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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, C07K 7/06	A1	(11) International Publication Number: WO 94/07519 (43) International Publication Date: 14 April 1994 (14.04.94)

(21) International Application Number: PCT/US93/08805

(22) International Filing Date: 17 September 1993 (17.09.93)

(30) Priority data:
07/951,500 25 September 1992 (25.09.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 07/951,500 (CIP)
Filed on 25 September 1992 (25.09.92)

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(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: GROWTH HORMONE RELEASING PEPTIDES

(57) Abstract

Peptides of the general formula (I): A-B-E-F-Ala-G-(D-Phe)-U-V-Y-Z wherein A is H or an amino protecting group or is absent; B is Gly, Ala, β-Ala, PyrAla, Gln or Lys, or is absent; E is absent or Het-$(CR'_2)_m CHR^1 (CR'_2)_p CO$; F is D-Trp($R^2$), D-Nal or D-Phe($R^2$); G is Nal($R^2$), Trp($R^2$) or Phe($R^2$); U is Lys, Arg or Orn; V is D- or L- Gln, $NH(CR'_2)_n CO$ or $NH(CR'_2)_n$-OH; Y is absent or Gly; Z is OR″ or NR′R″; R, R′ and R″ are H or C_{1-4}alkyl; R^1 is H, C_{1-4}alkyl or NR′; R^2 is H, F, Cl, Br, I or NO_2; m and n are 0 to 3; and p is 0 or 1; or stimulate the release of growth hormone from mammals.

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GROWTH HORMONE RELEASING PEPTIDES

5

Field of the Invention

This invention relates to compounds which cause enhanced release of growth hormone in animals, pharmaceutical compositions containing the compounds and
10 methods of using the compounds.

Background of the Invention

Growth Hormone (GH), a mediator released from the pituitary, causes
15 physiologic and metabolic changes in the tissues of the body. Effects such as tissue growth, increased rate of protein synthesis, increased mobilization and use of fatty acids for energy, and decreased use of carbohydrates, have been attributed to GH secretion. A deficiency of GH has been associated with certain types of medical disorders, such as certain instances of dwarfism.

20 GH is released and regulated by several natural secretagogues. In particular, GH is specifically released in response to growth hormone releasing hormone (GHRH), a 40-44 amino acid peptide which acts directly upon specific pituitary receptors to cause the release. Secretion of GH is inhibited by somatostatin (SRIF).

GH is also released *in vitro* and *in vivo* by certain smaller exogenous peptides.
25 Such peptides are described, for instance, in U.S. Patents 4,410,513; 4,839,344; 4,411,890; 4,880,777; and PCT patent application WO 88/09780 (PCT/US88/01947). Other related peptides and growth hormone releasing compositions are disclosed in U.S. Patents 4,223,019; 4,223,020; 4,223,021; 4,224,316; 4,226,857; 4,228,155; 4,228,156; 4,228,157; 4,228,158; 4,880,778 and 4,410,512. These peptides are
30 characterized by specific binding sites in the pituitary and the hypothalamus, and their

mechanism of action is distinct from that of GHRH. *See* Codd *et al.*, *Neuropharmacol.*, 28, 1139 (1989); Malozowski *et al.*, *J. Clin. Endocrin..Metab.*, 73, 314 (1991).

The action of one of these peptides, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, has been extensively studied in humans and other mammals. It has been reported to specifically release growth hormone in a broad range of species (*eg.*, monkey, sheep, pigs, chicks, steers) and not to affect the release of other mediators (*eg.*, TSH, LH, FSH and PRL). *See* Bowers *et al.*, *Endocrinology*, 128, 2027 (1991); Bowers *et al.*, *Endocrinology*, 114, 1537 (1984). Physiological effects such as enhanced milk production in cows, and weight gain in rats in response to administration of this peptide have been reported. *See* Baker *et al.*, *J. Anim. Sci.*, 59, 220 (1984); Croom *et al.*, *J. Anim. Sci.*, 67, 109 (1984). Bowers *et al.*, *J. Clin. Endocrin. Metab.*, 74, 292 (1992) also disclose that this peptide causes the release of GH in both normal men and short statured children, although bioavailability was low via oral administration. Novel, more potent or more bioavailable peptides which release growth hormone are an object of this invention.

Summary of the Invention

In one aspect this invention is a peptide compound comprising a hexa- to nonapeptide as described hereinafter by formula (I).

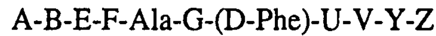
This invention is also a pharmaceutical composition for causing the release of growth hormone which comprises a compound of formula (I) and a pharmaceutically acceptable carrier.

This invention is further a method for causing the release of growth hormone, *in vivo* or *in vitro*, which comprises administering an effective amount of a compound of formula (I). This invention is also a method for treating pathologies caused by a deficiency in the release of growth hormone, or conditions in which the release of growth hormone is desirable.

Detailed Description of the Invention

This invention discloses novel peptides which cause the release of growth hormone (GH). Although the precise mechanism of action is not known, these peptides are believed to act directly upon the pituitary to cause this effect. The compounds of the present invention are novel and generally show greater potency at promoting the release of GH due to the residues incorporated in the residues V and Y.

This invention comprises compounds of formula (I):



A-B-E-F-Ala-G-(D-Phe)-U-V-Y-Z

(I)

wherein

5 A is H or an amino protecting group, or is absent when R^1 is H or C_{1-4}alkyl;

B is Gly, Ala, β-Ala, PyrAla, Gln or Lys, or is absent, when R^1 is H or C_{1-4}alkyl;

E is absent or Het-$(CR'_2)_m CHR^1 (CR'_2)_p$CO;

F is D-Trp(R^2), D-Nal or D-Phe(R^2);

10 G is Nal(R^2), Trp(R^2) or Phe(R^2);

U is Lys, Arg or Orn;

V is D- or L- Gln, $NH(CR'_2)_n$CO or, when Y and Z are absent, $NH(CR'_2)_n$-OH;

Y is absent or Gly;

15 Z is OR" or NR'R";

R is H or C_{1-4}alkyl;

R^1 is H, C_{1-4}alkyl or NR';

R^2 is H, F, Cl, Br, I or NO_2;

R' is H or C_{1-4}alkyl;

20 R" is H or C_{1-4}alkyl;

m and n are 0 to 3;

p is 0 or 1; or

a pharmaceutically acceptable salt thereof.

Also included in this invention are hydrates, complexes or prodrugs of the
25 compounds of formula (I). Prodrugs are considered to be any covalently bonded carriers which release the active parent drug according to formula (I) *in vivo*.

Suitably E is His, PyrAla, ImAc or ImPr.

Suitably F is D-Trp, β-D-Nal or D-Phe.

Suitably V-Y-Z is Gln-NH_2 or Gln-Gly-NH_2.

30 Suitably, A-B-E is ImAc, His or Ala-His.

Suitably, U is Lys.

Representative compounds of this invention are :

ImPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;

ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2;

35 ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-NH_2;

ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-Gly-NH_2; and

ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;

PyrPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;

His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2;
Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2; and
Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2.

5 Preferred compounds of this inventions are:
His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-NH_2;
ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-Gly-NH_2; and
Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2.

10 In cases wherein the compounds of this invention may have one or more chiral centers, unless specified, this invention includes each unique nonracemic compound or diastereomer which may be synthesized and resolved by conventional techniques, and all mixtures thereof. The meaning of any substituent at any one occurrence is independent of its meaning, or any other substituent's meaning, at any other 15 occurrence, unless specified otherwise.

Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe the compounds of this invention. In general, the amino acid abbreviations follow the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in *Eur. J. Biochem.*, 158, 9 (1984). Amino acid means the 20 D- or L- isomer of alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), valine (Val) or naphthylalanine (Nal). Nal may be 2-naphthylalanine (β-Nal) or 1- 25 naphthylalanine (α-Nal). In addition, Phe(R^2), Trp(R^2), Nal(R^2) and His(R^2) refer to the indicated amino acid substituted on the aromatic ring by the group R^2.

C_{1-4}alkyl as applied herein is meant to include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl. C_{1-6}alkyl additionally includes pentyl, n-pentyl, isopentyl, neopentyl and hexyl and the simple aliphatic isomers thereof.

30 Ar, or aryl, as applied herein, means phenyl or naphthyl, or phenyl or naphthyl substituted by one to three moieties R^{11}. In particular, R^{11} may be C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkthio, trifluoroalkyl, OH, Cl, Br or I.

Het indicates pyridyl, imidazolyl, pyrazolyl or thienyl.

t-Bu refers to the tertiary butyl radical, Boc refers to the t-butyloxycarbonyl 35 radical, Fmoc refers to the fluorenylmethoxycarbonyl radical, Ph refers to the phenyl radical, Cbz refers to the benzyloxycarbonyl radical, BrZ refers to the o-bromobenzyloxycarbonyl radical, ClZ refers to the o-chlorobenzyloxycarbonyl radical, Bzl refers to the benzyl radical, 4-MBzl refers to the 4-methyl benzyl radical,

Me refers to methyl, Et refers to ethyl, Ac refers to acetyl, Alk refers to C_{1-4}alkyl, Nph refers to 1- or 2-naphthyl and cHex refers to cyclohexyl. ImAc refers to 4-imidazolylacetyl, ImPr refers to 4-imidazolylpropanoyl, PyrAla refers to 4-pyridylalanine and PyrPr refers to 4-pyridylpropanoyl.

5 DCC refers to dicyclohexylcarbodiimide, DIC refers to diisopropylcarbodiimide, DMAP refers to dimethylamino-pyridine, DIEA refers to diisopropylethyl amine, EDC refers to N-ethyl-N'(dimethylaminopropyl)-carbodiimide.

HOBt refers to 1-hydroxybenzotriazole, THF refers to tetrahydrofuran, DIEA refers 10 to diisopropylethylamine, DMF refers to dimethyl formamide, Pd/C refers to a palladium on carbon catalyst, PPA refers to 1-propanephosphonic acid cyclic anhydride, DPPA refers to diphenylphosphoryl azide, BOP refers to benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate, HF refers to hydrofluoric acid, TEA refers to triethylamine, TFA refers to trifluoroacetic acid, 15 PCC refers to pyridinium chlorochromate, BHA refers to a benzhydrylamine polystyrene resin, CMR refers to a chloromethyl resin.

Coupling reagents as used herein denote reagents which may be used to form peptide bonds. Typical coupling methods employ carbodiimides, activated anhydrides and esters and acyl halides. Reagents such as EDC, DCC, DPPA, PPA, 20 BOP reagent and oxalyl chloride, optionally used in conjunction with HOBt and N-hydroxysuccinimide are typical.

The compounds of this invention are prepared by conventional techniques of chemistry and peptide bond formation by coupling the various residues B, E, F, G, U, V and Y together to form a linear peptide, and, if necessary, modifying the carboxy or 25 amino terminal group to add the group A and Z. Coupling methods to form peptide bonds are generally well known to the art and include both solid phase and solution phase synthesis. Typically, any sidechain reactive groups are protected as known in the art to prevent unwanted side reactions in the coupling process.

Thus, this invention includes a process for preparing a compound of the 30 formula:

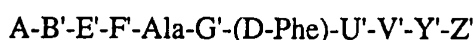
A-B-E-F-Ala-G-(D-Phe)-U-V-Y-Z

(I)

or a pharmaceutically acceptable salt thereof.

35 wherein A, B, E, F, G, U, V, Y and Z are as defined for formula (I), which comprises

a) coupling the protected amino acids corresponding to B, E, F, G, U, V, Y as defined in formula (I) to form a suitably protected compound of the formula:



wherein

A, B', E', F', G', U', V' and Y' are A, B, E, F, G, U, V and Y as defined in formula (I) with any sidechain reactive groups protected;

5 Z' is NR'R", OR", NH-T" or O-T"; and

T" is a chloromethyl, hydroxymethyl, benzhydrylamine or methylbenzhydryl amine resin;

b) removing any protecting groups and, if Z' is NH-T" or O-T", cleaving the peptide from the resin; and

10 c) optionally forming a pharmaceutically acceptable salt thereof.

The methods of solid phase peptide synthesis generally set forth by Stewart and Young, SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL, 1984 and Bodansky *et al.*, THE PRACTICE OF PEPTIDE SYNTHESIS, Springer-Verlag, Berlin, 1984, are generally illustrative of the technique and are incorporated herein by 15 reference. Typically, a benzhydrylamine or methylbenzhydrylamine resin is used if the desired peptide is to be a C-terminal amide and a chloromethyl or hydroxymethyl resin is used if the peptide is to be an ester or acid. The synthesis is commenced by attaching the required (α-amino-protected) alpha-amino acid to the resin. After the initial attachment, if the α-amino protecting group is an acid labile group, such as the 20 Boc group, the α-amino protecting group can be removed by a choice of acidic reagents, such as trifluoroacetic acid or hydrochloric acid solutions in an organic solvent, at room temperature. If the α-amino protecting group is a base labile group, such as the Fmoc group, the α-amino protecting group can be removed by a choice of a basic reagent, such as piperidine in an organic solvent, at room temperature. After 25 removal of the alpha-amino protecting group, the next (α-amino-protected) amino acid is coupled via its free carboxyl group to the free amino group. Each protected amino acid is reacted in about a three fold excess using an appropriate coupling reagent, typically in a solution of DMF or methylene chloride. Repeating the cycle of deprotection of the amino group and coupling, each of the remaining protected amino 30 acids is coupled stepwise in the desired order.

After the desired amino acid sequence has been completed, the desired peptide can be cleaved from the resin support by treatment with a suitable reagent, such as an acid. Typically hydrogen fluoride is used, since this reagent both cleaves the peptide from the resin and removes most common side-chain protecting groups.

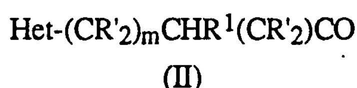
35 Modification of amino groups may be accomplished, if necessary, by alkylation, sulfonylation, cyanation or acylation as is generally known in the art. Similarly, the carboxy terminal group may be modified, if necessary, by coupling to an amine to form an amide or by esterification.

Solution synthesis for the formation of amide or peptide bonds is accomplished using conventional methods used to form amide bonds. Typically, the amine is coupled via its free amino group to an appropriate carboxylic acid substrate using a suitable carbodiimide coupling agent, such as N,N' dicyclohexyl carbodiimide (DCC), optionally in the presence of catalysts such as 1-hydroxybenzotriazole (HOBt) and dimethylamino pyridine (DMAP). Other methods, such as the formation of activated esters, anhydrides or acid halides, of the free carboxyl of a suitably protected acid substrate, and subsequent reaction with the free amine of a suitably protected amine, optionally in the presence of a base, are also suitable. For example, a protected Boc-amino acid or Cbz-amino acid is treated in an anhydrous solvent, such as methylene chloride or tetrahydrofuran (THF), in the presence of a base, such as N-methyl morpholine, DMAP or a trialkylamine, with isobutyl chloroformate to form the "activated anhydride", which is subsequently reacted with the free amine of a second protected amino acid.

The solution phase synthesis can proceed by sequential addition of the individual amino acid residues to form the linear peptide, or individual fragments may be synthesized and coupled together in a convergent fashion. Once the completed peptide sequence is formed, the sidechain protecting groups may be removed simultaneously by treatment with hydrofluoric acid.

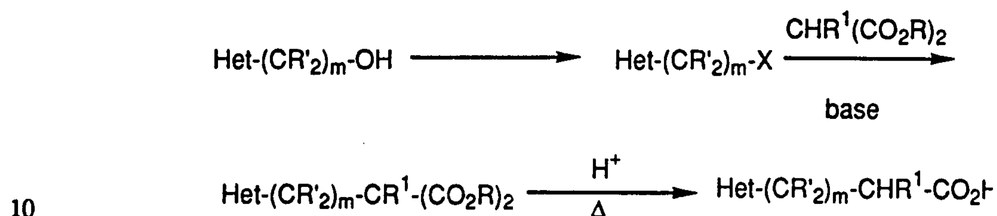
The reactive functional groups of the sidechains of each synthetic fragment are suitably protected as known in the art. Suitable protective groups are disclosed in Greene, PROTECTIVE GROUPS IN ORGANIC CHEMISTRY, John Wiley and Sons, New York, 1981. For example, a phthaloyl or Fmoc group or a suitably substituted alkyloxycarbonyl, benzyloxycarbonyl or benzyl group may be used for protection of an amino group. The Boc group is generally preferred for protection of an α-amino group. A t-Bu, cHex or benzyl ester may be used for the protection of the side chain carboxyl. A benzyl group or suitably substituted benzyl group (*eg*., 4-methoxy-benzyl or 2,4-dimethoxy-benzyl) is used to protect the mercapto group or the hydroxyl group. The tosyl group may be used for protection of the imidazolyl group and tosyl or nitro group for protection of the guanidino group. A suitably substituted benzyloxycarbonyl, silyl or benzyl group may also be used for the hydroxyl group. Suitable substitution of the benzyloxycarbonyl or benzyl protecting groups is ortho and/or para substitution with chloro, bromo, nitro or methyl, and is used to modify the reactivity of the protective group. Except for the Boc group, the protective groups for the amino moiety are, most conveniently, those which are not removed by mild acid treatment. These protective groups are removed by such methods as catalytic hydrogenation, sodium in liquid ammonia or HF treatment, as known in the art.

Compounds of formula (II):



which comprise the residue E are available commercially or are prepared by conventional methods known in the art from commercially available materials. For instance, compounds of formula (II) may be prepared according to Scheme 1, wherein X is a suitable displaceable group and R', R^1 and Het are as

Scheme 1



defined above for formula (I). Typically, the hydroxyl group of a heterocyclic carbinol is coverted to a suitable leaving group, such as a halogen (*eg*., by treatment with thionyl chloride or bromide) and reacted with the anion of a malonate ester. Hydrolysis, followed by decarboxylation produces the desired heterocyclic acid. If R^1 is an amino group, the heterocyclic acid may be converted to the acid bromide, brominated alpha to the carbonyl with thionyl bromide, and hydrolyzed to the alpha bromo carboxylic acid. Amination of the bromide produces the α-amino acid. Alternately, the bromide may be converted to a hydroxyl group, oxidized and reductively aminated, for instance with ammonium chloride and sodium cyanoborohydride.

Compounds of the formula Het-$(CR'_2)_{m-1}$CHO, and amino acids such as His and Trp are generally available commercially.

Acid addition salts of the peptides are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, maleic, succinic or methanesulfonic. The acetate salt form is especially useful. Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or alkoxide, containing the appropriate cation; or with an appropriate organic amine. Cations such as Li+, Na+, K+, Ca++, Mg++ and NH_4+ are specific examples of cations present in pharmaceutically acceptable salts.

This invention provides a pharmaceutical composition which comprises a compound according to formula (I) and a pharmaceutically acceptable carrier.

Preferably the pharmaceutical composition includes an effective amount of the compound to stimulate release of growth hormone, however, the administration of multiple doses of a composition containing suboptimal amounts of the compound may also be useful. Pharmaceutical acceptable carrier are well known in the art and are disclosed, for instance, in SPROWL'S AMERICAN PHARMACY, Dittert, L. (ed.), J.B. Lippincott Co., Philadelphia, 1974, and REMINGTON'S PHARMACEUTICAL SCIENCES, Gennaro, A. (ed.), Mack Publishing Co., Easton, Pennsylvania, 1985.

Pharmaceutical compositions of the compounds of formula (I) may be formulated as sterile solutions, suspensions or emulsions, or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, propylene glycol, polyethylene glycol, mannitol, sodium chloride or sodium citrate. Such compositions may also contain preserving, wetting, emulsifying and dispersing agents. Compositions for nasal administration may additionally contain enhancers, such as bile acids. Compositions for topical administration may also contain permeation enhancers, such as DMSO or formamide.

Alternately, these peptides may be encapsulated, tableted or prepared in a emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms.

When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a

sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

For rectal administration, the peptides of this invention may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and
5 molded into a suppository.

The compounds of this invention may be used to treat conditions in which growth hormone has been shown to exert a beneficial effect. Accordingly, this invention provides a method of treating: 1) disease states wherein an insufficiency of endogenous GH is a factor, 2) catabolic states in which it is desirable to increase the
10 level of circulating GH, 3) conditions in which it is beneficial to increase anabolic metabolism, or 4) enhanced development or production in commercial livestock. The method comprises administering a compound of formula (I) to a mammal in need thereof. For treating disease states or conditions in which an increase in GH is desirable, the compounds of formula (I) may be used in the manufacture of a
15 medicament.

Growth failure, pediatric GH deficiency, idiopathic short stature and dwarfism are instances wherein an insufficiency of growth hormone is a factor. Cancer-associated cachexia, acquired immune deficiency syndrome (AIDS) cachexia, chronic obstructive pulmonary disease, renal insufficiency, osteoporosis, chronic
20 hemodialysis, glucocorticoid excess, wound, burn and bone fracture healing, obesity and old age are examples of conditions in which it may be desirable to increase the level of circulating GH to combat a catabolic state or induce an anabolic state. Rapid weight gain, enhanced lean body mass and development in livestock, enhanced milk production in cows, and enhanced wool production in sheep are indications for use in
25 livestock. In addition, the compounds of this invention are useful for promoting release of insulin-like growth factor-1 (IGF-1).

The peptide is administered either orally, parenterally, rectally or intranasally, to the patient, in a manner such that the concentration of drug in the plasma is sufficient to cause the release of growth hormone. The peptide is administered one to
30 four times daily as is indicated by the effect being sought and the condition of the patient. The pharmaceutical composition containing the peptide is administered at a dose between about 0.01 to about 5000 µg/kg in a manner consistent with the condition of the subject, route of administration and species being treated. Typically, cows and humans require about the same dosages, while rats require a somewhat
35 higher dose. About 0.1 to 20 µg/kg is a typical intravensous dose. An intravenous infusion of the peptide in 5% dextrose in water or normal saline is most effective, although an intramuscular bolus injection may be sufficient. A somewhat higher dose

is used for oral administration, dosages in the range of 1 to 1000 μg/kg being useful, and in the range of 20 to 600 μg/kg being typical.

The compounds of this invention may also be combined with other compounds and compositions which cause the release of GH. Such combinations will generally allow the use of lower dosages of each component to cause an equivalent amount of GH to be released. In particular, the compounds of this invention may be combined with growth hormone releasing hormone (GHRH) and its bioactive analogs and inhibitors of somatostatin (SRIF). Suitable GHRHs and analogues are disclosed, for instance, in U.S. Patents 4,517,181; 4,585,756; 4,605,643; 4,610,976; and 4,801,456, which are incorporated herein by reference. Other suitable growth hormone releasing components are disclosed in U.S. Patent No. 4,880,778, which is incorporated herein by reference.

The pharmacological activity of the compounds of this invention is assessed by their ability to cause the release of growth hormone in rats. EC50 in rats for the compounds of this invention are in the range of 2-30 μg/kg. Preferred compounds have EC50 less than 10 μg/kg. As a reference His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 has an EC50 of about 42 μg/kg in the assay described hereinafter.

Assay for Determining Growth Hormone Release

The following buffers were used in the assay for GH release: Assay buffer - 0.1M sodium phosphate, 0.1% sodium azide, 1% RIA grade bovine serum albumin [Sigma Chemical, St. Louis, MO], pH 7.4-7.6; Ab buffer - 50 mM EDTA, 0.88% sodium chloride, 0.1% sodium azide, 0.1M disodium phosphate, and 1% normal monkey serum [Sigma], pH 7.5.

The assay demonstrating GH release in response to the administration of a compound of this invention is conducted as follows:

Female Sprague Dawley rats (300-400 g), approximately 3 months old, were anesthetized with pentabarbital. The test compound was dissolved in phosphate buffered saline. The rats were dosed i.v. in the tail vein with a volume of ~1 mL/kg at a dose of 0.1,0.5, 1.0, 10, 25, 50 and 100 μg/kg (n=5). After 10 min, 200 μL of blood was withdrawn via cardiac puncture into heparinized tubes, centrifuged (4000xG, 1 min), and cooled to 4°C. Growth hormone levels were determined by standard radioimmunoassay.

20 μL of plasma (or a rat Growth Hormone standard) was diluted to 500 μL with assay buffer, and incubated at room temperature overnight with 100 μL ^{125}I-rat-Growth Hormone (Chemicon Corp., Temecula, CA; ~20,000 cpm/tube) and 200 μL of monkey anti-rat Growth Hormone antiserum (diluted 1:4000 with Ab buffer; Pituitary Hormones and Antisera Center, Torrance, CA). On the following day, 200

μL of goat anti-monkey antisera (1:10 dilution P4; Antibodies Inc., Davis, CA) was added to each tube and the tubes were incubated for 2-4 h at room temperature. The assay tubes were then centrifuged (5000xG, 30 min), the supernatant was aspirated, and the resulting pellet was counted for radioactivity in a Packard Gamma Counter. The Growth Hormone content was calculated from the rat Growth Hormone standard curve. The lower limit of detection was 0.1 ng-GH/mL-plasma. Dose:response curves for the test compounds were determined from plasma growth hormone released after dosing. EC50 were calculated by fitting the data (average, n=5) to the equation $y=m1/(1+m2/x)^{m3}$, where m1 is the maximal GH release, m2 is the EC50 and m3 is the slope.

EXAMPLES

In the Examples, all temperatures are in degrees Centigrade. Mass spectra were performed using fast atom bombardment (FAB) or electro-spray (ES) ionization. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

NMR were recorded at 250 MHz using a Bruker AM 250 spectrometer, unless otherwise indicated. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane. Multiplicities for NMR spectra are indicated as: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, dt=doublet of triplets etc. and br indicates a broad signal. J indicates the NMR coupling constant in Hertz.

Both flash and gravity chromatography were carried out on Merck 60 (230-400 mesh) silica gel. ODS refers to an octadecylsilyl derivatized silica gel chromatographic support. Sephadex® is a cross-linked poly(dextran) manufactured by Pharmacia Fine Chemicals, Piscataway, N.J. PRP-1® is a polymeric (styrene-divinyl benzene) chromatographic support and is a registered trademark of Hamilton Co., Reno, Nevada. BondElut® C18 is an octadecyl silica manufactured Varian Associates, Palo Alto, California.

Preparation of 4-pyridylpropionic acid

a) diethyl 2-(4-pyridylmethyl)malonate

4-Pyridylcarbinol (5.46 g, 50 mmol) was dissolved in $CHCl_3$ (20 mL) and added dropwise to thionyl chloride (7.6 mL, 100 mmol) cooled to 0°C. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. The

reaction mixture was then evaporated and the residue triturated with $CHCl_3$ and dried in vacuum overnight to yield 4-chloromethylpyridine hydrochloride (8.0 g, 98%), which was used without further purification.

Sodium spheres (0.96 g, 42 mmol) were dissolved in 40 mL absolute ethanol and the resulting sodium ethoxide solution was cooled to 0°C. To this was added 4-chloromethylpyridine hydrochloride (2.42 g, 15 mmol) in ethanol (35 mL) dropwise. The reaction was allowed to warm to room temperature and was stirred overnight. The reaction mixture was evaporated to dryness and the residue dissolved in 6N HCl (100 mL) and extracted with EtOAc. The aqueous layer was then basified with sodium carbonate and extracted with ether. The ether extracts were combined, dried over $MgSO_4$ and evaporated to yield a crude product (2.93 g), which was purified by flash chromatography (silica gel, 1:1 EtOAc:hexane) to give diethyl 2-(4-pyridylmethyl)malonate (1.84 g, 49%). ^1H NMR($CDCl_3$) δ 1.19 (t, 6H), 3.24 (d, 2H), 3.73 (dd, 1H), 4.17 (q, 4H), 7.20 and 8.53 (dd, 4H).

b) 4-pyridylpropionic acid

Diethyl 2-(4-pyridylmethyl)malonate (1.84 g, 7.3 mmol) was dissolved in conc. HCl (30 mL) and heated on a steam bath for 8 h. The reaction mixture was evaporated to dryness and the solid residue triturated with water and dried under vacuum to yield 4-pyridylpropionic acid hydrochloride (1.43 g, 104%). MS(ES) 152.0 $[M+H]^+$.

Preparation of imidazolepropionic acid hydrochloride.

Urocanic acid (2.0 g, 14.5 mmol) was dissolved in 40 mL 1 N HCl (aq) to which was then added 30 mL methanol and 0.25 g 5% Pd/C catalyst. This mixture was hydrogenated on a Parr apparatus at 50 psig H_2 for 4 hr., then filtered through a pad of Celite and evaporated to dryness to yield the title compound (2.75 g, 108%). ^1H NMR(TFA) δ 3.0–3.3 (m, 4H), 7.37 (s, 1H), 8.67 (s, 1H); MS (DCI, NH_3) m/z 141 $[M+H]^+$.

Example 1

General procedure for solid phase peptide synthesis

Peptide amides were synthesized by solid phase peptide synthesis using benzhydrylamine resin as the support. Protected amino acids were added sequentially starting from the carboxyl terminus until the desired sequence was obtained. The t-butyloxycarbonyl (Boc) group was used for protection of the alpha-amino group.

Side chain functional groups were protected as follows: arginine and histidine, tosyl (Tos); cysteine, p-methylbenzyl (MeBzl); serine and threonine, benzyl ether (Bzl); lysine, p-chlorocarbobenzoxy (Clz); glutamic acid and aspartic acid, benzyl ester (OBzl); tyrosine, p-bromocarbobenzoxy (BrZ). Removal of the Boc group was accomplished by treatment with 50% trifluoroacetic acid (TFA) in methylene chloride. Neutralization of the amine-TFA salt was accomplished by treatment with 7% diisopropylethylamine (DIEA) in methylene chloride. Amino acids were coupled to the growing peptide using 3 equivalents of Boc-amino acid and 3 equivalents of 1-hydroxybenzotriazole (HOBt) in DMF and 3 equivalents of dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC) in methylene chloride. Completeness of coupling was checked by ninhydrin test and couplings were repeated as necessary. The general protocol is given below.

Step	Time
1. Wash with methylene chloride	1 x 1 min
2. Wash with 50% TFA	1 x 1 min
3. Deblock with 50% TFA	1 x 20 min
4. Wash with methylene chloride	6 x 1 min
5. Neutralize with 7% DIEA	3 x 2 min
6. Wash with methylene chloride	4 x 1 min
7. Wash with dimethylformamide	2 x 1 min
8. Boc-AA + HOBt in DMF-do not drain	
9. DCC in methylene chloride	2 h
10. Wash with dimethylformamide	2 x 1 min
11. Wash with methylene chloride	3 x 1 min

For attachment of the first (C-terminal) residue to the BHA resin, the synthesis was begun at step 5. For all subsequent amino acids, the synthesis was begun at step 1.

Typical procedure for coupling of ImAc hydrochloride to resin peptide

Imidazoleacetic acid hydrochloride (3 eq) is dissolved in 15 mL DMF with warming. This is added to the neutralized, free amine peptidyl resin, followed by DIC (3 eq) in CH_2CL_2 (15 mL), HOBt (3 eq) in DMF (5 mL) and then DIEA (3 eq). Alternatively, coupling is carried out using ImAc•HCl in DMF followed by DIC (3 eq) and DMAP (3 eq) in CH_2Cl_2 (15 mL) and then DIEA (3 eq). Coupling is allowed to proceed at room temperature for 2 h, after which the resin is washed as usual and checked for completeness of coupling by Kaiser ninhydrin test, recoupling, if necessary, until a negative test is obtained.

Preparation of Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2

Boc-Ala-His(Cbz)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-Gly-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (586 mg).

The crude peptide (480 mg) was dissolved in 0.1% TFA/water (3 mL) and chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/water-0.1% TFA fraction was evaporated to dryness and lyophilized from 1% HOAc/water to yield a partially purified peptide (285 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (47.9 mg). MS(ES) 1130.6 $[M+H]^+$, 585.8 $[M+2H]^{++}$; HPLC k' 2.72 (Hamilton PRP-1, gradient A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.77 (n-BuOH:HOAc:H_2O 1:1:1).

Example 2

Preparation of ImPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2

ImPr-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-Gly-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (531 mg).

The crude peptide was dissolved in 0.1% TFA/water (3 mL) and chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fraction was evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (171 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield a purified fraction

(47.1 mg). This was further purified by semi-preparative HPLC (Vydac C_{18}, 27% acetonitrile/water-0.1% TFA) to yield the title compound (42.4 mg). MS(ES) 1043.6 $[M+H]^+$, 523.2 $[M+2H]^{++}$; HPLC k' 3.25 (Hamilton PRP-1, gradient, A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.71 (n-BuOH:HOAc:H_2O 1:1:1).

Example 3

Preparation of ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2

ImAc-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (524 mg).

The crude peptide was dissolved in 0.1% TFA/water (3 mL) and chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fraction was evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (234 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (45.7 mg). MS(ES) 972.4 $[M+H]^+$, 487.0 $[M+2H]^{++}$; HPLC k' 3.50 (Hamilton PRP-1, gradient, A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.76 (n-BuOH:HOAc:H_2O 1:1:1).

Example 4

Preparation of 4-PyrPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2

4-PyrPr-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-Gly-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give a crude peptide (523 mg).

Crude peptide (480 mg) was dissolved in 0.1% TFA/water (3 mL) and chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA,

40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fraction was evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (259 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (55.8 mg). MS(ES) 1054 $[M+H]^+$, 527.8 $[M+2H]^{++}$; HPLC k' 3.42 (Hamilton PRP-1, gradient A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min).

Example 5

Preparation of His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2

Boc-His(Cbz)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-Gly-BHA resin (1.0 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give a crude peptide (1.20 g).

Crude peptide (900 mg) was dissolved in 0.1% TFA/water (6 mL) and aliquots (3 mL) were chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fractions were combined, evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (353 mg).

An aliquot (112 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (50.9 mg). MS(ES) 529.6 $[M+2H]^{++}$; HPLC k' 2.69 (Hamilton PRP-1, gradient, A:acetonitrile B:water-0.1% TFA, 20-50% acetonitrile over 15 min); TLC R_f 0.69 (n-BuOH:HOAc:H_2O 1:1:1).

Example 6

Preparation of ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2

ImAc-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-Gly-BHA resin (1.0 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (1.2 g).

Crude peptide (1.1 g) was dissolved in 0.1% TFA/water (6 mL) and aliquots (3 mL) were chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fractions were combined, evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (451 mg).

An aliquot (118 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (51.2 mg). MS(ES) 515.2 $[M+2H]^{++}$; HPLC k' 3.38 (Hamilton PRP-1, gradient A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.84 (n-BuOH:HOAc:H_2O 1:1:1).

Example 7

Preparation of His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2

Boc-His(Cbz)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (579 mg).

Crude peptide (480 mg) was dissolved in 0.1% TFA/water (3 mL) and chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fraction was evaporpated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (128 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (44.9 mg). MS(ES) 1001.6 $[M+H]^+$, 501.2 $[M+2H]^{++}$; HPLC k' 2.69 (Hamilton PRP-1, gradient, A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.80 (n-BuOH:HOAc:H_2O 1:1:1).

Example 8

Preparation of Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2

Boc-Ala-His(Cbz)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-Gln-BHA resin (0.5 mmol) was treated with a mixture of HF (10 mL) and anisole (1 mL) at 0°C for 1 h. After removal of HF by a stream of N_2 and drying under vacuum, the resin was washed with ether, air-dried and extracted with glacial acetic acid (3 x 30 mL). The acetic acid extracts were combined and lyophilized to give crude peptide (576 mg).

The crude peptide was dissolved in 0.1% TFA/water (6 mL) and aliquots (3 mL) were chromatographed (BondElut C_{18}, prewashed with 0.1% TFA/acetonitrile followed by 0.1% TFA/water, step gradient, 0.1% TFA/water, 20% acetonitrile/water-0.1% TFA, 40% acetonitrile/water-0.1% TFA). The 20% acetonitrile/0.1% TFA fractions were combined, evaporated to dryness and lyophilized from 1% HOAc to yield partially purified peptide (376 mg).

An aliquot (100 mg) of the partially purified peptide was dissolved in 1% HOAc/water (3 mL) and chromatographed (Sephadex® LH-20, 1% HOAc/water). The appropriate fractions were pooled and lyophilized to yield the title compound (61.0 mg). MS(ES) 1072.6 $[M+H]^+$, 537.0 $[M+2H]^{++}$; HPLC k' 2.81 (Hamilton PRP-1, gradient, A:acetonitrile B:water-0.1% TFA, 20-50% A over 15 min); TLC R_f 0.69 (n-BuOH:HOAc:H_2O 1:1:1).

Example 9

Parenteral Dosage Formulation

A parenteral dosage form containing 100 µg/mL of drug is prepared as follows:

1 mg of the compound of Example 1 is dissolved in 10 mL normal sterile saline solution and the pH is adjusted to 5.0 with either acetic acid or sodium hydroxide. This solution is filtered through a 0.5 µ filter into a sterile ampoule, the head space is filled with nitrogen and the ampoule is capped with a rubber septum.

The above description fully discloses how to make and use this invention. This invention, however, is not limited to the precise embodiments described herein, but encompasses all modifications within the scope of the claims which follow. The disclosures of the various publications which are cited herein are intended to describe the state of the art and are incorporated herein in their entirety as if fully set forth.

What is claimed is:

1. A compound of the formula (I):

5

$$A\text{-}B\text{-}E\text{-}F\text{-}Ala\text{-}G\text{-}(D\text{-}Phe)\text{-}U\text{-}V\text{-}Y\text{-}Z \quad (I)$$

wherein

A is H or an amino protecting group, or is absent when R^1 is H or C_{1-4}alkyl;

B is Gly, Ala, β-Ala, PyrAla, Gln or Lys, or is absent, when R^1 is H or

10 C_{1-4}alkyl;

E is absent or Het-$(CR'_2)_m CHR^1 (CR'_2)_p$CO;

F is D-Trp(R^2), D-Nal or D-Phe(R^2);

G is Nal(R^2), Trp(R^2) or Phe(R^2);

U is Lys, Arg or Orn;

15 V is D- or L- Gln, NH$(CR'_2)_n$CO or, when Y and Z are absent, NH$(CR'_2)_n$-OH;

Y is absent or Gly;

Z is OR", NR'R" or, when V is NH$(CR'_2)_n$-OH, absent;

R is H or C_{1-4}alkyl;

20 R^1 is H or C_{1-4}alkyl or NR';

R^2 is H, F, Cl, Br, I or NO_2;

R' is H or C_{1-4}alkyl;

R" is H or C_{1-4}alkyl;

m and n are 0 to 3;

25 p is 0 or 1; or

a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein U is Lys.

30 3. A compound according to claim 1 wherein E is His, PyrAla, ImAc or ImPr.

4. A compound according to claim 2 wherein V-Y-Z is Gln-NH_2 or Gln-Gly-NH_2.

35 5. A compound according to claim 4 wherein A-B-E is His or ImAc.

6. A compound according to claim 3 wherein A-B-E is Ala-His.

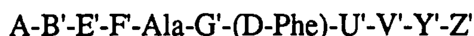
7. A compound according to claim 1 which is:
ImPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2;
ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-NH_2;
5 ImAc-D-Phe-Ala-Phe-D-Phe-Lys-Gln-Gly-NH_2; and
ImAc-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
PyrPr-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2;
His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2;
10 Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-NH_2; or
Ala-His-D-Trp-Ala-Trp-D-Phe-Lys-Gln-Gly-NH_2.

8. A process for preparing a compound of the formula:

15 A-B-E-F-Ala-G-(D-Phe)-U-V-Y-Z
(I)

wherein
A is H or an amino protecting group, or is absent when R^1 is H or C_{1-4}alkyl;
B is Gly, Ala, β-Ala, PyrAla, Gln or Lys, or is absent, when R^1 is H or
20 C_{1-4}alkyl;
E is absent or Het-$(CR'_2)_mCHR^1(CR'_2)_pCO$;
F is D-Trp(R^2), D-Nal or D-Phe(R^2);
G is Nal(R^2), Trp(R^2) or Phe(R^2);
U is Lys, Arg or Orn;
25 V is D- or L- Gln, $NH(CR'_2)_nCO$ or, when Y and Z are absent,
$NH(CR'_2)_n$-OH;
Y is absent or Gly;
Z is OR" or NR'R";
R is H or C_{1-4}alkyl;
30 R^1 is H or C_{1-4}alkyl or NR';
R^2 is H, F, Cl, Br, I or NO_2;
R' is H or C_{1-4}alkyl;
R" is H or C_{1-4}alkyl;
m and n are 0 to 3;
35 p is 0 or 1; or
a pharmaceutically acceptable salt thereof,
which comprises

a) coupling the protected amino acids corresponding to B, E, F, G, U, V, Y as defined in claim 1 to form a suitably protected compound of the formula:


A-B'-E'-F'-Ala-G'-(D-Phe)-U'-V'-Y'-Z'

5 wherein
A, B', E', F', G', U', V' and Y' are A, B, E, F, G, U, V and Y as defined in claim 1 with any sidechain reactive groups protected;
Z' is NR'R", OR", NH-T" or O-T"; and
T" is a chloromethyl, hydroxymethyl, benzhydrylamine or methylbenzhydryl amine
10 resin;

b) removing any protecting groups and, if Z' is NH-T" or O-T", cleaving the peptide from the resin; and

c) optionally forming a pharmaceutically acceptable salt thereof.

15 9. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier.

10. A method of stimulating growth hormone release in a mammal comprising administering a compound according to claim 1.

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11. The use of a compound according to claim 1 in the manufacture of a medicament.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08805

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 7/06
US CL :514/15, 16, 17; 530/328, 329
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/15, 16, 17; 530/328, 329

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 92/01711 (Bowers) 06 February 1992, See Summary of Invention.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 OCTOBER 1993	09 DEC 1993

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Lester L. Lee LESTER L. LEE
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08805

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CA, APS
S>Growth Hormone# or GH or GHRF or REL3
S>DPhe or D-Phe
S>Phe-Lys-Gln