₀ analogue.

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Table 8

Physicochemical data of 4'-nitro-phenylalanyl (npa)
and 4'-benzoylphenylalanyl (bpa) monosubstituted
analogues of hGRF(1-29)NH₂

5

No.	MW	% overall yield	t _R ^a , min	HPLC
				% homogeneity (214 nm/280 nm)
1	3358	24	24.0 ^b , 20.1 ^c	99/100 ^b , 99/100 ^c
2	3551	11	26.8 ^b , 24.2 ^c	100/100 ^b , 100/100 ^c
3	3386	18	25.4 ^b , 22.4 ^c	99/100 ^b , 100/100 ^c
4	3480	6	29.0 ^b , 23.6 ^c	100/100 ^b , 100/100 ^c
5	3440	18	29.2 ^b , 24.6 ^c	100/100 ^b , 100/100 ^c
6	3480	13	27.6 ^b , 22.2 ^c	99/100 ^b , 100/100 ^c
7	3438	21	28.0 ^b , 21.8 ^c	100/100 ^b , 100/100 ^c
8	3402	18	26.8 ^b , 20.6 ^c	100/100 ^b , 100/100 ^c
9	3449	20	30.2 ^b , 23.4 ^c	100/100 ^b , 100/100 ^c
10	3436	8	25.2 ^b , 23.8 ^c	99/100 ^b , 100/100 ^c
11	3463	25	27.8 ^b , 25.4 ^c	99/100 ^b , 100/100 ^c
12	3387	19	26.0 ^b , 23.8 ^c	99/100 ^b , 100/100 ^c
13	3505	12	27.8 ^b , 25.2 ^c	100/100 ^b , 100/100 ^c
14	3434	10	27.6 ^b , 23.8 ^c	100/100 ^b , 100/100 ^c
15	3418	15	30.2 ^b , 26.8 ^c	99/100 ^b , 100/100 ^c
16	3342	14	24.4 ^b , 25.2 ^c	99/100 ^b , 100/100 ^c

Identification of compounds corresponds to that of Table 6.

10

^a μ BondapakTM C18 (10- μ m particles) column (0.39cm x 15cm);
t_R retention time.

15

^b Linear gradient: solvent A consisted of 0.01% aqueous TFA (pH 2.9) and solvent B consisted of CH₃CN/0.01% TFA; 0.67% B/min for 45 min, initial condition 20% B, flow rate 1.5 ml/min, 23°C.

20

^c Linear gradient: solvent A consisted of 0.1 M aqueous NaClO₄ (pH 2.5) and solvent B consisted of CH₃CN; 0.67% B/min for 45 min, initial condition 30% B, flow rate 1.5 ml/min, 23°C.

25

Compound no. 1 is hGRF(1-29)NH₂.

Table 9

Quantitative amino acid analysis of 4'-nitro-phenylalanyl (npa) and 4-benzoylphenylalanyl (bpa) monosubstituted analogues of hGRF(1-29)NH₂

No.	Asx	Glx	Ser	Gly	Arg	Thr	Ala	Tyr	Val	Met	Ile	Leu	Phe	Lys	P.C. (%)
1	2.83(3)	2.11(2)	2.59(3)	1.05(1)	3.36(3)	0.87(1)	3.10(3)	2.02(2)	1.02(1)	0.90(1)	2.10(2)	4.07(4)	0.98(1)	1.94(2)	83
2	3.08(3)	2.07(2)	2.13(3)	1.09(1)	3.43(3)	0.91(1)	3.18(3)	2.18(2)	1.08(1)	0.69(1)	2.01(2)	4.00(4)	0.99(1)	1.79(2)	75
3	3.04(3)	2.07(2)	2.60(3)	1.06(1)	3.37(3)	0.96(1)	3.07(3)	0.80(1)	0.96(1)	1.00(1)	1.91(2)	3.69(4)	0.96(1)	2.42(2)	72
4	2.93(3)	2.08(2)	2.18(3)	1.03(1)	3.48(3)	0.82(1)	2.19(2)	1.65(2)	1.04(1)	0.99(1)	2.08(2)	3.92(4)	0.99(1)	1.97(2)	72
5	1.45(2)	1.88(2)	2.62(3)	1.24(1)	3.63(3)	1.05(1)	2.76(3)	1.81(2)	0.93(1)	1.09(1)	2.09(2)	4.41(4)	1.00(1)	1.90(2)	62
6	2.75(3)	2.07(2)	2.63(3)	1.21(1)	3.55(3)	1.05(1)	1.73(2)	1.88(2)	0.96(1)	1.09(1)	1.55(2)	4.16(4)	0.96(1)	2.14(2)	83
7	2.70(3)	2.08(2)	2.60(3)	1.23(1)	3.58(3)	1.00(1)	2.63(3)	1.70(2)	0.95(1)	1.05(1)	1.03(1)	4.18(4)	1.02(1)	2.15(2)	65
8	3.19(3)	1.95(2)	2.56(3)	1.05(1)	3.25(3)	0.96(1)	2.93(3)	1.74(2)	1.10(1)	1.17(1)	1.88(2)	4.04(4)		2.14(2)	72
9	2.96(3)	2.16(2)	2.24(3)	1.05(1)	3.36(3)		3.13(3)	1.45(2)	1.07(1)	0.82(1)	2.17(2)	4.23(4)	1.07(1)	2.05(2)	66
10	1.71(2)	2.00(2)	2.48(3)	1.09(1)	3.43(3)	0.87(1)	3.18(3)	1.52(2)	1.09(1)	1.08(1)	2.09(2)	4.15(4)	1.11(1)	2.06(2)	67
11	2.95(3)	2.14(2)	1.62(2)	1.03(1)	3.19(3)	0.89(1)	3.02(3)	1.56(2)	1.04(1)	1.13(1)	2.14(2)	4.10(4)	1.09(1)	2.03(2)	75
12	2.89(3)	2.08(2)	2.23(3)	1.05(1)	3.24(3)	0.67(1)	3.05(3)	0.73(1)	1.01(1)	0.88(1)	2.45(2)	4.07(4)	1.00(1)	1.98(2)	72
13	3.11(3)	2.07(2)	2.29(3)	1.02(1)	3.24(3)	1.12(1)	3.12(3)	1.71(2)	1.02(1)	1.12(1)	2.09(2)	4.02(4)	1.10(1)	1.78(2)	66
14	2.97(3)	2.04(2)	2.24(3)	1.03(1)	3.23(3)	0.90(1)	2.01(2)	1.55(2)	0.97(1)	1.13(1)	2.06(2)	4.13(4)	1.38(1)	2.31(2)	62
15	3.03(3)	2.05(2)	1.53(2)	1.05(1)	3.22(3)	0.90(1)	3.09(3)	1.65(2)	1.01(1)	1.04(1)	2.04(2)	4.06(4)	1.11(1)	1.80(2)	63
16	3.07(3)	2.04(2)	2.35(3)	1.08(1)	3.31(3)	0.95(1)	2.97(3)	0.80(1)	1.03(1)	1.15(1)	2.04(2)	4.14(4)	0.96(1)	1.99(2)	62

Identification of compounds corresponds to that of Table 6.

Amino acid composition was determined after acidic hydrolysis (6N HCl, 110°C, 18h) and PITC derivatization by reverse-phase HPLC (Waters PICO-TAG™ column, 0.39 X 15cm, 38°C) using a solvent system consisting of 140 mM sodium acetate (pH 6.4) and 60% aqueous CH₃CN.

Detection at 254 nm.

Example III

5 *In vitro* binding affinity of mono and polysubstituted analogues of hGRF(1-29)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary and their adenylate cyclase activity compared to hGRF(1-29)NH₂ in rat adenopituitary

10 The chemical integrity of the GRF analogs presented in Tables 10, 11 and 12 is reported in Tables 13 and 14. As shown in Tables 10 and 11, the biological activity of [D-Ala²,D-Tyr¹⁰], [D-Ala²,D-Tyr¹⁰,D-Ala¹⁵], [D-Ala²,D-Tyr¹⁰,D-Ala¹⁵,D-Lys²¹], [D-Ala²,D-Tyr¹⁰,D-Lys²¹], [Lys²²], [D-Ala²,D-Tyr¹⁰,Lys²²], [D-Ala²,D-Tyr¹⁰,D-Ala¹⁵,Lys²²], [D-Asn⁸,D-Leu²²], [Ala⁸,Ala¹⁵,Ala²²], [Ala⁸,Ala⁹,Ala¹⁵,Ala²²] and [octanoyl³⁰,Cys³¹] hGRF(1-29)NH₂ in rat adenopituitary was preserved or increased compared to that of native GRF, indicating that substitutions by D-amino acid to

20 increased *in vitro* and *in vivo* metabolic stability of GRF together with amino acid substitutions, that increase the biological activity of native GRF, are effective means to design potent GRF receptor agonists with a longer duration of action.

Table 10
Amino acid composition of compounds

	Number	Compound structure
5	1	Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln 1 5 10 15 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO:3)
10	2	Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln 1 5 10 15 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO:28)
15	3	Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln 1 5 10 15 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO:29)
20	4	Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln 1 5 10 15 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO:30)
25	5	Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln 1 5 10 15 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO:31)
30	6	Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln 1 5 10 15 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg-NH₂ 20 25 (SEQ ID NO: 35)
35	7	Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln 1 5 10 15
40		
45		
50		

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Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO:28)

5 8

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15

10 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO:29)

9

15 Tyr Ala Asp Ala Ile Phe Thr D-Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys D-Leu Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO:34)

10

20 Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15

25 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO:36)

11

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15

30 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg-NH₂
 20 25 (SEQ ID NO:10)

12

35 Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa-NH₂
 20 25 30 (SEQ ID NO:11)

40

Detailed chemical structure of (CH₂)_{n=8}

45 CH₂)_{n=8} is derived from 8-amino octanoic acid which
 was introduced in the peptide chain using the standard
 methods of solid-phase peptide synthesis.

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Table 11

Binding affinity of polysubstituted analogues of
hGRF(1-29)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding
sites in rat adenopituitary

5

No.	Substitution	IC ₅₀ (nM)	Relative affinity (%)	Hill coef.	SEQ ID No.
1	none	2.40±0.46	100±19	0.45±0.04	3
2	D-Ala ² ,D-Tyr ¹⁰	9.65±1.20	25±3	0.41±0.02	28
3	D-Ala ² ,D-Tyr ¹⁰ ,D-Ala ¹⁵	13.1±0.9	18±1	0.59±0.07	29
4	D-Ala ² ,D-Tyr ¹⁰ ,D-Lys ²¹	11.6±6.2	21±11	0.64±0.05	30
5	D-Ala ² ,D-Tyr ¹⁰ ,D-Ala ¹⁵ , D-Lys ²¹	31.6±11.7	8±3	0.68±0.03	31
6	Lys ²²	0.23±0.04	1043±181	0.62±0.06	40
7	D-Ala ² ,D-Tyr ¹⁰ ,Lys ²²	0.49±0.27	490±270	0.62±0.06	32
8	D-Ala ² ,D-Tyr ¹⁰ ,D-Ala ¹⁵ , Lys ²²	2.49±0.98	104±40	0.52±0.06	33
9	D-Asn ⁸ ,D-Leu ²²	4.90±3.25	49±33	0.48±0.04	34
10	Ala ⁸ ,Ala ¹⁵ ,Ala ²²	0.25±0.07	960±269	0.48±0.05	36
11	Ala ⁸ ,Ala ⁹ ,Ala ¹⁵ ,Ala ²²	0.31±0.14	774±349	0.47±0.01	10
12	Octanoyl ³⁰ , Cys ³¹	6.84±2.12	35±11	0.43±0.14	11

Values represent the mean±SD of 2 experiments
or the mean±SE of 3 experiments performed in tripli-
cate for the analogues and the mean±SE of 11 experi-
ments performed in triplicate for hGRF(1-29)NH₂. IC₅₀
is the concentration of peptide inhibiting 50% of
¹²⁵I-GRF-specific binding as determined by the LIGAND
program for analysis of competition studies. The
relative affinity was obtained by taking the ratio
IC₅₀ of hGRF(1-29)NH₂/IC₅₀ analogue.

- 40 -

Table 12

5 Adenylate cyclase activity of polysubstituted analogues of hGRF(1-29)NH₂ for [¹²⁵I-Tyr¹⁰]hGRF(1-44)NH₂ binding sites in rat adenopituitary

Number	Substitutions	IC ₅₀ (nM)	Relative activity (%)	SEQ ID NO.
1	none	36.7±5.5	100±15	3
2	D-Ala ² ,D-Tyr ¹⁰ ,D-Ala ¹⁵	176±87	21±10	29
3	D-Ala ² ,D-Tyr ¹⁰ ,D-Ala ¹⁵ ,D-Lys ²¹	136±50	27±10	31
4	Ala ⁸ ,Ala ¹⁵ ,Ala ²²	2.60±0.70	1412±380	36
5	Ala ⁸ ,Ala ⁹ ,Ala ¹⁵ ,Ala ²²	2.88±1.01	1274±448	10
6	Lys ²²	2.63±0.64	1395±339	40
7	D-Asn ⁸ ,D-Leu ²²	67±24	55±36	34

10 Values represent the mean ± SD of 2 experiments performed in triplicate or the mean ± SE of 3 to 6 experiments performed in triplicate for the analogues and the mean ± SE of 10 experiments performed in triplicate for hGRF(1-29)NH₂. EC₅₀ is the concentration of peptide inducing 50% of maximal cAMP accumulation induced by 1 μM hGRF(1-29)NH₂. The relative activity
15 was obtained by taking the ratio EC₅₀ of hGRF(1-29)NH₂/EC₅₀ analogue. The maximal activity of the analogues was not significantly different from that of hGRF(1-29)NH₂.

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Table 13

**Physicochemical data of mono and polysubstituted
analogues of hGRF(1-29)NH₂**

5

No.	MW	% overall yield	t_R^a , min	HPLC
				% homogeneity (214 nm/280 nm)
1	3358	24	24.0 ^b , 20.1 ^c	99/100 ^b , 99/100 ^c
2	3358	18	23.6 ^b , 21.4 ^c	100/100 ^b , 100/100 ^c
3	3372	20	23.8 ^b , 20.6 ^c	99/100 ^b , 100/100 ^c
4	3372	14	21.2 ^b , 20.0 ^c	100/100 ^b , 100/100 ^c
5	3372	7	20.4 ^b , 18.8 ^c	100/100 ^b , 100/100 ^c
6	3373	11	14.0 ^b , 13.6 ^c	100/100 ^b , 100/100 ^c
7	3373	10	13.8 ^b , 12.8 ^c	100/100 ^b , 100/100 ^c
8	3387	10	14.0 ^b , 11.8 ^c	100/100 ^b , 100/100 ^c
9	3358	6	21.0 ^b , 19.0 ^c	100/100 ^b , 100/100 ^c
10	3287	13	21.0 ^b , 18.2 ^c	100/100 ^b , 100/100 ^c
11	3271	12	23.2 ^b , 20.2 ^c	100/100 ^b , 100/100 ^c
12	3602	9	27.4 ^b , 23.4 ^c	96/100 ^b , 100/100 ^c

Identification of compounds corresponds to that of Table 10.

- 10 a μ BondapakTM C18 (10- μ m particles) column (0.39-cm x 15-cm);
 t_R retention time.
- 15 b Linear gradient: solvent A consisted of 0.01% aqueous TFA (pH 2.9) and solvent B consisted of CH₃CN/0.01% TFA; 0.67% B/min for 45 min, initial condition 20% B, flow rate 1.5 ml/min, 23°C.
- 20 c Linear gradient: solvent A consisted of 0.1 M aqueous NaClO₄ (pH 2.5) and solvent B consisted of CH₃CN; 0.67% B/min for 45 min, initial condition 30% B, flow rate 1.5 ml/min, 23°C.
- 25 Compound no. 1 is hGRF(1-29)NH₂.

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Table 14
Quantitative amino acid analysis of mono and polysubstituted analogues of hGRF(1-29)NH₂

No.	Asx	Glx	Ser	Gly	Arg	Thr	Ala	Tyr	Val	Met	Cys	Ile	Leu	Phe	Lys	P.C. (%)
1	2.83(3)	2.11(2)	2.59(3)	1.05(1)	3.36(3)	0.87(1)	3.10(3)	2.02(2)	1.02(1)	0.90(1)		2.10(2)	4.07(4)	0.98(1)	1.94(2)	83
2	3.08(3)	2.11(2)	2.50(3)	1.02(1)	3.36(3)	0.87(1)	3.17(3)	1.50(2)	1.04(1)	1.00(1)		2.10(2)	4.23(4)	1.15(1)	1.96(2)	77
3	3.12(3)	2.10(2)	2.33(3)		3.40(3)	0.82(1)	4.07(4)	1.72(2)	1.04(1)	1.07(1)		2.08(2)	4.16(4)	1.02(1)	2.03(2)	69
4	2.92(3)	2.09(2)	2.32(3)		3.41(3)	0.82(1)	4.15(4)	1.49(2)	1.10(1)	1.19(1)		2.16(2)	4.17(4)	1.08(1)	2.06(2)	71
5	2.87(3)	2.13(2)	2.37(3)		3.41(3)	0.89(1)	4.07(4)	1.57(2)	1.06(1)	1.20(1)		2.08(2)	4.14(4)	1.02(1)	2.07(2)	68
6	3.21(3)	2.10(2)	2.11(3)	1.02(1)	3.11(3)	0.83(1)	2.83(3)	1.57(2)	1.05(1)	1.01(1)		2.00(2)	3.02(3)	0.99(1)	2.96(3)	77
7	3.13(3)	2.10(2)	2.17(3)	1.02(1)	3.13(3)	0.83(1)	2.85(3)	1.49(2)	1.05(1)	1.02(1)		2.00(2)	3.05(3)	0.98(1)	2.95(3)	78
8	2.93(3)	1.93(2)	2.06(3)		3.03(3)	0.81(1)	3.74(4)	1.69(2)	1.03(1)	1.18(1)		1.93(2)	2.90(3)	1.08(1)	2.93(3)	69
9	3.22(3)	2.23(2)	1.99(3)	1.08(1)	3.49(3)	0.75(1)	3.23(3)	1.82(2)	1.10(1)	0.97(1)		2.13(2)	4.10(4)	1.05(1)	1.96(2)	65
10	1.81(2)	2.12(2)	2.64(3)		3.79(3)	0.93(1)	5.60(6)	1.70(2)	1.13(1)	1.18(1)		1.79(2)	3.36(3)	0.99(1)	1.96(2)	84
11	2.01(2)	2.03(2)	1.78(2)		3.55(3)	0.98(1)	7.18(7)	1.69(2)	0.93(1)	1.07(1)		1.90(2)	3.16(3)	0.97(1)	2.04(2)	77
12	2.79(3)	2.04(2)	2.51(3)	1.13(1)	3.45(3)	0.93(1)	3.61(3)	1.34(2)	0.88(1)	0.97(1)	0.56	1.99(2)	4.22(4)	1.03(1)	2.01(2)	72

Identification of compounds corresponds to that of Table 10.

Amino acid composition was determined after acidic hydrolysis (6N HCl, 110°C, 18h) and PITC derivatization by reverse-phase HPLC (Waters PICO-TAG™ column, 0.39 X 15cm, 38°C) using a solvent system consisting of 140 mM sodium acetate (pH 6.4) and 60% aqueous CH₃CN.

Detection at 254 nm.

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Example IV**In vitro labeling of GRF receptors on human and rat pituitary tissue sections**

5

For optical immunocytochemistry, adult male Sprague-Dawley rats were perfused with 4% 2 in 100 mM phosphate buffer, pH 7.4. Their pituitaries were removed and immersed in the same fixative for 24 h. 10 Once fixed, the tissues were dehydrated and embedded in paraffin. For ultrastructural immunocytological studies, the anterior pituitaries were removed, cut into 1mm³ pieces, and fixed by immersion in 4% buffered paraformaldehyde. Ultrathin frozen sections (100 15 nm) were cut at -120°C on a UltracutTM microtome equipped with a FO4D cryosectioning system. Additional pituitaries were embedded in LowicrylTM K4M resin. Dehydration through graded ethanol and polymerization were performed at -20°C. The tissue sections 20 were incubated with the anti-GRF-receptor antisera of interest at a dilution of 1/50 to 1/10000, in 100 mM phosphate buffer saline for 1 h at room temperature.

Sections were then incubated with a secondary antibody coupled to horseradish peroxidase or gold 25 particles and labeling revealed according to the manufacturer's specifications.

At the optical level, GRF receptor immunoreactivity appeared as a brown deposit. The human (Fig. 2A) and rat (Fig. 2B) pituitary sections immunostained 30 for the GRF receptor showed numerous immunoreactive cells (40-50%). The reaction was localized in the cytoplasm and in the nucleus of ~30% of positive cells. This immunostaining was specific since no signal was observed with corresponding preimmune sera or 35 when the primary sera were omitted.

In rat pituitary ultrathin cryosections, the immunocytochemical labeling obtained with these antisera was selective for the somatotrophs. The ultrastructural distribution of gold particles correlated with the reported distribution of ^{125}I -GRF. Highest densities were associated to the plasma membrane and secretory granules, moderate densities were found in the cytoplasmic matrix and nucleus.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

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SEQUENCE LISTING

GENERAL INFORMATION

APPLICANT: UNIVERSITE DE MONTREAL

TITLE OF INVENTION: MARKER FOR GROWTH HORMONE-RELEASING FACTOR RECEPTORS

NUMBER OF SEQUENCES: 40

CORRESPONDENCE ADDRESS: Janique Forget, BCF LLP 1100 Boul. René-Lévesque
Ouest, 25ème étage Montréal, Québec, H3B 5C9

COMPUTER READABLE FORM

COMPUTER: IBM PC Compatible

OPERATING SYSTEM: MS-DOS

SOFTWARE: PATENTIN VERSION 3.4

CURRENT APPLICATION DATA

APPLICATION NUMBER: 2,158,782

FILING DATE: 1995-09-21

CLASSIFICATION: C07K 14/60 (2006.01)

PRIOR APPLICATION DATA

APPLICATION NUMBER: 08/312,244

FILING DATE: 1994-09-23

PATENT AGENT INFORMATION

NAME: Janique Forget

REFERENCE NUMBER: 09832-222

INFORMATION FOR SEQ ID NO.:1

SEQUENCE CHARACTERISTICS

LENGTH: 44 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:1

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg	Gln	Gln	Gly
			20					25					30		

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Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
35 40

INFORMATION FOR SEQ ID NO.:2

SEQUENCE CHARACTERISTICS
LENGTH: 15 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:2

Gln Gln Gly Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
1 5 10 15

INFORMATION FOR SEQ ID NO.:3

SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:3

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:4

SEQUENCE CHARACTERISTICS
LENGTH: 44 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 1
OTHER INFORMATION: Xaa is N-alpha-FTC-Tyr

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SEQUENCE DESCRIPTION: SEQ ID NO.:4

Xaa Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly
20 25 30

Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
35 40

INFORMATION FOR SEQ ID NO.:5

SEQUENCE CHARACTERISTICS

LENGTH: 44 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 31

OTHER INFORMATION: Xaa is N epsilon-CF-Lys

SEQUENCE DESCRIPTION: SEQ ID NO.:5

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Xaa Gly
20 25 30

Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
35 40

INFORMATION FOR SEQ ID NO.:6

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 1

OTHER INFORMATION: Xaa is Tyr or 4'-nitro-L-phenylalanine

FEATURE

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NAME/KEY: Modified site
 LOCATION: 2
 OTHER INFORMATION: Xaa is Ala or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 3
 OTHER INFORMATION: Xaa is Asp or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 4
 OTHER INFORMATION: Xaa is Ala or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 5
 OTHER INFORMATION: Xaa is Ile or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 6
 OTHER INFORMATION: Xaa is Phe or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 7
 OTHER INFORMATION: Xaa is Thr or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 8
 OTHER INFORMATION: Xaa is Asn or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 9
 OTHER INFORMATION: Xaa is Ser or 4'-nitro-L-phenylalanine

FEATURE

NAME/KEY: Modified site
 LOCATION: 10
 OTHER INFORMATION: Xaa is Tyr or 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:6

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5						10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:7

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
 TYPE: amino acid
 STRANDEDNESS: single
 TOPOLOGY: linear

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MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 4

OTHER INFORMATION: Xaa is Ala or 4'-benzoyl-L-phenylalanine

FEATURE

NAME/KEY: Modified site

LOCATION: 9

OTHER INFORMATION: Xaa is Ser or 4'-benzoyl-L-phenylalanine

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is Tyr or 4'-benzoyl-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:7

Tyr	Ala	Asp	Xaa	Ile	Phe	Thr	Asn	Xaa	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20				25					

INFORMATION FOR SEQ ID NO.:8

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 2

OTHER INFORMATION: Xaa is Ala or D-Ala

FEATURE

NAME/KEY: Modified site

LOCATION: 8

OTHER INFORMATION: Xaa is Asn or D-Asn

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is Tyr or D-Tyr

FEATURE

NAME/KEY: Modified site

LOCATION: 15

OTHER INFORMATION: Xaa is Gly or D-Ala

FEATURE

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NAME/KEY: Modified site
LOCATION: 21
OTHER INFORMATION: Xaa is Lys or D-Lys

FEATURE

NAME/KEY: Modified site
LOCATION: 22
OTHER INFORMATION: Xaa is Leu or D-Leu

SEQUENCE DESCRIPTION: SEQ ID NO.:8

Tyr Xaa Asp Ala Ile Phe Thr Xaa Ser Xaa Arg Lys Val Leu Xaa Gln
1 5 10 15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:9

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 22
OTHER INFORMATION: Xaa is Lys or Ala

SEQUENCE DESCRIPTION: SEQ ID NO.:9

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1 5 10 15

Leu Ser Ala Arg Lys Xaa Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:10

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:10

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
1 5 10 15

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Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:11

SEQUENCE CHARACTERISTICS
LENGTH: 30 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 30
OTHER INFORMATION: Xaa is (CH₂)₈-Cys

SEQUENCE DESCRIPTION: SEQ ID NO.:11

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa
20 25 30

INFORMATION FOR SEQ ID NO.:12

SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 1
OTHER INFORMATION: Xaa is N-alpha-FTC-Tyr

SEQUENCE DESCRIPTION: SEQ ID NO.:12

Xaa Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:13

SEQUENCE CHARACTERISTICS
LENGTH: 30 amino acids
TYPE: amino acid

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STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 1
OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:13

Xaa	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly
1				5					10					15	

Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25					30

INFORMATION FOR SEQ ID NO.:14

SEQUENCE CHARACTERISTICS
LENGTH: 30 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 1
OTHER INFORMATION: Xaa is 4'-benzoyl-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:14

Xaa	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly
1				5					10					15	

Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25					30

INFORMATION FOR SEQ ID NO.:15

SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

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LOCATION: 1

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:15

Xaa	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:16

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 2

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:16

Tyr	Xaa	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:17

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 3

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:17

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

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Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:18

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 4
OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:18

Tyr Ala Asp Xaa Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:19

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 5
OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:19

Tyr Ala Asp Ala Xaa Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:20

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single

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TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 6

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:20

Tyr	Ala	Asp	Ala	Ile	Xaa	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:21

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 7

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:21

Tyr	Ala	Asp	Ala	Ile	Phe	Xaa	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:22

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 8

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OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:22

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

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:23

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 9

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:23

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Xaa	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:24

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is 4'-nitro-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:24

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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20

25

INFORMATION FOR SEQ ID NO.:25

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 4

OTHER INFORMATION: Xaa is 4'-benzoyl-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:25

Tyr	Ala	Asp	Xaa	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:26

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 9

OTHER INFORMATION: Xaa is 4'-benzoyl-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:26

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Xaa	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:27

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

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MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is 4'-benzoyl-L-phenylalanine

SEQUENCE DESCRIPTION: SEQ ID NO.:27

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20				25					

INFORMATION FOR SEQ ID NO.:28

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 2

OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is D-Tyr

SEQUENCE DESCRIPTION: SEQ ID NO.:28

Tyr	Xaa	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20				25					

INFORMATION FOR SEQ ID NO.:29

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

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FEATURE

NAME/KEY: Modified site
LOCATION: 2
OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site
LOCATION: 10
OTHER INFORMATION: Xaa is D-Tyr

FEATURE

NAME/KEY: Modified site
LOCATION: 15
OTHER INFORMATION: Xaa is D-Ala

SEQUENCE DESCRIPTION: SEQ ID NO.:29

Tyr	Xaa	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Xaa	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:30

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 2
OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site
LOCATION: 10
OTHER INFORMATION: Xaa is D-Tyr

FEATURE

NAME/KEY: Modified site
LOCATION: 21
OTHER INFORMATION: Xaa is D-Lys

SEQUENCE DESCRIPTION: SEQ ID NO.:30

Tyr	Xaa	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Xaa	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:31

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SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 2
OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site
LOCATION: 10
OTHER INFORMATION: Xaa is D-Tyr

FEATURE

NAME/KEY: Modified site
LOCATION: 15
OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site
LOCATION: 21
OTHER INFORMATION: Xaa is D-Lys

SEQUENCE DESCRIPTION: SEQ ID NO.:31

Tyr	Xaa	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Xaa	Arg	Lys	Val	Leu	Xaa	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Xaa	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:32

SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site
LOCATION: 2
OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site
LOCATION: 10
OTHER INFORMATION: Xaa is D-Tyr

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SEQUENCE DESCRIPTION: SEQ ID NO.:32

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1 5 10 15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:33

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 2

OTHER INFORMATION: Xaa is D-Ala

FEATURE

NAME/KEY: Modified site

LOCATION: 10

OTHER INFORMATION: Xaa is D-Tyr

FEATURE

NAME/KEY: Modified site

LOCATION: 15

OTHER INFORMATION: Xaa is D-Ala

SEQUENCE DESCRIPTION: SEQ ID NO.:33

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1 5 10 15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
20 25

INFORMATION FOR SEQ ID NO.:34

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

FEATURE

NAME/KEY: Modified site

LOCATION: 8

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OTHER INFORMATION: Xaa is D-Asn

FEATURE

NAME/KEY: Modified site

LOCATION: 22

OTHER INFORMATION: Xaa is D-Leu

SEQUENCE DESCRIPTION: SEQ ID NO.:34

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

Leu	Ser	Ala	Arg	Lys	Xaa	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:35

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:35

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Ala	Ser	Tyr	Arg	Lys	Val	Leu	Ala	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Lys	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:36

SEQUENCE CHARACTERISTICS

LENGTH: 29 amino acids

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:36

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Ala	Ser	Tyr	Arg	Lys	Val	Leu	Ala	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Ala	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

INFORMATION FOR SEQ ID NO.:37

SEQUENCE CHARACTERISTICS

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LENGTH: 12 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:37

Asp Phe Ile Thr Gln Leu Arg Asp Asp Glu Leu Ala
1 5 10

INFORMATION FOR SEQ ID NO.:38

SEQUENCE CHARACTERISTICS
LENGTH: 10 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:38

Pro Ala Gln Gly Gly Leu His Thr Arg Ala
1 5 10

INFORMATION FOR SEQ ID NO.:39

SEQUENCE CHARACTERISTICS
LENGTH: 13 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO.:39

Tyr Gly His Asp Pro Glu Leu Leu Pro Ala Arg Arg Thr
1 5 10

INFORMATION FOR SEQ ID NO.:40

SEQUENCE CHARACTERISTICS
LENGTH: 29 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

MOLECULE TYPE: peptide

HYPOTHETICAL: NO

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SEQUENCE DESCRIPTION: SEQ ID NO.:40

Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly	Gln
1				5					10					15	

Leu	Ser	Ala	Arg	Lys	Lys	Leu	Gln	Asp	Ile	Met	Ser	Arg
			20					25				

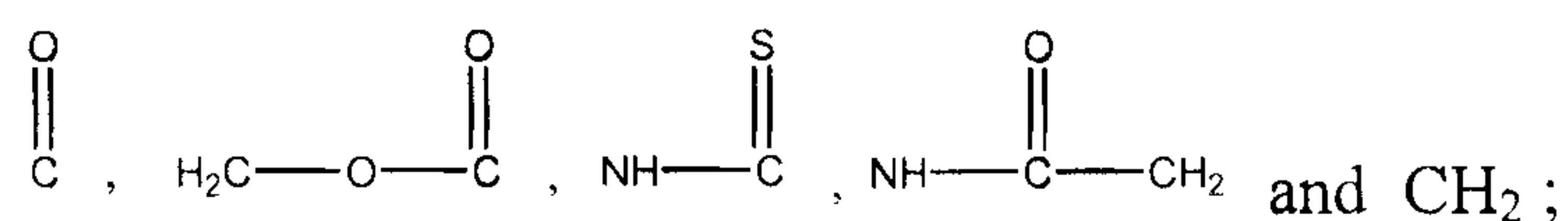
The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A compound having the formula:

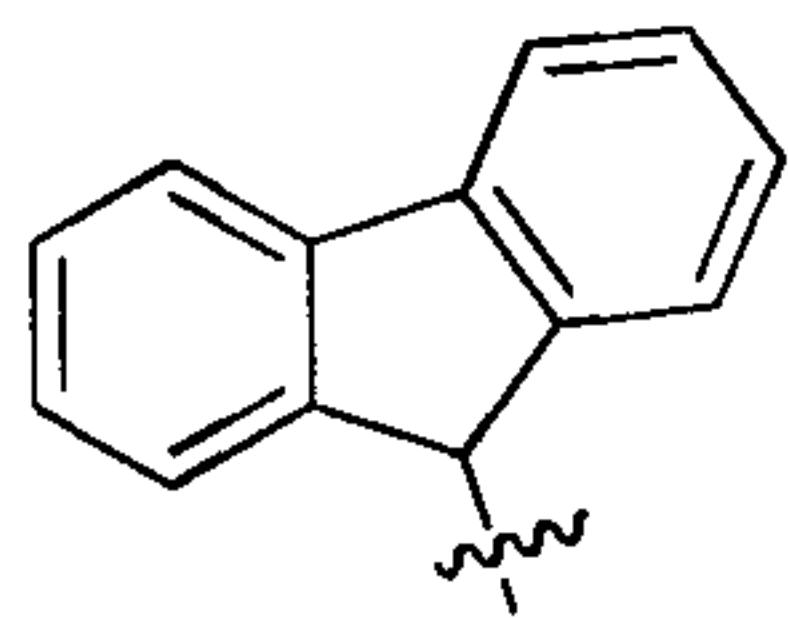


or a pharmaceutically acceptable salt thereof,

X is selected from the group consisting of



Ra is a fluorophore selected from the group consisting of fluorescein, rhodamine, Texas redTM, BODIPYTM, CASCADE BLUETM, coumarin, phycoerythryn, eosin, rosamine



and a fluorenyl group having the structure

Rb is a polypeptide moiety selected from the group consisting of

```
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1           5           10           15
Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
                20           25
SEQ ID NO.:3;
```

```
Nα-FTC-Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1           5           10           15
Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
                20           25
SEQ ID NO.:12;
```

```
Nα-FTC-Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1           5           10           15
Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly
                20           25           30
Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
                35           40
SEQ ID NO.:4;
```

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Nε-CF-Lys Gly
 20 25 30
 Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
 35 40

SEQ ID NO.:5;

npa Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly
 1 5 10 15
 Gln Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 30

SEQ ID NO.:13;

npa Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:15;

Tyr npa Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:16;

Tyr Ala npa Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:17;

Tyr Ala Asp npa Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:18;

Tyr Ala Asp Ala npa Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:19;

Tyr Ala Asp Ala Ile npa Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:20;

Tyr Ala Asp Ala Ile Phe npa Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:21;

Tyr Ala Asp Ala Ile Phe Thr npa Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25

SEQ ID NO.:22;

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Tyr Ala Asp Ala Ile Phe Thr Asn npa Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:23;

Tyr Ala Asp Ala Ile Phe Thr Asn Ser npa Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:24;

bpa Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly
 1 5 10 15
 Gln Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 30 SEQ ID NO.:14;

Tyr Ala Asp bpa Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:25;

Tyr Ala Asp Ala Ile Phe Thr Asn bpa Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:26;

Tyr Ala Asp Ala Ile Phe Thr Asn Ser bpa Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:27;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:28;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:29;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:30;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:31;

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Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:35;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:32;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:33;

Tyr Ala Asp Ala Ile Phe Thr D-Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys D-Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:34;

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:36;

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:10;

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg (CH₂)₈-Cys
 20 25 30 SEQ ID NO.:11; and

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:40.

2. A polypeptide selected from the group consisting of

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:10;

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg (CH2)8-Cys
 20 25 30 SEQ ID NO.:11;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:28;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:29;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:30;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg D-Lys Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:31;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:32;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:33;

Tyr Ala Asp Ala Ile Phe Thr D-Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys D-Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:34;

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:35;

- 70 -

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:36; and

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:40.

3. The polypeptide of claim 2, wherein said polypeptide is selected from the group consisting of

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:10;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:32;

Tyr D-Ala Asp Ala Ile Phe Thr Asn Ser D-Tyr Arg Lys Val Leu D-Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:33;

Tyr Ala Asp Ala Ile Phe Thr D-Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys D-Leu Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:34;

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:36; and

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
 1 5 10 15
 Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
 20 25 SEQ ID NO.:40.

2158782

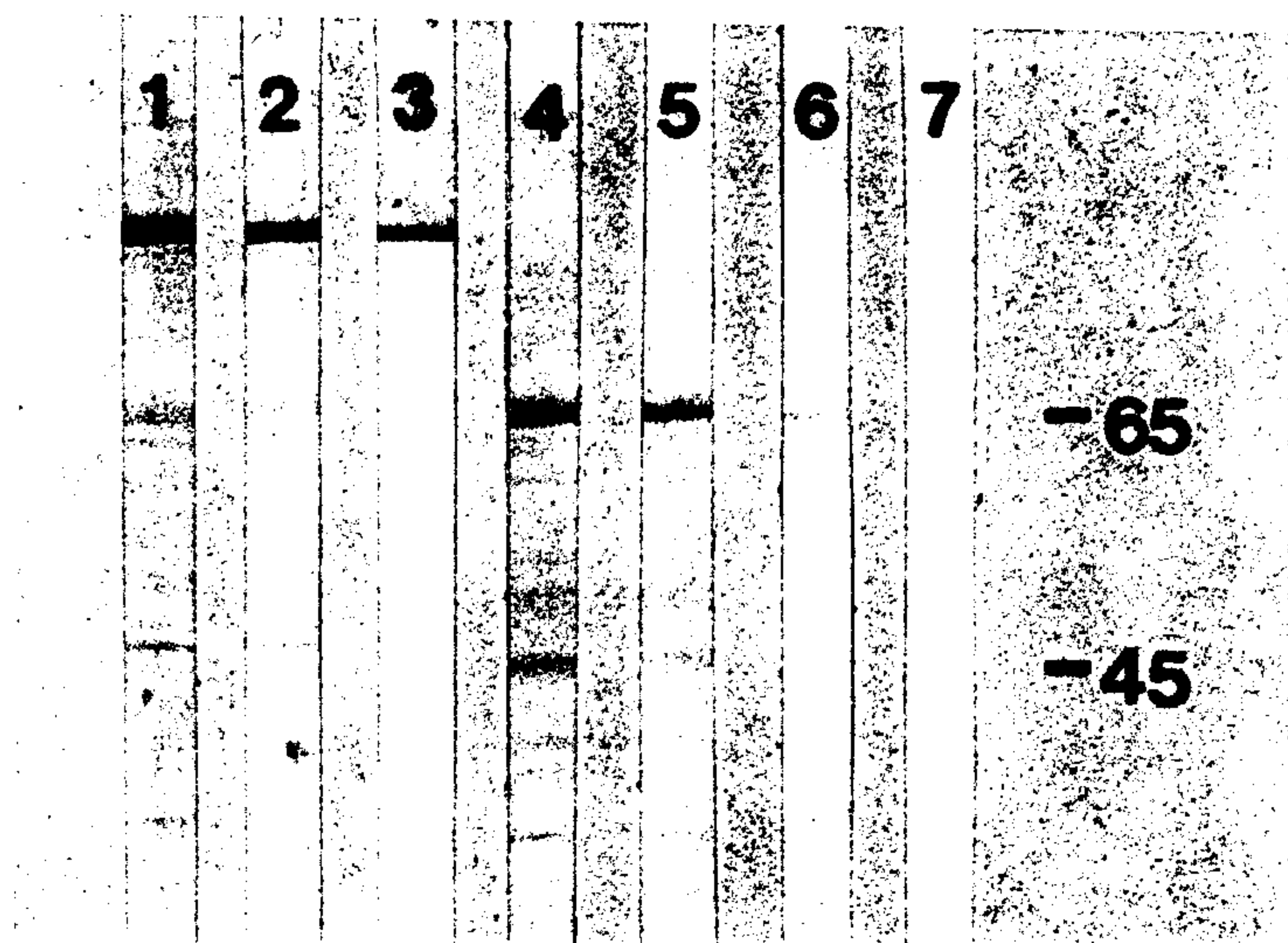


Fig. 1A

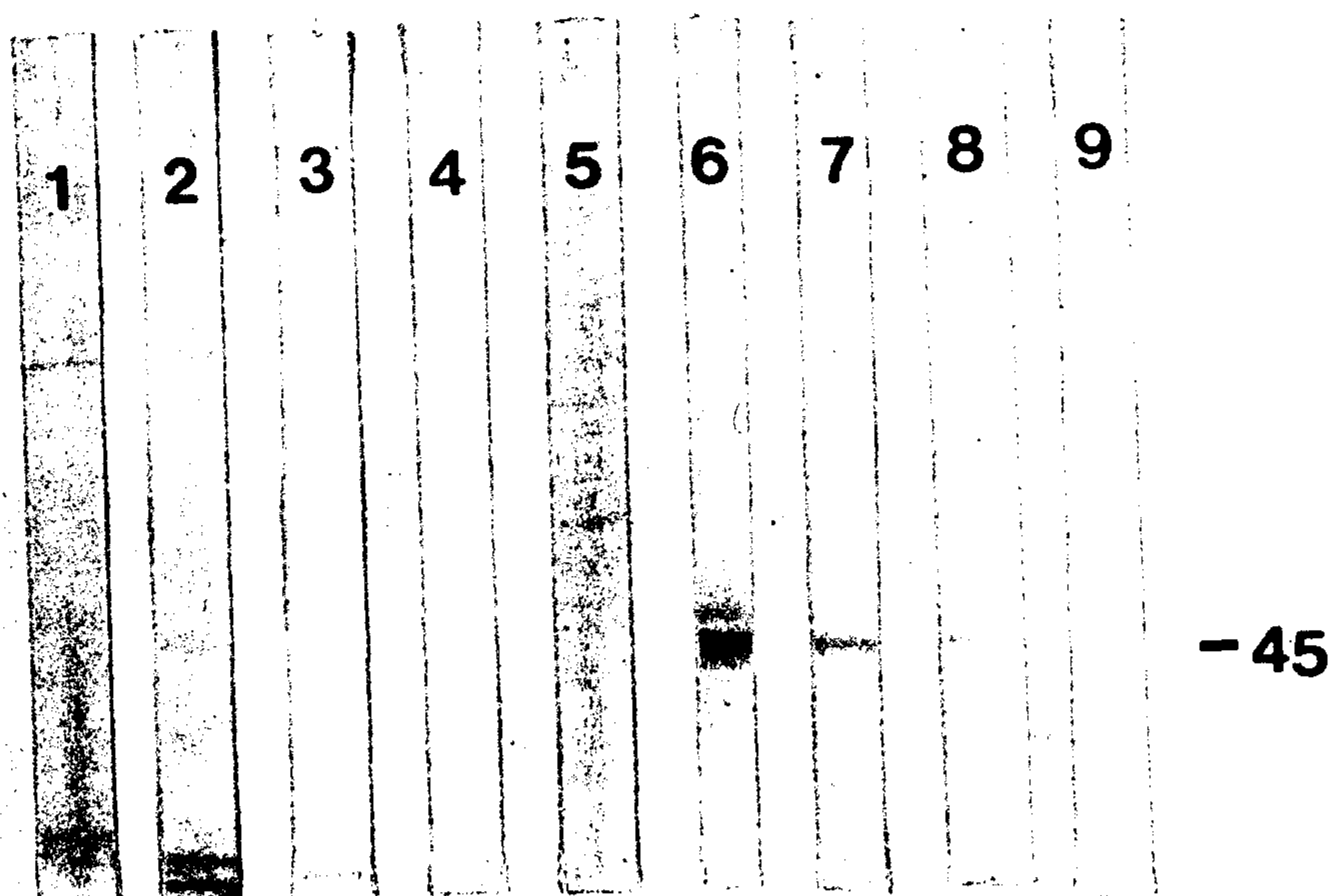


Fig. 1B

PATENT AGENTS

Dwight Ogilvy Renault

Fig. 2A

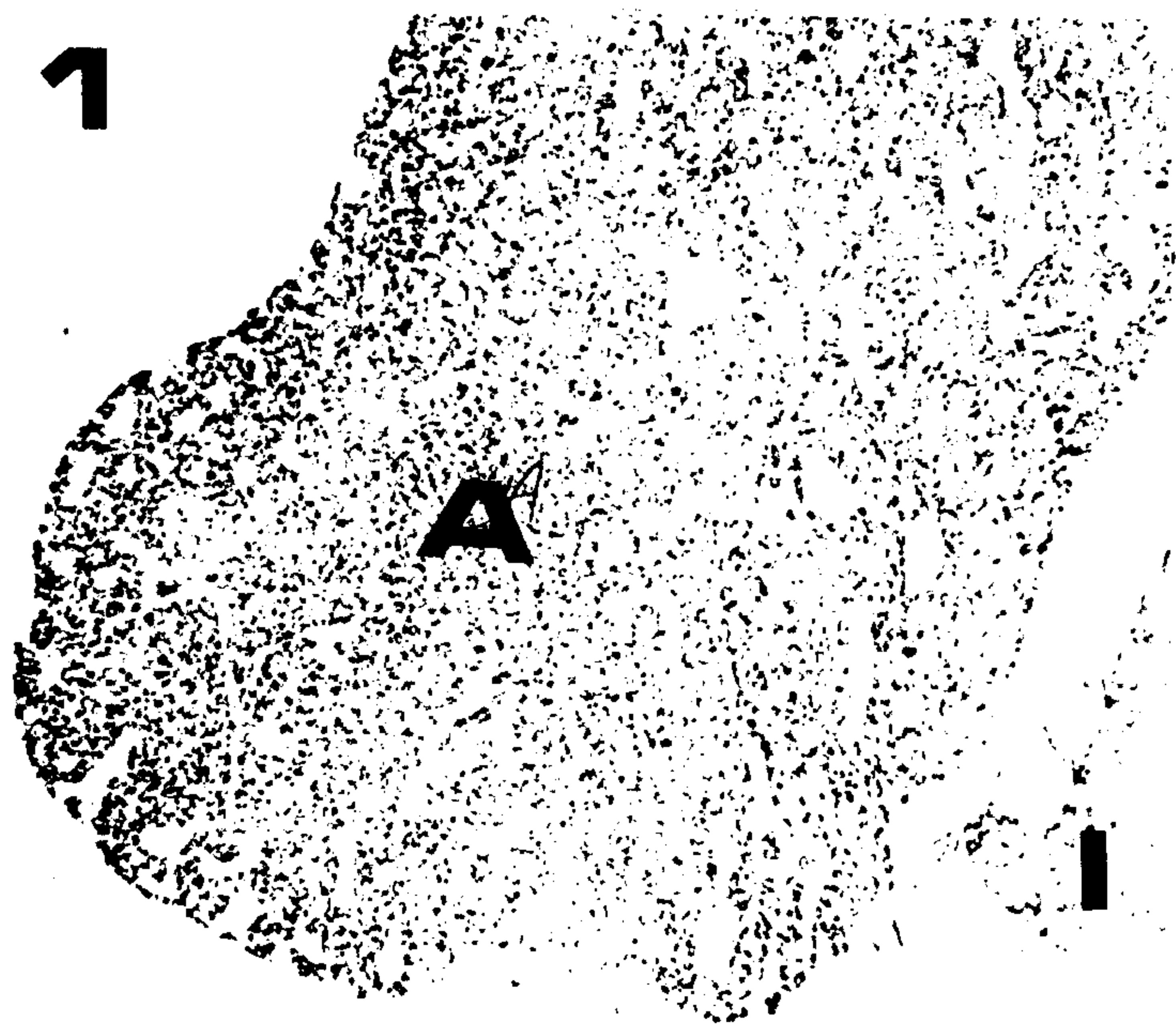
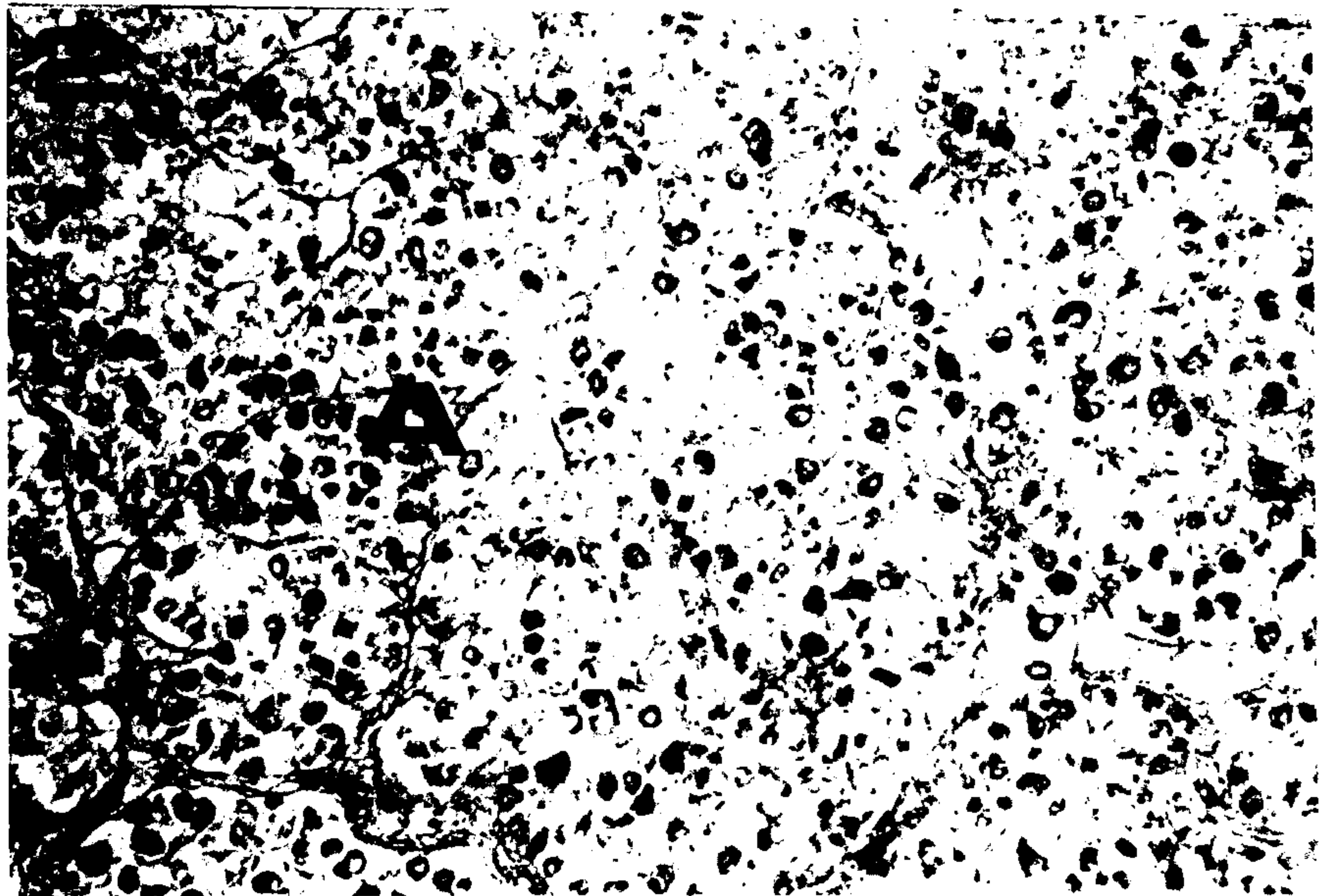


Fig. 2B



PATENT AGENTS

Swaney Ogilvy Renault

