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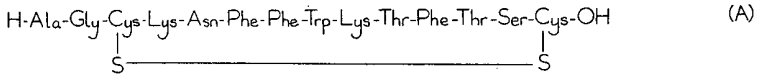
(54) SOMATOSTATIN PEPTIDE ANALOGUES

(71) We, AMERICAN HOME PRODUCTS CORPORATION, a corporation organised and existing under the laws of the State of Delaware, United States of America, of 685, Third Avenue, New York 10017, New York, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to peptide derivatives of somatostatin, to processes for their preparation, to pharmaceutical compositions containing them and to intermediates used in their preparation.

The cyclic somatotropin-release inhibiting factor (SRIF), known as somatostatin, has been shown [Brazeau et al., Science, *179*, 77 (1973)] to have the following structure:

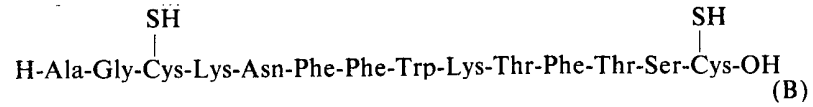


(A)

all amino acids being of the "natural" or L configuration.

Several methods for synthesizing somatostatin have been reported in the literature including the solid phase method of Rivier, J. Am. Chem. Soc., *96*, 2986 (1974) and the solution methods of Sarantakis et al., Biochemical Biophysical Research Communications, *54*, 234 (1973), and Immer et al., Helv. Chim. Acta, *57*, 730 (1974); and there is much peptide research whose goal is to enhance somatostatin's pharmacological activity by synthetically modifying its structure.

The reduced form (open chain form) of somatostatin (RS) is the linear tetradecapeptide of the formula:



(B)

The reduced form (B) has been prepared by total synthesis, [see Rivier et al., C.R.Acad.Sci.Ser.p.Sci. Natur. (Paris), *276*, 2737 (1973) and Sarantakis and McKinley, *Biochem. and Biophys. Res. Communications, 54*, 234 (1973)] and (B) can be converted to somatostatin (A) by oxidation whereby a bridging bond is formed between the two sulfhydryls of the two cysteinyl amino acid residues in the tetradecapeptide.

Various polypeptides which may be regarded as structural modifications of somatostatic have been prepared synthetically and are reported in the chemical literature. Such polypeptides have certain structural features in common with somatostatin and differ from somatostatin in that specific amino acid residues or functional groups originally present in the somatostatic molecule are either missing or are replaced by other amino acid residues or functional groups. The present invention relates to synthetic biologically active polypeptides which may be regarded as a structural modification of somatostatin. The polypeptides of the invention differ from somatostatin in the following respects:

(a) the Ala^1-Gly^2 segment is either present, missing, or replaced by Gly-Gly, Gly-Gly-Gly, Ala-D-Ala, acetyl, or benzoyl;

(b) The Lys^4 residue is replaced by Arg or His;

(c) The Asn^5 residue is replaced by His, Glu, or Asp; and

(d) The Trp^8 residue is either present or replaced by D-Trp or 6-F-D-Trp;

(e) The Cys^{14} residue is either present or replaced by D-Cys.

All optically active amino acid residues and amino acids are of the L-configuration unless specified otherwise.

Accordingly this invention provides compounds of Formula I:

$$\begin{array}{l} X\text{-Cys-}X_1\text{-}X_2\text{-Phe-Phe-}X_3\text{-Lys-Thr-Phe-Thr-Ser-}X_4\text{-OH} \qquad (I) \\ \quad | \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad | \\ \quad SA \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad SA \end{array}$$

wherein A is hydrogen or the two A groups form a direct bond between the sulfur atoms; X is hydrogen, H-Ala-Gly, H-Gly-Gly, H-Ala-D-Ala, H-Gly-Gly-Gly, acetyl or benzoyl; X_1 is His or Arg; X_2 is His, Glu or Asp, X_3 is Trp, D-Trp or 6-F-D-Trp and X_4 is Cys or D-Cys and the pharmaceutically acceptable salts thereof.

The symbols identifying the amino acids and the amino acid residues in the polypeptides described herein are those adopted by the IUPAC-IVB Committee on Biochemical Nomenclature Recommendation (1971), and are described in the *Archives of Biochemistry and Biophysics, 150*, 1—8 (1972). The symbol "6-F-D-Trp" means D-tryptophan in which the 6-position is substituted by fluorine.

The cyclic compounds of formula I possess the inherent physical properties of being white to light tan coloured solids, are substantially insoluble in chloroform and benzene, but exhibit solubility in water and aqueous acid solutions such as hydrochloric and acetic. The compositions of the invention display no clearly discernible melting points and may be purified by, for example, chromatographic means. Hydrolysis of the compositions of the invention in, for example 4 N methanesulfonic acid allows determination of their amino acid content, which is consistent with the structures as hereinbefore set forth.

The compounds of formula I possess pharmaceutical activity in particular the applied use characteristic of inhibiting the release of one or more of the hormones somatotropin, glucagon, insulin as evidenced by standard pharmacological test procedures. By dosage control, selectivity of action permit inhibition of somatotropin and glucagon release without affecting endogenous insulin secretion levels.

In addition, the compounds of the formula I may be utilized in admixture with insulin for treating a warm-blooded animal suffering from diabetes mellitus.

The invention in a subgeneric aspect provides compounds of Formula I, wherein X is hydrogen. Preferably X_3 is D-Trp. Preferably X_4 is Cys.

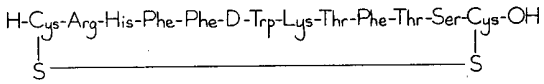
Preferred compounds of the invention include

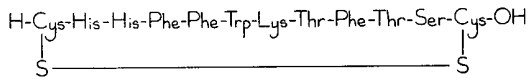
a) a compound of formula I wherein X is hydrogen, H-Ala-Gly, H-Ala-D-Ala, H-Gly-Gly or H-Gly-Gly-Gly; X_1 is His; X_2 is His; X_3 is Trp or D-Trp; and X_4 is Cys.

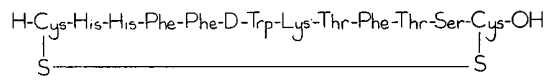
b) a compound of formula I wherein X is hydrogen, H-Ala-Gly, H-Ala-D-Ala, H-Gly-Gly or H-Gly-Gly-Gly; X_1 is Arg; X_2 is His; X_3 is Trp or D-Trp and X_4 is Cys or D-Cys.

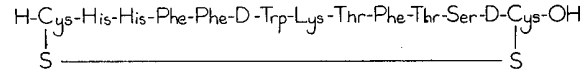
c) a compound of formula I wherein X is hydrogen, X_1 is His, X_2 is His, X_3 is Trp or D-Trp, and X_4 is Cys or D-Cys.

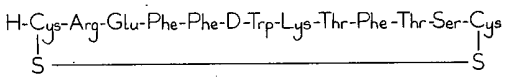
d) a compound of formula I wherein X is hydrogen, H-Ala-Gly, H-Gly-Gly-Gly, H-Ala-D-Ala, acetyl or benzoyl;
X_1 is Arg or His;
X_2 is Glu or Asp;
X_3 is Trp or D-Trp; or 6-F-D-Trp; and
X_4 is Cys or D-Cys;
Preferably X_1 and X_2 are both His or X_1 is Arg and X_2 is Glu.
Especially preferred compounds of this invention are



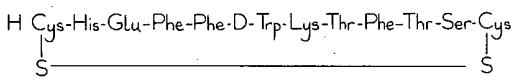






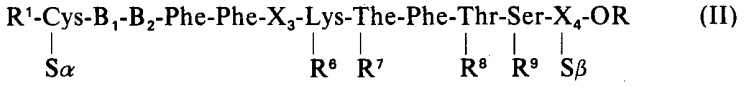


and



and the reduced (open chain) forms thereof.

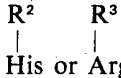
This invention also provides protected intermediates for the compounds of formula I, i.e. compounds having the amino acid sequence of formula I in which one or more amino acid residues have protected side chains and/or the terminal amino and carboxy groups are protected, including compounds having the formula



wherein R represents a carboxyl protecting group (e.g. lower alkyl of 1 to 6 carbon atoms or aralkyl such as benzyl) or a polystyrene resin support linked via a methylene group;

R represents —CH_2— [polystyrene resin support] ; R^1 represents an

α-amino protecting group or an α-amino protected Ala-Gly, Gly-Gly, Ala-D-Ala or Gly-Gly-Gly group, acetyl or benzoyl; B_1 represents



wherein R^3 is a protecting group for the side chain nitrogen atoms of arginine and R^2 is hydrogen or a protecting group for the imidazole nitrogen atom of histidine; B_2 represents

as defined above,

Glu or Asp
| |
R^4 R^5

where R^4 and R^5 are respectively protecting groups for the side chain carboxy groups of glutamic and aspartic acid; R^6 is a protecting group for the side chain amino group of lysine; R^7, R^8 and R^9 are each protecting groups for the hydroxyl groups of threonine and serine; X_3 is Trp, D-Trp or 6-F-D-Trp, X_4 is Cys or D-Cys, and α and β each represent a sulphydryl protecting group or hydrogen, or α and β represent a direct bond between the sulfur atoms.

Examples of α-amino protecting groups for R^1 are (1) acyl, type protecting groups illustrated by the following; formyl, trifluoroacetyl, phthalyl, *p*-toluenesulfonyl (tosyl) and o - nitrophenylsulfenyl; (2) aromatic urethane type protecting groups illustrated by benzyloxycarbonyl and substituted benzyloxycarbonyl such as *p* - chlorobenzyloxycarbonyl, *p* - nitrobenzyloxycarbonyl; (3) aliphatic urethane protecting groups illustrated by *tert.* - butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, allyloxycarbonyl, 2,2,2 - trichloroethoxycarbonyl, amyloxycarbonyl; (4) cycloalkyl urethane type protecting groups illustrated by cyclopentyl - oxycarbonyl; adamantylcarbonyl, cyclohexyloxycarbonyl; (5) thiourethane type protecting groups such as phenylthiocarbonyl; (6) alkyl type protecting groups as illustrated by triphenylmethyl (trityl); (7) trialkylsilane groups such as trimethylsilane. The preferred α-amino protecting group is *tert* - butyloxycarbonyl.

Examples of R^2 are tosyl, benzyloxycarbonyl, adamantyloxycarbonyl and *tert* - butyloxycarbonyl; preferably R^3 is tosyl.

Protection via the nitro or tosyl group is on either the N^{ω} or $N^{\omega 1}$ nitrogen atoms of arginine, while the oxycarbonyl type protecting groups protect the N^{δ} and either one of the N^{ω} or $N^{\omega 1}$ nitrogen atoms.

Examples of R^2 are tosyl, benzyloxycarbonyl, adamantylloxycarbonyl and *tert* - butyloxycarbonyl. Preferably R^2 is the tosyl group.

The side chain amino group of lysine is protected by R^6 which is exemplified by tosyl, *t* - amyloxycarbonyl, *t* - butyloxycarbonyl, diisopropyloxycarbonyl, benzyloxycarbonyl, 2 - halobenzyloxycarbonyl and p - nitrobenzyloxycarbonyl the 2 - chlorobenzyloxycarbonyl group being preferred.

Examples of R^7, R^8 and R^9 protecting groups for the hydroxyl group of threonine and serine are acetyl, benzoyl, *tert* - butyl, and benzyl. Benzyl group is preferred for this purpose.

Examples of R^4 and R^5 are benzyl and t - butyl, the benzyl group being preferred.

Examples of the protecting groups α and β for the sulfhydryl group of the cysteinyl amino acid residue are benzyl; substituted benzyl wherein the substituent is at least one of methyl, methoxy, nitro, or halo (e.g. 3,4 - dimethylbenzyl, *p* - methoxybenzyl, *p* - chlorobenzyl and *p* - nitrobenzyl); trityl, benzyloxycarbonyl, benzhydryl, *p* - methoxybenzyloxycarbonyl, benzylthiomethyl, ethyl carbamoyl, ethylthio, tetrahydropyranyl, acetamidomethyl, benzoyl and S - sulfonic salt, the *p* - methoxybenzyl group being preferred.

Preferred compounds of formula II are those wherein

a) R^1 is an α-amino protecting group or an α-amino protected Ala-Gly-Gly-Gly, Ala-D-Ala, or Gly-Gly-Gly group,

B_1 is His, B_2 is His,
| |
R_2 R^2

X_3 is Trp or D-Trp, X_4 is Cys and R is a polystyrene resin support linked by methylene;

b) R^1 is an α-amino protecting group or an α-amino protected Ala-Gly, Gly-Gly, Ala-D-Ala, or Gly-Gly-Gly group,

B_1 is Arg, B_2 is His,
| |
R^3 R^2

X_3 is Trp or D-Trp, X_4 is Cys or D-Cys and R is a polystyrene resin support linked by methylene;

c) R^1 is an α-amino protecting group,

$$B_1 \text{ is } \underset{\underset{R^2}{|}}{\text{His}}, \quad B_2 \text{ is } \underset{\underset{R^2}{|}}{\text{His}},$$

X_3 is Trp or D-Trp and X_4 is Cys or D-Cys and R is a polystyrene resin support linked by methylene;

d) R^1 is an α-amino protecting group, an α-amino protected Ala-Gly, Gly-Gly-Gly, Ala-D-Ala group, acetyl or benzoyl,

$$B_2 \text{ is } \underset{\underset{R^4}{|}}{\text{Glu}} \text{ or } \underset{\underset{R^5}{|}}{\text{Asp}},$$

and R is a polystyrene resin support linked by methylene.

This invention also provides processes for preparing the compounds of the invention.

The compounds of formula I may be prepared by removing all the protecting groups and the polystyrene resin support when present, from a compound of formula II as defined above; if desired oxidising or reducing the product to give the cyclic or open chain form, and further if desired isolating as the free base or as a pharmaceutically acceptable salt.

Removal of the protecting groups may be effected by methods known in the art for the respective protecting groups. Preferably the protecting groups are removed by using hydrogen fluoride in the presence of anisole.

The polystyrene resin support may be cleaved at the same time as removal of the protecting groups, e.g. using hydrogen fluoride in the presence of anisole or it may be cleaved by transesterification to give a carboxyl protected intermediate of formula II wherein R is an ester function. For example, methanolysis gives a methyl ester intermediate of formula II wherein R is methyl. Hydrolysis of such esters provides the required C-terminal carboxyl function.

By appropriate choice of protecting groups the protecting groups, α and β may be selectively removed from the compounds of formula II to give the free disulfhydryl derivatives of formula II wherein α and β represent hydrogen. For example, when α and β are trityl or acetamido, they may be selectively removed by the action of a mercuric or silver salt to obtain the corresponding disulfhydryl derivative in the form of its corresponding mercuric or disilver salt. The latter salt may be subjected to the action of hydrogen sulfide to obtain the corresponding free disulfhydryl derivative of formula II.

The free disulfhydryl derivatives of formula I and II may each be cyclised by oxidizing using an oxidizing agent, for example using iodine, oxygen (e.g. air), 1,2 - diiodoethane, or sodium or potassium ferricyanide, to obtain the corresponding compounds of formula I wherein the two A groups form a direct bond and formula (II) wherein α and β form a direct bond.

The polypeptide final products and their requisite intermediates may be prepared by the well-known solid phase method as described, for example, by Merrifield, J. Am. Chem. Soc. *85*, 2149 (1963) or by classical synthesis. Hence this invention also provides a process for preparing a compound of formula II wherein α and β are protecting groups and R represents a polystyrene resin support which comprises sequentially coupling the requisite amino acids, protected and/or activated as necessary, to a chloromethylated or hydroxymethylpolystyrene resin support. Alternatively the compounds of formula II wherein α and β are protecting groups and R is a carboxyl protecting group may be prepared by coupling the requisite amino acids or groups of amino acids, protected and/or activated as necessary, to give the desired sequence of amino acids. The α and β protecting groups can then be selectively removed and the product oxidized to give the cyclic disulfide of formula II wherein α and β form a direct bond.

Methods of activating amino acids prior to coupling and coupling methods themselves are well known in the art—see for example the textbook of Schroder and Lubke mentioned below.

In selecting a particular side chain protecting group to be used in the synthesis of the peptides of this invention, the following rules should be followed: (a) the side chain protecting group must be stable to the reagent and under the reaction

conditions selected for removing the α-amino protecting group at each step of the synthesis. (b) the protecting group must retain its protecting properties (i.e. not be split off under coupling conditions), and (c) the side chain protecting group must be removable upon the completion of the synthesis containing the desired amino acid sequence under reaction conditions that will not alter the peptide chain.

In the classical method as applied to the compounds of this invention the desired peptide is built up by condensing amino acids or groups of amino acids which are protected if necessary. The condensation reactions may be carried out using methods generally known to form amide bonds in peptide and penicillin chemistry. To promote facile condensation of the amino acids it is preferred to employ a condensing agent. Examples of condensing agents are carbodiimides; e.g. N,N' - dicyclohexylcarbodiimide (DCC), N,N' - diisopropylcarbodiimide. Alternatively the condensation may be effected by activating one or both of the terminal groups. Examples of the activated form of the terminal carboxyl are the acid chloride, anhydride, azide and activated ester. It will be apparent to those skilled in the art that the proposed method of carrying out the condensation reactions should be compatible with the protecting groups on the amino acids.

In building up the molecule via the classical method it is preferred to condense a protected peptide of formula

$$\begin{array}{l} R^1\text{-Cys-}B_1\text{-}B_2\text{-Phe-OH} \\ | \\ S\alpha \end{array} \qquad \text{(VIa)}$$

with a protected peptide of formula

$$\begin{array}{l} H\text{-Phe-}X_3\text{-Lys-Thr-Phe-Thr-Ser-}X_4\text{-OR} \\ \quad\;\; | \quad | \qquad\quad | \quad | \quad | \\ \quad\;\; R^6 \;\; R^7 \qquad\; R^8 \;\; R^9 \;\; S\beta \end{array}$$

wherein R is a carboxy protecting group (preferably benzyl); R^1, R^6, R^7, R^8, R^9, B_1, B_2, X_3 and X_4 are as hereinbefore defined and α and β are protecting groups, preferably p-methoxybenzyl, to give a compound of formula II which is deprotected and cyclised.

A preferred solid phase method as applied to the compounds of this invention is as follows: α-amino and sulfhydryl protected cysteine is first attached to a chloromethylated polystyrene resin followed by removal of the α-amino protecting group with trifluoroacetic acid in methylene chloride, trifluoroacetic acid alone or HCl in dioxane. The deprotection is conducted at a temperature between 0°C and room temperature. Other standard cleaving reagents and conditions for removal of specific α-amino protecting groups may be used as described in Schroder and Lubke, "The Peptides", *1*, 72—75 (Academic Press, 1965). After removal of the α-amino protecting group, the next desired protected amino acids are coupled individually to the resin supported sequence, *seriatim*. Alternatively, small peptide fragments may be prepared by, for example, the solution method and introduced into the solid phase reactor in the desired order. Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a four fold excess. The coupling is carried out in dimethylformamide, methylene chloride, or a mixture of the two solvents. The success of each coupling reaction at each stage of the synthesis is determined by the ninhydrin reaction as described by E. Kaiser et al., Analyt. Biochem., *34*, 595 (1970). Where incomplete coupling has occurred, the reaction is repeated before the α-amino protecting group is removed for introduction of the next amino acid or amino acid sequence.

The preferred coupling reagents are 1 - hydroxybenzotriazole and diisopropylcarbodiimide; other such reagents will be familiar to those skilled in the art.

After the desired amino acid sequence has been synthesized, the polypeptide is removed from the resin support by treatment with, for example, hydrogen fluoride and anisole to obtain the fully deprotected linear polypeptide. The cyclic disulfide may be produced by air oxidation, or, for example, by oxidation with $K_3Fe(CN)_6$.

Non-toxic addition salts of the linear and cyclic polypeptides are produced by methods well-known in the art from hydrochloric, hydrobromic, sulfuric, phosphoric, polyphosphoric, maleic, acetic, citric, benzoic, succinic, malonic, or ascorbic acid. The acetic acid salt is preferred.

The protecting groups employed throughout the solid phase synthesis are well-known to the art. The α-amino protecting groups employed with each amino acid introduced in sequence of the ultimate polypeptide are of the (1) acyl type protecting groups illustrated by the following: formyl, trifluoroacetyl, phthalyl, *p* - toluenesulfonyl (tosyl) and o - nitrophenylsulfenyl; (2) aromatic urethane type protecting groups illustrated by benzyloxycarbonyl and substituted benzyloxycarbonyl such as *p* - chlorobenzyloxycarbonyl, *p* - nitrobenzyloxycarbonyl; (3) aliphatic urethane protecting groups illustrated by *tert* - butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, allyloxycarbonyl, 2,2,2 - trichloroethoxycarbonyl, amyloxycarbonyl; (4) cycloalkyl urethane type protecting groups illustrated by cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl; (5) thiourethane type protecting groups such as phenylthiocarbonyl; (6) alkyl type protecting groups as illustrated by triphenylmethyl (trityl); (7) trialkylsilane groups such as trimethylsilane. The preferred α-amino protecting group is *tert* - butyloxycarbonyl.

The imidazole nitrogen atom of histidine, denoted N^{im} is protected by a group which may be tosyl, benzyloxycarbonyl, adamantyloxycarbonyl or *tert* - butyloxycarbonyl, preferably the tosyl group.

Protection for the side chain amino group of lysine may be by tosyl, *t* - amyloxycarbonyl, *t* - butyloxycarbonyl, diisopropyloxycarbonyl, benzyloxycarbonyl, 2 - halobenzyloxycarbonyl and *p* - nitrobenzyloxycarbonyl, the 2 - chlorobenzyloxycarbonyl group being preferred.

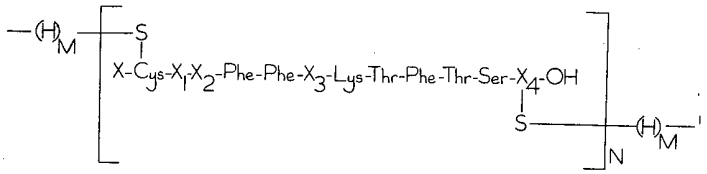
The side chain nitrogen atoms of arginine, denoted N^{g} are protected by a group which may be nitro, tosyl, benzyloxycarbonyl, adamantyloxycarbonyl or *tert* - butyloxycarbonyl, preferably the tosyl group. Protection via the nitro or tosyl group is on either the N^{ω} or $N^{\omega'}$ nitrogen atoms, while the oxycarbonyl type protecting groups protect the N^{δ} and either one of the N^{ω} or $N^{\omega'}$ nitrogen atoms.

Protection for the hydroxyl group of threonine and serine may be with the acetyl, benzoyl, *tert* - butyl, benzyl. The benzyl group is preferred for this purpose.

The protecting group for the sulfhydryl group of the cysteinyl amino acid residue may be selected from benzyl; substituted benzyl wherein the substituent is at least one of methyl, methoxy, nitro, or halo (e.g. 3,4 - dimethylbenzyl, *p* - methoxybenzyl, *p* - chlorobenzyl, *p* - nitrobenzyltrityl, benzyloxycarbonyl, benzhydryl, *p* - methoxybenzyloxycarbonyl, benzylthiomethyl, ethylcarbamoyl, ethylthio, tetrahydropyranyl, acetamidomethyl, benzoyl and S - sulfonic salt; the *p* - methoxybenzyl group being preferred.

The compositions of the invention, similar to somatostatin itself, may exist in either the monomeric open chain form (the so-called "reduced" form), or the monomeric cyclic form (the so-called "oxidized" form). Each of these forms may be produced by a procedure substantially identical to that utilized to obtain the corresponding form of somatostatin itself. These procedures will be familiar to those skilled in the art. The "reduced" form is herein represented by the structure wherein "A" is hydrogen; thus there are free thiol substituents on the two Cys amino acid residues. The "oxidized" form is herein represented by the same structure when the two "A" groups represent a direct bond, i.e. there is a single bond between the sulfur atoms borne on the two Cys amino acid residues, thus a monomeric cycle is formed.

In addition, the compounds of the invention can exist in a so-called "polymeric reduced" form. (see, for example, U.S. Patent 3,926,937), which form can be obtained by the procedure described in the art for the obtention of polymeric reduced somatostatin. Said polymeric form can be described by the formula:



wherein X, X^1, and X^2 are as in Formula I; M is 0 or 1; and N is an integer of from 2 to 100 inclusive. In the bracketed structure, where X is other than H, the sulfur-sulfur bonds are randomly formed between Cys^3-Cys^3, Cys^3-Cys^{14}, and Cys^{14}-Cys^{14} (where X is H, Cys^1 instead of Cys^3, etc.). The structure is cyclic when M is 0, i.e. the compound contains no free SH groups and there is a bond between the sulfur

atoms borne on the terminal Cys residues. For the purposes of this invention, the polymeric reduced forms are qualitatively the full equivalents of the compounds particularly claimed.

The pharmacological activity of the peptides of the invention characterizes the compounds as useful in the treatment of acromegaly and diabetes in the same manner as somatostatin itself. Administration of the peptides may be by conventional routes common to somatostatin and related polypeptides, under the guidance of a physician in an amount dictated by the extent of the dysfunction as determined by the physician. The compounds may be administered alone or in conjunction with conventional pharmaceutically acceptable carriers and adjuvents, in unit dosage form.

As hereinabove disclosed, the compositions of the invention are also useful in admixture with insulin for the treatment of a warm-blooded animal suffering from diabetes mellitus. See, for example, U.S. Patent 3,912,807 which teaches the use of an effective amount of a composition comprising somatostatin admixed with insulin for treating a warm-blooded animal suffering from diabetes mellitus.

In therapeutic use as agents for treating acromegaly, juvenile diabetes, and diabetes mellitus, the treatment is initiated with small dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compounds of the invention are administered at a dosage level which will generally afford effective results without causing any harmful or deleterious side effects. The dosages, however, may be varied depending upon the requirements of the patient and the compound being employed. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

Accordingly a further aspect of this invention provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable carrier. Preferably the pharmaceutical composition is in unit dosage form. The composition may also comprise insulin.

The following examples further illustrate the invention.

Example 1

tert - Butyloxycarbonyl - S - *p* - Methoxybenzyl - L - Cysteinyl - N^{Im} - Tosyl - L - Histidyl - N^{Im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - N^{ϵ} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - *p* - Methoxybenzyl - L - Cysteinyl - Hydroxymethylpolystyrene

Chloromethylated polystyrene resin (Lab Systems, Inc.), in which the degree of cross-linking by divinylbenzene is 1%, was esterified with BOC-Cys-(MBzl)-OH according to Gisin, Helv. Chim. Acta, *56*, 1976 (1973). The polystyrene resin ester was treated according to Schedule A for the incorporation of BOC-Ser(Bzl)-OH, BOC-Thr(Bzl)-OH, BOC-Phe-OH, BOC-Thr(Bzl)-OH, BOC-Lys(Clz)-OH, BOC-D-Trp-OH, BOC-Phe-OH, BOC-Phe-OH, BOC-His(Tos)-OH, BOC-His(Tos)-OH, and BOC-Cys(MBzl)-OH to afford the tittle peptidoresin.

Schedule A

1. Wash with CH_2Cl_2×3.
2. Treat with TFA-CH_2-Cl_2 (1:1 v/v) containing 5% (v/v) 1,2-ethanedithiol for 5 minutes.
3. Treat as in 2 for 25 minutes.
4. Wash with CH_2Cl_2×3.
5. Wash with DMF.
6. Treat with 12% (v/v) TEA in DMF twice for 3 minutes.
7. Wash with DMF.
8. Wash with CH_2Cl_2×3.
9. Treat with 4 equivalents of the corresponding amino acid derivative in CH_2Cl_2-DMF and stir for 5 minutes.
10. Add in two portions 5 equivalents of Diisopropylcarbodiimide (DIC) dissolved in CH_2Cl_2 and over a period of 30 minutes. Reaction time 6 hours.
11. Wash with DMF×3.

12. Wash with $CH_2Cl_2 \times 3$.
13. Test ninhydrin reaction according to Kaiser et al., Annal. Biochem., *34*, 595 (1970). In case of incomplete reaction repeat lines 9 to 13 above.

Example 2

L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - L - Lysyl - L - Threonyl - L - Phenylalanyl - L - Threonyl - L - Seryl - L - Cystein Cyclic (1—12) Disulfide

The peptidoresin of Example 1 (9 g.) was mixed with anisole (18 mg.) and treated with liquid HF (180 ml.) for 45 minutes in an ice bath. The excess HF was removed *in vacuo* as fast as possible (ca. 1 hour) and the residue was extracted with deaerated 2M-glAcOH then filtered. The filtrated was washed with diethyl ether and the aqueous layer was oxidized with $K_3Fe(CN)_6$ at a pH 7. The excess oxidant was removed by ion exchange resin Bio-Rad AG-3 then the peptide was absorbed on an ion exchange resin Bio-Rex 70 and eluted with a pyridine buffer pH 7 to give after lyophilization 1.5 g of material. This material was applied onto a column of Sephadex G 15 (2.5 cm.×160 cm.) and eluted with 15% aq. AcOH. (Bio-Rad, Bio-Rex and Sephadex are Registered Trade Marks). The fractions (5.2 ml. each) in tubes 88—90 were pooled and lyophilized to yield the title compound, 174 mg.
R_f (BWA, 4:1:1 by volume) 0.45 (BWA=n-butanol:water:acetic acid);
R_f (BWAP, 30:24:6:20 by volume) 0.64 (BWAP=n-butanol:water:acetic acid:pyridine).

Amino Acid Analysis: Thr(2)1.87, Ser(1)0.89, Cys(2)1.16, Phe(3)3, Lys(1)1.05, His(2)1.76, Trp ND.

Example 3

The *in vivo* pharmacological activity of the dodecapeptide prepared in Example 2 was established by the following procedures with the indicated results:

Suppression of Growth Hormone

A subcutaneous (sc) injection of peptide solubilized or suspended in physiological saline, is given to Charles River CD nonfasted male rats. (Charles River is a Registered Trade Mark). Matched saline control solution sc injected rats serve as control animals so that every experimental rat is paired with a control rat. The rats are kept in separate cages and 20 minutes before the end of the test time period they are given an intraperitoneal (i.p.) injection of Nembutal at a dose of 50 mg/kg. (Nembutal is a Registered Trade Mark). Blood samples are obtained by cardiac puncture and the plasma separated for the radioimmunoassay of growth hormone (GH) concentration (ng/ml.). Time periods after injection of 2 and 4 hours are used to test the duration of the activity of the peptide to suppress circulating peripheral GH levels. Comparisons between control and experimental GH values at each time are evaluated by the Student "t" test and statistical significance (p) at the 0.05 level or lower is used as the index of activity.

Compound (Dose)	2 Hr	4 Hr
Control	118±20	115±23
Example 2 (1 mg/kg.)	32±6	62±13
	p=<0.01	p=<0.01

Suppression of Growth Hormone, Glucagon and Insulin

Albino male rats are arranged in the three groups (nine rats/group) and injected i.p. with membutal at 50 mg/kg. Fifteen minutes after the nembutal injection they are injected s.c. according to group with (a) test compound, typically 10—2000 μg/kg.; (b) SRIF 200 μg/kg.; or (c) physiological saline. Ten minutes later 0.5 ml. of arginine (300 mg/ml. pH 7.2) is injected into the heart. The rats are decapitated five minutes after receiving the arginine, and the blood is collected into Trasylol-EDTA. Appropriate aliquots are then assayed for growth hormone (GH), glucagon and insulin. An active compound is one which significantly changes the plasma level of any of these hormones from that of the saline controls. Comparisons between control and experimental values are statistically evaluated

by the analysis of variants method and statistical significance (p) at 0.05 or lower is used as the index of activity.

Compound (Dose μg/kg.)	GH(ng/ml.)	Insulin (μU/ml.)	Glucagon (pg/ml.)
Control	163±29	278±32	69±13
Example 2 (100)	20±8	202±49	20±8
	p=<0.01	p=>0.05	p=<0.01

Example 4

tert - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - N^{gn} - Tosyl - L - Arginyl - N^{im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - N^{ϵ} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - p - Methoxybenzyl - L - Cysteinyl - oxymethylpolystyrene

Chloromethylated polystyrene resin (Lab Systems, Inc.), in which the degree of cross-linking by divinylbenzene is 1%, was esterified with Boc-Cys(MBzl)-OH according to Gisin, Helv. Chim. Acta, *56*, 1976 (1973). The polystyrene resin ester was treated according to Schedule A for the incorporation of Boc-Ser(Bzl)-OH, Boc-Thr(Bzl)-OH, Boc-Phe-OH, Boc-Thr(Bzl)-OH, Boc-Lys(ClZ)-OH, Boc-D-Trp-OH, Boc-Phe-OH, Boc-Phe-OH, Boc-His(Tos)-OH, Boc-Arg(Tos-OH) and Boc-Cys(MBzl)-OH, to afford the title peptido resin.

Schedule A

1. Wash with CH_2Cl_2×3.
2. Treat with TFA-CH_2Cl_2 (1:1 v/v) containing 5% (v/v) 1,2-ethanedithiol for 5 min.
3. Treat as in 2 for 25 min.
4. Wash with CH_2Cl_2×3
5. Wash with DMF
6. Treat with 12% (v/v) TEA in DMF twice for 3 min.
7. Wash with DMF
8. Wash with CH_2Cl_2×3
9. Treat with 4 equivalents of the corresponding amino acid derivative in CH_2Cl_2-DMF and stir for 5 min.
10. Add in two portions 5 equivalents of DIC dissolved in CH_2Cl_2 and over a period of 30 min. Reaction time 6 hours.
11. Wash with DMF×3
12. Wash with CH_2Cl_2×3
13. Test ninhydrin reaction according to Kaiser et al., Anal. Biochem., *34*, 595 (1970). In case of incomplete reaction repeat lines 9 to 13 above.

Example 5

L - Cysteinyl - L - Arginyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - L - Lysyl - L - Threonyl - L - Phenylalanyl - L - Threonyl - L - Seryl - L - Cysteine Cyclic (1—12) Disulfide

The peptidoresin of the previous example, 8.5 g., was mixed with anisole (16 ml.) and treated with liquid HF (100 ml.) for 45 minutes. The excess HF was removed *in vacuo* as fast as possible and the residue was taken in 25% (v/v) aq. AcOH. The polymeric support was filtered off and the filtrate was washed with diethyl ether. The aqueous layer was poured into 3.5 l. water, the pH was adjusted with NH_4OH to 7 and then the sulfhydryl compound was oxidized with $K_3Fe(CN)_6$. The pH was adjusted to 5 with gl. AcOH and the inorganic oxidant was removed with Bio-Rad AG 3. The peptide material was absorbed on Bio-Rex 70 and eluted with pyridine buffer pH 7 to afford the crude compound, 2 g. This material was passed through a column of Sephadex G-15 (2.5×160 cm.) and eluted with 15% (v/v) aq. AcOH. The material in fractions 47 to 93 (5.2 ml. each fraction) 886 mg. was applied onto a column of CM-Sephadex G-25 and eluted with stepwise NH_4OAc gradient (0.1 to 0.3 molar NH_4OAc) to afford the title compound. This material was applied onto a column of Sephadex LH 20 (2.5×92 cm.) and eluted with 10% aq. AcOH. The pure title compound emerged in fractions 55 to 63 (4.1 ml. each). Yield 159 mg.

R_f (BWA, 4:1:1 by volume) 0.46; R_f (BWAP, 30:24:6:20 by volume) 0.65

Amino Acid Analysis: Thr(2) 1.94, Ser(1) 0.91, Cys(2) 1.79, Phe(3) 3, His(1) 1.02, Lys(1) 0.85, Arg(1) 0.95, Trp. N.D.

Example 6

The *in vivo* pharmacological activity of the title compound prepared in Example 5 was established by the following procedure with the indicated results:

Suppression of Growth Hormone, Glucagon and Insulin

Albino male rats are arranged in two groups (nine rats/group) and injected i.p. with Nembutal at 50 mg/kg. Fifteen minutes after the Nembutal injection they are injected s.c. according to group with test compound, or physiological saline. Ten minutes later 0.5 ml. of arginine (300 mg/ml. pH 7.2) is injected into the heart. The rats are decapitated five minutes after receiving the arginine, and the blood is collected into Trasylol-EDTA. Appropriate aliquots are then assayed for growth hormone (GH), glucagon (GLUN), and insulin (INS). An active compound is one which significantly changes the plasma level of any of these hormones from that of the saline controls. Comparisons between control and experimental values are statistically evaluated by the analysis of variants method and statistical significance (p) at 0.05 or lower is used as the index of activity.

Results

Experiment	Dose μg/Kg	GH ng/ml	INS μN/ml	GLUN pg/ml
1	—	257±49	177±19	61±9
	100	61±5*	52±16*	0±0*
2	—	131±43	262±26	42±6
	10	71±21*	238±72	4±3*

* $p<0.01$

EXAMPLE 7

tert - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - N^{im} - Tosyl - L - Histidyl - N^{im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - tryptophyl - N^{ε} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - p - Methoxybenzyl - D - Cysteinyl - oxymethylpolystyrene

Chloromethylated polystyrene resin (Lab Systems, Inc.), in which the degree of cross-linking by divinylbenzene is 1%, was esterified with Boc-D-Cys-(MBzl)-OH according to Gisin, Helv.Chim.Acta, *56* 1976 (1973). The polystyrene resin was treated according to Schedule A for the incorporation of Boc-Ser(Bzl)-OH, Boc-Thr(Bzl)-OH, Boc-Phe-OH, Boc-Thr(Bzl)-OH, Boc-Lys(ClZ)-OH, Boc-D-Trp-OH, Boc-Phe-OH, Boc-Phe-OH, Boc-His(Tos)-OH, Boc-His(Tos)-OH and Boc-Cys(MBzl)-OH, to afford the title peptidoresin.

Example 8

L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - L - Lysyl - L - Threonyl - L - Phenylalanyl - L - Threonyl - L - Seryl - D - Cystein Cyclic (1—12) Disulfide, Diacetate Salt

The peptidoresin of the previous example (10 g.) was mixed with anisole (20 ml.) and treated with liquid HF (150 ml.) in an ice-bath for 45 minutes and with exclusion of air. The excess liquid HF was removed under vacuo and the residue was taken in 50% (v/v) aqueous AcOH. The mixture was filtered and the filtrate was diluted with water to 3.5 liters. The pH was adjusted to 7 with dilute NH_4OH and the disulfhydryl peptide was oxidized with $K_3Fe(CN)_6$, to cyclic disulfide. The pH of the mixture was adjusted to 5 with glacial AcOH and treated with Bio-Rad AG 3. The peptidic material was absorbed onto Bio-Rex 70 (H^+ form) and eluted with pyridine buffer (Py-H_2O-AcOH, 30:66:4, v/v/v). The fractions containing peptidic material were pooled and lyophilized to yield 1.6 g. of crude material. This crude

product was applied onto a column of Sephadex LH-20 (2.5×90 cm.) and eluted with 10% (v/v) aqueous AcOH (fractions 5.8 ml. each). The material which emerged in fractions 97—101 was pooled and lyophilized to yield the title dodecapeptide, 171 mg.

TLC, Avicel precoated glass plates, Rf (BWA, 4:1:1, v/v/v) 0.32 Rf (BWAP, 30:24:6:20, v/v/v/v) 0.56.

Amino Acid Analysis: Thr(2)1.85, Ser(1)0.84, Cys(2)1.29, Phe(3)3, Lys(1)1.05, His(2)1.41, Trp(1)0.75.

(Avicel is a Registered Trade Mark).

The *in vivo* pharmacological activity of the dodecapeptide prepared in Example 8 was established by the procedures detailed at the conclusion of Example 3. The data obtained is as follows:

Suppression of Growth Hormone

Compound (Dose)	2 Hr.	4 Hr.
Control	127±22	59±20
Example 8 (1 mg/kg.)	20±3**	107±34

**p<0.001

Suppression of Growth Hormone, Glucagon and Insulin

Compound (Dose (μg/kg.)	GH (ng/ml.)	Insulin (μU/ml.)	Glucagon (pg/ml.)
Control	279±53	323±30	42±6
Example 8 (100)	64±20*	165±21*	5±2*
Control	201±35	397±36	117±11
Example 8 (100)	110±22+	318±54	70±7*

* p<0.01
+ p<0.05

From this it is seen that at low doses, the peptide of Example 8 is specific for the suppression of glucagon and growth hormone without affecting insulin secretion.

Example 9

tert - Butyloxycarbonyl - S - p - methoxybenzyl - L - cysteinyl - N^{gn} - tosyl - L - arginyl - γ - benzyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - N^{ϵ} - 2 - chlorobenzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - Methoxybenzyl - L - cysteinyl - oxymethylpolystyrene

Chloromethylated polystyrene resin is esterified with Boc-Cys(MBzl)-OH, according to the procedure of Gisin, *Helv. Chim. Acta., 56,* 1976 (1973). The amino acid resin ester is then treated according to Schedule A (set forth below), a Boc-Ser(Bzl)-OH being employed as the protected amino acid in Step 9 thereof. Schedule A is then repeated in order to incorporate consecutively the following amino acids into the peptido resin:

Boc-Thr(Bzl)-OH
Boc-Phe-OH
Boc-Thr(Bzl)-OH
Boc-Lys(ClZ)-OH
Boc-D-Trp-OH
Boc-Phe-OH
Boc-Phe-OH
Boc-Glu(OBzl)-OH
Boc-Arg(Tos)-OH
Boc-Cys(MBzl)-OH

Schedule A: (for treatment of the resin ester)

1. Wash with methylene chloride (CH_2Cl_2), three times.
2. Treat with trifluoroacetic acid-methylene chloride (1:1, v/v) containing 5% (v/v) 1,2 - ethanedithiol for 5 minutes.

3. Repeat Step 2 for 25 minutes.
4. Wash with CH_2Cl_2, three times.
5. Wash with dimethylformamide (DMF).
6. Treat with 12% (v/v) triethylamine in DMF for 3 minutes.
7. Wash with DMF.
8. Wash with CH_2Cl_2, three times.
9. Treat with 4 equivalents of the appropriate protected amino acid in CH_2Cl_2-DMF and stir for 5 minutes
10. Add in two portions over a 30 minute period, 5 equivalents of diisopropylcarbodiimide dissolved in CH_2Cl_2. Allow reaction to proceed for 6 hours.
11. Wash with DMF, three times.
12. wash with CH_2Cl_2, three times.
13. Test by ninhydrin reaction according to the procedure of Kaiser et al., *Annal. Biochem., 34,* 595 (1970). In case of incomplete reaction, repeat Steps 9 to 13, as above.

Example 10

L - Cysteinyl - L - arginyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide

The peptido resin of Example 9 (8.5 g.) was suspended in anisole (16 ml.) and treated with anhydrous liquid hydrogen fluoride (HF) for 45 minutes in an ice-bath, after which time the excess HF was removed under vacuo and the residue extracted with 10% (v/v) aq. acetic acid. The filtrate was washed with diethyl ether and the aqueous layer was poured into 5 liters of water, then the pH was brought to 7 with dilute ammonium hydroxide. The mixture was stirred in the open air for 48 hours then the pH was adjusted to 5 with gl. acetic acid and the peptide absorbed onto Amberlite CG-50 (H^+ form). The peptidic material was eluted with 50% (v/v) aq. acetic acid and lyophilized to yield 850 mg. solid material. (Amberlite is a Registered Trade Mark).

The crude product was applied onto a column of Sephadex LH 20 (2.5×150 cm.) and eluted with 10% acetic acid. The fractions 94—130 were pooled and lyophilized to yield 483 mg. of material. This material was applied onto a column of Sephadex G25 (1.5×115 cm.) and eluted with 10% (v/v) aq. acetic acid. The fractions 43—53 were pooled and lyophilized to give the title peptide, 286 mg.

TLC, Avicel precoated glass plates, R_f (BWA, 4:1:1, v/v/v) 0.40, R_f (BWAP, 30:24:6:20, v/v/v/v) 0.65.

Amino acid analysis: Thr(2) 1.92, Ser(1) 0.89, Glu(1) 1.03, Cys(2) 1.49, Phe(3) 3, Lys(1) 1.05, Trp(1) 0.81, Arg(1) 1.02.

Example 11

tert - Butyloxycarbonyl - S - p - methoxybenzyl - L - cysteinyl - N^{im} - tosyl - L - histidyl - *p* - benzyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - N^{ε} - 2 - chlorobenzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteinyl - oxymethylpolystyrene

Chloromethylated polystyrene resin is esterified with Boc-Cys(MBzl)-OH, according to the procedure of Gisin, *Helv. Chim. Acta., 56,* 1976 (1973). The amino acid resin is then treated according to Schedule A (see Example 9), Boc-Ser(Bzl)-OH being employed as the protected amino acid in Step 9 thereof. Schedule A is then repeated in order to incorporate consecutively the following amino acids into the peptido resin:

Boc-Thr(Bzl)-OH
Boc-Phe-OH
Boc-Thr(Bzl)-OH
Boc-Lys(ClZ)-OH
Boc-D-Trp-OH
Boc-Phe-OH
Boc-Phe-OH
Boc-Glu(OBzl)-OH
Boc-His(Tos)-OH
Boc-Cys(SMBzl)-OH

Example 12

L - Cysteinyl - L - histidyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide

The peptido resin of Example 11(8 g.) is treated with liquid anhydrous HF as described in Example 10, and the linear disulfhydryl dodecapeptide is cyclized by oxidation with $K_3Fe(CN)_6$ at pH 7.3 and in high dilution. The pH is brought to 5 with gl. acetic acid, and the mixture is treated with Bio-Rad AG3-X4 (Cl form) ion exchange resin, and then absorbed onto Amberlite CG 50 (H^+ form). The peptidic material is eluted with 30% (v/v) aq. acetic acid and lyophilized to yield 500 mg. crude material. This material is chromatographed through a column of Sephadex LH 20 to give the title dodecapeptide.

TLC, Avicel precoated glass plates R_f (BWA, 4:1:1, v/v/v) 0.43, R_f (*tert*-AmOH-Py-W, 7:7:6, v/v/v) 0.74.

Amino acid analysis: Thr(2) 1.92, Ser(1) 0.93, Glu(1) 0.94, Cys(2) 1.59, Phe(3) 3, Lys(1) 1.02, His(1) 0.91, Trp(1) 0.81.

Example 13

The biological activity of the peptides of Examples 10 and 12 were determined by the following procedure:

Albino male rats are administered Nembutal intraperitoneally at a dose of 50 milligrams per kilogram. Fifteen minutes later a subcutaneous injection of the test compound of physiological saline is administered. Ten minutes later 0.5 milliliters of arginine (300 milligrams per milliliter, pH 7.2) is injected into the heart. Five minutes after receipt of the arginine the rats are decapitated and blood is collected into trasylol-EDTA. An appropriate aliquot is assayed for growth hormone (GH), insulin, and glucagon by radioimmunoassay. The results of the assay are as follows:

Compound	Dose (ug/kg)	GH ng/kg	Insulin μU/ml.	Glucagon pg/ml.	No. of Animals
Control	—	277±69	301±27	46±8	10
Example 10	200	42±6*	115±16*	1.5±1*	10
Control	—	216±35	283±28	55±5	10
Example 10	40	28±5*	158±57	12±2*	10
Control	—	454±106	269±37	60±10	10
Example 12	200	107±29*	205±36	4±2*	10
Control	—	233±35	342±45	44±4	10
Example 12	50	84±17*	322±40	10±4*	10

*—$p<0.01$

The data show that the peptides of Examples 10 and 12, representative of the other peptides of the invention, are effective agents for reducing growth hormone and glucagon without materially affecting insulin levels at a dose of 40 mg/kg and 50 mg/kg, respectively. The peptides of Example 10 and 12 were also tested for duration of their effect on GH secretion in rats treated with Nembutal. The peptide of Example 10 showed significant inhibition of GH for at least 4 hours at a dose of 1,000 mg/kg (S.C.). The peptide of Example 12 showed no significant inhibition of GH at 2 and 4 hours.

The compounds described herein may be administered to warm-blooded mammals, either intravenously, subcutaneously, intramuscularly, or orally to control serum glucose in the treatment of diabetes. The required dosage will vary with the particular condition being treated, the severity of the condition and the duration of treatment.

The active ingredient may be administered alone or in combination with pharmaceutically acceptable carriers or excipients. Suitable pharmaceutical compositions will be apparent to those skilled in the art.

EXAMPLE 14

Classical Synthesis of L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanyl D - Tryptophyl - L - Lysyl - L - Threonyl - L - Phenylalanyl - L - Threonyl - L - seryl - L - Cysteine cyclic (1→12) disulfide

(a) N^{α} - Benzyloxycarbonyl - N^{im} - Benzyloxycarbonyl - L - Histidyl - L - Phenylalanine Methyl Ester (I)

A suspension of 42.3 g. (0.1 mole) Z-His(Z)-OH and 21.8 g. (0.1 mole) H-Phe-OMe · HCl (98%) in 1000 ml. CH_2Cl_2 was cooled to 0°C., then 22 g. (0.12 mole) DCC and 10.1 g. (0.10 mole) of triethylamine were added. The mixture allowed to reach room temperature overnight.

The dicyclohexylurea which separated was filtered off, and the filtrate was concentrated to gummy material. The material was dissolved in ethyl acetate, insoluble material was filtered off. The EtOAc was washed with 5% (w/v) citric acid solution, H_2O, ice-cold 5% (w/v) sodium bicarbonate solution and H_2O. The organic layer was dried over sodium sulfate, concentrated 1/3 to its original volume, diethyl ether (500 ml.) added until solution became cloudy and left in a cold room overnight. White solid formed was separated and dried. 32g.

m.p. 131—133°C.

TLC: silica gel F-254, CH_2Cl_2:MeOH (9:1 by volume) R_f=0.8.

(b) N^{im} - Benzyloxycarbonyl - L - Histidyl - L - Phenylalanylmethyl Ester Dihydrobromide Salt (II)

30 g. (0.5 mole) of Z-His(Z)-Phe-OMe (I) was dissolved in 300 ml. HBr-HOAc (32% w/w)*, and the mixture was left at room temperature for 30 min., and diethyl ether added to cause precipitation. The soft solid was filtered and dried overnight to yield 32 g. of solid.

m.p. 135—137°C. TLC: does not move.

* solution became cloudy after 2 minutes.

(c) N - Benzyloxycarbonyl - N^{im} - Benzyloxycarbonyl - L - Histidyl - N^{im} - Benzyloxycarbonyl - L - Histidyl - L - Phenylalanine Methyl Ester (III)

A suspension of 30.5 g (0.05 mole) H-His(Z)-Phe-OMe-2HBr (II), 21.7 g. (0.05 mole) Z-His(Z)-OH in 500 ml. CH_2Cl_2 was cooled to 0°C. Then 11 g. of DCC and 10.1 g. (0.10 mole) of triethylamine were added. The mixture allowed to reach room temperature overnight.

The dicyclohexylurea formed was filtered off and the filtrate was concentrated to a glassy material. The residue was dissolved in ethyl acetate, and the EtOAc was washed with 10% (w/v) citric acid solution, H_2O, ice-cold 5% (w/v) sodium bicarbonate solution and H_2O. The EtOAc was dried over sodium sulfate, concentrated to about 1/3 its volume, and diethyl ether added until solution became cloudy and left in a cold room. White solid was separated, 29.5 g.

(d) L - Histidyl - L - Histidyl - L - Phenylalanine Methyl Ester Trihydrochloride Salt (IV)

The mixture of 29 g.

Z Z
| |
Z-His-His-Phe-OMe (IV),

10.8 ml. conc. HCl, 180 ml. MeOH and two spatula full of 10% by weight Pd-C was hydrogenated for 5 hours using Parr hydrogenator.

After filtering, the methanol was concentrated to an oil and diethyl ether was added to form solid. 18 g, of crude material was used for next reaction without further purification.

(e) *tert* - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanine - Methyl Ester (V)

BOC-Cys(MBzl)-OH 13 g. (0.038 mole), 1-hydroxybenztriazole (5.4 g., 0.040 mole) and dicyclohexylcarbodiimide (8.3 g., 0.040 moles) were stirred in 1 l. of ice-cold methylene chloride for thirty minutes. H-His-His-Phe-OMe · 3HCl (19.5 g., 0.0345 mole) and N-methylmorpholine (10.5 ml., 0.096 mole) were added and the mixture was stirred overnight as it reached room temperature. The suspension was

filtered to remove dicyclohexylurea and the filtrate was washed successively with water, 10% (w/v) Na_2CO_3, H_2O and dried over Na_2SO_4. After filtering, the solvent was removed *in vacuo* leaving 21.0 g. of crude product. This material was stirred in 100 ml. of acetonitrile for thirty minutes at room temperature and filtered. The precipitate was washed with acetonitrile, then diethyl ether, and dried. Yield 11 g. TLC: R_f 0.12 silica gel 60, $CHCl_3$:CH_3OH:AcOH, 9:11:0.5 v/v/v.

(f) *tert* - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanine (VI)

BOC-Cys(MBzl)-His-His-Phe-OMe (11.0 g., 0.0142 mole) was dissolved in 200 ml. of methanol and 30 ml. of 1N potassium hydroxide was added. The solution was stirred at room temperature for two hours and the methanol was removed *in vacuo*. The residue was diluted with 200 ml. of water and filtered. The filtrate was adjusted to pH 5 with 10% (w/v) citric acid and the gummy precipitate was allowed to stand as it crystallized.

The product was filtered, washed with water and dried *in vacuo* over P_2O_5. Yield 7.3 g. 67%.

TLC: R_f 0.52 silica gel 60 n - butanol:ethyl acetate:acetic acid:H_2O, 1:1:1:1 v/v/v/v

R_f 0.43 silica gel 60 *tert* - amylalcohol:pyridine:H_2O, 7:6:7 v/v/v.

(g) N - *tert* - butyloxycarbonyl - O - benzyl - L - threonyl - L - phenylalanine methyl ester. (VII)

A solution of Boc-Thr(Bzl)-OH (61.8 g, 0.2 moles) and N-methylmorpholine (22.4 ml, 0.2 moles) in THF was cooled to −15°C. Isobutylchloroformate (26.2 ml, 0.2 moles) was added in portions, keeping the temperature between −15° and −10°C. After stirring at −15°C for 15 minutes, a cold mixture of H-Phe-OMe HCl (43.1 g 0.2 moles) and N - methylmorpholine (22.4 ml, 0.2 moles) in DMF was added in portions keeping the temperature between −10° and −5°C. The mixture was stirred at 0°C for 2 hours, and then at room temperature overnight. The filtered reaction mixture was concentrated in vacuo, and the residue taken up in ethyl acetate. The ethyl acetate solution was washed consecutively with 5% (w/v) $KHSO_4$, 5% (w/v) $KHCO_3$, saline, and dried over Na_2SO_4. After concentrating in vacuo an oil was obtained which crystallized on standing. The solid was recrystallized from isopropyl ether - n - hexane, 74.9 g (80 percent). m.p. 78°—81°C; $[\alpha]_D^{24}$+10.75° (c 1.023, MeOH); R_f ($CHCl_3$) 0.35.

Anal. Calc. for $C_{26}H_{34}N_2O_6$ (470.55) C 66.36, H 7.28, N 5.95
Found: C 66.72, H 7.32, N 5.85.

(h) N - *tert* - Butyloxycarbonyl - O - Benzyl - L - Threonine - L - Phenylalanine. (VIII)

Boc-Thr(Bzl)-Phe-OMe (23.5 g, 0.05 moles) was dissolved in a mixture of MeOH-dioxane (100 ml, 1:1) and treated with 1N-NaOH (55 ml) for 3 hours (until starting material cannot be detected by TLC). Most of the solvent was evaporated *in vacuo* and the residue was diluted with water then it was acidified with 5 percent (w/v) citric acid. The gum which separated was taken in EtOAc and washed with water (brine) then evaporated to dryness. The residue was crystallized from Et_2O-n-hexane, 19.8 g. (87 percent) m.p. 118°—119°C.

R_f (Chloroform-methanol-glacetic acid, 85:10:5) 0.80.

(i) N - *tert* - Butyloxycarbonyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Serine methyl ester (IX)

N - *tert* - butyloxycarbonyl - O - benzyl - L - threonine (30.9 g, 0.1 mole) was dissolved in dry tetrahydrofuran (200 ml) cooled at −20°C and treated with N - methylmorpholine (11 ml) followed by isobutylchloroformate (13.4 ml). The cold reaction mixture was stirred for 5 minutes at −20°C then treated with a solution of O - benzyl - L - serine, methyl ester, hydrochloride (25 g ca. 0.1 moles) containing N - methylmorpholine (11 ml), in DME, and the mixture was allowed to reach room temperature overnight.

The solvent was removed in vacuo and the residue was partitioned between water-ethyl acetate. The organic phase was washed with 5 (w/v) percent citric acid, water, aq. $KHCO_3$, water and dried over $MgSO_4$, then evaporated to dryness to afford an oily residue which crystallized from diethyl ether-n-hexane to a jelly

like solid (29 g), R_f ($CHCl_3$-MeOH, 25:1, v/v) 0.85, R_f (EtOAc-n-hexane, 1:1, v/v) 0.65.

(j) N - *tert* - Butyloxycarbonyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Serine (X)

Boc-Thr(Bzl)-Ser(Bzl)-OMe (28.2 g, 0.0563 moles) was dissolved in methanol (ca. 50 ml) and treated with 1 N sodium hydroxide (75 ml) for 1.5 hours at room temperature. The alkaline solution was neutralized to pH 7 with 10 percent (w/v) citric acid and most of the methanol was removed in vacuo. The residue was diluted with some water and acidified with 5 (w/v) percent aq. $KHSO_4$ then extracted with EtOAc. The organic phase was washed with H_2O dried over Na_2SO_4 and evaporated to dryness to an oil. Yield quantitative.

R_f (EtOAc-n-hexane, 1:1, v/v) 0.15 (long spot).

(k) N - tert - butyloxycarbonyl - O - benzyl - L - threony - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteine benzyl ester. (XI)

Boc-Thr(Bzl)-Ser(Bzl)-OH (24.3 g, 50 m moles) was dissolved in acetonitrile and dichloromethane (250 ml, 2:3 v/v) and was mixed with H-Cys(MBzl)-O Bzl TosOH (25 g 50 m moles), then with triethylamine (6.8 ml) and N - hydroxybenzotriazole (6.8 g) and the mixture was cooled in an ice-bath.

A solution of DCC (11 g, 53 m moles) in acetonitrile (50 ml) was added and the reaction mixture was stirred for two hours in the cold then for 2 days at room temperature. The DCU which separated was filtered off and the filtrate was evaporated to dryness. The oily residue was partitioned between ethyl acetate-water and the organic phase was washed with 10 (w/v) percent citric acid, water, 5% (w/v) $KHCO_3$, brine and dried over Na_2SO_4. The solvent was evaporated and the oily residue was crystallized from Et_2On-hexane to afford a white solid, 24.5 g m.p.=87°—90°C. R_f (chloroform-methanol, 25:1, v/v) 0.54, traces at 0.4 and 0.3 (n-heptane-EtOAc, 1:1, v/v) 0.64, traces at 0.25, I_2 positive $[\alpha]_D^{25}$—14.7° (C 0.98, DMF)

Elemental Analysis for $C_{44}H_{52}N_3SO_9$ (798.9)

Calcd.	C 66.15,	H 6.56, N 5.26
Found	C 66.22,	H 6.95, N 5.29

(l) O - Benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteine benzyl ester, trifluoroacetate. (XII)

Boc-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl (8 g, 10 m moles) was mixed with anisole (100 m moles and treated with TFA (100 ml) for 45 minutes. The solvent was evaporated in vacuo and the residue was dissolved in dry Et_2O, then evaporated to dryness in high vacuo to give an oily compound, 7.2 g (90 percent) R_f n - butanol - water - glacial acetic acid, 4:1:1, v/v/v) 0.9, R_f (n - heptane - EtOAc) 0.0—0.1 long spot, I_2 positive and ninhydrin positive.

(m) N - tert - butyloxycarbonyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteine benzyl ester. (XIII)

Boc-Thr(Bzl)-Phe-OH (Prepared by the method of Example III) (9.2 g 20 m moles) was dissolved in DMF (100 ml) and mixed with N - hydroxysuccinimide (3.4 g) and a solution of H-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl · TFA salt (20 m moles) in DMF (20 ml) neutralized with triethylamine to pH 7. The mixture was cooled in an ice-bath and treated with DCC (4.5 g) for 2 hours in the cold then for 2 days at room temperature. The DCU was filtered and the filtrate evaporated to dryness. The residue was triturated with water to give a precipitate which was taken in EtOAc, washed with 5% (w/v) citric acid, water, 5% (w/v) Na_2CO_3, water, dried over Na_2SO_4 and evaporated to dryness. The residue was solidified from Et_2O - n - hexane, it can be crystallized from MeOH, very soluble in $CHCl_3$. 17.3 g (70%) m.p. 105°—107°C R_f $CHCl_3$-MeOH, 10:1, v/v) 0.90 $[\alpha]_D^{25}$—3.6° (C 1, DMF)

Anal. Calc. for $C_{64}H_{75}N_5SO_{12}H_2O$ (1156.2) C 66.46, H 6.71, N 6.05.
Found: C 66.68, H 6.59, N 6.20.

(n) - tert - Butyloxycarbonyl - D - tryptophyl - N^{ε} - benzyloxycarbonyl - L - lysine methyl ester (XIV)

Boc-D-Trp-OH, 30.4 g. (0.1 mole), H-Lys(Z)-OMe · HCl 33 g. (0.1 mole) and triethylamine, 13.6 ml., was dissolved in DMF (400 ml.) and cooled in an ice-bath

then dicyclohexylcarbodiimide, 22 g. was added and the mixture allowed to reach room temperature overnight. The dicyclohexylurea which separated was filtered and the filtrate was concentrated to a small volume then excess water was added to afford a gummy material. This material was taken in ethyl acetate and washed with 10% (w/v) aq. citric acid, H_2O, sat. $NaHCO_3$ and H_2O and dried over Na_2SO_4, then evaporated to dryness. The residue was triturated with diethyl ether followed by n-hexane to afford crystalline solid, 28 g.

TLC: silgel G, n-hexane-EtOAc, 1:1, v/v, Rf 0.3.

(o) tert - Butyloxycarbonyl - L - phenylalanyl - D - tryptophyl - N^{ϵ} - benzyloxycarbonyl) - L - lysine (XV)

A. Boc-*D*-Trp-Lys(Z)-OMe (22 g., 44 mmoles) was added to a prechilled mixture of 220 ml. of TFA and 25 ml. of anisole and stirred for thirty minutes in an ice bath. The TFA was evaporated as rapidly as possible at 27°C on the rotary and the remaining oil was triturated several times with a solution of n - hexane - diethylether 2:1 v/v, decanted, and finally pumped to a gum. Yield 21.3 g., 36 mmoles.

B. Boc-Phe-OH (9.6 g. 36 mmoles), HOBT (5.3 g., 39 mmoles) and DCC (8 g. 39 mmoles) were stirred in 500 ml. of cold dichloromethane for twenty minutes. H-D-Trp-Lys(Z)-OMe · TFA salt, (21.3 g., 36 mmoles) in 200 ml. of dichloromethane was added, followed by N - methylmorpholine 4.25 ml., 39 mmoles. The reaction stirred overnight as it warmed to room temperature. The mixture was filtered to remove DCC and the filtrate was washed with H_2O twice, 10% (w/v) citric acid twice, sat. $NaHCO_3$ twice, water twice and dried over Na_2SO_4. After filtering and evaporating, the residue weighed 21.4 g. 82% and was used without further purification. TLC: silgel F-254/$CHCl_3$-CH_3OH-HOAc, 9:1:0.5, v/v/v. Rf 0.76 plus traces of minor impurities.

C. Boc-Phe-*D*-Trp-Lys(Z)-OMe (22.9 g., 31.4 mmoles) was dissolved in 450 ml. dioxane and 100 ml. methanol and 39 ml. of 1N KOH aq. was added and stirred overnight at room temperature. The solution was evaporated on a rotovap. The residue was dissolved in 500 ml. of warm water, acidified to pH 3 with 10% (w/v) citric acid, stirred for 30 minutes and filtered. After drying *in vacuo* overnight, 21.6 g. 96% were obtained. M.p. 85—90°. $[\alpha]_D^{26}$=+7.09° (c, 0.987 MeOH). TLC:$CHCl_3$-CH_3OH-HOAc, 9:1:0.5, v/v/v, Silgel F-254. Rf 0.63.

(p) tert - Butyloxycarbonyl - L - phenylalanyl - D - tryptophyl - N^{ϵ} - benzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteine benzyl ester (XVI)

A. Boc-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl (XIII) (40.5 g., 35 mmoles) was added to a cold solution of 400 ml. of TFA and 40 ml. of anisole and stirred cold for 30 minutes. The TFA was evaporated on the rotovap and the residue was triturated with 1 liter of 1:1 (v/v)diethylether - n - hexane to a white powder. After filtering, washing with diethylether - n - hexane and drying *in vacuo* over P_2O_5 and NaOH pellets, 34.6 g. 82% was obtained. M.p. 136—139°C, softens 120°C. $[\alpha]_D^{26}$=—9.93° (c, 1.04 MeOH). TLC:silgel $CHCl_3$-CH_3OH-HOAc, 9:1:0.05, v/v/v, Rf 0.63. small more polar impurity Rf 0.53.

B. Boc-Phe-*D*-Trp-Lys(Z)-OH (20.0 g., 27.9 mmoles), HOBT (4.15 g., 30.8 mmoles), and DCC (6.3 g., 30.8 mmoles) were stirred in 2.0 liters of cold dichloromethane in an ice bath for thirty minutes. H-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl. TFA salt 32.6 g. 27.9 mmoles was added followed by N - methylmorpholine (3.35 ml., 30.8 mmoles), and stirring was continued overnight as the mixture warmed to room temperature. TLC showed essentially complete reaction after two hours.

The mixture was filtered, and the filtrate was washed with water, 10% (w/v) citric acid, water, sat. $NaHCO_3$, water and dried. After filtration and evaporation, TLC showed product and a more polar impurity which was removed by dissolving the crude product in 200 ml. of warm DMF and adding 1 liter of 2% (w/v) aq. $NaHCO_3$. The product was filtered, washed with dilute $NaHCO_3$ and water and dried in vacuo over P_2O_5. Yield 36.5 g. 75% M.p. 182—184°C (shrinks 150°C). $[\alpha]_D^{26}$=0° (c, 0.988 $CHCl_3$), TLC silgel $CHCl_3$-CH_3OH-HOAc, 9:1:0.05 v/v/v, Rf (single spot) 0.76.

(q) Preparation of H-Phe-*D*-Trp-Lys(Z)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl trifluoroacetate salt. (XVII)

Boc-Phe-*D*-Trp-Lys(Z)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl (32 g.) and anisole (33 ml.) were dissolved in 50 ml. of dichloromethane and 100 ml. of trifluoroacetic acid were added and stirred thirty minutes at room temperature. The solution was evaporated on a rotary evaporator at 30°C. The residue was triturated with anhydrous diethyl ether to a powder which was filtered, washed with diethyl ether, and dried *in vacuo* over P_2O_5 and sodium hydroxide pellets.

TLC on silica gel 60 in $CHCl_3$:CH_3OH:HOAc 9:1:0.5, v/v/v showed a single spot Rf 0.65 HNMR showed no peak for t-butyl.

(r) *Tert* - Butyloxycarbonyl - S - p - methoxybenzyl - L - cysteinyl - L - histidyl - L - histidyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - N^{ϵ} - benzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteine benzyl ester (XVIII)

Boc-Cys(MBz)-His-His-Phe-OH (5.5 g, 7.2 mmoles), 1 - hydroxybenzatriazole monohydrate (1.07 g., 7.9 mmoles), and dicyclohexylcarbodiimide (1.65 g., 7.9 mmoles) were stirred in 100 ml. of dry dimethylformamide in an ice bath for thirty minutes. H-Phe-*D*-Trp-Lys(Z)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl trifluoroacetate salt (12.5 g., 7.2 mmoles) and N - methylmorpholine (0.855 g., 7.65 mmoles) were added and the mixture was allowed to attain room temperature overnight with stirring. The suspension was filtered to remove dicyclohexylurea and the filtrate was evaporated to 50 ml. and diluted with 500 ml. of saturated aqueous sodium bicarbonate solution. The resulting solid was filtered, washed with water and redissolved in 200 ml. of DMF. The solution was diluted with 1 l. of 5% (w/v) aq. citric acid and filtered. The solid was washed with water, dissolved in 200 ml. of DMF and precipitated with 1 l. of sat. sodium bicarbonate solution, filtered, washed with water and dried *in vacuo* over P_2O_5 at room temperature. Yield 15. g. Amino acid Analysis:

His, 1.66(2.0); Lys, 1.05(1.0); Phe, (3.0); Ser 1.02(1.0); Thr 2.2 (2.0).

(s) L - Cysteinyl - L - histidyl - L - histidyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide. (XIX)

Boc-Cys(MBzl)-His-His-Phe-Phe-D-Trp-Lys(Z)-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Cys(MBzl)-OBzl. (5 g.) was mixed with anisole (10 ml.) and treated with liquid HF (100 ml) under exclusion of air and in our ice-bath for 60 minutes. The excess HF was removed *in vacuo* and the residue was taken in 10% (v/v) aq. AcOH (200 ml.) and poured into 7 liters of deaerated water. The pH was adjusted to 7 with dilute NH_4OH and stirred in the open air for 24 hours then the pH was brought to 5 with gl. AcOH and the solutions were passed through a column of Amberlite CG 50 (H^+ form). The peptidic material which was absorbed onto the ion exchange resin was eluted with 50% (v/v) aq. acetic acid and the fractions containing the peptidic material, were pooled and lyophalysed to yield 2 g. of the crude material. This crude dodecapeptide was purified by columns chromatography through Sephadex G25 and elutions with 10% (v/v) aq. AcOH and partitions chromatography through Sephadex G25 with the biphasic system n - BuOH - Water - gl AcOH, 4:5:1, v/v/v.

TLC, Silica gel Merck A.G., Chlorine peptide spray, D.E. Nitecki et al. Biochem. *5* 665 (1966). (Merck is a Registered Trade Mark). Rf (BWA, 4:1:1, v/v/v) 0.45 Rf (Rf (BWAP, 30:24:6:20, v/v/v/v) 0.64 Amino acid analysis: Thr(2) 1.85; ser(1) 0.79; Phe(3) 3; Lys(1) 1.02; His(2) 1.75; Cys, Trp N.D.

It will be apparent to those skilled in the art that different coupling agents and protecting groups can be employed in the classical synthesis described in Example 14. Further it will be apparent that different sequences of building up the amino acid residues can be employed to give the desired sequence.

WHAT WE CLAIM IS:—

1. A compound having the formula

$$\underset{\displaystyle\text{SA}}{\text{X-}\overset{}{\underset{|}{\text{Cys}}}}\text{-X}_1\text{-X}_2\text{-Phe-Phe-X}_3\text{-Lys-Thr-Phe-Thr-Ser-}\underset{\underset{\displaystyle\text{SA}}{|}}{\text{X}_4}\text{-OH} \qquad \text{(I)}$$

wherein A is hydrogen or the two A groups form a direct bond between the sulphur atoms;

X is hydrogen, H-Ala-Gly, H-Gly-Gly, H-Ala-D-Ala, H-Gly-Gly-Gly, acetyl or benzoyl;

X_1 is His or Arg;

X_2 is His, Glu or Asp;

X_3 is Trp, D-Trp or 6-F-D-Trp as hereinbefore defined; and

X_4 is Cys or D-Cys;

and the pharmaceutically acceptable salts thereof.

2. A compound as claimed in Claim 1 wherein X is hydrogen, H-Ala-Gly, H-Ala-D-Ala, H-Gly-Gly or H-Gly-Gly-Gly; X_1 is His; X_2 is His; X_3 is Trp or D-Trp; and X_4 is Cys.

3. A compound as claimed in Claim 1 wherein X is hydrogen, H-Ala-Gly, H-Ala-D-Ala, H-Gly-Gly or H-Gly-Gly-Gly; X_1 is Arg, X_2 is His; X_3 is Trp or D-Trp and X_4 is Cys or D-Cys.

4. A compound as claimed in Claim 1 wherein X is hydrogen, X_1 is His, X_2 is His, X_3 is Trp or D-Trp, and X_4 is Cys or D-Cys.

5. A compound as claimed in Claim 1 wherein X is hydrogen, H-Ala-Gly, H-Gly-Gly-Gly, H-Ala-D-Ala, acetyl or benzoyl;

X_1 is Arg or His;

X_2 is Glu or Asp;

X_3 is Trp, D-Trp or 6-F-D-Trp; and

X_4 is Cys or D-Cys.

6. A compound as claimed in Claim 1 or 6 wherein X_1 is Arg and X_2 is Glu.

7. A compound as claimed in any one of Claims 1 to 6 wherein X is hydrogen.

8. A compound as claimed in any one of Claims 1 to 7 wherein X_3 is D-Trp.

9. A compound as claimed in any one of Claims 1 to 8 wherein X_4 is Cys.

10. L - Cysteinyl - L - histidyl - L - histidyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulphide.

11. L - Cysteinyl - L - arginyl - L - histidyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide.

12. L - Cysteinyl - L - histidyl - L - histidyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - D - cysteine cyclic (1—12) disulfide.

13. L - Cysteinyl - L - arginyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide.

14. L - Cysteinyl - L - histidyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - L - lysyl - L - threonyl - L - phenylalanyl - L - threonyl - L - seryl - L - cysteine cyclic (1—12) disulfide.

15. A compound of formula I as claimed in any one of Claims 1, 5 to 9, 13 and 14 which is in the form of a pharmaceutically acceptable acid addition salt selected from the hydrochloride, hydrobromide, sulphate, phosphate, polyphosphate, maleate, acetate, citrate, benzoate, succinate, malonate and ascorbate.

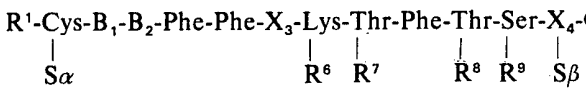
16. A compound of Formula I as claimed in Claim 2 or Claim 10 which is in the form of a pharmaceutically acceptable acid addition salt as defined in Claim 15.

17. A compound of formula I as claimed in Claim 3 or Claim 11 which is in the form of a pharmaceutically acceptable acid addition salt as defined in Claim 15.

18. A compound of formula I as claimed in Claim 4 or Claim 12 which is in the form of a pharmaceutically acceptable acid addition salt as defined in Claim 15.

19. A compound having the amino acid sequence of a compound of formula I as claimed in any one of Claims 1 to 15 in which one or more amino acid residues have protected side chains and/or the terminal amino and carboxy groups are protected.

20. A compound of formula:



wherein R represents a carboxy protecting group or a polystyrene resin support linked via a methylene group; R^1 represents an α-amino protecting group or an α-

amino protected Ala-Gly, Gly-Gly, Ala-D-Ala or Gly-Gly-Gly group, acetyl or benzoyl;
B_1 represents

$$\begin{array}{cc} \text{His or Arg} & \\ | & | \\ R^2 & R^3 \end{array}$$

wherein R^3 is a protecting group for the side chain nitrogen atoms of arginine and R^2 is hydrogen or a protecting group for the imidazole nitrogen atom of histidine;
B_2 represents

$$\begin{array}{c} \text{His} \\ | \\ R^2 \end{array}$$

as defined above,

$$\begin{array}{cc} \text{Glu or Asp} & \\ | & | \\ R^4 & R^5 \end{array}$$

where R^4 and R^5 are respectively protecting groups for the side chain carboxy group of glutamic and aspartic acid; R^6 is a protecting group for the side chain amino group of lysine; R^7, R^8 and R^9 are each protecting groups for the hydroxyl groups of threonine and serine;
X_3 is Trp, D-Trp or 6-F-D-Trp;
X_4 is Cys or D-Cys; and
α and β each represent a sulfhydryl protecting group or hydrogen, or α and β represent a direct bond between the sulphur atoms.

21. A compound of formala II as claimed in Claim 20 wherein R^1 represents formyl, trifluoroacetyl, phthalyl, tosyl, o - nitrophenylsulfenyl, benzyloxycarbonyl, *p* - chlorobenzyloxycarbonyl, *p* - nitrobenzyloxycarbonyl, *tert* - butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, allyloxycarbonyl, 2,2,2 - trichloroethoxycarbonyl, amyloxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl, trityl or trimethylsilyl protecting group or Ala-Gly, Gly-Gly, Ala-D-Ala, Gly-Gly-Gly protected by one of the aforementioned protecting groups.

22. A compound of formula II as claimed in Claim 20 or Claim 21 wherein R^2 is tosyl, benzyloxycarbonyl, adamantyloxycarbonyl or *tert* - butyloxycarbonyl.

23. A compound of formula II as claimed in any one of Claims 20 to 22 wherein R^3 is nitro or tosyl in which case protection is on either the N^{ω} or $N^{\omega 1}$ nitrogen atom of arginine, or benzyloxycarbonyl, adamantyloxycarbonyl or *tert* - butyloxycarbonyl in which case protection is on the N^{δ} and either one of the N^{ω} or $N^{\omega 1}$ nitrogen atoms of argine.

24. A compound of formula II as claimed in any one of Claims 20 to 23 wherein R^4 and/or R^5 represent(s) benzyl.

25. A compound of formula II as claimed in any one of Claims 20 to 24 wherein R^6 is tosyl, *t* - amyloxycarbonyl, *t* - butyloxycarbonyl, diisopropyloxycarbonyl, benzyloxycarbonyl, 2 - halobenzyloxycarbonyl or p - nitrobenzyloxycarbonyl.

26. A compound of formula II as claimed in any one of Claims 20 to 25 wherein at least one of R^7, R^8 and R^9 is acetyl, benzoyl, *tert*-butyl or benzyl.

27. A compound of formula II as claimed in any one of Claims 20 to 26 wherein α and β are benzyl, 3,4 - dimethylbenzyl, p - methoxybenzyl, p - chlorobenzyl, p - nitrobenzyl, trityl, benzyloxycarbonyl, benzhydryl, *p* - methoxybenzylcarbonyl, benzylthiomethyl, ethylcarbamoyl, ethylthio, tetrahydropyranyl, acetamidomethyl, benzoyl or S - sulfonic salt.

28. A compound as claimed in any one of Claims 20 to 27 wherein R is lower alkyl of 1 to 6 carbon atoms or benzyl.

29. A compound of formula II as claimed in any one of Claims 20 to 22, 26 and 27 wherein R^1 is an α-amino protecting group or an α-amino protected Ala-Gly, Gly-Gly, Ala-D-Ala, or Gly-Gly-Gly group,

$$\begin{array}{cc} B_1 \text{ is His,} & B_2 \text{ is His,} \\ | & | \\ R^2 & R^2 \end{array}$$

X_3 is Trp or D-Trp, X_4 is Cys and R is a polystyrene resin support linked by methylene.

30. A compound of formula II as claimed in any one of Claims 20 to 23, 26 and 27 wherein R^1 is an α - amino protecting group or an α - amino protected Ala-Gly, Gly-Gly, Ala-D-Ala, or Gly-Gly-Gly group,

$$B_1 \text{ is } \underset{\underset{R^3}{|}}{\text{Arg}},\ B_2 \text{ is } \underset{\underset{R^2}{|}}{\text{His}},$$

X_3 is Trp or D-Trp, X_4 is Cys or D-Cys and R is a polystyrene resin support linked by methylene.

31. A compound of formula II as claimed in any one of Claims 20 to 22, 26 and 27 wherein R^1 is an α-amino protecting group,

$$B_1 \text{ is } \underset{\underset{R^2}{|}}{\text{His}},\ B_2 \text{ is } \underset{\underset{R^2}{|}}{\text{His}},$$

X_3 is Trp or D-Trp and X_4 is Cys or D-Cys and R is a polystyrene resin support linked by methylene.

32. A compound of formula II as claimed in any one of Claims 20, 21 and 23 to 27 wherein R^1 is an α-amino protecting group, an α-amino protected Ala-Gly, Gly-Gly-Gly, Ala-D-Ala group, acetyl or benzoyl,

$$B_2 \text{ is } \underset{\underset{R^4}{|}}{\text{Glu}} \text{ or } \underset{\underset{R^5}{|}}{\text{Asp}},$$

and R is a polystyrene resin support linked by methylene.

33. A compound of formula II as claimed in Claim 32 wherein R^1 is *tert* - butyloxycarbonyl, R^3 is tosyl, R^4, R^5, R^7, R^8 and R^9 are benzyl, R^6 is 2 - chlorobenzyloxycarbonyl and α and β represent *p* - methoxybenzyl.

34. A compound of formula II as claimed in Claim 29 wherein R^1 is tert - butyloxycarbonyl or a tert - butyloxycarbonyl protected Ala-Gly, Gly-Gly, Ala-D-Ala or Gly-Gly-Gly group, R^2 is tosyl, R^6 is 2 - chlorobenzyloxycarbonyl, R^7, R^8 and R^9 are benzyl and α and β represent p - methoxybenzyl.

35. A compound of formula II as claimed in Claim 30 wherein α, β, R^1, R^2, R^6, R^7 and R^8 are as defined in Claim 34 and R^3 is tosyl.

36. A compound of formula II as claimed in Claim 31 wherein R^1 is *tert* - butyloxycarbonyl and R^2, R^6, R^7, R^8, R^9, α and β are as defined in Claim 34.

37. *tert* - Butyloxycarbonyl - S - *p* - Methoxybenzyl - L - Cysteinyl - L - N^{im} - Tosyl - L - Histidyl - N^{im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - N^{ϵ} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - *p* - Methoxybenzyl - L - Cysteinyl - oxymethylpolystyrene.

38. *tert* - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - N^{gn} - Tosyl - L - Arginyl - N^{im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - N^{ϵ} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - p - Methoxybenzyl - L - Cysteinyl - oxymethylpolystyrene.

39. *tert* - Butyloxycarbonyl - S - p - Methoxybenzyl - L - Cysteinyl - N^{im} - Tosyl - L - Histidyl - N^{im} - Tosyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - L - N^{ϵ} - 2 - Chlorobenzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - Seryl - S - p - Methoxybenzyl - D - Cysteinyl - oxymethylpolystyrene.

40. *tert* - Butyloxycarbonyl - S - p - methoxybenzyl - L - cysteinyl - N^{gn} - tosyl - L - arginyl - γ - benzyl - L - glutamyl - L - phenylalanyl - L - phenylalanyl - D - tryptophyl - N^{ϵ} - 2 - chlorobenzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteinyl - oxymethylpolystyrene.

41. *tert* - Butyloxycarbonyl - S - p - methoxybenzyl - L - cysteinyl - N^{im} - tosyl - L - histidyl - γ - benzyl - L - glutamyl - L - phenylalanyl - L -

phenylalanyl - D - tryptophyl - N^{ϵ} - 2 - chlorobenzyloxycarbonyl - L - lysyl - O - benzyl - L - threonyl - L - phenylalanyl - O - benzyl - L - threonyl - O - benzyl - L - seryl - S - p - methoxybenzyl - L - cysteinyl - hydroxymethylpolystyrene.

42. *tert* - Butyloxycarbonyl - S - *p* - Methoxybenzyl - L - Cysteinyl - L - Histidyl - L - Histidyl - L - Phenylalanyl - L - Phenylalanyl - D - Tryptophyl - N^{ϵ} - benzyloxycarbonyl - L - Lysyl - O - Benzyl - L - Threonyl - L - Phenylalanyl - O - Benzyl - L - Threonyl - O - Benzyl - L - seryl - S - *p* - Methoxybenzyl - L - Cysteine, benzyl ester.

43. A process for preparing a compound of formula I as claimed in Claim 1 which comprises deprotecting a compound as claimed in Claim 19.

44. A process for preparing a compound of formula I as defined in Claim 1 which comprises removing all the protecting groups and the polystyrene resin support when present from a compound of formula II as defined in any one of Claims 20 to 28, if desired oxidising or reducing the compound of formula I produced to give the cyclic or open chain form, and further if desired isolating as the free base or a pharmaceutically acceptable salt.

45. A process as claimed in Claim 44 wherein the polystyrene resin support and/or protecting groups are removed by treatment with hydrogen fluoride in the presence of anisole.

46. A process for preparing a compound of formula I as defined in Claim 1 wherein the two A groups from a direct bond between the sulphur atoms which comprises oxidising using an oxidising agent a corresponding compound of formula I as defined in Claim 1 wherein A represents hydrogen.

47. A process for preparing a compound of formula II as claimed in Claim 20 wherein α and β are protecting groups and R represents a polystyrene resin support which comprises sequentially coupling the requisite amino acids, protected and/or activated as necessary, under solid phase synthesis conditions to a chloromethylated or hydroxymethylpolystyrene resin support.

48. A process for preparing a compound of formula II as defined in any one of Claims 20 to 28 wherein α and β represent a direct bond which comprises oxidising a corresponding compound of the formula II wherein α and β each represent hydrogen.

49. A process as claimed in Claim 44, 45 or Claim 48 in which the oxidation is effected using oxygen or potassium ferricyanide.

50. A process for preparing a compound of formula II as defined in any one of Claims 20 to 26 and 28 wherein α and β are hydrogen which comprises selectively removing the sulfhydryl protecting groups from a corresponding compound of formula II wherein α and β represent trityl or acetamido.

51. A process for preparing a compound of formula II as claimed in Claim 20 wherein α and β are protecting groups and R represents a carboxy protecting group, which comprises coupling the requisite amino acids or groups of amino acids, protected and/or activated as necessary, to give the desired sequence.

52. A process as claimed in Claim 44 or Claim 45 wherein the compound of formula II as defined in Claim 20 prepared by a process as claimed in any one of Claims 48 to 51.

53. A process as claimed in Claim 45 in which the compound of formula II is as defined in Claim 29 or Claim 34.

54. A process as claimed in Claim 45 in which the compound of formula II is as defined in Claim 30 or Claim 35.

55. A process as claimed in Claim 46 in which the compound of formula II is as defined in Claim 31 or Claim 36.

56. A process as claimed in Claim 46 in which the compound of formula II is as defined in Claim 32 or Claim 33.

57. A process for preparing a compound of formula II as defined in Claim 20 which comprises condensing a peptide of formula

```
R¹-Cys-B₁-B₂-Phe-OH          (VIa)
   |
   Sα
```

with a peptide of formula

```
H-Phe-X₃-Lys-Thr-Phe-Thr-Ser-X₄-OR
      |   |       |   |   |
      R⁶  R⁷      R⁸  R⁹  Sβ
```

in which formulae R is a carboxy protecting group; R^1, R^6, R^7, R^8, R^9, B_1, B_2, X_3 and X_4 are as defined in Claim 20 and α and β are sulfhydryl protecting groups.

58. A process as claimed in Claim 57 which is effected using dicyclohexylcarbodiimide.

59. A process for preparing a compound of formula I as defined in Claim 1 substantially as hereinbefore described with reference to Example 2.

60. A process for preparing a compound of formula I as defined in Claim 1 substantially as hereinbefore described with reference to Example 5.

61. A process for preparing a compound of formula I as defined in Claim 1 substantially as hereinbefore described with reference to Example 8.

62. A process for preparing a compound of formula I as defined in Claim 1 substantially as hereinbefore described with reference to any one of Examples 10, 12 and 14 (s).

63. A process for preparing a compound of formula II as defined in Claim 20 substantially as hereinbefore described with reference to Example 1.

64. A process for preparing a compound of formula II as defined in Claim 20 substantially as hereinbefore described with reference to Example 4.

65. A process for preparing a compound of formula II as defined in Claim 20 substantially as hereinbefore described with reference to Example 7.

66. A process for preparing a compound of formula II as defined in Claim 20 substantially as hereinbefore described with reference to any one of Examples 9, 11 and 14 (r).

67. A compound of formula I as defined in Claim 1 or formula II as defined in Claim 20, whenever prepared by a process as claimed in any one of Claims 43 to 52.

68. A compound of formula I as defined in Claim 1 or formula II as defined in Claim 20 whenever prepared by a process as claimed in any one of Claims 53, 59 and 63.

69. A compound of formula I as defined in Claim 1 or formula II as defined in Claim 20, whenever prepared by a process as claimed in any one of Claims 54, 60 and 64.

70. A compound of formula I as defined in Claim 1 or formula II as defined in Claim 20, whenever prepared by a process as claimed in any one of Claims 55, 61 and 65.

71. A compound of formula I as defined in Claim 1 or formula II as defined in Claim 20, whenever prepared by a process as claimed in any one of Claims 56 to 58, 62 and 66.

72. A compound of formula I as claimed in any one of Claims 1 to 14 which is the polymeric-reduced form, as hereinbefore defined.

73. A pharmaceutical composition comprising a compound of formula I as defined in any one of Claims 1 to 18 and 72, or a pharmaceutically acceptable salt thereof in conjunction with a pharmaceutically acceptable carrier.

74. A pharmaceutical composition as claimed in Claim 73 when in unit dosage form.

75. A pharmaceutical composition as claimed in Claim 73 or Claim 74 also comprising insulin.

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