594652 49820/85

COMMONWEALTH OF AUSTRALIA

Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

LODGED AT SUB-OFFICE
12 NOV 1985
Melbourne

(1) Here insert (in full) Name or Names of Applicant or Applicants, followed by Address (es).

~~I~~ We (1) SYNTEX (U.S.A.) INC.,
of 3401 Hillview Avenue, Palo Alto,
California 94303, United States of America

(2) Here insert Title of Invention.

hereby apply for the grant of a Patent for an invention entitled: (2)
NONA AND DECAPEPTIDE ANALOGS OF LHRH USEFUL AS LHRH ANTAGONISTS

(3) Here insert number(s) of basic application(s)

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered (3)
671,153

(4) Here insert Name of basic Country or Countries, and basic date or dates

for a patent or similar protection made in (4) United States of America
on 13th November 1984

APPLICATION ACCEPTED AND AMENDMENTS
ALLOWED 12-1-90

~~My~~ Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys, 50 Queen Street, Melbourne, Victoria, Australia.

DATED this 11th day of November 1985.

(5) Signature (s) of Applicant (s) or Seal of Company and Signatures of its Officers as prescribed by its Articles of Association.

(5) SYNTEX (U.S.A.) INC.
by [signature]
W. F. Dancer
Reg'd. Patent Attorney

To:
THE COMMISSIONER OF PATENTS.

Edwd. Waters & Sons, Melbourne.

Patents Act 1952-1969

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION

(1) Here insert (in full) Name of Company.

In support of the Convention Application made by[1] SYNTEX (U.S.A.) INC.

(hereinafter referred to as the applicant) for a Patent

(2) Here insert title of Invention.

for an invention entitled:[2] Nona and Decapeptide Analogs of LHRH Useful as LHRH Antagonists

(3) Here insert full Name and Address, of Company official authorized to make declaration.

I,[3] Herwig von Morze of 3401 Hillview Avenue, Palo Alto, California 94303, United States of America

do solemnly and sincerely declare as follows:

1. I am authorised by the applicant for the patent to make this declaration on its behalf.

(4) Here insert basic Country or Countries followed by date or dates and basic Applicant or Applicants.

2. The basic application as defined by Section 141 of the Act was made in[4] the United States of America on the 13th day of November 19 84 , by John Nestor and Brian Vickery

~~on the~~ ~~day of~~ ~~19 , by~~

(5) Here insert (in full) Name and Address of Actual Inventor or Inventors.

3.[5] The said John Nestor, of 677 Kirk Glen Drive, San Jose, California 95133, U.S.A.; and the said Brian Vickery, of 10678 Farallone Drive, Cupertino, California 95014

~~is~~/are the actual inventor s of the invention and the facts upon which the applicant is entitled to make the application are as follow:

The applicant is the assignee of the said John Nestor and the said Brian Vickery

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Palo Alto, California, U.S.A.

this 17th day of October 1985

(6) Signature. (6)

To: THE COMMISSIONER OF PATENTS.

SYNTEX (U.S.A.) INC.

By [signature]

HERWIG VON MORZE

MANAGER, INTERNATIONAL PATENT OPERATIONS

(AUTHORIZED SIGNATURE)

Edwd. Waters & Sons, Melbourne.

(12) PATENT ABRIDGMENT (11) Document No. AU-B-49820/85
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 594652

(54) Title
NONA AND DECAPEPTIDE ANALOGS OF LHRH AS ANTAGONISTS THEREOF

International Patent Classification(s)
(51)[4] C07K 007/20 A61K 037/02 A61K 037/43

(21) Application No. : 49820/85 (22) Application Date : 12.11.85

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671153 13.11.84 US UNITED STATES OF AMERICA

(43) Publication Date : 22.05.86

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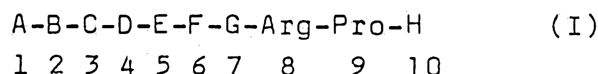
(71) Applicant(s)
SYNTEX (U.S.A.) INC.

(72) Inventor(s)
BRIAN VICKERY; JOHN NESTOR

(74) Attorney or Agent
WATERMARK MELBOURNE

(57) Claim

1. A compound of the formula



A-B-C-D-E-F-G-Arg-Pro-H (I)
1 2 3 4 5 6 7 8 9 10

and the pharmaceutically acceptable salts thereof, wherein:

A is an amino acyl residue selected from the group consisting of N-Ac-D,L-$\Delta^{3,4}$-prolyl, N-Ac-D,L-prolyl, N-Ac-L-alkylprolyl, N-Ac-D,L-phenylalanyl, N-Ac-D,L-p-Cl-phenylalanyl, N-Ac-D,L-seryl, N-Ac-D,L-threonyl, N-Ac-D,L-alanyl, N-Ac-3-(1-naphthyl)-D,L-alanyl, N-Ac-3-(2-naphthyl)-D,L-alanyl, N-Ac-3-(2,4,6-trimethylphenyl)-D,L-alanyl, and N-Ac-3-(4-trifluoromethylphenyl)-D,L-alanyl;

B is an amino acyl residue selected from the group consisting of D-phenylalanyl, D-p-Cl-phenylalanyl, D-p-Br-phenylalanyl, D-p-F-phenylalanyl, D-p-nitrophenylalanyl, 3-(3,4,5-trimethoxyphenyl)-D-alanyl, 2,2-diphenylglycine, D-α-methyl-p-Cl-phenylalanyl and

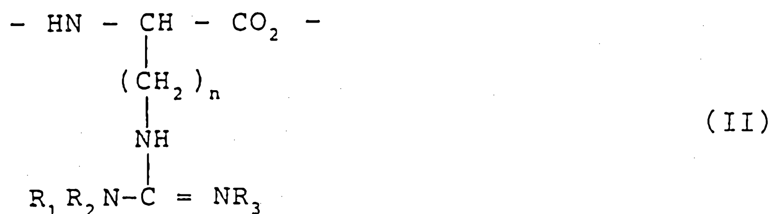
3-(2,4,6-trimethylphenyl)-D-alanyl;

C is an amino acyl residue selected from the group consisting of D-tryptophanyl, D-phenylalanyl, D-pentamethyl-phenylalanyl, 3-(3-pyridyl)-D-alanyl, 3-(1-naphthyl)-D-alanyl, and 3-(2-naphthyl)-D-alanyl;

D is an amino acyl residue selected from the group consisting of L-seryl, and D-alanyl;

E is an amino acyl residue selected from the group consisting of L-phenylalanyl and L-tyrosyl;

F is an amino acyl selected from the group consisting of the radicals represented by the following structural formulas:



(II)

wherein

n is 1 to 5;

R_1 is halo lower alkyl;

R_2 is hydrogen, methyl or ethyl;

R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$;

G is an amino acyl residue selected from the group consisting of L-leucyl, L-norleucyl, L-tryptophanyl, L-Nal(2) and L-norvalyl;

H is ' -alaninamide, D-leucinamide, glycinamide or $-NHR_5$ wherein R_5 is lower alkyl or $NHCONH_2$;

or a pharmaceutically acceptable salt thereof.

594652

Form 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class | Int. Class

Application Number: 49820

Lodged:

Complete Specification Lodged:

Accepted:

Published:

Priority :

Related Art :

This document contains the amendments made under Section 49 and is correct for printing.

Name of Applicant : SYNTEX (U.S.A.) INC.

Address of Applicant : 3401 Hillview Avenue, Palo Alto, California 94303, United States of America

Actual Inventor: BRIAN HENRY VICKERY and JOHN JOSEPH NESTOR

Address for Service : EDWD. WATERS & SONS, 50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

NONA AND DECAPEPTIDE ANALOGS OF LHRH USEFUL AS LHRH ANTAGONISTS

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

NONA AND DECAPEPTIDE ANALOGS OF LHRH USEFUL AS LHRH ANTAGONISTS

BACKGROUND OF THE INVENTION

Luteinizing hormone (LH) and follicular stimulating hormone (FSH) are released from the anterior pituitary gland under the control of the releasing hormone LHRH produced in the hypothalamic region. LH and FSH act on the gonads to stimulate the synthesis of steroid hormones and to stimulate gamete maturation. The pulsatile release of LHRH, and thereby the release of LH and FSH, controls the reproductive cycle in domestic animals and humans.

LHRH also affects the placenta, and the gonads indirectly, in causing the release of chorionic gonadotropin (hCG).

Antagonists of LHRH are useful for the control of fertility. Such antagonists block ovulation in the female and suppress spermatogenesis in the male. Related to these effects is a suppression of normal circulating levels of sexual steroids of gonadal origin, resulting in reduction in accessory organ weight and function in the male and the female. In domestic animals this effect

promotes weight gain in a feed-lot situation, stimulates abortion in pregnant animals and in general, acts as a chemical sterilant.

The natural releasing hormone LHRH is a decapeptide comprised of naturally occurring amino acids (which have the L-configuration except for the achiral amino acid glycine). Its sequence is as follows:

(pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
1 2 3 4 5 6 7 8 9 10

Many analogs of this natural material have been studied and the very large majority of them have proven to be of insufficient biological activity to be clinically useful. Certain select modifications have proven to have an agonist effect on biological activity. By far the most significant enhancement is obtained by changing the 6-position residue from Gly to a D-amino acid.

In addition to agonists, analogs have been prepared which are competitive antagonists to LHRH; all of which require deletion or replacement of the histidine residue at position 2; Vale, W., et al, Science, 176: 933 (1972). In general, it appears that a D-amino acid placed in the sequence at that position gives the best activity; Rees, R. W. A., et al, J. Med. Chem., 17: 1016 (1974).

It has also been shown that adding a modification at the 6 position, which, without the modification at position 2, results in the agonist activity cited above, enhances the antagonist activity of the 2-modified analogs; Beattie, C. W., et al, J. Med. Chem., 18: 1247 (1975); Rivier, J., et al, Peptides 1976 p. 427, Editions de l'Universite de Bruxelles, Belgium (1976).

Against the background of these two major alterations, which result in a potent series of LHRH

antagonists; additional increments in antagonist activity may be had by modifying positions 1, 3 and/or 10 in the already 2, 6 modified peptide. Coy, D. H., et al Peptides 1976, p. 462, Editions de l'Universite de Bruxelles, Belgium (1976); Rivier, J. E., et al, Life Sci. 23: 869 (1978); Dutta, A. S., et al, Biochem. Biophys. Res. Commun. 81: 382 (1978), Humphries, J., et al, Biochem. Biophys. Res. Commun., 85: 709 (1978). It has also been shown that N-acylation of the amino acid at position 1 is helpful; Channabasavaia, K., et al, Biochem. Biophys. Res. Commun. 81: 382 (1978); Coy, D. H., et al, Peptides. - Structure and Biological Function p. 775, Pierce Chemical Co. (1979). Additionally, (N-Ac-D-p-Cl-Phe^1, D-p-Cl-Phe^2, D-Trp^3, D-Arg^6, D-Ala^{10})LHRH has been published by D.H. Coy, Endocrinology, 110, 1445 (1982). In another instance D-Ala^4 modification to LHRH has been reported to retain antagonist activity. See E. Pedroza, J.A. Martinez, D.H. Coy, A. Arimura and A.V. Schally; Int. J. Fert.; 23, 294 (1978). Also, a modification at position 7 to D-Trp has been shown to retain antiovulatory activity, see Folkers, F.A. Bowers, C.Y.; Shieh, H.M., Yin-Zeng, L., Shao-Bo, X., Tang., D.F. L., Li-Yu, C; Biochem. Biophys. Res. Conven. 123:1221 (1984).

Since antagonists function by competing with LHRH for the appropriate receptors, high dosages of these compounds are required in order to block out the natural peptide. It is especially desirable, in view of this, to obtain antagonists with a very high degree of potency and prolonged activity. The ability to be slowly released from depot formulations will also be important.

SUMMARY OF THE INVENTION

The present invention refers to novel, highly potent nonapeptide and decapeptide analogs of LHRH in which a

replacement at position 2, (thus converting the peptide to the antagonist series) is made more effective by replacement of the glycine residue at position 6 by a novel halo-lower-alkyl guanidino-substituted amino acid residue which does not occur in nature. These compounds are potent LHRH antagonists. Other enhancements by substitutions at 1, 2, 3, 4, 7 and/or 10 are also disclosed. The invention is also directed to various methods of use of these compounds and to pharmaceutical compositions therefor. A further aspect of the invention involves processes for the preparation of the novel compounds described above.

DETAILED DESCRIPTION OF THE INVENTION

Description of the Analogs

The present invention relates to novel nonapeptide and decapeptide analogs of LHRH which have the formula

$$\underset{1}{A}-\underset{2}{B}-\underset{3}{C}-\underset{4}{D}-\underset{5}{E}-\underset{6}{F}-\underset{7}{G}-\underset{8}{Arg}-\underset{9}{Pro}-\underset{10}{H} \qquad (I)$$

and the pharmaceutically acceptable salts thereof, wherein:

A is an amino acyl residue selected from the group consisting of N-Ac-D,L-$\Delta^{3,4}$-prolyl, N-Ac-D,L-prolyl, N-Ac-L-alkylprolyl, N-Ac-D,L-phenylalanyl, N-Ac-D,L-p-Cl-phenylalanyl, N-Ac-D,L-seryl, N-Ac-D,L-threonyl, N-Ac-D,L-alanyl, N-Ac-3-(1-naphthyl)-D,L-alanyl, N-Ac-3-(2-naphthyl)-D,L-alanyl, N-Ac-3-(2,4,6-trimethylphenyl)-D,L-alanyl, and N-Ac-3-(4-trifluoromethylphenyl)-D,L-alanyl;

B is an amino acyl residue selected from the group consisting of D-phenylalanyl, D-p-Cl-phenylalanyl, D-p-Br-phenylalanyl, D-p-F-phenylalanyl,

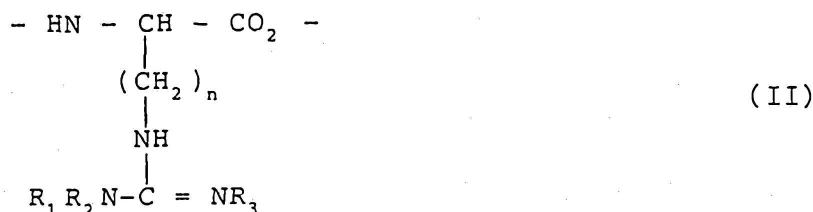
D-p-nitrophenylalanyl, 3-(3,4,5-trimethoxyphenyl)-D-alanyl, 2,2-diphenylglycine, D-α-methyl-p-Cl-phenylalanine and 3-(2,4,6-trimethylphenyl)-D-alanyl;

C is an amino acyl residue selected from the group consisting of D-tryptophanyl, D-phenylalanyl, D-pentamethyl-phenylalanyl, 3-(3-pyridyl)-D-alanyl, 3-(1-naphthyl)-D-alanyl, and 3-(2-naphthyl)-D-alanyl;

D is an amino acyl residue selected from the group consisting of L-seryl, and D-alanyl;

E is an amino acyl residue selected from the group consisting of L-phenylalanyl and L-tyrosyl;

F is an amino acyl selected from the group consisting of the residues represented by the following structural formulas:



(II)

wherein

n is 1 to 5;

R_1 is halo lower alkyl;

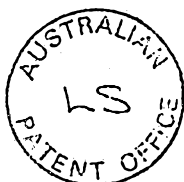
R_2 is hydrogen, methyl or ethyl;

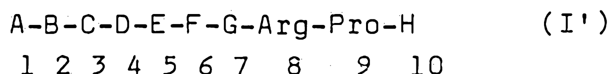
R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$;

G is an amino acyl residue selected from the group consisting of L-tryptophanyl, L-Nal(2), L-leucyl, L-norleucyl and L-norvalyl;

H is D-alaninamide, D-leucinamide, glycinamide or $-NHR_5$ wherein R_5 is lower alkyl or $NHCONH_2$; and the pharmaceutically acceptable salts thereof.

The present invention also comprises compounds of the formula





A-B-C-D-E-F-G-Arg-Pro-H (I')
1 2 3 4 5 6 7 8 9 10

and the pharmaceutically acceptable salts thereof, wherein:

A is an amino acyl residue selected from the group consisting of N-Ac-D,L-$\Delta^{3,4}$-prolyl, N-Ac-D,L-prolyl, N-Ac-L-alkylprolyl, N-Ac-D,L-phenylalanyl, N-Ac-D,L-p-Cl-phenylalanyl, N-Ac-D,L-seryl, N-Ac-D,L-threonyl, N-Ac-D,L-alanyl, 3-(1-naphthyl)-D,L-alanyl, 3-(2-naphthyl)-D,L-alanyl, 3-(2,4,6-trimethylphenyl)-D,L-alanyl, and 3-(4-trifluoromethylphenyl)-D,L-alanyl;

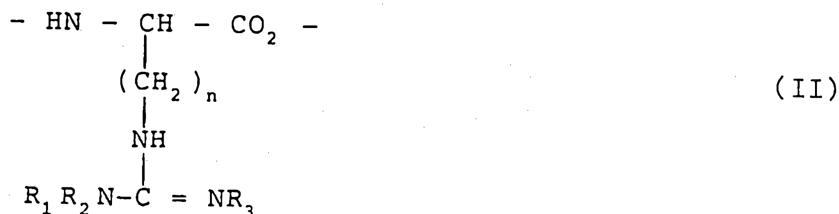
B is an amino acyl residue selected from the group consisting of D-phenylalanyl, D-p-Cl-phenylalanyl, D-p-Br-phenylalanyl, D-p-F-phenylalanyl, D-p-nitrophenylalanyl, 3-(3,4,5-trimethoxyphenyl)-D-alanyl, 2,2-diphenylglycine, D-α-methyl-p-Cl-phenylalanine and 3-(2,4,6-trimethylphenyl)-D-alanyl;

C is an amino acyl residue selected from the group consisting of D-tryptophanyl, D-phenylalanyl, D-pentamethyl-phenylalanyl, 3-(3-pyridyl)-D-alanyl, 3-(1-naphthyl)-D-alanyl, and 3-(2-naphthyl)-D-alanyl;

D is an amino acyl residue selected from the group consisting of L-seryl, and D-alanyl;

E is an amino acyl residue selected from the group consisting of L-phenylalanyl and L-tyrosyl;

F is an amino acyl selected from the group consisting of the radicals represented by the following structural formulas:



(II)

5

wherein

n is 1 to 5;

R_1 is halo lower alkyl;

R_2 is hydrogen, methyl or ethyl;

10 R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$;

G is an amino acyl residue selected from the group consisting of L-tryptophanyl, L-Nal(2), L-leucyl, L-norleucyl and L-norvalyl;

H is D-alaninamide, D-leucinamide, glycinamide or
15 $-NHR_5$ wherein R_5 is lower alkyl or $NHCONH_2$; and the pharmaceutically acceptable salts thereof.

The replacement of the L-histidyl residue which is at position 2 in LHRH with one of the residues herein specified is a requirement to convert the peptide to an
20 LHRH antagonist. The replacement of the glycyl residue at position 6 in LHRH with one of the residues specified as F gives a dramatic enhancement of the antagonist effect. The substitutions disclosed herein at positions 1, 2, 3, 4, 7 and 10 are further helpful in enhancing the
25 antagonist activity.

Abbreviations and Definitions

As set forth above, and for convenience in describing this invention, the conventional abbreviations
30 for the various common amino acids are used as generally accepted in the peptide art as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry, 11, 1726 (1972). These represent L-amino acids, with the exception of the achiral amino acid

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glycine, and with the further exception of any unnatural or natural amino acids which are achiral, or are otherwise designated as D-. All peptide sequences mentioned herein are written according to the generally accepted convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

Certain other abbreviations will be useful in describing the invention. The present invention employs replacements by amino acids which do not occur in nature. Particularly commonly employed among these are the following:

Amino acid residue	Abbreviation
3-(2-naphthyl)-D-alanyl	D-Nal(2)
3-(p-fluorophenyl)-D-alanyl	D-p-F-Phe
3-(p-chlorophenyl)-D-alanyl	D-p-Cl-Phe
3-(p-bromophenyl)-D-alanyl	D-p-Br-Phe
3-(2,3,4,5,6-pentamethylphenyl)-D-alanyl	D-Me_5Phe
3-(2,4,6-trimethylphenyl)-D-alanyl	D-Tmp
3-(3,4,5-trimethoxyphenyl)-D-alanyl	D-Tmo
3-(P-(trifluoromethylphenyl)-D-alanyl	D-Ptf
3-(3-pyridyl)-D-Alanyl	D-Pal(3)
N^G,$N^{G'}$-bis(2,2,2-trifluoroethyl)-D-homoarginine	D-FDeh
N^G-methyl,$N^{G'}$-(2,2,3,3,3-pentafluoropropyl)-D-homoarginine	D-mPfh

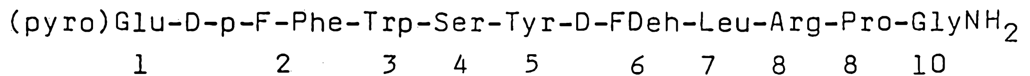
As a further convenience, since the amino acid sequence of LHRH has been shown to be

(pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,
1 2 3 4 5 6 7 8 9 10

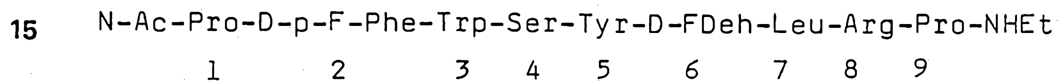
nona- and decapeptides in which the amino acid residues at particular places in the sequence have been replaced

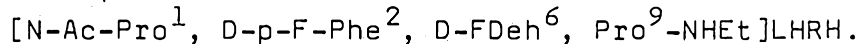
by other amino acid residues or other moieties are abbreviated by showing the nature of the substitution, superscribed by the location, followed by LHRH as the parent.

Thus, for example, the sequence,


(pyro)Glu-D-p-F-Phe-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-GlyNH$_2$
1 2 3 4 5 6 7 8 8 10

in which the Gly at position 6 has been replaced by D-FDeh and the His at position 2 has been replaced by D-p-F-Phe, is represented [D-p-F-Phe^2, D-FDeh^6]LHRH; and the sequence


N-Ac-Pro-D-p-F-Phe-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-NHEt
1 2 3 4 5 6 7 8 9

is represented:

[N-Ac-Pro^1, D-p-F-Phe^2, D-FDeh^6, Pro^9-NHEt]LHRH.

As used herein, the term "pharmaceutically acceptable salts" refers to salts that retain the desired biological activity of the parent compound and do not impart any undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, polygalacturonic acid; (b) salts with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper,

cobalt, nickel, cadmium, and the like; or with an organic cation formed from N,N'-dibenzylethylene-diamine or ethylenediamine; or (c) combinations, of (a) and (b), e.g., a zinc tannate salt and the like.

5 "Halo lower alkyl" refers to a lower alkyl radical substituted with halo groups, especially those having one, two or three halo groups on the ω-carbon. The halo grop may be fluoro, chloro or bromo. This group is exemplified by trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoro-

10 propyl, 2,2,2-trichloroethyl and the like. The term "lower alkyl" used herein refers to an alkyl radical having 1 to 6 carbon atoms.

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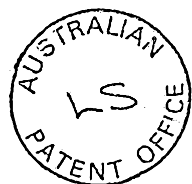


cobalt, nickel, cadmium, and the like; or with an organic cation formed from N,N'-dibenzylethylene-diamine or ethylenediamine; or (c) combinations, of (a) and (b), e.g., a zinc tannate salt and the like.

"Halo lower alkyl" refers to a lower alkyl radical substituted with halo groups, especially those having one, two or three halo groups on the ω-carbon. The halo group may be fluoro, chloro or bromo. This group is exemplified by trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoropropyl, 2,2,2-trichloroethyl and the like.

For the purpose of this invention the abbreviation "alkylPro" refers to cis-5-alkyl-L-prolyl residue wherein alkyl is the same as "lower alkyl" defined above. More specifically "MePro" is cis-5-methyl-L-Prolyl, "EtPro" is cis-5-ethyl-L-Prolyl and "ButPro" is cis-5-n-butyl-L-Prolyl.

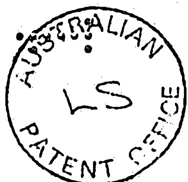
The abbreviation "N-Ac" refers specifically to the N-acetyl amino acid residue in conformance with generally accepted nomenclature.

Preferred Embodiments of the Compounds

Compounds which are preferred embodiments of the present invention are those wherein A is N-Ac-L-Pro, N-Ac-D-Ser, N-Ac-D-p-Cl-Phe, N-Ac-D-Nal(2); B is D-p-F-Phe or D-p-Cl-Phe; C is D-Trp, D-Nal(2), D-Phe or D-Pal(3); D is Ser; E is Tyr; F is the compound of Formula II wherein n is 3 or 4, and halo lower alkyl is trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoropropyl, 2,2,3,3,3-pentafluoropropyl, or 3,3,3-trichloropropyl.

More preferred embodiments herein are:

A is N-Ac-D-Nal(2) or N-Ac-D-p-Cl-Phe, B is D-p-F-Phe or D-p-Cl-Phe, C is D-Nal(2), D-Pal(3) or D-Trp, D is Ser, E is Tyr, F is D-FDeh or D-mPfh, G is



D-Leu, D-Trp, or D-Nal(2), and H is D-AlaNH$_2$, GlyNH$_2$ or NHEt;

Most preferred are the compounds

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH$_2$,

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Trp-Arg-Pro-D-AlaNH$_2$,

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Nal(2)-Arg-Pro-D-AlaNH$_2$,

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Pal(3)-Ser-Tyr-D-FDeh-L-Leu-Arg-Pro-D-AlaNH$_2$,

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Pal(3)-Ser-Tyr-D-FDeh-L-Trp-Arg-Pro-D-AlaNH$_2$, and

N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-mPfh-Nal(2)-Arg-Pro-D-AlaNH$_2$.

In all the above embodiments, the compound may be prepared as the corresponding pharmaceutically acceptable salt.

Assay Procedures

The compounds of this invention and, particularly, the salts thereof, exhibit surprisingly potent and long lasting LHRH antagonist activity.

Primary measures of potency are ability to inhibit ovulation in rats, as assayed by the procedure of Corbin, A. and Beattie, C. W., Endocrine Res. Commun., 2:1 (1975) and ability to inhibit LH release and ovulation in the rabbit, as per Phelps, C. P., et al, Endocrinology 100: 1526 (1977).

Other bioassays which are used for LHRH antagonists and for the compounds of the present invention are:

(a) inhibition of LHRH induced FSH and LH release in the rat, in vivo; Vilchez-Martinez, J.A., et al, Endocrinology, 96: 1130 (1975); and,

(b) inhibition of LH and FSH release by dispersed anterior pituitary cell cultures as measured

by radioimmuno assay. (Vale, W., et al, Endocrinology 91: 562 (1972).

Antagonist Effects and Utilities

The following utilities flow from the antagonist effect of the compounds herein:

- female contraception;
- ovulation suppression or delay;
- induction of parturition;
- synchronization of ovulation;
- estrus suppression;
- growth promotion in female animals;
- luteolysis, menses induction;
- early, first trimester abortifacient;
- therapy for endometriosis;
- therapy for mammary and cysts
- therapy for polycystic ovary syndrome (Stein-Leventhal);
- therapy for benign prostatic hypertrophy;
- male contraception;
- gonadal protection during cancer therapy;
- therapy for diseases which result from excessive gonadal hormone production in either sex;
- pregnancy termination in pets;
- functional castration in male food producing animals;
- suppression of proestrous bloody discharge in dogs;
- suppression of menopausal symptoms.

The aspect of the present invention which relates to particular uses for the above-described compounds is concerned with these utilities, most particularly: inhibition of ovulation and treatment of endometriosis in the female, and inhibition of spermatogenesis and treatment of prostatic tumors in the male.

In the practice of the method of this invention an effective amount of a compound of the invention or a

pharmaceutical composition containing same is administered to the subject in need of, or desiring, such treatment. These compounds or compositions may be administered by any of a variety of routes depending upon the specific end use, including orally, parenterally (including subcutaneous, intramuscular and intravenous administration), vaginally (particularly for contraception), rectally, buccally (including sublingually), transdermally or intranasally. The most suitable route in any given case will depend upon the use, particular active ingredient, the subject involved, and the judgment of the medical practitioner. The compound or composition may also be administered by means of slow-release, depot or implant formulations as described more fully herein below.

In general for the uses herein above described, it is expedient to administer the active ingredient in amounts between about 0.01 and 10 mg/kg body weight per day, preferably between about 0.01 and 5.0 mg/kg body weight per day. This administration may be accomplished by a single daily administration, by distribution over several applications or by slow release in order to achieve the most effective results.

The exact dose and regimen for administration of these compounds and compositions will necessarily be dependent upon the needs of the individual subject being treated, the type of treatment, the degree of affliction or need and, of course, the judgment of the medical practitioner. In general, parenteral administration requires lower dosage than other methods of administration which are more dependent upon absorption.

A further aspect of the present invention relates to pharmaceutical compositions containing as active ingredient a compound of the present invention which compositions comprise such compound in admixture with a

pharmaceutically acceptable, non-toxic carrier. As mentioned above, such compositions may be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as creams and suppositories; for oral or buccal administration particularly in the form of tablets or capsules; or intranasally particularly in the form of powders, nasal drops or aerosols.

The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA., 1970. Formulations for parenteral administration may contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for vaginal or rectal administration, e.g. suppositories, may contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for inhalation administration may be solid and contain as excipients, for example, lactose or may be aqueous or oily solutions for administration in the form of nasal drops. For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

It is particularly desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms may be utilized. For example, a dosage form may contain a pharmaceutically acceptable non-toxic salt of the

compound which has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid,
5 alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic
10 cation formed from e.g., N,N'-dibenzylethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, may be formulated in a
15 gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the
20 compound or salt dispersed or encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those
25 described above may also be formulated in cholesterol matrix pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. liposomes, are well known in the literature. See, for example, Sustained and Controlled Release Drug
30 Delivery Systems, J. R. Robinson ed., Marcel Dekker, Inc., New York, 1978. Particular reference with respect to LHRH type compounds may be found, for example, in U.S. 4,010,125.

35

Synthesis of the Peptides

The polypeptides of the present invention may be synthesized by any techniques that are known to those skilled in the peptide art. An excellent summary of the many techniques so available may be found in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, 1969, and J. Meienhofer, Hormonal Proteins and Peptides, Vol. 2, p. 46., Academic Press (New York), 1973 for solid phase peptide synthesis and E. Schroder and K. Lubke, The Peptides, Vol. 1, Academic Press (New York), 1965 for classical solution synthesis.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide.

Preferred Embodiments of Synthesis

A particularly preferred method of preparing compounds of the present invention involves solid phase peptide synthesis.

5 In this particularly preferred method the α-amino function of the amino acids is protected by an acid or base sensitive group while the side chain functional groups may be free or protected. Such protecting groups for the α-amino function should have the properties of 10 being stable to 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 therein. Suitable protecting groups are t-butyloxycarbonyl (Boc), 15 benzyloxycarbonyl (Cbz), biphenylisopropyloxycarbonyl, t-amyloxycarbonyl, isobornyloxycarbonyl, 1,1-dimethyl-3,5-dimethoxybenzyloxycarbonyl, o-nitrophenylsulfenyl, 2-cyano-t-butyloxycarbonyl, 9-fluorenylmethyloxycarbonyl and the like, especially 20 t-butyloxycarbonyl (Boc).

Particularly preferred side chain protecting groups are, for arginine:nitro, p-toluenesulfonyl, 4-methoxybenzenesulfonyl, Cbz, Boc and adamantyloxycarbonyl; or the guanidino function may be unprotected and 25 incorporated as Boc-Arg-OH (the tetraphenylborate salt); for tyrosine:benzyl, bromobenzyl, 2,6-dichlorobenzyl, isopropyl, cyclohexyl, cyclopentyl and acetyl; for serine:benzyl and tetrahydropyranyl; for histidine:benzyl, p-toluenesulfonyl and 2,4-dinitrophenyl.

30 The C-terminal amino acid is attached to a suitable solid support. Suitable solid supports useful for the above synthesis are those materials which are inert to the reagents and reaction conditions of the stepwise condensation-deprotection reactions, as well as being 35 insoluble in the media used. Suitable solid supports are

chloromethylpolystyrene-divinylbenzene polymer, hydroxy-methyl-polystyrene-divinylbenzene polymer, and the like, especially chloromethyl-polystyrene-1% divinylbenzene polymer. For the special case where the C-terminus of the compound will be glycinamide, a particularly useful support is the benzhydrylamino-polystyrene-divinyl-benzene polymer described by P. Rivaille, et al, Helv. Chim. Acta., 54, 2772 (1971). The attachment to the chloromethyl polystyrene-divinylbenzene type of resin is made by means of the reaction of the N^{α}-protected amino acid, especially the Boc-amino acid, as its cesium, tetramethylammonium, triethylammonium, 1,5-diazabicyclo-[5.4.0]undeca-5-ene, or similar salt in ethanol, acetonitrile, N,N-dimethylformamide (DMF), and the like, especially the cesium salt in DMF, with the chloromethyl resin at an elevated temperature, for example between about 40 and 60°C, preferably about 50°C, for from about 12 to 48 hours, preferably about 24 hours. The N^{α}-Boc-amino acid is attached to the benzhydrylamine resin by means of an N,N'-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HBT) mediated coupling for from about 2 to about 24 hours, preferably about 12 hours at a temperature of between about 10 and 50°C, preferably 25°C in a solvent such as dichloromethane or DMF, preferably dichloromethane. The coupling of successive protected amino acids can be carried out in an automatic polypeptide synthesizer as is well known in the art. The removal of the N^{α}-protecting groups may be performed in the presence of, for example, a solution of trifluoro-acetic acid in methylene chloride, hydrogen chloride in dioxane, hydrogen chloride in acetic acid, or other strong acid solution, preferably 50% trifluoroacetic acid in dichloromethane at about ambient temperature. Each protected amino acid is preferably introduced in

approximately 2.5 molar excess and the coupling may be carried out in dichloromethane, dichloromethane/DMF mixtures, DMF and the like, especially in methylene chloride at about ambient temperature. The coupling agent is normally DCC in dichloromethane but may be N,N'-di-iso-propylcarbodiimide (DIC) or other carbodiimide either alone or in the presence of HBT, N-hydroxysuccinimide, other N-hydroxyimides or oximes. Alternately, protected amino acid active esters (e.g. p-nitrophenyl, pentafluorophenyl and the like) or symmetrical anhydrides may be used.

At the end of the solid phase synthesis the fully protected polypeptide is removed from the resin. When the linkage to the resin support is of the benzyl ester type, cleavage is by means of aminolysis with an alkylamine or fluoroalkylamine for peptides with a proline C-terminus, or by aminolysis with, for example, ammonia/methanol or ammonia/ethanol for peptides with a glycine C-terminus at a temperature between about 10 and 50°C, preferably about 25°C, for between about 12 and 24 hours preferably about 18 hours. Alternatively, the peptide may be removed from the resin by transesterification, e.g., with methanol, followed by aminolysis. The protected peptide may be purified at this point by silica gel chromatography. The removal of the side chain protecting groups from the polypeptide is performed by treating the aminolysis product with, for example, anhydrous liquid hydrogen fluoride in the presence of anisole or other carbonium scavenger, treatment with hydrogen fluoride/pyridine complex, treatment with tris(trifluoroacetyl)boron and trifluoroacetic acid, by reduction with hydrogen and palladium on carbon or polyvinylpyrrolidone, or by reduction with sodium in liquid ammonia, preferably with liquid hydrogen fluoride, and anisole at a temperature between about -10 and +10°C,

preferably about 0°C, for between about 15 minutes and 1 hour, preferably about 30 minutes. For the glycine terminal peptides on the benzyhydrylamine resins, the resin cleavage and deprotection steps may be combined in a single step utilizing liquid hydrogen fluoride and anisole as described above. The fully deprotected polypeptide is then purified by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on underivatized polystyrene-divinylbenzene (for example Amberlite XAD); silica gel adsorption chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, e.g., on Sephadex G-25, or countercurrent distribution; high performance liquid chromatography (HPLC), especially reverse phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

If a racemic amino acid is used in the 1, 2, 3 or 6 position, the diastereomeric nonapeptide or decapeptide final products are separated, and the desired peptide containing a D-amino acid in the appropriate position is isolated and purified, preferably during the above-described chromatographic process.

The preparation of peptides having C-terminal azaglycine amides is preferably done using classical peptide solution synthesis using known peptide intermediates. This is described in more detail in Example 3.

Thus, in another aspect the present invention relates to a method for preparing compounds of the invention and of the pharmaceutically acceptable salts thereof which process comprises:

removing protecting groups and, optionally, covalently bound solid support from a protected

polypeptide to afford a compound of Formula (I) or a salt thereof; or coupling together in the required sequence two fragments of the desired compound of formula (I); or

(a) converting a compound of Formula (I) to a pharmaceutically acceptable salt, or

(b) converting a salt of a compound of Formula (I) to a pharmaceutically acceptable salt, or

(c) converting a salt of a compound of Formula (I) to a free polypeptide of Formula (I).

It will be appreciated that the novel halo-lower alkyl guanidino-substituted amino acids used in this invention to replace the glycine residue at position 6 of LHRH are useful intermediates and as such form an important part of this invention.

Preferred intermediates include those of formula (II) wherein n is 3 or 4, R is trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoropropyl, 2,2,3,3,3-pentafluoropropyl or 3,3,3-trichloropropyl.

Such intermediates of formula II may be prepared by two methods. One method follows classic peptide synthesis techniques. An ω-amino-α-amino acid is treated with appropriate protecting groups in such a way that the acid function and the α-amino group are protected but leaving the ω-amino function available for further treatment. This protected compound is then reacted with N,N'-dialkylcarbodiimide in an appropriate solvent. The reaction is carried out at temperature between about 22-150°C for up to about 6 hours. The solvent is then removed. In order to remove the N,N'-dialkylurea by-product, the residue is suspended in a second solvent such as dimethylformamide and the suspension filtered to recover the desired product as a solid. Alternatively, the corresponding N,N'-halodialkylthiourea may be reacted with the

ω-function of a suitably protected amino acid (eg. CBZ-Lys-OBZl) in the presence of $HgCl_2$.

Alternatively, lysine dihydrochloride or appropriate homolog is reacted with an S-methyl-dialkyl-iso-thiourea•HI or the corresponding free base in the presence of a solution of a strong base such as sodium hydroxide, potassium hydroxide or the like. The reaction is best effected at from room temperature to 90°C, preferably 60°C over several days, i.e., 2-6 days at a pH of ca. 10.5. Additional thiourea may be added if needed after the initial reaction period. A dialkyl dicarbonate and base such as magnesium oxide is then added in an organic solvent, such as dioxane, to react with the α-amino function of the product and the α,ω-functions of excess starting material. The reaction product is then worked up by extraction, an ion exchange resin treatment and other appropriate chromatographic means. The following examples illustrate the preparation of compounds within the scope of this invention.

PREPARATION 1

A mixture of 17.5g $NaHCO_3$, 125ml methylene chloride and 2.65 ml thiopho gene was cooled to 0°C and a solution of 9.4g of CF_3CH_2N .HCl in 50ml of water was added dropwise. The r tion mixture was stored at 0° for 2 hr. and then at n temperature overnight.

The mixture was partitioned between methylene chloride and water. The methylene chloride layer was drie over magnesium sulfate. The methylene chloride solution was filtered and concentrated to an oil. The oil was crystallized from ethyl acetate/hexane to yield 6.5g of N,N'-bis-(2,2,2-trifluoroethyl)thiourea of mp. 154-5°C.

A solution of 3.36 g of the above thiourea in 10 ml of methanol was treated with 0.96 ml of CH_3I. The reaction mixture was heated at 70°C for 1 hr. An additional 0.96 ml of CH_3I was added and stirring was continued for 2 hr. at 70°C, then overnight at room temperature.

The solvent was evaporated in vacuo and the residue was crystallized from MeOH/diethyl ether to yield S-methyl N,N'-bis-(2,2,2-trifluoroethyl)-iso-thiouronium iodide of mp 145-6°C,

In a similar fashion substituting:

2,2,2-trichloropropylamine,

trifluoromethylamine,

2,2,3,3,3-pentafluoropropylamine and the like there are obtained:

S-methyl N,N'-bis(2,2,2-trichloropropyl)-iso-thiouronium iodide,

S-methyl N,N'-bis(trifluoromethyl)-iso-thiouronium iodide, and

S-methyl N,N'-bis(2,2,3,3,3-pentafluoropropyl)-iso-thiouronium iodide.

PREPARATION 2

A mixture of 5.42 g of benzyl N^{α}-benzyloxy-carbonyl-D-lysinate toluenesulfonate (B. Bezas and L. Zervas, J. Am. Chem. Soc., 83, 719 (1961)) and 1.72 ml of diisopropylethyamine in 60 ml of dioxane is created with 3.6 g of S-methyl-N,N'-bis(2,2,2-trifluoroethyl)-isothiourea. The reaction mixture is stirred at 100°C for 6 hours, cooled to room temperature and concentrated to a solid. The solid is suspended in 20 ml of warm DMF, filtered and the filtrate concentrated to a solid. Benzyl N^{α}-benzyloxycarbonyl-N,N'-guanidino-bis(2,2,2-trifluoroethyl)-D-homoargininate toluenesulfonate is obtained as a white solid by crystallization from methanol/ethyl acetate.

Similarly, by using the above procedure, but substituting:

N,N'-bis(2,2,2-trichloropropyl)carbodiimide;

N,N'-bis(trifluoromethyl)carbodiimide;

5 N,N'-bis(2,2,3,3,3-pentafluoropropyl)carbodiimide;

and the like, there are obtained:

benzyl N^{α}-benzyloxycarbonyl-N,N'-guanidino-bis(2,2,2-trichloropropyl)-D-homoargininate;

benzyl N^{α}-benzyloxycarbonyl-N,N'-guanidino-

10 bis(trifluoromethyl)-D-homoargininate; and

benzyl N^{α}-benzyloxycarbonyl-N,N'-guanidino-bis(2,2,3,3,3-pentafluoropropyl)-D-homoargininate.

Similarly, by substituting benzyl N^{α}-benzyloxycarbonyl-D-ornithinate for the D-lysinate

15 there may be obtained the corresponding D-arginine analogs as their toluenesulfonate salts.

PREPARATION 3

A solution of 6g of benzyl N^{α}-benzyloxycarbonyl-

20 N,N'-guanidino-bis-(2,2,2-trifluoroethyl)-D-homoarginate in 150 ml of ethanol containing 1 g of 10% Pd/C catalyst was treated with hydrogen gas for 3 hr at ambient pressure. An additional 0.4 g of 10% Pd/C was added and hydrogenolysis continued for an additional 3 hr.

25 The reaction mixture was filtered through Celite and concentrated to dryness to yield N,N'-guanidino-bis-(2,2,2-trifluoroethyl)-D-homoarginine as a white foam of $[\alpha]_D^{25}$ -6.1° (C 0.6, MeOH).

A solution of 1.96 g of the above named free amino

30 acid in a mixture of 8 ml 1N NaOH and 8ml dioxane was treated with 1.05 g di-t-butyldicarbonate and 0.16 g MgO at 0°C for 1 hr and at room temperature for 3 hr. The mixture was filtered, concentrated to dryness, diluted with water and washed with diethyl ether. The aqueous

35 layer was acidified at 0°C with 1N HCl to a pH of 3.5 and

was then extracted with ethyl acetate. The ethyl acetate layer was washed with water, sat. NaCl, and dried over magnesium sulfate. The ethyl acetate extract was filtered and concentrated to give a white foam. The foam
5 was triturated with Ag3 (Cl^-) resin to give 1.4 g of Nα-t-butoxycarbonyl-N,N'-guanidino-bis-(2,2,2-trifluoroethyl)-D-homoarginine hydrochloride of mp 122-130°C, $[\alpha]_D^{25}$ -2.2°(C 0.5, MeOH).

In a similar fashion, substituting the products of
10 Preparation 2 were obtained the corresponding Boc protected homoarginine and arginine derivatives.

PREPARATION 4

Cis-5-alkylproline compounds may be prepared by the
15 following method:

To a 200-ml round-bottomed flask is added (S)-3-(benzyloxycarbonyl)-5-oxo-4-oxazolidinepropionic acid and 63 ml of anhydrous benzene. To this solution is
20 added 13.9 g of phosphorus pentachloride at 0°C. The reaction mixture is stirred at 0°C for 1 hr during which time all of the phosphorus pentachloride dissolves. The benzene solvent is removed under vacuum and coevaporation with two 25 ml samples of dry benzene, and the residue
25 dried under vacuum to give a light solid. The light solid is suspended in 30 ml of hexamethylphosphoramide and 9.4 ml of tetramethyltin and 40 mg of $PhCH_2Pd(PPh_3)_2Cl$ is added. The reaction mixture is heated at 65°C for 4 hours. An additional 2 ml of tetramethyltin is added at
30 the end of that period and the reaction mixture is stirred over night at room temperature.) After dilution with water and extraction with ethyl acetate, the ethyl acetate layer is washed with water, 5% sodium bicarbonate, water, 5% sodium bisulfate, water, and
35 saturated sodium chloride and dried over anhydrous magnesium sulfate. The solution is filtered and

concentrated to give 16 g of a yellow oil, which is passed through a silica gel column using ethyl acetate/hexane(4/6) as eluent. Concentration of the appropriate fractions gives 15 g of a light yellow oil
5 which is recrystallized from ethyl acetate-hexane to produce 14.3 g of (S)-3-(benzyloxycarbonyl)-4-(3-oxobutyl)-5-oxazolidinone as a white solid (74% yield), having a mp of 64-65°C, $[\alpha]_D^{25}$= + 102° (c = 1.1, CH_2Cl_2).

10 Anal: Calcd. for $C_{18}H_{17}NO_5$:
C, 61.85; H, 5.84; N, 4.81.
Found: C, 61.54; H, 5.89; N, 4.84.

By repeating the above procedure in a similar manner, and, by replacing the tetramethyltin with a
15 stoichiometrically equivalent of the appropriate tetraalkyltin the following compounds are prepared:

(a) With tetraethyltin:
(S)-3-(Benzyloxycarbonyl)-4-(3-oxopentyl)-5-oxazolidinone having a mp of 45-46°C;
20 $[\alpha]_D^{25}$= 82.5°(c 0.7, CH_3OH).
Anal: Calcd. for $C_{16}H_{19}NO_5$(305.336):
C,62.94; H,6.37; N,4.59.
Found: C,63.02; H,6.15; N,4.48.

(b) With tetrabutyltin:
25 (S)-3-(Benzyloxycarbonyl)-4-(3-oxoheptyl)-5-oxazolidinone as an oil; $[\alpha]_D^{25}$ 67.9°(c 0.12, CH_3OH).
Anal: Calcd. for $C_{18}H_{23}NO_5$ EtOAc(421.494):
C,62.69; H,7.41; N,3.32.
Found: C,62.50; H,7.29; N,3.39.

30 Ten grams of the (S)-3-(benzyloxycarbonyl)-4-(3-oxobutyl)-5-oxazolidinone from Example 4 is dissolved in 480 ml of distilled tetrahydrofuran, followed by 160 ml of ammonia at 0°C. The reaction mixture is stirred at 0° for 5 hours, then at ambient temperature overnight.
35 After stripping under vacuum to dryness, the reaction

mixture yields a white solid which is recrystallized from hot ethyl acetate to give 8.8 g of (S)-2-(benzyloxy-carbonylamino)-5-oxo-hexanamide as a white solid (82% yield), mp 142-144°;
5 $[\alpha]_D^{25}$= -4.0° (c 0.4, CH_3OH).

Anal.: Calcd. for $C_7H_9NO_2$:
C, 60.4; H, 6.4; N, 10.0.
Found: C, 60.44; H, 6.53; N, 10.05.

By repeating the above procedure in a similar manner
10 and substituting a stoichiometrically equivalent of the corresponding intermediates from the second previous paragraph, the following compounds are prepared :

(S)-2-Benzyloxycarbonylamino-5-oxo-heptanamide having a mp of 133-135°C; $[\alpha]_D^{25}$ -4.17°(c 0.8, CH_3OH).
15 Anal: Calcd. for $C_{15}H_{20}N_2O_4$(292.341):
C,61.63; H,6.90; N,9.58.
Found: C,61.51; H,6.75; N,9.16.

(S)-2-Benzyloxycarbonylamino-5-oxo-nonamide having a mp of 162-163°C;$[\alpha]_D^{25}$ -4.32° (c 0.6, CH_3OH).
20 Anal: Calcd. for $C_{17}H_{24}N_2O_4$(320.395):
C,63.73; H,7.55; N,8.74.
C,63.62; H,7.56; N,8.82.

To a solution of 2.8 g of (S)-2-benzyloxycarbonyl-amino)-5-oxohexanamide from the preceeding paragraph in a
25 mixture of 60 ml of methanol and 7.5 ml of glacial acetic acid is added, under nitrogen, 1.5 g of palladium diacetate. This reaction mixture is hydrogenated under atmospheric pressure for 4 hrs, at which time a thin layer chromatographic analysis shows the reaction had
30 gone to completion. The reaction mixture is then filtered through Celite and washed with methanol. The reaction mixture and washings are concentrated to dryness to give 1.7 g of a yellow oil, which is treated with 1 ml of a mixture of hydrochloric acid and ethyl acetate to
35 produce the hydrochloride salt. This oil is triturated

with methanol/ethyl ether to produce 1.3 g of a yellow

solid; mp 174-176°C; $[\alpha]_D^{25}$= -33° (c 0.96, CH_3OH). The yellow solid of (S)-cis-5-methylprolinamide (as the
5 hydrochloride salt) is passed through a Bio-Rex 70 column (a weakly acid carboxylic acid ion-exchange resin) with elution first with 300-ml of water, followed by 1% solution of ammonium hydroxide. Concentration of the appropriate fractions gives a 0.9 g of a yellow solid,
10 which is recrystallized from methylene chloride to produce 0.64 g of (S)-cis-S-methylprolinamide as a yellow solid (50% yield); mp 55-56°C.

By repeating the above procedure in a similar fashion, and substituting a stoichiometrically equivalent
15 of the corresponding intermediate, the following compounds are prepared after reduction:

(S)-cis-5-ethylprolinamide, mp 63-65°C; and
(S)-cis-5-butylprolinamide, mp 74-75°C.

20 PREPARATION 5

4.9 g of Boc-glycine was dissolved in a mixture of 50 ml. ethanol and 50 ml. distilled water. The pH of the solution was brought to 7 with aqueous cesium bicarbonate. The solvent was then removed under vacuum.
25 After 18 hours of drying under high vacuum, the residue was dissolved in 150 ml. dry DMF. 25 g chloromethylated polystyrene - 1% divinylbenzene (Merrifield) resin (corresponding to 25 mmole chloride) was added. The mixture was shaken at 50°C for 24 hours,
30 filtered, and the resin was then washed sequentially with DMF, water, and ethanol. The resin was dried under vacuum for 3 days to yield 28.34 g of Boc-Gly-O-Resin.

Example 1

35 In the reaction vessel of a Beckman 990 Peptide Synthesizer was placed 0.5 g. (0.5 mmol.) of

benzhydrylamine resin (Beckman). Amino acids were added sequentially to this resin by means of a synthesis program, as follows:

Step			
Step 1	CH_2Cl_2 wash	1 time	1.5 min
2	50% CF_3CO_2H/CH_2Cl_2--deprotection	1 time	1.5 min
3	50% CF_3CO_2H/CH_2Cl_2--deprotection	1 time	30 min
4	CH_2Cl_2 wash	3 times	1.5 min
5	10% triethylamine/CH_2Cl_2	2 times	1.5 min
6	CH_2Cl_2 wash	3 times	1.5 min
7	N^{α}-Boc-amino acid solution	1 time	add
8	N,N'-dicyclohexylcarbo-diimide solution	1 time	add
9	CH_2Cl_2 rinse and hold--coupling	1 time	coupling reaction 2 hr
10	CH_2Cl_2--rinse add	1 time	1.5 min
11	CH_2Cl_2 wash	3 times	1.5 min
12	ethanol wash	3 times	1.5 min
13	CH_2Cl_2 wash	3 times	1.5 min

Steps 1-13 complete a coupling cycle for one amino acid and completeness of the reaction is checked by the ninhydrin method of E. Kaiser, et al., Anal. Biochem., 34, 595 (1970).

The resin was coupled sequentially with a 2.0 to 2.5 molar excess of each protected amino acid and DCC with or without additions such as 1-hydroxybenzotriazol (HBT). Thus, the resin was treated during successive coupling cycles with 0.237 g of Boc-D-Ala-OH and 0.155 g of BTH

0.269 g. Boc-Pro-OH,

0.536 g. Boc-Arg(Tos)-OH,

0.312 g. Boc-Leu-OH·H_2O

0.488 g. Boc-D-FDeh-OH·HCl and 0.155g BTH,

0.44 g. Boc-Tyr(2,6-dichlorobenzyl)-OH and 0.155g BTH,
0.375 g. Boc-Ser(Benzyl)-OH,
0.380 g. Boc-D-Trp-OH,
5 0.375 g. Boc-D-p-Cl-Phe-OH and 0.155g BTH,
0.275 g. Boc-D-Nal(2)-OH and 0.155g BTH, and
2.0 ml. acetic anhydride.

The resin was removed from the reaction vessel, washed with CH_2Cl_2, and dried *in vacuo* to yield 10 1.64 g. of protected polypeptide resin. The protected peptide was removed from the resin and deprotected by treatment with 25 ml. anhydrous liquid HF in the presence of 3.2 ml. of anisole (scavenger) in a Kel-F reaction vessel at 0°C for 1 hour. The HF was evaporated under 15 vacuum and the residue of N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH_2, as its HF salt, was washed with ether. The residue was then extracted with glacial acetic acid. The acetic acid extract was lyophilized to yield the crude material.

20 The crude material was converted to the acetate salt by passage in water through a column of AG3X (a weakly basic tertiary amine resin) which had been converted to the acetate form. Lyophilization of the eluate yielded 0.6 g. of the crude peptide acetate salt as a white solid.

25 The crude peptide was purified by high performance liquid chromatography on a 2.5 x 100 cm. column of Licroprep Rp-18 (25-40 micron) equilibrated to the running buffer 55% CH_3CN/45%H_2O (0.06 M in NH_4OAc, pH 7). The major UV absorbing (280 nm) peak eluting at 30 approximately 4 column volumes was collected, concentrated to dryness, and lyophilized 3 times from distilled water to yield 124 mg of pure N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH_2, $[\alpha]_D^{22}$ = -15.4° (C 0.5, HOAc).

35 Proceeding in a similar manner but substituting the appropriate A, B, C, D, E, G or F amino acid for those

recited, there are prepared corresponding D-AlaNH$_2$ decapeptides.

Example 2

5 For the synthesis of analogues with a C-terminal Pro-NHCH$_2$CH$_3$, a synthesis program identical to that described in Example 1 was used. The Beckman 990 Synthesizer reaction vessel was loaded with 2.13 g. of Boc-Pro-O-Resin, prepared by the reaction of equimolar 10 ratios of the dry cesium salt of Boc-Pro-OH with chloromethyl-polystyrene/1% divinylbenzene (Lab Systems, Inc.). The quantity of Boc-Pro-O-Resin taken contained 1.4 mmol. of proline.

The resin was coupled sequentially with a 2.0 to 2.5 15 molar excess of each protected amino acid and DCC. Thus, the resin was reacted during successive coupling cycles with

1.49 g. Boc-Arg(Tos)-OH,
0.87 g. Boc-Leu-OH H_2O,
20 1.34 g. Boc-FDeh,
0.38 g. HBT,
1.23 g. N-Boc-O-2,6-dichlorobenzyl-L-tyrosine and
0.38g HBT,
1.03 g. Boc-Ser(Benzyl)-OH,
25 1.07 g. Boc-D-Trp-OH,
1.05 g. Boc-D-p-Cl-Phe-OH
1.10 g. Boc-D-Nal(2)-OH and
2 ml of acetic anhydride.

The resin was removed from the reaction vessel, 30 washed with CH_2Cl_2, and dried *in vacuo* to yield the protected polypeptide resin.

The protected polypeptide was cleaved from the resin by aminolysis with 50 ml. of ethylamine for 18 hours at 2°C. The ethylamine was allowed to evaporate and the 35 resin was extracted with methanol. The methanol was

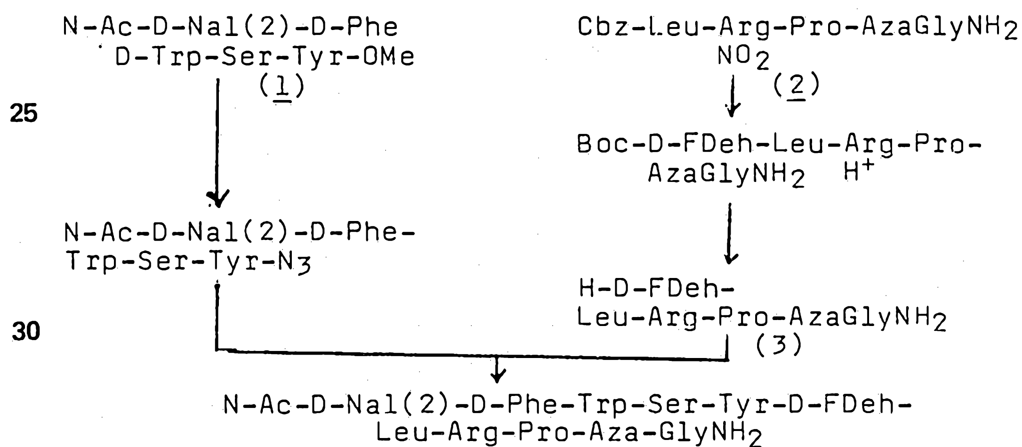
evaporated to yield the protected peptide ethylamide. The peptide was deprotected by treatment of the residue with 3 ml of anisole and 30 ml. redistilled (from CoF_3) anhydrous liquid HF at 0°C. for 30 minutes in a Kel-F reaction vessel. The HF was evaporated under vacuum and the residue was washed with ether. The residue was dissolved in 2 M acetic acid and lyophilized to yield 0.82 g. of crude N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-NHEt as its acetic acid salt.

Final purification was achieved by preparative high performance liquid chromatography of a 200 mg. sample on a 2.5 x 100 cm. column of octadecylsilylated silica (Merck, Lichroprep C_{18} 40-50 microns) using 55%CH_3CN/45% H_2O eluent which was 0.06 M in NH_4OAc (pH 7).

Example 3

Compounds of Formula I wherein H is $-NH-CONH_2$ may be prepared by classical solution synthesis.

For example, the following approach may be used wherein "$AzaGlyNH_2$" is $-NH-NH-CONH_2$, to prepare the peptide as the free peptide or salt.



The coupling of the individual fragments may proceed by the acyl azide method (J. Honzel, et al, Coll. Czech. Chem. Comm., 26, 2333 (1971), by DCC/HBT coupling or other racemization free fragment coupling techniques.
5 Compound (2) is known: A.S. Dutta, et al., J. Chem. Soc. Perkin I, 1979, 379, and compound (1) may be prepared by methods analogous to those in Example 1. Compound (3) is prepared from (2) by removal of the Cbz and nitro groups by hydrogenolysis, followed by coupling with N-Boc-
10 FDeh using DCC/HBT or other coupling agent known in the art. See Dutta, et al, supra, for a similar LHRH analogue synthesis.

Often, the fragments coupled in this way will be peptides or amino acids. Alternatively, the N-terminal
15 nonapeptide acid may be prepared by the solid phase or solution method and subsequently coupled to semicarbazide-HCl by the dicyclohexylcarbodiimidehydroxy-benzotriazole or other coupling method.

20 Example 4

A. A solution of 0.1 g of the hydrogen fluoride salt of N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH$_2$, (See Example 1) is dissolved in 50 ml of water and passed through a column of 50 g Dowex 3
25 anion exchange resin which had previously been equilibrated with acetic acid and washed with deionized water. The column is eluted with deionized water and the effluent is lyophilized to yield the corresponding acetic acid salt.

30 Repeating the above, substituting other acids for acetic acid during the equilibration of the resin, there may be obtained, for example, the corresponding salts

35

with hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, benzoic acid, and the like.

Similarly there may be prepared the acid addition salts of the other peptides analogous to LHRH, described herein.

5 B. In the case of salts of low water solubility, these may be prepared by precipitation from water utilizing the desired acid. For example:

Zinc tannate salt - a solution of 10 mg of N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-

10 D-AlaNH$_2$ acetic acid salt in 0.1 ml of water was treated with a solution of 8 mg of tannic acid in 0.08 ml of 0.25 M NaOH. A solution of 5 mg of $ZnSO_4$ heptahydrate in 0.1 ml of water was immediately added to the solution of the LHRH analogue.

15 The resultant suspension was diluted with 1 ml water and the precipitate was centrifuged. The supernatant was decanted and the residue was washed twice with 1 ml portions of water by centrifugation of the precipitate

20 and decantation of the supernatant. The precipitate was dried in vacuo to yield 15 mg of the mixed zinc tannate salt of the above named LHRH analogue.

Example 5

25 A solution of 10 mg of N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH$_2$ in 25 ml. of water is passed through a 50 g column of Dowex 1 (strongly basic quaternary ammonium anion exchange resin) which had been equilibrated with NaOH solution to

30 make the counter ion hydroxide. The column is eluted with 150 ml of water and the eluant is lyophilized to yield 45 mg of the corresponding polypeptide as the free base.

Similarly other acid addition salts of compounds of

35 the peptides herein may be converted to the corresponding free bases.

Example 6

Biological Activity

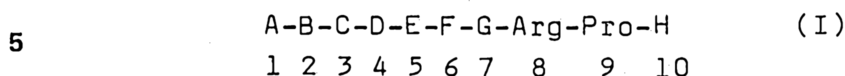
The useful activity of the compounds of the invention is illustrated by the following results obtained in the standard-ovulation test of A. Corbin and C. W. Beattie, Endocr. Res. Commun., Vol. 2, page 1, 1975.

Compound Structure	ED50 in µg Proestrus Propylene Glycol/Saline	ED50 in µg Diestrus Corn Oil
N-Ac-D-Nal(2)^1, D-p-Cl-Phe^2, D-Trp^3, D-FDeh^6, L-Leu^7, D-AlaNH$_2$ ^{10}	0.35	10.8
N-Ac-D-Nal(2)^1, D-p-Cl-Phe^2, D-Trp^3, D-FDeh^6, L-Trp^7, D-AlaNH$_2$ ^{10}	2.0-4.0	-

IN THE TESTS REPORTED ABOVE, NO TOXIC EFFECTS WERE OBSERVED.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:
xWHATxISxCLAIMEDxISxx

1. A compound of the formula



A-B-C-D-E-F-G-Arg-Pro-H (I)
1 2 3 4 5 6 7 8 9 10

and the pharmaceutically acceptable salts thereof, wherein:

A is an amino acyl residue selected from the group consisting of N-Ac-D,L-$\Delta^{3,4}$-prolyl, N-Ac-D,L-prolyl, N-Ac-L-alkylprolyl, N-Ac-D,L-phenylalanyl, N-Ac-D,L-p-Cl-phenylalanyl, N-Ac-D,L-seryl, N-Ac-D,L-threonyl, N-Ac-D,L-alanyl, N-Ac-3-(1-naphthyl)-D,L-alanyl, N-Ac-3-(2-naphthyl)-D,L-alanyl, N-Ac-3-(2,4,6-trimethylphenyl)-D,L-alanyl, and N-Ac-3-(4-trifluoromethylphenyl)-D,L-alanyl;

B is an amino acyl residue selected from the group consisting of D-phenylalanyl, D-p-Cl-phenylalanyl, D-p-Br-phenylalanyl, D-p-F-phenylalanyl, D-p-nitrophenylalanyl, 3-(3,4,5-trimethoxyphenyl)-D-alanyl, 2,2-diphenylglycine, D-α-methyl-p-Cl-phenylalanyl and 3-(2,4,6-trimethylphenyl)-D-alanyl;

C is an amino acyl residue selected from the group consisting of D-tryptophanyl, D-phenylalanyl, D-pentamethyl-phenylalanyl, 3-(3-pyridyl)-D-alanyl, 3-(1-naphthyl)-D-alanyl, and 3-(2-naphthyl)-D-alanyl;

D is an amino acyl residue selected from the group consisting of L-seryl, and D-alanyl;

E is an amino acyl residue selected from the group consisting of L-phenylalanyl and L-tyrosyl;

F is an amino acyl selected from the group consisting of the radicals represented by the following

structural formulas:

$$- HN - CH(-(CH_2)_n - NH - C(=NR_3)-NR_1R_2) - CO_2 - \qquad (II)$$

5

wherein

n is 1 to 5;

10 R_1 is halo lower alkyl;

R_2 is hydrogen, methyl or ethyl;

R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$;

G is an amino acyl residue selected from the group consisting of L-leucyl, L-norleucyl, L-tryptophanyl,

15 L-Nal(2) and L-norvalyl;

H is D-alaninamide, D-leucinamide, glycinamide or $-NHR_5$ wherein R_5 is lower alkyl or $NHCONH_2$;

or a pharmaceutically acceptable salt thereof.

20 2. A compound of the formula

A-B-C-D-E-F-G-Arg-Pro-H (I')
1 2 3 4 5 6 7 8 9 10

25 and the pharmaceutically acceptable salts thereof, wherein:

A is an amino acyl residue selected from the group consisting of N-Ac-D,L-$\Delta^{3,4}$-prolyl, N-Ac-D,L-prolyl, N-Ac-L-alkylprolyl, N-Ac-D,L-phenylalanyl,

30 N-Ac-D,L-p-Cl-phenylalanyl, N-Ac-D,L-seryl, N-Ac-D,L-threonyl, N-Ac-D,L-alanyl, 3-(1-naphthyl)-D,L-alanyl, 3-(2-naphthyl)-D,L-alanyl, 3-(2,4,6-trimethylphenyl)-D,L-alanyl, and 3-(4-trifluoromethylphenyl)-D,L-alanyl;

35 B is an amino acyl residue selected from the group consisting of D-phenylalanyl, D-p-Cl-phenylalanyl,

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D-p-Br-phenylalanyl, D-p-F-phenylalanyl, D-p-nitrophenylalanyl, 3-(3,4,5-trimethoxyphenyl)-D-alanyl, 2,2-diphenylglycine, D-α-methyl-p-Cl-phenylalanyl and 3-(2,4,6-trimethylphenyl)-D-alanyl;

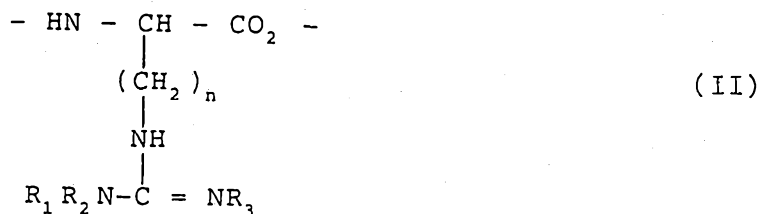
5 C is an amino acyl residue selected from the group consisting of D-tryptophanyl, D-phenylalanyl, D-pentamethyl-phenylalanyl, 3-(3-pyridyl)-D-alanyl, 3-(1-naphthyl)-D-alanyl, and 3-(2-naphthyl)-D-alanyl;

D is an amino acyl residue selected from the group consisting of L-seryl, and D-alanyl;

10 E is an amino acyl residue selected from the group consisting of L-phenylalanyl and L-tyrosyl;

F is an amino acyl selected from the group consisting of the radicals represented by the following structural formulas:

15



20

wherein

n is 1 to 5;

R_1 is halo lower alkyl;

25 R_2 is hydrogen, methyl or ethyl;

R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$;

G is an amino acyl residue selected from the group consisting of L-leucyl, L-norleucyl, L-tryptophanyl, L-Nal(2) and L-norvalyl;

30 H is D-alaninamide, D-leucinamide, glycinamide or $-NHR_5$ wherein R_5 is lower alkyl or $NHCONH_2$;

or a pharmaceutically acceptable salt thereof.

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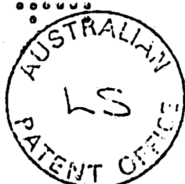


3. The compound of claim 1 or 2 wherein n is 3 or 4.

4. The compound of claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Leu-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

5. The compound of claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Trp-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

6. The compound of claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-FDeh-Nal(2)-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

7. The compound of claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-mPfh-Nal(2)-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

8. The compound of Claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Trp-Ser-Tyr-D-mPfh-Leu-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

9. The compound of Claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Pal(3)-Ser-Tyr-D-FDeh-L-Leu-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

10. The compound of Claim 3 which is N-Ac-D-Nal(2)-D-p-Cl-Phe-D-Pal(3)-Ser-Tyr-D-FDeh-L-Trp-Arg-Pro-D-AlaNH_2 or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising a compound of any one of the Claims 1 to 10 in admixture with at least one pharmaceutically acceptable excipient.

12. A compound according to Claim 1 to 10 when ~~for~~ used as a LHRH antagonist.

13. A process for preparing ~~a~~ compounds of Formula (I) or (I') of ~~according to~~ Claim 1 or 2 which process comprises

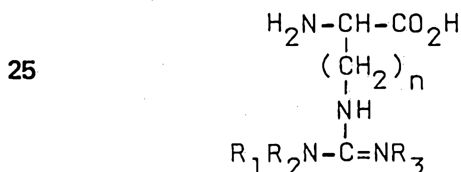
removing protecting groups and, optionally, covalently bound solid support from a protected polypeptide to afford a compound of Formula (I) or a salt thereof; or coupling together in the required sequence two fragments of the desired compound of formula (I); or

(a) converting a compound of Formula (I) to a pharmaceutically acceptable salt, or

(b) converting a salt of a compound of Formula (I) to a pharmaceutically acceptable salt, or

(c) converting a salt of a compound of Formula (I) to a free polypeptide of Formula (I).

14. A compound of formula (II):



wherein

n is 1 or 5;

R_1 is halo lower alkyl;

R_2 is hydrogen, methyl or ethyl; and

R_3 is R_1, methyl, ethyl or $-CH_2CH_2OH$.

DATED this 11th day of November 1985.

SYNTEX (U.S.A.) INC.

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