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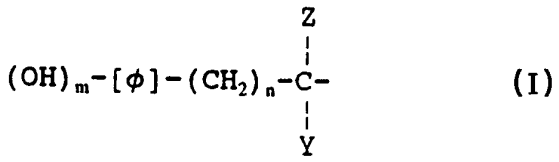

(I)

(57) Abstract

Synthetic peptides which have the sequence: Q^1-CO-R^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-R^8-R^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-R^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-R^{27}-R^{28}-NH-Q^2 wherein Q^1 is an omega or alpha-omega substituted alkyl of structure (I), where: [Φ] is phenyl; Y is H, $-NH_2$, CH_3CONH- or CH_3NH; Z is H or CH_3; m is 1 or 2; n is 0, 1 or 2; R^2 is Ala, Abu or Aib; R^8 is Asn, Ser, Ala, or Abu; R^9 is Ser, Ala or Abu; R^{15} is Gly, Ala, or Abu; R^{27} is Met, Tba or Nle; R^{28} is Asp or Ser; and Q^2 is a lower omega-guanidino-alkyl group having a formula: $-(CH_2)_p$-NH-C(NH_2) = NH wherein p is 2 - 6, and at least one of R^2, R^8, R^9 and R^{15} is Abu or Aib, and the pharmaceutically acceptable addition salts thereof with the pharmaceutically acceptable organic or inorganic bases or acids. Also included in the invention are a pharmaceutical dosage form comprising said peptide and an excipient; methods of treating human growth deficiency comprising administering said peptide; a diagnostic test; and a method for ascertaining endogenous physiological ability to produce hGH.

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GHRH AGONISTS

This invention was made in part with Government support. The Government has certain rights in this application.

FIELD OF THE INVENTION

The present invention relates to peptides having influence on the function of the pituitary gland in humans and other animals. In particular, the present invention is directed to a synthetic peptide which promotes the release of growth hormone by the pituitary gland.

BACKGROUND OF THE INVENTION

Physiologists have long recognized that the hypothalamus controls the secretory functions of the adenohypophysis with the hypothalamus producing special substances which stimulate or inhibit the secretion of each pituitary hormone. Hypothalamic releasing factors have been characterized for the pituitary hormone thyrotropin (the tripeptide TRF), for the pituitary gonadotropins luteinizing hormone and follicle stimulating hormone (the decapeptide LRF, LH-RH or GnRH) and for the pituitary hormone adrenocorticotropin (the 41-amino acid polypeptide CRF). An inhibitory factor, called somatostatin, has also been characterized in the form of a tetradecapeptide which inhibits the secretion of growth hormone (GH). GH releasing hormones or GH-releasing factor (GHRHs or GRFs) have been isolated from human pancreatic tumor as well as rat, porcine, bovine, ovine, caprine and human hypothalami. With the exception of the rat, all characterized GHRHs contain 44 amino acids with an amidated carboxy-terminus. Each of these hypophysiotropic factors has been reproduced by total synthesis. Analogs of the native structures have also been synthesized in order to elucidate structure-activity relationships and, eventually, to provide synthetic congeners with improved properties, such as increased growth hormone release and/or increased metabolic stability. Studies with the synthetic human growth hormone releasing hormone (hGHRH or hGRF) and its analogs have revealed that:

a) deletion of the NH_2-terminal tyrosine residue of hGRF causes its activity to drop to 0.1%; N-acetylation of this residue or change of replacement of L by

D isomers lowers the in vitro bioactivity of hGRF (1-40)-OH to 2-3% and causes a 10-12 fold increase in the in vivo potency of hGRF (1-29)-NH_2; these findings indicate that the presence of said residue is essential for imparting the hGRF molecule with high bioactivity;

b) fragments containing the first 29 residues at least and being terminated with an amide group, e.g., hGRF (1-29)-NH_2 and hGRF (1-37)-NH_2, have at least 50% of the potency of hGRF; removal of the C-terminal amide group or further deletion of amino acids results in a marked decrease in bioactivity even if the C-terminus is amidated, e.g., hGRF (1-29)OH, hGRF (1-27)-NH_2 and hGRF (1-23)NH_2 exhibit 12.6%, 1.12% and 0.24% of the potency of hGRF, respectively; these findings indicate the significance of the arginine amide, i.e., -Arg-NH_2 residue at position 29 of hGHRH; and

c) substituting different amino acids at several sites throughout hGHRH analogs alters the potency of the analogs with respect to hGHRH: U. S. Patent Nos. 4,518,586 (residues 1-3,8,10,15,17,23,25 and 27); 4,528,190 (residues 2,14,27 and 29); and 4,622,312 (residues 1,2,12,15 and 27).

With respect to deamidation of the C-terminal carboxamide function, e.g., transformation of hGRF (1-29)NH_2 into hGRF (1 -29)OH, which can easily occur by the action of hydrolytic enzymes under physiological conditions or in storage in solution, and which is accompanied with substantial loss of bioactivity. There may be taken into consideration previous observations that the 4-guanidino-butylamino group, the so-called agmatine (Agm) residue, can play the role of the arginine residue in certain tripeptide inhibitors of trypsin-like enzymes (S. Bajusz, et al., in: PEPTIDES, 1982 (K. Blaha and P. Malon, eds.), Walter deGruyter, Berlin-New York, 1983, pp. 643-647 . Only classical (solution) methods have been employed for the preparation of agmatine peptides to date; it is desired to provide methodology for the solid phase synthesis of these peptides using techniques of peptide synthesis which have proven to be suitable for preparing GRFs and their analogs.

SUMMARY OF THE INVENTION

Synthetic peptides.

The novel synthetic peptides of this invention are extremely potent in stimulating the release of pituitary GH in animals, including humans. These synthetic peptides retain their physiological activity in solution for an extended period of time and are resistant to enzymatic degradation in the body. Without in any way limiting the invention or its scope, Applicants wish to express their understanding that this retention of activity in vitro and resistance to in vivo degradation are due to the omega-guanidino lower alkyl group at the terminal 29-position of the peptide and the presence non-coded amino acids in the enzymic cleavage site(s) of the synthetic peptides.

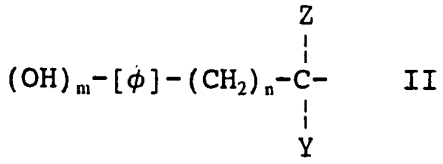
The synthetic peptides (abbreviated [PeP]) have the sequence:

Q^1-CO-R^2-R^3-Ala^4-Ile^5-Phe^6-Thr^7-R^8-R^9-R^{10}-Arg^{11}-R^{12}-R^{13}-R^{14}-R^{15}-Gln^{16}-R^{17}-R^{18}-Ala^{19}-Arg^{20}-R^{21}-R^{22}-R^{23}-R^{24}-R^{25}-Ile^{26}-R^{27}-R^{28}-NH-Q^2 I

wherein

Q^1 is 3-(5-hydroxyindolyl)-methyl or

an omega or alpha-omega substituted alkyl of the structure:



II

where:

[ϕ] is phenyl;

Y is H, -NH_2, CH_3CONH- or CH_3NH-;

Z is H or CH_3;

m is 1 or 2; and n is 0, 1, or 2;

R^2 is Ala, D-Ala, D-N-Methyl-Ala,Abu or Aib

R^3 is Asp, D-Asp, Glu or D-Glu;

R^8 is Asn, D-Asn, Ser, D-Ser, or Abu

R^9 is Ser, Ala,or Abu

R^{10} is Tyr, D-Tyr,or 5-HTP

R^{12} is Lys, D-Lys, Arg or Orn

R^{13} is Val, Ile, or Tbg

R^{14} is Leu or D-Leu

R^{15} is Gly, Ala, Abu or Tbg

R^{17} is Leu or D-Leu

R^{18} is Tyr,or Ser

R^{21} is Lys, D-Lys, Arg or D-Arg

R^{22} is Leu, D-Leu, Ala or Abu

R^{23} is Leu or D-Leu

R^{24} is Gln or His

R^{25} is Asp, D-Asp, Glu or D-Glu;

R^{27} is Met, Nle, Ile, Leu, Tga or Tba

R^{28} is Asp, Asn or Ser; and

Q^2 is a lower omega-guanidino-alkyl group having a formula:

$-(CH_2)_p-NH-C(NH_2) = NH$ III.

wherein p is 2 - 6 and the pharmaceutically acceptable addition salts thereof with the pharmaceutically acceptable organic or inorganic bases and organic or inorganic acids. Preferably, at least one of R^2, R^8, R^9 and R^{15} is Abu or Aib.

(As used herein the symbol -ϕ- signifies para-substituted phenyl, except when used as -[ϕ]- wherein random, i.e., ortho, meta, para or bis-substitution is signified.)

Synthetic Methods.

The synthetic peptides are synthesized by a suitable method such as by exclusively solid phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis.

When the analogs of this invention are synthesized by solid-phase method, the C-terminal residue (here, Q^2) is appropriately linked (anchored) to an inert solid

support (resin). Then, an amino acid bearing protecting groups for its alpha amino group (and, where appropriate, for its amino acid side chain) is coupled in the C-->N direction. After completion of this coupling step, the alpha amino protecting group is removed from this newly added amino acid residue and the next amino acid (suitably protected) is added, and so forth. The N-terminal protecting groups are removed after each residue is added, but the side chain protecting groups, e.g., X^2, X^3 etc. in formula [PR][PeP] below are not yet removed. After all the desired amino acids have been linked in the proper sequence, the peptide is cleaved from the support and then freed from any side chain protecting group(s) under conditions that are minimally destructive towards residues in the sequence. This must be followed by a careful purification and scrupulous characterization of the synthetic product, so as to ensure that the desired structure is indeed the one obtained.

It is particularly preferred to protect the alpha amino function of the amino acids during the coupling step with an acid or base sensitive protecting group. Such protecting groups should have the properties of being stable in the conditions of peptide linkage formation, while being readily removable without destruction of the growing peptide chain or racemization of any of the chiral centers contained herein. Suitable alpha amino protecting group are Boc and Fmoc.

There are also provided several methods of attaching said omega-guanidino alkyl group to a support phase with improved efficiency and yield, procedures for building up the aforesaid peptide by means of the solid phase syntheses both with BOC and with the very fast Fmoc/BOP strategy and procedures for cleaving said peptide from said support phase with liquid HF and with TFA/scavengers.

Synthetic Intermediates.

Intermediates formed during synthesis of the peptides, attached to the support phase and having protective groups which inhibit reaction by the side

chains of certain amino acid residues, also constitute part of the invention.

Medical Applications.

The synthetic peptides of Formula I may be formulated in pharmaceutical dosage form and administered to humans or animal for therapeutic or diagnostic purposes. More particularly, the peptides may be used to promote the growth of warm-blooded animals, as, in humans, to treat human growth deficiency by stimulating in vivo synthesis and/or release of endogenous GH; to treat certain physiological conditions such as severe growth retardation due to chronic renal insufficiency; to offset certain effects of aging, e.g., reducing loss of muscle and bone loss; to accelerate healing and tissue repair; to improve feed utilization, thereby increasing lean/fat ratio favoring muscle gain at the cost of fat; and also to enhance milk production in lactating cattle. Further, the synthetic peptides may be used in a method to ascertain endogenous physiological ability to produce hGH and a diagnostic kit for carrying out this method.

DESCRIPTION OF THE FIGURE

Figure 1 is a graphical representation of selected data from Table 4 in Example XX herein, where "A" indicates the peptide of Example XVI -- i.e., (Dat^1, Abu^2, Ser^8, Abu^{15}, Nle^{27}, Agm^{29})hGH-RH(1-29) and "B" indicates the peptide of Example XI -- i.e., (Dat^1, Aib^2, Ser^8, Ala^{15}, Nle^{27}, Agm^{29})hGH-RH(1-29).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. SYNTHETIC PEPTIDES

1. Nomenclature. The nomenclature used to define the peptides is that specified by the IUPAC-IUB Commissioner on Biochemical Nomenclature (European J. Biochem., 1984, 138, 9-37). By natural amino acid is meant one of common, naturally occurring amino acids found in proteins comprising Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met Phe, Tyr, Pro, Trp and His. By Nle is meant norleucine, by Nva is meant norvaline, by Abu is meant alpha amino butyric acid, by Aib meant alpha iso-butyric acid, by Tba is meant

alpha-t-butylalanine, by Tbg is meant alpha-t-butylglycine, by 5-Hiaa is meant 5-Hydroxy-indolylacetic acid, by C-MeTyr is meant 1-methyl-DL-Tyrosine, by N-MeAla and N-MeTyr are meant N-methyl-alanine and N-methyl-tyrosine respectively. When the amino acid residue has isomeric forms, it is the L-form of the amino acid that is represented unless otherwise expressly indicated.

Other abbreviations used are:

AcOH	acetic acid
AcOEt	ethyl acetate
Ac_2O	acetic anhydride
Boc-	tert-butyloxycarbonyl-
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate
Cbz-	benzyloxycarbonyl
Chx-	cyclohexyl-
2-Cl-Cbz-	2-chloro-benzyloxycarbonyl-
DCB-	2,6 dichlorobenzyl-
DCCI	dicyclohexylcarbodiimide
DIC	diisopropylcarbodiimide
DCM	dichloromethane
DIEA	diisopropylethylamine
DMF	dimethylformamide
Fmoc-	fluorenylmethyloxycarbonyl
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
MeOH	methyl alcohol
NMM	N-Methyl-morpholine
Pmc-	2,2,5,7,8-Pentamethyl-chroman-6-sulphonyl
TEA	triethylamine
TFA	trifluoroacetic acid
Tos-	p-toluensulfonyl-
tBu-	tert-butyl-

The amino acid sequences of the synthetic peptides are numbered to in correspondence with the amino acid residues in hGH-RH(1-29). That is, the Ala^4 and R^8 of the Formula I peptide occupy the same position in the sequence as the Ala^4 and R^8 residues in hGH-RH(1-29).

The convention under which the N-terminal of a peptide is placed to the left, and the C-terminal to the right is also followed herein. Strictly speaking however, the synthetic peptides of Formula I have neither an N nor a C terminal since the Q^1 or Q^2 moieties, at what would otherwise be the N and C termini, lack an N- and a C- terminus respectively. In order to remove any question therefore, it should be understood that the terms N- and C-terminal used with respect to the synthetic peptides of Formula I mean Q^1 and Q^2.

2 . Preferred Embodiments.

The synthetic peptides of the present invention have the sequence:

Q^1-CO-R^2-R^3-Ala^4-Ile^5-Phe^6-Thr^7-R^8-R^9-R^{10}-Arg^{11}-R^{12}-R^{13}-R^{14}-R^{15}-Gln^{16}-R^{17}-R^{18}-Ala^{19}-Arg^{20}-R^{21}-R^{22}-R^{23}-R^{24}-R^{25}-Ile^{26}-R^{27}-R^{28}-NH-Q^2 I

wherein Q^1, R^2, R^3, R^8, R^9, R^{10}, R^{12}, R^{13}, R^{14}, R^{15}, R^{17}, R^{18}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{27}, R^{28} and Q^2 are as defined above. Preferably, at least one of R^2, R^8, R^9 and R^{15} is Abu or Aib.

Depending on the identity of the functional groups in Q^1, the Q^1-CO moieties may when taken together form an amino acid residue: thus, when in Q^1 m is 1, n is 1 and y is NH_2, Q^1-CO forms tyrosyl ("Tyr-"). Even when the Q^1 functional groups do not cause Q^1 to form an amino acid with CO-, the two constituents of the Formula I peptide may together form a readily recognized group. Thus, when in Q^1, m is 1, n is 1 and Y and Z are H, Q^1-CO together form 3-(4-hydroxyphenyl)propionyl, known as des-amino-Tyr (or "Dat"). Thus, for convenience's sake, the Q^1-CO moieties may be taken as a unitary moiety R^1 and replaced in the Formula I peptides by abbreviations such as Tyr^1, Dat^1, etc. as appropriate.

Similarly at the N-terminal of the Formula I peptides, the NH-Q^2 moieties may be taken as a unitary moiety R^{29} when together they form a single recognized functional group. Thus, when in Q^2, p is 4, the combination of NH and Q^2 forms -NH-$(CH_2)_4$-NH-C(NH_2)=NH, or an agmatine ("Agm") residue.

The use of these abbreviations in Formula I peptides is optional. Thus in an especially preferred peptide, this format is not used. This peptide has the structure:

Q^1-CO-R^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-R^8-R^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-R^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-R^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-R^{27}-R^{28}-NH-Q^2

wherein

R^2 is Ala, D-Ala, Abu or Aib

R^8 is Asn, Ser, ,or Abu

R^9 is Ser, Ala, or Abu

R^{15} is Abu,Ala or Gly

R^{27} is Met, Tba, or Nle,

R^{28} is Ser or Asp;

Especially preferred embodiments include peptides of Formula I in which Q^1-CO is Dat, Ac-Tyr, N-Me-Tyr, C-Me-Tyr and Tyr; and NH-Q^2 is Agm. Further preferred embodiments include peptides of Formula I in which Q^1-CO is Dat or Tyr; R^{15} is Abu; R^{27} is Nle; R^{28} is Asp; and NH-Q^2 form Agm.

In one very preferred analog, in Q^1, m is 1, n is 1 and Y and Z are H, so that Q^1-CO is Dat; R^2 is Aib, R^8 is Ser, R^9 is Ser, R^{15} is Ala, R^{27} is Nle, R^{28} is Ser; and in Q^2, p is 4. The peptide has the formula:

3-(4-hydroxyphenyl)propionyl^1-Aib^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Ser^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Gly^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}(X^7)-NH-(CH_2)4-NH-C(NH_2) = NH.

This analog may be expressed under a well known convention as follows:

3-(4-hydroxyphenyl)propionyl^1-Aib^2-Ser^8-Nle^{27}-Ser^{28}-4-guanidino-butylamide^{29}

hGHRH(1-29). This is the analog described in Example VIII below; it may be further abbreviated as (Dat^1, Aib^2, Ser^8, Nle^{27}, Agm^{29})hGHRH(1-29).

Similarly, where Q^1-CO is Dat, R^2 is Abu, R^8 is Ser, R^9 is Thr, R^{15} is Abu, R^{27} is Nle, R^{28} is Ser and in Q^2 p is 4, the peptide may be written as: (Dat^1, Abu^2, Ser^8, Abu^{15}, Nle^{27}, Agm^{29})hGHRH (1-29) described in Example XIV.

In another preferred embodiment where Q^1-CO is Dat, R^2 is Aib, R^8 and R^{15} are Abu, R^9 is Thr, R^{27} is Nle, R^{28} is Ser and in Q^2 p is 4, the peptide may be written: (Dat^1, Aib^2, Abu^8, Abu^{15}, Nle^{27}, Agm^{29})hGHRH (1-29) described in Example XIII.

B. SYNTHETIC METHODS

1. Overview of Synthesis.

The peptides are synthesized by a suitable method such as by exclusively solid phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis. For example, the techniques of exclusively solid-phase synthesis are set forth in the textbook "Solid Phase Peptide Synthesis", J.M. Stewart and J.D. Young, Pierce Chem. Company, Rockford, 111.,1984 (2nd. ed.), G. Barany and R.B. Merrifield, "The Peptides", Ch. 1, 1-285, pp. 1979, Academic Press, Inc., and M. Bodanszky, "Principles of Peptide Synthesis", SpringerVerlag, 1984. The synthetic peptides of Formula I are preferably prepared using solid phase synthesis, such as that generally described by Merrifield, J.Am.Chem.Soc., 85, p. 2149 (1963), although other equivalent chemical syntheses known in the art can also be used as previously mentioned.

In solid phase synthesis, the moiety which forms the C or N-terminal group of the resulting peptide is joined to a polymeric resin support phase via a chemical link. Amino acid residues or oligopeptide fragments are then added sequentially to the alpha amino functional group of the C or N-terminal moiety until the desired

peptide sequence is obtained. Because the amino acid residues or oligopeptide fragments are added to the C-terminal Q^2 group here, growth of the synthetic peptides of Formula I begins at the C terminal and progresses toward the N-terminal. When the desired sequence has been obtained, it is removed from the support phase.

When reactive side chain functional groups of the various amino acid moieties or peptide fragments that bear them, suitable protecting groups prevent a chemical reaction from occurring at said side chains until the protecting group is ultimately removed. Accordingly, it is common that, as a step in 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 residues.

The initial synthetic sequence utilized herein is disclosed in US Patent 4 914 189 which is incorporated by reference herein. Reference is particularly made to Examples I through VII therein.

2. Joining NH_2-Q^2 to the Support Phase.

The peptides of Formula I may be synthesized on a variety of support phases. These support phases may be amino or hydroxy resins such as amino-methyl resins (suitably 1% cross linked), benzhydrylamine resins (suitably 2% cross linked) p-methylbenzhydrylamine resins (suitably 2% cross linked) and the like. It is generally preferred that the support phase [SP] is an amino type resin of the formula: H_2N-CH_2-ϕ-[Pm] IX where [Pm] is a polymeric substrate.

The initial material joined to the support phase is suitably the C-terminal Q^2 group. Preferably, the adjacent NH functional group is already affixed to Q^2. Agm or 4-guanidino butylamine are especially preferred.

The NH-Q^2 group is joined to the support phase via a stable but readily cleavable bridging group. It has been found that such a bridge may be readily provided by a sulfonyl phenoxy acetyl moiety. Thus,

Boc-NH-Q^2-SO_2-ϕ-(M_s)O-CH_2-CO-[SP] or

Fmoc-NH-Q^2-SO_2-[ϕ]-(M_s)O-CH_2-CO-[SP]

constitute the material joined to the support phase for the synthetic sequence. They are prepared and linked to the support phase as follows.

2(a). Preparing Boc-NH-Q^2-SO_2-ϕ-(M_s)O-CH_2-CO-[SP].

The primary amino group of agmatine (i.e., the -NH- group preceding Q^2) is protected with a tert-butyloxy carbonylating agent, suitably ditert-butyl dicarbonate, by reaction. This reaction may take place preferably at ambient temperatures or at 5-10°C in the presence of a base. The protected material is then reacted in a similar solvent system with the arylsulfonyl halide

Hal-SO_2-ϕ-O-CH_2-COOH

wherein Hal is chloro or bromo to form

Boc-NH-Q^2-SO_2-ϕ-O-CH_2-COOH.

The protected aminoalkyl guanidino-sulfonyl phenoxyacetic acid is then coupled to the support phase [SP] by the action of a diloweralkyl carbodiimide or BOP on:

H_2N-CH_2-ϕ-[Pm]

to yield:

Boc-NH-Q^2-SO_2-ϕ-O-CH_2-CO-[SP]

which represents Q^2 linked to the polymeric substrate support phase via the sulfonyl phenoxy acetyl moiety, more fully expressed as:

Boc-NH-Q^2-SO_2-ϕ-O-CH_2-CO-NH-CH_2-ϕ-[Pm]

In the preferred procedure, the coupling is carried out using any of the known dialkyl carbodiimide coupling procedures. For example, there may be utilized diisopropyl-carbodiimide in the presence of 1-hydroxybenzotriazole hydrate

in dimethylformamide at ambient temperatures. Alternatively, there may be employed N,N'dicyclohexyl-carbodiimide. In this procedure, the aforesaid Boc-aminoalkyl guanidino-sulfonyl-phenoxyacetic acid is mixed with the carbodiimide in 2 to 1 molar ratio, the formed N,N'dicyclohexyl urea is removed by filtration and the resulting solution is added to the support phase.

When BOP is used as the carboxyl activating agent, the protected aminoalkyl guanidinosulfonyl phenoxyacetic acid is mixed with equimolar BOP and dissolved in DMF containing twice molar NMM in 10% concentration. The activation can be enhanced in the presence of 1-3 molar excess of HOBt yielding an efficient coupling rate to the solid support preferably to an aminomethyl resin at room temperature.

Where the ultimate synthetic sequence is intended to follow the Boc protocol, the aromatic ring of the arylsulfonyl halide is not substituted since such a situation will permit the use of trifluoroacetic acid as the intermediate deprotecting agent and hydrogen fluoride as the agent for final cleavage from the resin.

2(b). Preparing Fmoc-NH-Q^2-SO_2-[ϕ](M_s)-O-CH_2-CO-[SP].

In place of utilizing Boc to protect the aminoalkyl guanidino moiety, N-benzyloxycarbonyl chloride is used in the presence of an aqueous alkali, suitably sodium hydroxide, preferably at about 4N under agitation and cooling to between about 5 and about 15°C. Upon sodium hydrogen carbonate treatment, the protected product is precipitated and thereafter is reacted with an aryl-sulfonyl chloride as previously.

More particularly, the Fmoc procedure comprises the sequential steps of reacting N-benzyloxy carbonyl chloride with NH_2-Q^2 in the presence of a base to form Cbz-NH-Q^2, reacting said Cbz-NH-Q^2 with a substituted arylsulphonyl halide in the presence of a strong aqueous base and removing the Cbz group by catalytic

hydrogenation. The substituted arylsulphonyl halide has the formula:

$$Hal\text{-}SO_2\text{-}[\phi](M_s)\text{-}O\text{-}CH_2\text{-}COOH$$

wherein Hal is chloro or bromo; M_s is lower alkyl or lower alkoxy of 1 to 5 carbon atoms located at the positions of the phenyl nucleus other than 1 and 4; and s is 1-3. The reaction yields $H_2N\text{-}Q^2\text{-}SO_2\text{-}[\phi](M_s)\text{-}O\text{-}CH_2\text{-}COOH$.

After removal of the Cbz group, the product is reacted with Fmoc-Cl in the presence of a base to yield the corresponding Fmoc protected aminoalkyl guanidino-sulfonyl aryloxy acetic acid, i.e.

$$Fmoc\text{-}NH\text{-}Q^2\text{-}S02\text{-}[\phi](M_s)\text{-}O\text{-}CH_2\text{-}COOH.$$

This product is then coupled to said support phase [SP] to yield:

$$Fmoc\text{-}NH\text{-}Q^2\text{-}SO_2\text{-}[\phi](M_s)\text{-}O\text{-}CH_2\text{-}CO\text{-}[SP],$$

which may be expressed in more detailed fashion:

$$Fmoc\text{-}NH\text{-}Q^2\text{-}SO_2\text{-}[\phi](M_s)\text{-}O\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}\phi\text{-}[Pm].$$

As noted above, the arylsulfonyl halide should preferably carry between 1 and 3 M substituents which may be alkyl or lower alkoxy, suitably methyl or methoxy. This is in contrast to the Boc procedure: here, it is desirable to substitute the phenyl moiety with up to three alkyl or alkoxy groups. These groups weaken the strength of sulfonamide bridge and enable it to be cleaved by trifluoroacetic acid, once all the amino acid residues have been added and one wishes to remove the intermediate from the support phase. This reaction is carried out in the presence of a strong but dilute aqueous base, suitably about 1N sodium hydroxide. The reaction mixture is acidified and the product extracted, suitably with a water immiscible organic solvent such as ethyl acetate. The extract is then concentrated and hydrogenated, suitably at atmospheric pressure and room temperature in the presence of a catalyst such as palladium on charcoal in a reduction inert solvent. If ethyl acetate is used as the extractant, this may be used as the hydrogenation solvent.

3. Stepwise Addition of Amino Acid Residues.

Utilizing the aforementioned Boc- or Fmoc-protected amino-alkyl-guanidino sulfophenoxyacetyl as the anchored C-terminal, the peptide itself may then suitably be built up by solid phase synthesis in the conventional manner. Thus, the alpha amino-protecting group of the next amino acid residue is protected by Boc or Fmoc to prevent reaction between the N-terminal of R^{28} while this is being reacted with NH_2-Q^2. These are attached to the NH_2-Agm or H_2N-Q^2-SO_2-[ϕ](M_s)-O-CH_2-COOH respectively, as described above. The amino acid residues of the synthetic peptides are added sequentially . Because the peptide is constructed from the C-terminal, the residues are added in reverse numerical order, i.e., R^{28}, R^{27}, and so forth.

The protected amino acids are coupled sequentially, with Q^1 finally being added. As an alternative to adding each amino acid separately in the synthesis, some may be coupled to one another in a separate vessel and added as an oligopeptide in the solid phase reaction. The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as coupling reagents are N,N'-diisopropyl carbodiimide (DIC) or the BOP carboxyl activating reagent.

Each protected amino acid or amino acid sequence is introduced into the solid phase reaction in about a three-fold molar excess, with respect to NH_2-Q^2, etc., and the coupling may be carried out in as medium such as DMF: CH_2Cl_2 (1:1) or in DMF or CH_2Cl_2 alone. In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the alpha amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis is preferably monitored by the ninhydrin reaction.

4. Removal of Complete Intermediate from the Support Phase.

When the synthesis is complete, the Q^2 guanidino alkyl end of the Formula I peptide is cleaved from the support phase at the sulfonyl group. If the Boc

protocol is utilized, then the cleaving agent is anhydrous hydrofluoric acid; whereas, in the Fmoc protocol the cleaving agent is trifluoroacetic acid.

Removal of the intermediate peptide from the resin support is performed by treatment with a reagent such as liquid hydrogen fluoride or trifluoroacetic acid. This also cleaves all remaining side chain protecting groups X^3, X^7, X^{11}, X^{12} and and the anchoring guanidinosulfo bond, if present, and also the alpha amino or alpha-hydroxyl protecting group X^1. These groups have different values depending on whether Boc or Fmoc protocols are used.

C. SYNTHETIC INTERMEDIATES

1. Side Chain Protecting Groups.

The protection of reactive side chain functional groups of the various amino acid moieties or peptide fragments is common to solid phase synthesis. These side chain functional groups are protected in order to prevent an undesirable chemical reaction from occurring at said chains. 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 (e.g., X^1, X^7, etc.) linked to the appropriate residues.

The selection of a side chain amino protecting group is not critical except that generally one is chosen which is not removed during deprotection of the alpha amino groups during the synthesis.

In selecting a particular side chain protecting group to be used in the synthesis of the peptides, the following general rules are followed: (a) the protecting group preferably retains its protecting properties and is not split off under coupling conditions, (b) the protecting group should be stable to the reagent, is preferably stable under the coupling reaction conditions selected for removing the alpha amino protecting group at each step of the synthesis and, (c)

the side chain protecting group must be removable upon the completion of the synthesis of the desired amino acid sequence, under reaction conditions that will not undesirably alter the peptide chain.

The side chain protecting groups are attached to the amino acid residues by steps well known in the art.

2. Synthetic Intermediates.

Also considered to be within the scope of the present invention are intermediates of the formula: [PR] [PeP] attached to a support phase [SP] selected from the group consisting of amino type resins, said combination having the structure:

[PR] [PeP]-SO_2-[ϕ](M_s)-O-CH_2-CO-SP

where M is hydrogen, lower alkyl or lower alkoxy of 1 to 5 carbon atoms, suitably methyl or methoxy, s is 1-3, [SP] is an amino type resin. Suitably [PR][PeP] has the structure:

X^1-Q^1-CO-R^2-Asp^3(X^3)-Ala^4-Ile^5-Phe^6-Thr^7(X^7)-R^8(X^7or Nil)-Ser^9(X^7or Nil)-Tyr^{10}(X^{10} or 10)-Arg^{11}(X^{11})-Lys^{12}(X^{12})-Val^{13}-Leu^{14}-R^{15}-Gln^{16}-Leu^{17}-Ser^{18}(X^7)-Ala^{19}-Arg^{20}(X^{11})-Lys^{21}(X^{12})-Leu^{22}-Leu^{23}-Gln^{24}(H or X^{24})-Asp^{25}(X^3)-Ile^{26}-R^{27}-R^{28}(X^3 or X^7)-NH-Q^2

wherein

X^1 is either hydroxyl, or an amino protecting group or nil, depending on the meaning of Y in Formula II (the formula for one of the Q^1 moieties).

X^3 is a suitable ester-forming protecting group for the carboxyl group of Asp or Glu. In the Boc protocol, it may form cyclohexyl esters; in the Fmoc protocol tert-butyl esters are preferred.

X^7 is a suitable protecting group for the hydroxyl group of Thr or Ser, such as tert-butyl, Bzl. The preferred protecting group in the Boc protocol is Bzl and in the Fmoc protocol it is tert-butyl.

X^{10} is a suitable protecting group for the phenolic hydroxyl group of Tyr or N-MeTyr, such as tert-butyl, Bzl, 4Br-Cbz and 2,6-dichlorobenzyl (DCB). The preferred protecting group in the Boc protocol is 2,6-dichlorobenzyl and in the

Fmoc protocol it is tert-butyl.

X^{11} is a suitable protecting group for the guanidino group of Arg, such as nitro, Tos, Pmc, Mtr; in the Boc protocol Tos is the preferred group and 2,2,5,7,8-pentamethyl chroman-6-sulfonyl in the Fmoc protocol.

X^{12} is a suitable protecting group for the side chain amino group of Lys. Illustrative of suitable side chain amino protecting groups are 2-chloro-benzyloxycarbonyl (2-Cl-CBz) and tert-butyloxycarbonyl (Boc). In the Boc protocol, 2-chlorobenzyloxycarbonyl is the preferred protecting group and in the Fmoc protocol Boc is thus utilized.

X^{24} is hydrogen or a protecting group for the imidazole nitrogen of His, such as Tos in the Boc or Trt in the Fmoc protocol.

MEDICAL APPLICATIONS

The products of the present invention may be utilized to promote the growth of warm-blooded animals (e.g., humans) and also enhance the milk production of females of milk producing mammals, suitably but not exclusively goats and cows, preferably cows.

The peptides of the invention may be administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginate, pamoate, malate, ascorbate, tartrate, and the like.

The compounds of the present invention are suitably administered to the subject humans or animals s.c., i.m., or i.v; intranasally or by pulmonary inhalation; or in a depot form (e.g., microcapsules, microgranules, or cylindrical rod like implants) formulated from a biodegradable suitable polymer (such as D,L-lactide-co-glycolide), the former two depot modes being preferred. Other equivalent modes of administration are also within the scope of this invention, i.e., continuous drip, depot injections, infusion pump and time release modes such

as microcapsules and the like. Administration is in any physiologically acceptable injectable carrier, physiological saline being acceptable, though other carriers known to the art may also be used.

The dosage level should be between 0.2 and 2 micrograms/kg body weight per injection, except for depot form where the amount injected would be calculated to last from about 15 to about 30 days or longer. These dosage ranges are merely preferred. Administration of non-depot forms may be between 1 and 4 times per day. It is convenient to inject the animal whenever it is milked.

Until the production of growth hormone by recombinant-DNA methods began, the small supply of pituitary-derived human growth hormone limited its use to the treatment of children with growth hormone deficiency. The wide availability of synthetic human growth hormone has made possible long-term studies of other potentially beneficial uses of growth hormone and its more physiologic actions. Although synthetic GH is currently approved in USA only for treatment of growth failure due to lack of endogenous growth hormone, this therapy has also been used to treat short children not classically GH deficient. However the cost of long-term treatment with hGH and the need of daily sc administration are important considerations. Currently, the cost of growth hormone therapy for a child with growth deficiency ranges from $10,000 to 30,000 a year depending on body weight. Treatment of a 70-kg adult with hGH three times a week costs $13,800 a year. Vance, M.L., N.Eng.J.Med 323: 52-54 (1990). Thus, long-term growth hormone treatment in elderly adults with diminished growth hormone secretion would require a considerable personal and financial investment. In addition there are many children all over the world with growth retardation due to the lack of GH who cannot be treated with hGH because of the cost of this therapy. Consequently there is an urgent need to develop a drug that releases GH and with an affordable price. This alternative method to increase endogenous growth hormone secretion is through the administration of agonistic analogs of growth hormone-releasing hormone. The therapy with GHRH agonistic analogs

should be much less expensive than that utilizing hGH. In addition, the development of long-acting delivery systems for these analogs can make this new modality of treatment more practical and convenient.

The ability to produce synthetic growth hormone (GH) by recombinant DNA technology has enabled the manufacture of GH in GH in potentially unlimited quantities. This greatly facilitated the treatment of GH-deficient children. As stated above, synthetic hGH is currently approved only for the treatment of growth failure due to a lack of adequate endogenous growth hormone, but hGH has also been used to treat short children who are not classically GH-deficient such as girls with Turner's syndrome; prepubertal children with chronic renal insufficiency and severe growth retardation; and children with non-GH deficient short stature.

Of the 3 million children born in the USA annually, 90,000 will below the third percentile for height. These children may be labeled as having short stature and may be candidates for GH treatment. Therapy with human growth hormone currently costs about $20,000 per year and the average length of treatment is about 10 years. The treatment will usually be stopped when the patient reaches an acceptable adult size (a height of well over five feet) or when the patient matures sexually and the epiphyses close, at which time linear growth ceases, or if the patient fails to respond to the treatment. If all children who are less than the third percentile for height receive a five year course of hGH therapy, hGH for height augmentation therapy will cost at least $8 billion to 10 billion per year. Lantos J. et al., JAMA 261:1020-1024, (1989).

It is desirable to ascertain the endogenous physiological ability of children having short stature to produce hGH. This may be done with a diagnostic test containing a 50-100 microgram dose of GH-RH; a 50-100 microgram dose of a GH-RH analog of Formula I; and means for assaying the GH response evoked by each dose.

The assay means may be any conventional means which will indicate the quantitative amount of hGH present in a blood sample drawn from the patient. The concentration of GH in serum is determined using standard radioimmunoassay ("RIA") procedures as set forth in e.g., Miles I.E.M. et al., Lancet ii, 492-493 (1968) or O'Dell W et al., J.Lab.Clin.Med. 70, 973-80 (1967).

The test is used as follows. First, the GH-RH dose is administered. Thirty minutes later, a blood sample is taken for RIA of GH. Various commercially available kits (e.g., Nichols Institute of Diagnostics, San Juan Capistrano, CA) or reference preparations of hGH (e.g., NIAMDD-hGH-RP-1) can be used for RIA of GH.

After waiting 6-24 hours for the effect of GH-RH to wear off, the dose of GH-RH analog is administered. Blood again is drawn for radioimmunoassay of GH.

The presence of a normal hGH response in the first assay reveals that endogenous hGH producing ability is present. This result also suggests a short, mild course of GH-RH therapy, if any, may be suitable. A low GH response, or no response, to the first dose reveals only that GH-RH must be evaluated in view of the second test result. If a good hGH response follows the second dose, there is clear physiological hGH producing ability which is not evoked by GH-RH. This indicates that a therapy with the GH-RH analog may be desirable. Finally, no or low response to both tests reliably reveals lack of physiological ability to produce hGH, and so suggests therapy with hGH is probably needed.

A diagnostic kit for testing endogenous physiological ability to produce GH may incorporate a 50-100 microgram dose of GH-RH; a 50-100 microgram dose of GH-RH analog peptide of Formula I; and means for assaying the GH response evoked by each dose.

As indicated above, short stature in children may result from many causes, none of which are immediately apparent. Use of the diagnostic test on all children with this condition would greatly clarify the cause of short stature. Such a widespread screening test would also provide earlier indications for desirable treatment.

Thus, the invention further includes a method for ascertaining the endogenous physiological ability to produce hGH and a diagnostic kit for carrying out this method.

Glucocorticoids are potent inhibitors of linear growth in man and growth suppression is a well known risk of long term treatment of asthmatic children with steroids. Thus stunted growth is an important consequence of chronic administration of glucocorticoids in childhood. The inhibition of GH secretion is due in some extent to the fact that chronic administration of glucocorticoids suppresses GHRH. This inhibition occurs at the level of the hypothalamus or above and in this situation only the treatment with GHRH agonists will stimulate linear growth.

Growth hormone tends to decline with the aging process and may lead to decrease in muscle mass and adiposity. Studies have shown that healthy older men and women with growth hormone deficiency had increases lean body mass and decreases in the mass of adipose tissue after six months of hGH administration. Other effects of long-term administration of hGH on body composition included increase in vertebral-bone density and increase in skin-fold thickness. In addition, it has been reported that daily GHRH injection to menopausal women, for 8 days augments GH responses and IGF-I levels and raises serum osteocalcin levels. Thus the therapy with GHRH agonistic analogs reduces the loss of muscle, bone and skin mass and lessen the increase of body fat that normally accompanies the aging process.

Growth hormone is a potent anabolic hormone that enhances protein synthesis and nitrogen retention and chronic administration of agonistic analogs of GHRH increase the endogenous growth hormone secretion. The therapy with GHRH agonistic analogs has uses in other areas of medicine such as catabolic states causing accelerated weight loss; tissue repair in patients with severe body surface burn, in accelerating healing of nonunion fractures and in some cases of cardiac failure.

Although long term follow-up is necessary before all treatment responses can be ascribed to GH, there has been improvement in cardiac mass and an increase in both cardiac mass and contractility. The therapy with hGH interrupts the cardiac-cachexia cycle. This response is in keeping with other observations that the therapy with GH has a major role in catabolic states in adults. An alternative method to increase endogenous growth hormone secretion in these conditions is the administration of GHRH agonistic analogs.

These agonistic analogs of GH-RH can replace hGH for many applications.

GH-deficient children respond to GH-RH(1-40), GH-RH(1-29) or GH-RH(1-44), with an increase in growth. Thorner M.O. et al., N.Engl.J.Med. 312:4-9, 1985; Ross R.J.M. et al., Lancet 1:5-8, 1987; Takano K et al., Endocrinol. Japan 35;775-781 (1988). Most children who respond to hGH, will respond to GH-RH. This is because most GH-deficient children have a hypothalamic defect in GH release, and will show a GH response after the administration of analogues of the hypothalamic hormone GH-RH. Thus repeated administration of GH-RH promotes linear growth. GH-RH(1-29)NH_2 given subcutaneously twice a day promoted linear growth in approximately 50% of a group of GH-deficient children (Ross et al, cited above). A small group of severely GH-deficient children will respond to GH-RH after 6 (six) months of treatment.

FURTHER CLINICAL APPLICATIONS OF AGONISTIC ANALOGUES OF GHRH IN CHILDREN WITH GROWTH RETARDATION:

1. As a screening test for growth hormone deficiency
2. Treatment of Hypothalamic GHRH deficiency
3. Constitutional growth delay
4. Turner Syndrome
5. Familial short stature
6. Prepubertal children with chronic renal insufficiency and severe growth retardation
7. Infants and children with intrauterine growth retardation
8. Children with GH deficiency following radiotherapy for pituitary or hypothalamic lesions
9. Children on long-term treatment with glucocorticoids and growing at subnormal rate

FURTHER CLINICAL APPLICATIONS OF AGONISTIC ANALOGUES OF GHRH IN ADULTS:

1. Geriatric Patients: To reduce the loss of muscle, bone and skin mass and lessen the increase of body fat that normally accompanies the aging process.
2. Catabolic states
3. Wound healing
4. Delayed healing of fractures
5. Osteoporosis
6. Obesity
7. As an adjunct to total parenteral nutrition in malnourished patients with chronic obstructive pulmonary disease
8. Cardiac failure
9. GHRH agonists could be used during and after space flights to

counteract the decrease in GH secretion. Weightlessness of space flight significantly decreases the release of growth hormone. This finding could explain the bone loss and muscle weakness many astronauts experience after prolonged space flights.

5

The following Examples set forth suitable methods of synthesizing by the solid-phase technique, several fragments and novel analogs of hGH-RH which have improved potency.

EXAMPLE I

Synthesis of Boc-agmatine

EXAMPLE II

Synthesis of 4-Chlorosulfonyl Phenoxyacetic Acid (Cl-SPA)

EXAMPLE III

Boc-agmatine-[SPA]

EXAMPLE IV

Coupling of Boc-agmatine-[SPA] to Support Phase

EXAMPLE V

Synthesis of Cbz-agmatine

EXAMPLE VI

Synthesis of Agmatine-Sulfonyl- 2,3,5 (and 2,3,6)-Trimethylphenoxy Acetic Acids

EXAMPLE VII

Preparing of Fmoc-Agmatine-Sulfonyl-2,3,5 (and 2,3,6)-Trimethylphenoxy Acetic Acid and coupling to support phase

The initial synthetic sequence utilized herein and indicated by headings above is disclosed in Examples I through VII of US Patent 4 914 189 and are incorporated by reference herein.

EXAMPLE VIII

(Dat^{1},Aib^{2},Ser^{8},Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

3-(4-hydroxyphenyl)propionyl^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Gly^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}(X^{7})-NH-(CH_2)4-NH-C(NH_2) = NH

was carried out in a stepwise manner on a PS-2755 (Protein Technologies) synthesizer starting with the appropriate Boc-NH-$(CH_2)_4$-NH-C(NH_2) = N-SO_2-ϕ-OCH_2-CO-[SP] in accordance with the procedures set forth below in Schedule A.

Deblocking and neutralization are preferably carried out in accordance with Schedule A as follows:

SCHEDULE A Deprotection

Step	Reagent	Mixing (min)
1.	Deprotect: 100% TFA	1+4
2.	DMF wash	1
3.	Neutralization: 5% DIEA in DMF	2
4.	DMF wash	1
5.	Neutralization: 5% DIEA in DMF	2
6.	DMF wash (3 X)	1

The couplings are preferably carried out as set out in Schedule A for coupling:

SCHEDULE A Coupling

Step	Reagent	Mixing (min)
1.Coupling	Boc-amino acid (3 eq) in DMF (or preformed HOBt ester of Boc protected amino acid) plus DIC (3eq)	20 (+ 20)
2.	DCM wash (3 X)	1
3.	Ac_2O(30%) in DMF	10+20
4.	DMF wash (3 X)	1

Briefly, BOC is used for the alpha amino protection. Benzyl ether is used as the hydroxyl side chain protecting group for Ser and Thr. The phenolic hydroxyl group of Tyr is protected with DCB and the side chain carboxyl group of Asp is

protected in the form of the cyclohexyl ester. Tos is used to protect the guanidino group of Arg and 2-Cl-Cbz is used as the protecting group for the Lys side-chain. Due to the decreased reaction rate of the 3-(4-hydroxyphenyl)propionic acid N-hydroxy-succinimide ester, a prolonged coupling time 60 minutes was used.

The following compound is obtained:

3-(4-hydroxyphenyl)propionyl^{1}-Aib^{2}-Asp^{3}(X^{3})-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(X^{7})-Ser^{8}(X^{7})-Ser^{9}(X^{7})-Tyr^{10}-(X^{10})-Arg^{11}(X^{11})-Lys^{12}(X^{12})-Val^{13}-Leu^{14}-Gly^{15}-Gln^{16}-Leu^{17}-Ser^{18}(X^{7})-Ala^{19}-Arg^{20}(X^{11})-Lys^{21}(X^{12})-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(X^{3})-Ile^{26}-Nle^{27}-Ser^{28}(X^{7})-NH-$(CH_2)_4$-NH-C(NH_2) = N-SO_2-ϕ-OCH_2-CO-[SP]

wherein X^{3} is O-cyclohexyl ester, X^{7} is O-benzyl ether, X^{10} is O-2,6-DiCl-Bzl ether, X^{11} is tosyl, X^{12} is 2-Cl-Cbz.

In order to cleave and deprotect the protected peptide-resin, it is treated with 1 ml m-cresol and 10 ml hydrogen fluoride (HF) per gram of peptide resin, at 0°C for 45 min. After elimination of the HF under high vacuum and evaporating the traces of the scavenger, the cleaved peptide and resin remainder is washed with dry diethyl ether and ethyl acetate. The peptide is then extracted with 50% aqueous acetic acid, separated from the resin by filtration and lyophilized.

Depending on the amount gained, the crude peptide is purified using either a Beckman Prep-350 preparative HPLC system (with a Beckman Type 163 variable wavelength UV detector) or a Beckman semipreparative HPLC system (with a Beckman 420 Gradient Controller, two 114M Solvent Delivery Modules, a 165 Variable Wavelength Detector and a Kipp and Zonen BD41 Recorder. In the preparative system, separations are achieved on a 41.5 x 150 mm. DYNAMAX column packed with spherical C18 silica gel (300 A pore size, 12 um Particle size) (RAININ Inc., Co., Woburn, MA). In the semipreparative system, purification can be performed on a VYDAC C18 reversed phase column (10 x 250 mm., 300 A pore size, 5 um particle size).

Gradient elution is used. Solvent A consists of 0.1% aqueous TFA and solvent B is 0.1% TFA in 70% aqueous acetonitrile. The column eluate is monitored at 220 nm and 280 nm.

The peptide is judged to be substantially (95%) pure by using a Hewlett-Packard HP-1090 liquid chromatograph. The peptides are chromatographed on a 4.6 x 250 mm. W-Porex 5 um C18 column (Phenomenex, Rancho Palos Verdes, CA) at a flow rate of 1.2 ml/min. with a mixture of Solvent A and Solvent B using a gradient (from 40 to 70% B in 30 min).

EXAMPLE IX

(Ac-Tyr^{1},Abu^{8},Ala^{15},Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Ac-Tyr^{1}-Ala^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

was carried out in a stepwise manner on a PS-2755 (Protein Technologies) synthesizer starting with the appropriate Fmoc-NH-$(CH_2)_4$-C(NH_2) = N-SO_2-(2,3,5)-trimethyl-[ϕ]-OCH_2-CO-[SP] (i.e., Fmoc-agmatine-sulfonyl-2,3,5,-trimethylphenoxy-acetic acid amino-methyl resin). Deblocking and washing are preferably carried out in accordance with modified Schedule A as follows:

SCHEDULE B FOR FMOC SYNTHESIS

DEPROTECTION

Step	Reagent	Mixing (min)
1.	20%(v/v)piperidin in DMF	2x5
2.	washing with DMF	6x1

SCHEDULE B FOR FMOC SYNTHESIS

COUPLING

1.	washing with DMF	3x0.5
2.	coupling with 3eq amino acid + 3eq BOP in DMF containing 6 eq NMM + 0-6eq HOBt. repeated coupling: 1 eq	20 (+10)
3.	washing with DMF	6x1

The following compound is thus obtained:

Ac-Tyr^{1}(X^{1})-Ala^{2}-Asp^{3}(X^{3})-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(X^{1})-Abu^{8}-Ser^{9}(X^{1})-Tyr^{10}(X^{1})-Arg^{11}(X^{11})-Lys^{12}(X^{12})-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}(X^{1})-Ala^{19}-Arg^{20}(X^{11})-Lys^{21}(X^{12})-Leu^{22}-Leu^{23}-Ile^{26}-Nle^{27}-Ser^{28}(X^{1})-NH-$(CH_2)_4$-NH-C-(NH_2) = N-SO_2-2,3,5,trimethyl[ϕ]-OCH_2-CO-[SP]

X^{1} is t-butyl ether, X^{3} is t-butyl ester, X^{11} is 2,2,5,7,8-pentamethyl-chroman-6-sulphonyl, X^{12} is Boc.

The protected peptide produced as above is utilized as starting material. The cleavage of the peptide from the solid support and the removal of side chain protecting groups was carried out simultaneously by the reaction of the protected peptide resin with a mixture of TFA(90%)-thioanisol(5%)-ethandithiol (2.5%)-water(2.5%) at a temperature 35-40°C for 2-3 hours (10 ml liquid per each gram of resin was used). After completion of the reaction, the reagent was removed on centrifugation in high vacuo at room temperature. The remaining material was washed with ether. The peptide is then extracted with 50% aqueous acetic acid, separated from the resin by filtration and lyophilized. After lyophilization the crude peptide was subjected to purification as described in Example VIII.

EXAMPLE X

Ac-Tyr^{1},Aib^{2},Ser^{8},Ala^{15},Nle^{27},Agm^{29}hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Ac-Tyr^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C-(NH_2) = NH

was carried out as described in Example VIII, to give another protected peptide-resin having the formula:

Ac-Tyr^{1}-Aib^{2}-Asp^{3}(OChx)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(Bzl)-Ser^{8}(Bzl)-Ser^{9}(Bzl)-Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OChx)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-NH-$(CH_2)_4$-NH-C(NH_2) = N-[SPA]-[SP], which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XI

(Dat^{1},Aib^{2},Ser^{8},Ala^{15},Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}--Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

was carried out as described in Example VIII to give: Dat^{1}(DCB)-Aib^{2}-Asp^{3}(OChx)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(Bzl)-Ser^{8}(Bzl)-Ser^{9}(Bzl)-Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OChx)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-NH$(CH_2)_4$-NH-C-(NH_2) = N-[SPA]-[SP], which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XII

(Dat^{1},Abu^{8},Ala^{15},Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^{1}-Ala^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-

Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-$C(NH_2)$=NH was carried out as described in Example VIII to give another protected peptide-resin having the formula:

Dat^1-Ala^2-Asp^3(COHx)-Ala^4-Ile^5-Phe^6-Thr^7(Bzl)-Abu^8-Ser^9(Bzl)-Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OChx)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-NH-$(CH_2)_4$-NH-$C(NH_2)$=N-[SPA]-[SP]

which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XIII

(Dat^1,Aib^2,$Abu^{8,15}$,Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^1-Aib^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Abu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-$C(NH_2)$ = NH

is carried out as described in Example VIII to give another protected peptide-resin having the formula:

Dat^1-Aib^2-Asp^3(COhx)-Ala^4-Ile^5-Phe^6-Thr^7(Bzl)-Abu^8-Ser^9(Bzl)-Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OCH_x)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-NH-$(CH_2)_4$-NH-$C(NH_2)$=NH-[SPA]-[SP],

which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XIV

(Dat^1,Aib^2,$Abu^{8,9,15}$,Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

3-(4-hydroxyphenyl)propionyl^1-Aib^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Abu^8-Abu^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C-(NH_2)=NH

is carried out as described in Example IX, with the exception that Dat is incorporated as its HOSu ester in place of Ac-Tyr-OH in position 1 to give another protected peptide-resin having the formula:

Dat^{1}-Aib^{2}-Asp^{3}(OtBu)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(tBu)-Abu^{8}-Abu^{9}-Tyr^{10}(tBu)-Arg^{11}(Pmc)-Lys^{12}(Boc)-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}(tBu)-Ala^{19}-Arg^{20}(Pmc)-Lys^{21}(Boc)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OtBu)-Ile^{26}-Nle^{27}-Ser^{28}(tBu)-NH-$(CH_2)_4$-NH-$C(NH_2)$ = N-{2,3,5,Trimethyl-[SPA]}-[SP], which is then similarly converted to the desired peptide in accordance with the procedure of Example IX .

EXAMPLE XV

(Dat^{1},$Abu^{2,8,9,15}$,Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^{1}-Abu^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Abu^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-$C(NH_2)$ = NH

was carried out as described in Example VIII, to give another protected peptide-resin having the formula:

Dat^{1}-Abu^{2}-Asp^{3}(OChx)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(Bzl)-Abu^{8}-Abu^{9}-Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OChx)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-NH-$(CH_2)_4$-NH-$C(NH_2)$= N-[SPA]-[SP],

which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XVI

(Dat^{1},Abu^{2},Ser^{8},Abu^{15},Nle^{27},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^{1}-Abu^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-$C(NH_2)$ = NH

was carried out as described in Example VIII in position 1 to give another

protected peptide-resin having the formula:

Dat^{1}-(DCB)-Abu^{2}-Asp^{3}(COHx)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(Bzl)-Ser^{8}(Bzl)-Ser^{9}(Bzl)--Tyr^{10}(DCB)-Arg^{11}(Tos)-Lys^{12}(2-Cl-Cbz)-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}(Bzl)-Ala^{19}-Arg^{20}(Tos)-Lys^{21}(2-Cl-Cbz)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OCHx)-Ile^{26}-Nle^{27}-Ser^{28}(Bzl)-$(CH_2)_4$-NH-$C(NH_2)$ = N-[SPA]-[SP]

which is then similarly converted to the desired peptide in accordance with the procedure of Example VIII.

EXAMPLE XVII

(Dat^{1},Aib^{2},Ser^{8},Ala^{9},Abu^{15},Nle^{27},Asp^{28},Agm^{29})hGH-RH(1-29)

The synthesis of an hGHRH analog of the formula:

Dat^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ala^{9}-Tyr^{10}-$Arg^{11}Lys^{12}$-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Asp^{28}-NH-$(CH_2)_4$-NH-$C(NH_2)$ = NH

is carried out as described in Example IX, with the exception that Fmoc-Asp(OtBu)-OH was coupled instead of Fmoc-Ser(tBu)-OH in position 28, Fmoc-Ser(tBU) was coupled instead of Fmoc-Asn-OH in position 8, Fmoc-Aib-OH was coupled instead of Fmoc-Ala-OH in position 2, and 3-(4-hydroxyphenyl propionic acid (Dat) was coupled in place of Ac-Tyr(tBu)-OH in position 1, to form:

Dat^{1}-Aib^{2}-Asp^{3}(OtBu)-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}(tBu)-Ser^{8}(tBu)-Ala^{9}-Tyr^{10}(tBu)-Arg^{11}(Pmc)-Lys^{12}(Boc)-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}(tBu)-Ala^{19}-Arg^{20}(Pmc)-Lys^{21}-(Boc)-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}(OtBu)-Ile^{26}-Nle^{27}-Asp^{28}(OtBu)-NH-$(CH_2)_4$-NH-$C(NH_2)$ = N-[SPA]-[SP], which is then similarly converted to the desired peptide in accordance with the procedure of Example IX.

Test Procedures

The compounds of the present invention were tested both in vitro and in vivo. Growth hormone releasing activities in vitro are summarized in Table 1. In vivo effect of the compounds on the release of GH in rats after intravenous (i.v.) and subcutaneous (sc.) injections are given in Tables 2 and 3 and Tables 4 and

5, respectively.

EXAMPLE XVIII

Growth hormone releasing activity in vitro was assayed by using a superfused rat pituitary cell system as described in S. Vigh and A.V. Schally, Peptides 5, Suppl: 241-347,1984, which is incorporated by reference. Each peptide, hGH-RH (1-29)NH_2 (as control) and hGHRH analogs of the present invention, was administered for 3 minutes (1 ml. perfusate) at various concentrations as shown below. GH content of the 1 ml. fractions collected was determined by RIA. The potencies of the peptides, based on 4-point assays, were calculated in all the Examples of this specification, by the factorial analyses of Bliss and Marks with 95% confidence limits.

TABLE 1

In vitro effect of hGHRH analogs on the GH Release in a Superfused Rat Pituitary Cell System.

Peptide	Test conditions(nM)	Potency Relative to hGH-RH(1-29)NH2 =1
Example VIII	0.03-1.00	7.5
Example IX	0.03-1.00	8.0
Example X	0.03-1.00	25.0
Example XI	0.03-1.00	40.0
Example XII	0.03-1.00	8.0
Example XIII	0.03-1.00	10.0
Example XIV	0.03-1.00	3.0
Example XV	0.03-1.00	4.0
Example XVI	0.03-1.00	25.0

EXAMPLE XIX

For in vivo tests based on intravenous administration, adult male Sprague-Dawley rats were anesthetized with pentobarbital (6 mg./100/g., b.w.), injected i.p.; 20 minutes after the injection of pentobarbital, blood samples were taken

from the jugular vein (pretreated level) and immediately thereafter hGH-RH (1-29)NH_2 (as a control) or hGH-RH(1-29)NH_2 analogs were injected i.v. Blood samples were taken from the jugular vein 5 and 15 minutes after the injection. The blood samples were centrifuged, plasma was removed and the GH level was measured by RIA.

The result tabulated in Table 2 below are expressed as the mean ± SEM; statistical significance is assessed by Duncan's Multiple Range Test. These results, expressed as potency, appear in Table 3.

TABLE 2

In vivo effect of hGH-RH analogs on the GH release in the rat after i.v. injection

Peptide	ug/kg	0 min	5 min	15 min
Saline		32.2±20.9	29.8±1.7	23.5±1.7
hGH-RH				
(1-29)NH_2	1.0	39.8±7.0	278.0±57.9	76.7±22.2
	2.5	46.0±18.4	635.6±75.5	113.7±22.1
Exmp XI	0.4	52.6±10.2	813.4±117.2	227.7±58.1
	1.0	54.8±9.4	1550.3±89.3	474.2±17.2
Exmp XIII	0.4	34.5±7.8	477.0±109.2	122.6±14.4
	1.0	37.6±9.4	892.0±178.7	443.2±95.1
Exmp XIV	0.4	25.4±3.1	327.2±74.4	92.7±14.7
	1.0	59.6±10.4	648.2±45.6	301.5±95.1
Exmp XVI	0.4	58.8±23.1	487.5±42.8	191.6±29.5
	1.0	38.5±3.8	773.0±128.5	429.5±132.6

TABLE 3

Potency of hGH-RH Analogs Relative to hGH-RH(1-29)NH_2(=1) as calculated from the data of Table 2 by using the 4-Point Assay Method

hGH-RH Analog	After(min)	Potency
Example XI	5	8.49
	15	12.9
Example XIII	5	4.3
	15	6.5
Example XIV	5	2.7
	15	5.3
Example XVI	5	4.1
	15	10.4

EXAMPLE XX

Subcutaneous evaluation

Adult male rats were used and were anesthetized with pentobarbital (6 mg/100g., b.w.), injected i.p.; 20 minutes after the injection of pentobarbital, blood samples were taken from the jugular vein (pretreated level) and immediately thereafter hGH-RH(1-29)NH_2 (as a control) or hGH-RH analogs were injected s.c Blood samples were taken from the jugular vein 15 and 30 minutes after the injection. The blood samples were centrifuged, plasma was removed and the GH level was measured by RIA.

The results are tabulated in Table 4 as mean ± SEM below and are summarized in terms of potency in Table 5.

TABLE 4

In vivo effect of hGHRH analogs on the GH release in the rat after subcutaneous (SC) injection

Peptide	Dose ug/kg	0 min	15 min	30 min
Saline		32.1±7.2	22.3±3.7	12.3±3.7
hGH-RH(1-29)NH2 (control)	50	30.1±4.4	116.8±14.7	25.7 ±2.3
	150	21.8±3.3	199.6±30.5	38.3±3.6
Example XI	1.0	36.1±4.4	199.8±30.5	50.5±16.2
	3.0	49.01	662.0±84.0	137.5±36.7
Example XIII	1.0	43.8±7.7	104.6±28.3	30.2±3.4
	3.0	37.2±11.3	213.2±23.2	54.2±12.3
Example XIV	1.0	25.6±3.5	33.5±3.8	24.2±5.4
	3.0	31.1±3.4	103.0±15.1	39.5±7.7
Example XVI	1.0	40.3±8.7	148.6±22.4	37.2±4.6
	3.0	38.6±10.2	196.0±60.2	46.7±3.1
In another subcutaneous experiment:				
Saline		41.2±11.9	46.0±2.0	55.0±13.0
hGH-RH (1-29)NH2	50	35.2±23.6	765.5±73.4	112.7±11.3
	100	45.0±7.4	1018±7.0	219.5±28.2
Example VIII	1.0	58.0±10.1	448.1±77.0	194.1±53.8
	3.0	38.2±8.2	862.1±117.2	228.8±55.3

TABLE 5

Potency of hGHRH analogs after subcutaneous administration (SC) relative to hGHRH(1-29)NH_2 (=1) as calculated from the data of table 4 by the 4-point assay method

hGH-RH Analog	After (min)	Potency
Example VIII	15	22.79
	30	109.5
Example XI	15	149.3
	30	196.2
Example XIII	15	51.8
	30	93.7
Example XIV	15	13.8
	30	49.5
Example XVI	15	62.4
	30	136.2

Following intravenous administration, the analogs gave growth hormone levels greater than those from hGHRH alone. The effect was long lasting which indicates that the analogs have not only higher receptor affinity but increased peptidase resistance too.

Following subcutaneous administration, again all of the analogs gave greater growth hormone levels than hGHRH. Here the analogs from Example VIII, XVI and especially XI produced unusually good results with a prolonged release rate.

The in vitro and in vivo results show different biological activity pattern.

It is believed that in vitro activity depends primarily on binding capacity of the peptide to its receptor, whereas in vivo potency relies also on favorable transport properties, suitable binding to plasma proteins and metabolic

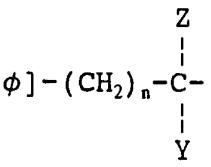
stability. The above findings therefore indicate that the analogs tested are resistant to local degradation at the injection site and they may also be less susceptible to enzyme degradation in the blood stream and/or have more affinity for GH-RH receptors than hGHRH(1-29)NH_2.

Claims

1. A peptide having the formula:

Q^1-CO-R^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-R^8-R^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-R^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-R^{27}-R^{28}-NH-Q^2

wherein Q^1 is an omega or alpha-omega substituted alkyl of the structure:



where:

[ϕ] is phenyl;

Y is H, -NH_2, CH_3CONH- or CH_3NH-;

Z is H or CH_3;

m is 1 or 2;

n is 0, 1 or 2;

R^2 is Ala, Abu or Aib;

R^8 is Asn, Ser, Ala, or Abu;

R^9 is Ser, Ala,or Abu

R^{15} is Gly, Ala, or Abu;

R^{27} is Met, Tba or Nle;

R^{28} is Asp or Ser;

Q^2 is a lower omega-guanidino-alkyl group having a formula:

-$(CH_2)_p$-NH-C(NH_2) = NH

wherein p is 2 - 6 and at least one of R^2, R^8, R^9 and R^{15} is Abu or Aib, and the pharmaceutically acceptable addition salts thereof with the pharmaceutically acceptable organic or inorganic bases or acids.

2. A peptide according to Claim 1 wherein

Q^1-CO is Dat or Ac-Tyr,

NH-Q^2 is Agm.

3. A peptide according to Claim 1 wherein Q^1-CO is Dat.

4. A peptide according to Claim 1 wherein Q^1-CO is Ac-Tyr.

5. A peptide according to Claim 2 wherein

R^2 is Abu or Aib;

R^8 is Ser or Abu;

R^9 is Ser, Ala or Abu

R^{15} is Gly, Ala, or Abu;

R^{27} is Nle;

R^{28} is Asp or Ser.

6. A peptide according to Claim 5 having the formula:

3-(4-hydroxyphenyl)propionyl^1-Aib^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Ser^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Gly^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)4$-NH-C(NH_2) = NH

7. A peptide according to Claim 4 having the formula:

Ac-Tyr^1-Ala^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Abu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

8. A peptide according to Claim 5 having the formula:

Ac-Tyr^1-Aib^2-Asp^3-Ala^4-Ile^5-Phe^6-Thr^7-Ser^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C-(NH_2) = NH

9. A peptide according to Claim 5 having the formula:

Dat^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

10. A peptide according to Claim 3 having the formula:

Dat^{1}-Ala^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

11. A peptide according to Claim 5 having the formula:

Dat^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

12. A peptide according to Claim 5 having the formula:

3-(4-hydroxyphenyl)propionyl^{1}-Aib^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Abu^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C-(NH_2) = NH

13. A peptide according to Claim 5 having the formula:

Dat^{1}-Abu^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Abu^{8}-Abu^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

14. A peptide according to Claim 5 having the formula:

Dat^{1}-Abu^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe^{6}-Thr^{7}-Ser^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-NH-$(CH_2)_4$-NH-C(NH_2) = NH

15. A peptide according to Claim 5 having the formula:
Dat[1]-Aib[2]-Asn[3]-Ala[4]-Ile[5]-Phe[6]-Thr[7]-Ser[8]-Ala[9]-Tyr[10]-Arg[11]Lys[12]-Val[13]-Leu[14]-Abu[15]-Gln[16]-Leu[17]-Ser[18]-Ala[19]-Arg[20]-Lys[21]-Leu[22]-Leu[23]-Gln[24]-Asp[25]-Ile[26]-Nle[27]-Asp[28]-NH-$(CH_2)_4$-NH-C(NH_2) = NH

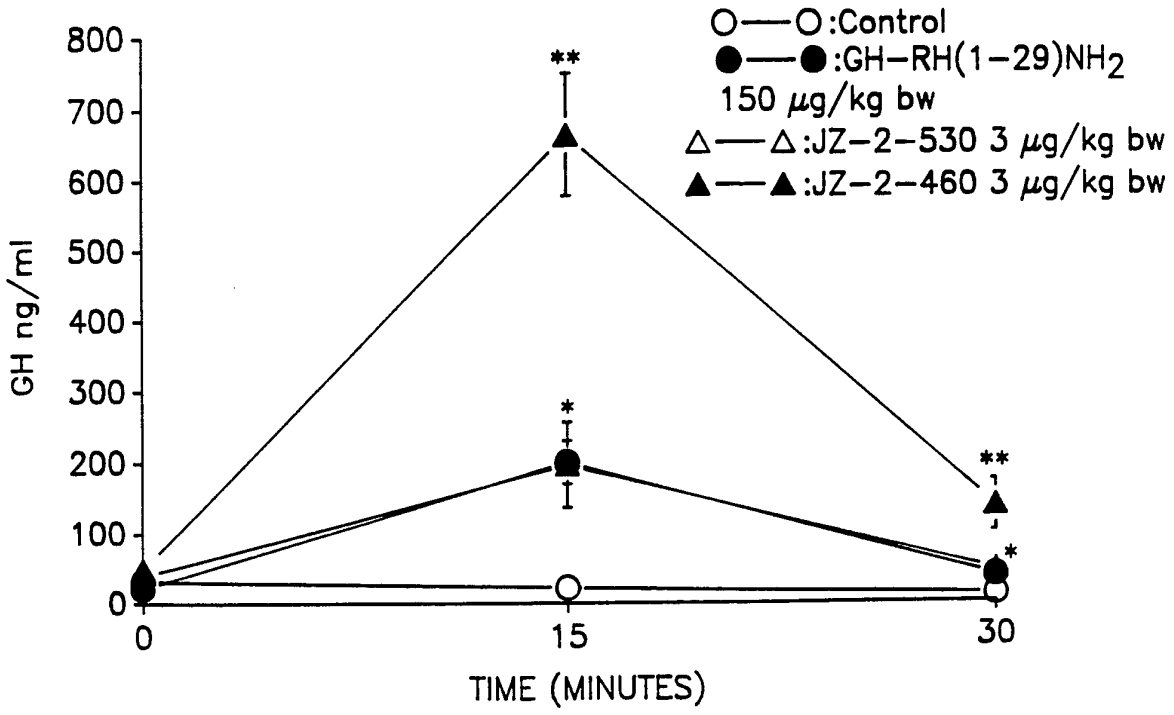
16. A pharmaceutical dosage form comprising a peptide according to Claim 1 and an excipient.

17. A method of treating human growth deficiency comprising administering from 0.2 mg to 2 mg of a peptide according to Claim 1 per day per kg body weight.

18. A method for ascertaining the endogenous physiological ability to produce hGH in a subject in need of GH-RH level amplification comprising administration to said subject of an effective amount of a GH-RH peptide according to Claim 1.

19. A method for ascertaining the endogenous physiological ability to produce hGH in patients apparently deficient in hGH, wherein the steps are administering a 50-100 microgram dose of GH-RH to a patient and measuring the hGH response evoked by said GH-RH by radioimmunoassay; and, after the hGH dose has worn off, administering a 50-100 microgram dose of peptide according to Claim 1 and measuring the hGH response evoked by said peptide by radio-immunoassay.

20. A diagnostic kit for ascertaining the endogenous physiological ability to produce hGH comprising a 50-100 microgram dose of GH-RH; a 50-100 microgram dose of peptide of Claim 1; and means for assaying the GH response evoked by each dose.


O—O:Control
●—●:GH-RH(1-29)NH2
150 μg/kg bw
△—△:JZ-2-530 3 μg/kg bw
▲—▲:JZ-2-460 3 μg/kg bw
GH ng/ml
800
700
600
500
400
300
200
100
0
0
15
30
TIME (MINUTES)
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*
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*

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 93/11057

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/10 A61K37/43 G01N33/74

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)
IPC 5 C07K A61K G01N

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 413 839 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 27 February 1991 see the whole document ---	1-20
Y	INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH vol. 39, no. 3 , March 1992 , COPENHAGEN DK page 211-217 M.ZARANDI ET AL 'POTENT AGONISTS OF ghrh' see the whole document ---	1-20
Y	WO,A,91 16923 (THE ADMINISTRATORS OF THE TULANE UNIVERSITY EDUCATIONAL FUND) 14 November 1991 see the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

"E" earlier document but 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
1 March 1994	07 -04- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BICHIMICA ET BIOPHYSICA ACTA vol. 1122, no. 2 , 31 July 1992 , AMSTERDAM pages 147 - 153 J.BONGERS 'KINETICS OF DIPEPTIDYL PEPTIDASE IV PROTEOLYSIS OF GHRH AND ANALOGS' see the whole document	1-5,13, 14,16-20
Y	WO,A,87 06835 (EASTMAN KODAK COMPANY) 19 November 1987 see page 78, line 25 - page 79, line 32	1-12, 15-20

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11057

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 17-19 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Interna... ...l Application No
PCT/US 93/11057

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0413839	27-02-91	NONE		
WO-A-9116923	14-11-91	AU-A-	7882291	27-11-91
		EP-A-	0527914	24-02-93
WO-A-8706835	19-11-87	US-A-	4880778	14-11-89
		AU-B-	600952	30-08-90
		AU-A-	7433287	01-12-87
		DE-A-	3781275	24-09-92
		EP-A,B	0305401	08-03-89
		JP-T-	1502586	07-09-89