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(54) PEPTIDES

(71) We, SANDOZ LTD., of 35 Lichtstrasse, 4002 Basle, Switzerland, a Swiss Body Corporate, 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:—

The present invention relates to polypeptides.

The present invention provides a compound of formula I,

H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2. I

The present invention also provides a process for the production of the compound of formula I comprising joining two peptide units by an amide linkage, the peptide units being such that when joined the amino acid sequence of the compound of formula I is achieved.

Preferably one of the peptide units incorporates the group of formula II,

H-Cys-Ser-Asn-Leu-Ser-Thr-Cys- II

wherein an S—S bridge exists between the L-cysteinyl units, and preferably also. the S—S bridge is formed in the last stage in the production of the peptide unit.

It is to be understood that the expression "peptide unit" includes within its meaning a single amino acid, and a plurality of amino acids joined by amide linkages in the form of a peptide.

The peptide units comprising more than two amino acids may be produced by

generally known methods for the synthesis of compounds joining together the constituent amino acids in the desired order one at a time, or when appropriate, by first forming two suitable peptides and joining these together.

The peptide units may, for example, be joined by reacting an amino acid or, as the case may be, a peptide, having a protected α-amino group and an activated terminal carboxyl radical, with an amino acid or, as the case may be, a peptide, having a free α-amino group and a protected terminal carboxyl radical, with an amino acid or, as the case may be, a peptide, having a free terminal carboxyl radical and a protected α-amino group.

The carboxyl radical may, for example, be activated by conversion into an acid azide, anhydride, imidazolide, isoxazolide or an activated ester, or by reaction with a carbodiimide or N,N'-carbonyl-diimidazole. The preferred condensation methods are the carbodiimide method, the azide method, the method of the activated esters, the anhydride method and the Merrifield method.

In the last stage of the condensation it is desirable to employ methods avoiding or minimizing racemization, preferably by using the azide or activated ester method, whereby activation is conveniently effected with N-hydroxysuccinimide.

The free functional groups which do not participate in the reaction may be protected as follows during the building up of the peptides:

With reference to partial sequence B, described hereinafter, a suitable radical for blocking the guanidino radical of the arginine radical is the nitro radical, but other suitable protective radicals, such as the tosyl radical, the *p*-nitrocarbobenzoxy radical or the 2-(isopropyloxycarbonyl)-3,4,5,6-tetrachlorobenzoyl radical, may likewise be used. It is also possible to use the protective effect of the protonization of the guanidino radical during the synthesis.

With reference to partial sequence D, described hereinafter, a suitable radical for blocking the γ-carboxyl radical is the tert. butyloxy radical, but other protective radicals, such as the methoxy, ethoxy, tert. amyloxy, the amide or the benzyloxy radical, may likewise be used.

With reference to partial sequence C, described hereinafter, a suitable radical for blocking the ω-amino radical of the lysyl radical is a carbo-tert. alkoxy radical, preferably the carbo-tert. butoxy radical.

With reference to partial sequence EF, described hereinafter, the benzyl or trityl radical is preferably used as the mercapto-protective radical. When benzyl or trityl radicals are used for the protection of SH radicals, it is usual to split them off at the end of the synthesis by treatment with sodium in liquid ammonia. It has now been found that the splitting off of the benzyl or trityl-protective radicals and the formation of the S—S bridge at the stage immediately before the last stage of the build up lead to particularly high yields of final product.

The conversion of a protected mercapto or amino radical into a free radical, as well as the conversion of a functionally modified carboxyl radical into a free carboxyl radical during the course of the process for the production of the polypeptide is effected in conventional manner by treatment with hydrolyzing or reducing agents.

The polypeptide of formula I may exist in free form or in acid addition salt or heavy metal complex form. Such latter forms may be produced from the free form or *vice versa* in manner known *per se*. By free form is meant the compound of formula I in non-protonated, or non-complexed form. Examples of acids for acid addition salt formation are organic acids, e.g. acetic, lactic, succinic, benzoic, salicylic, methanesulphonic or toluenesulphonic acid, and inorganic acids, such as hydrohalic acids, e.g. hydrochloric acid, or sulphuric acid or phosphoric acid. An example of a heavy metal complex formed with zinc$^{\oplus\oplus}$.

The compound of formula I exhibits interesting pharmacological effects in tests in animals. In particular, the compound lowers the calcium content in blood plasma in the calcium lowering test in the rat. The compound is therefore indicated for use in the treatment of conditions in which a lowering of the calcium content in plasma is desired. For such use an indicated suitable daily dose is in the range of 7.0 to 700 MRC units which may be administered in divided doses 2 or 3 times daily or in sustained release form. An indicated suitable unit dosage form is in the range of 2 to 700 MRC units of the compound.

Free forms and pharmaceutically acceptable acid addition salt or heavy metal complex forms of the compound of formula I possess the same order of activity. Examples of pharmaceutically acceptable acid addition salt forms are those with organic acids, e.g. acetic, lactic or succinic acids and with inorganic acids, e.g. hydrochloric, sulphuric or phosphoric acids. An example of a pharmaceutically acceptable heavy metal complex is a complex with zinc ($Zn^{\oplus\oplus}$).

The invention accordingly also provides a pharmaceutical composition comprising a compound of formula I, in free form or in pharmaceutically acceptable acid addition salt or heavy metal complex form as active agent, in association with a pharmaceutical diluent or carrier. The pharmaceutical composition may, for example, be in unit dosage form comprising from 2 to 700 MRC units of active agent.

The following abbreviation are used herein, viz.

Z	=	Carbobenzoxy (Benzyloxycarbonyl)
Bzl	=	Benzyl
BOC	=	tert. Butyloxycarbonyl
Trt	=	Trityl=Triphenylmethyl
OTB	=	tert. Butyloxy
ONP	=	*p*-Nitrophenyl ester
OCP	=	2,4,5-Trichlorophenoxy
OMe	=	Methoxy
OEt	=	Ethoxy
NO_2	=	Nitro
Ser	=	L-Seryl
Asn	=	L-Asparaginyl
Leu	=	L-Leucyl
Thr	=	L-Threonyl
Val	=	L-Valyl
Tyr	=	L-Tyrosyl
Arg	=	L-Arginyl
Gln	=	L-Glutaminyl
Glu	=	L-Glutamyl
His	=	L-Histidyl
Pro	=	L-Prolyl
Gly	=	Glycyl
Lys	=	L-Lysyl
Cys	=	L-Cysteinyl
Piv	=	Pivaloyl
MCP	=	β-Mercaptopropionyl
OSu	=	N-Oxysuccinimide
EOC	=	Ethoxycarbonyl
DCHA	=	Dicyclohexylamine

One representative process for the production of the compound of formula I will now be described in the following Example.

In the following description all temperatures are indicated in degrees Centigrade. The value c for the optical rotation amounts to 1.

EXAMPLE

Production of H - Cys - Ser - Asn - Leu - Ser - Thr - Cys - Val - Leu - Gly - Lys - Leu - Ser - Gln - Glu - Leu - His - Lys - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr - Gly - Ser - Gly - Thr - Pro - NH_2 . hexaacetate . decahydrate

a) BOC - Cys - Ser - Asn - Leu - Ser - Thr - Cys - Val - Leu - Gly - Lys(BOC) - Leu - Ser - Gln - Glu(OTB) - Leu - His - Lys(BOC) - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr - Gly - Ser - Gly - Thr - Pro - NH_2 . 2 CH_3COOH . 3 H_2O

1.0 g of nonapeptide (partial sequence EF described below) is dissolved in 10 cc of dimethyl formamide, 1.5 g of N-hydroxysuccinimide and 0.52 g of dicyclohexyl carbodiimide are added, the mixture is stirred at 25° for 6 hours, filtration is effected and the filtrate is evaporated to dryness. The residue is washed with ethyl acetate and diethyl ether and drying is effected.

BOC-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-OSu,

having a M.P. of 242°, is obtained and is dissolved in 10 cc of dimethyl formamide.

3.1 g of tricosapeptide diacetate (partial sequence ABCD described below) and 1.2 g of N-hydroxysuccinimide are added to this solution and the mixture is stirred at 25° for 16 hours. The reaction mixture is evaporated to dryness, the residue is washed with diethyl ether, chloroform and acetone. The crude, protected dotriacontapeptide is obtained and is dissolved in 50 cc of a mixture of chloroform/methanol/water (70:30:5). The resulting solution is arranged in layers on a silica gel column (5×100 cm), which has been balanced with the mixture indicated above. Elution is effected with an increasing concentration of methanol. The combined fractions which contain the pure peptide are evaporated, subsequently washed with diethyl ether and dried over potassium hydroxide in a high vacuum. The protected compound, indicated in the heading to part a) above, having a M.P. of 230° (decomp.), $[\alpha]_D^{20} = -45°$ in 50% acetic acid, is obtained.

b) H - Cys - Ser - Asn - Leu - Ser - Thr - Cys - Val - Leu - Gly - Lys - Leu - Ser - Gln - Glu - Leu - His - Lys - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr - Gly - Ser - Gly - Thr - Pro - NH_2 . hexaacetate . decahydrate

3.3 g of the protected dotriacontapeptide . diacetate . trihydrate of part a) are dissolved under an atmosphere of nitrogen in 100 cc of trifluoroacetic acid, the solution is allowed to stand at 20° for 15 minutes and is evaporated to dryness. The residue is dissolved in 300 cc of 0.2 N acetic acid, the solution is treated with 20 cc of Amberlite-IRA-410 (Registered Trade Mark) (acetate), lyophilization is effected, the residue is washed with diethyl ether and dried over potassium hydroxide in a high vacuum. The title compound of the Example, having a M.P. of 220° (decomp.), $[\alpha]_D^{20} = -46°$ in 50% acetic acid, is obtained.

PRODUCTION OF STARTING MATERIALS EMPLOYED IN THE EXAMPLE

PARTIAL SEQUENCE A: *H-Ser-Gly-Thr-Pro-NH₂*

a) H-Thr-Pro-NH_2 . HCl

134 g of Z-Thr-$NHNH_2$ are dissolved at −5° in 2 liters of 1 N hydrochloric acid, and 0.55 liters of 1 N sodium nitrate are added. After 5 minutes potassium carbonate is added until the pH of the solution amounts to 9, the resulting azide is extracted with ethyl acetate, and a solution of 80 g of H-Pro-NH_2 . hydrochloride in 100 cc of water, 500 cc of dimethyl formamide and 77 cc of triethyl amine is added. The ethyl acetate is evaporated at 20° in a vacuum and the reaction mixture is allowed to stand over night at 25°. The remaining solution is evaporated in a vacuum, the residue is dissolved in ethyl acetate, the solution is washed with water, dilute hydrochloric acid and an aqueous calcium carbonate solution and is dried over sodium sulphate. The reaction mixture is concentrated by evaporation in a vacuum, the residue is dissolved in warm ethyl acetate and is cooled. Z-Thr-Pro-NH_2, having a M.P. of 148°, $[\alpha]_D^{20} = -72°$ in 95% acetic acid, is obtained. 90 g of Z-Thr-Pro-NH_2 are subsequently dissolved in 2 liters of dioxane and 260 cc of 1 N hydrochloric acid, and hydrogenation is effected at 20° and normal pressure in the presence of a palladium catalyst. Filtration is effected, the solution is evaporated in a vacuum and the crystalline residue is washed with ethyl acetate, whereby H-Thr-Pro-NH_2 . HCl, having a M.P. of 216°, $[\alpha]_D^{20} = -64°$ in 95% acetic acid, is obtained.

b) *Z-Ser-Gly-NHNH₂*

14.5 g of Z-Ser-OH are dissolved in 100 cc of chloroform and 6.1 g of N-methyl morpholine are added to the solution; 8.2 g of chloroformic acid isobutyl ester are subsequently added dropwise. After 10 minutes a solution of 6.6 g of glycine ethyl ester in 50 cc of chloroform is added, and the mixture is stirred at room temperature for 1 hour. The reaction mixture is washed with dilute ammonia, subsequently with hydrochloric acid solution, is dried over sodium sulphate, and the organic phase is removed by evaporation. The resulting Z-Ser-Gly-OEt is recrystallized from ethyl acetate. M.P. 103°, $[\alpha]_D^{20} = +3°$ in dimethyl formamide. 19.4 g of Z-Ser-Gly-OEt are dissolved in 270 cc of ethanol, 27 cc of hydrazine hydrate are added and the mixture is allowed to stand at room temperature for 2 days. The crystalline mass is filtered off, and washing with methanol and drying are then effected. Z-Ser-Gly-$NHNH_2$, having a M.P. of 180°, $[\alpha]_D^{20} = +2°$ in dimethyl formamide, is obtained.

c) *H-Ser-Gly-Thr-Pro-NH₂*

25 cc of a 4 N hydrogen chloride solution in ethyl ether are added to 200 cc of dimethyl formamide, and 11 g of Z-Ser-Gly-NHNH₂ are dissolved therein. 5.4 cc of tert. butyl nitrite are subsequently added dropwise at −10°, 18 cc of triethyl amine are added to the solution, and filtration is subsequently effected. 12 g of H-Thr-Pro-NH₂ . HCl are simultaneously dissolved in 100 cc of dimethyl formamide, 6.2 cc of triethyl amine are added, the precipitated triethyl amine hydrochloride is filtered off, and the dipeptide azide solution obtained above is added to the filtrate. The reaction mixture is allowed to stand at 0° for 2 hours, is evaporated to dryness, the residue is dissolved in a mixture of 500 cc of methanol and 100 cc of water, and the resulting solution is filtered through a 100 cc Dowex-50 column (Registered Trade Mark (H^+ form). The solution is concentrated by evaporation and the residue is crystallized from methanol/ethyl acetate Z-Ser-Gly-Thr-Pro-NH₂, having a M.P. of 132° (decomp.), $[\alpha]_D^{20} = -22°$ in dimethyl formamide, is obtained.

22 g of Z-Ser-Gly-Thr-Pro-NH₂ are dissolved in a mixture of 440 cc of methanol and 88 cc of water, and hydrogenation is effected at 20° and normal pressure in the presence of palladium charcoal. Filtration is subsequently effected, the filtrate is evaporated to dryness, the residue is washed with ether and dried.

H-Ser-Gly-Thr-Pro-NH₂, having a M.P. of 95° (decomp.), $[\alpha]_D^{20} = -27°$ in dimethyl formamide, is obtained.

PARTIAL SEQUENCE B: *BOC-Arg-Thr-Asn-Thr-Gly-NHNH₂*

a) *Z-Asn-Thr-Gly-OEt*

43 g of Z-Thr-Gly-OEt are dissolved in 500 cc of dimethyl formamide, and hydrogenation is effected at normal pressure and room temperature in the presence of palladium charcoal. Filtration is effected, 60 g of Z-Asn-OCP are added, the mixture is allowed to stand at 25° for 16 hours, is concentrated to about 250 cc, ethyl acetate is added, the reaction mixture is washed with dilute hydrochloric acid, dried over sodium sulphate and evaporated to dryness. The residue is washed with ethyl acetate and dried. Z-Asn-Thr-Gly-OEt, having a M.P. of 225°, $[\alpha]_D^{20} = -4°$ in dimethyl formamide, is obtained.

b) *Z-Thr-Asn-Thr-Gly-OEt*

29 g of Z-Asn-Thr-Gly-OEt are dissolved in 700 cc of dimethyl formamide, hydrogenation is effected at normal pressure and room temperature in the presence of palladium charcoal, filtration is effected, and Z-Thr-N_3, produced from 30 g of Z-Thr-NHNH₂ [see partial sequence A, a)], is added to the filtrate. After allowing to stand at 0° for 12 hours the reaction mixture is evaporated to dryness, the residue is washed with water, ether and ethanol.

Z-Thr-Asn-Thr-Gly-OEt, having a M.P. of 230°, $[\alpha]_D^{20} = -8°$ in dimethyl formamide, is obtained.

c) *BOC-Arg(NO₂)-Thr-Asn-Thr-Gly-NHNH₂*

22 g of Z-Thr-Asn-Thr-Gly-OEt are dissolved in 700 cc of dimethyl formamide, hydrogenation is effected at normal pressure and room temperature in the presence of palladium charcoal and filtration is effected. 23 g of BOC-Arg(NO₂)-OH are dissolved in 500 cc of dimethyl acetamide, 12 cc of triethyl amine are added, the solution is cooled to −10°, 9.4 cc of chloroformic acid isobutyl ester are added, the mixture is allowed to react for 10 minutes, and the tetrapeptide solution obtained above is added. After standing at 25° for 30 minutes 100 cc of water are added, the resulting solution is treated with Amberlite-IRA-410 (Registered Trade Mark) (OH form) until a negative chlorine reaction is obtained, filtration and evaporation to dryness are effected. The residue is washed with chloroform, ethyl acetate and ether and is dried. BOC-Arg(NO₂)-Thr-Asn-Thr-Gly-OEt, having a M.P. of ~100°, $[\alpha]_D^{20} = -4°$ in dimethyl formamide, is obtained.

12 g of BOC-Arg(NO₂)-Thr-Asn-Thr-Gly-OEt are dissolved in a mixture of 200 cc of acetic acid and 40 cc of water, hydrogenation is effected in the presence of palladium charcoal at 20° and normal pressure, filtration and evaporation to dryness in a vacuum at 20° are effected. The residue is dissolved in dimethyl formamide and is evaporated. This treatment is repeated until no acetic acid can be detected. The residue is dissolved in 120 cc of dimethyl formamide, 6 cc of hydrazine hydrate are added, the solution is allowed to stand at 25° for 16 hours, is evaporated, the residue is washed with ether, methanol/ether (1:1) and then again with ether, and drying is effected. BOC-Arg-Thr-Asn-Thr-Gly-NHNH₂, having a M.P. of 175° (decomp.), $[\alpha]_D^{20} = -42°$ in dimethyl formamide/1 N HCl (1:1), is obtained.

PARTIAL SEQUENCE AB: *H-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH₂ . 2 CH₃COOH*

13.5 g of BOC-Arg-Thr-Asn-Thr-Gly-NHNH₂ (partial sequence B) are dissolved in 270 cc of dimethyl formamide/water (8:2), cooling is effected to −15°, 40 cc of a 4 N solution of hydrochloric acid in dioxane and 2.5 cc of tert. butyl nitrate are added. The solution is stirred at −15° for 10 minutes, 28 cc of triethyl amine are added, filtration is effected, 10.5 g of H-Ser-Gly-Thr-Pro-NH₂ (partial sequence A) are added and stirring is effected at 0° for 16 hours. The reaction solution is subsequently evaporated to dryness, the residue is washed with ether and dried. The residue is dissolved in methanol, one tenth by volume of water and a sevenfold quantity of chloroform are added, and the resulting solution is filtered through a column of 500 g of silica gel. After elution with an increasing amount of methanol and concentrating by evaporation, BOC-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH₂ is obtained This is suspended in 200 cc of a 4 N solution of hydrochloric acid in dioxane and the solution is stirred at 25° for 2 hours. The solution is evaporated to dryness, the residue is dissolved in 0.2 N acetic acid, the solution is treated with Amberlite-IRA-410 (acetate form), and the aqueous solution is lyophilized. The residue is subsequently washed with ether, ethyl acetate and again with ether and drying is effected over sodium hydroxide shavings. H-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH₂ . 2 CH_3COOH, having a M.P. of 195° (decomp.), $[\alpha]_D^{20} = -64°$ in dimethyl formamide/1 N HCl (1:1), is obtained.

PARTIAL SEQUENCE C: *Z-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-OH*

a) *H-Thr-Tyr-Pro-OH*

176 g of Z-Tyr(Z)-Pro-OMe are dissolved in 1000 cc of methanol/1 N HCl, hydrogenation is effected in the presence of palladium charcoal at 20° and 1 atmosphere, and the solution is concentrated by evaporation. The residue is dissolved in 400 cc of dimethyl formamide, 100 cc of triethyl amine are added at 0°, the triethyl amine hydrochloride which crystallizes is filtered off, and Z-Thr-N_3 [produced from 87 g of Z-Thr-NHNH₂, see partial sequence A, a)], is added to the filtrate. The reaction mixture is subsequently allowed to stand at 0° for 16 hours, is evaporated to dryness, the residue is dissolved in ethyl acetate, the solution is successively washed with dilute hydrochloric acid and dilute ammonia, is dried and concentrated by evaporation. The residue is pulverized in heptane, and washing with petroleum ether and drying are effected. Z-Thr-Tyr-Pro-OMe, having a M.P. of 80° (decomp.), $[\alpha]_D^{20} = -23°$ in dimethyl formamide, is obtained.

53 g of Z-Thr-Tyr-Pro-OMe are dissolved in 530 cc of methanol, 100 cc of a 2 N caustic soda solution are added, the solution is allowed to stand at 25° for 1 hour, 50 cc of 2 N hydrochloric acid are added, the solution is concentrated to about 200 cc, 100 cc of water are added, the pH value of the solution is adjusted to 10, the aqueous solution is washed twice with ethyl acetate, the pH value of the solution is subsequently adjusted to 1 with 4 N hydrochloric acid, the precipitated tripeptide Z-Thr-Tyr-Pro-OH is extracted with ethyl acetate, is dried and concentrated by evaporation. M.P. 80°, $[\alpha]_D^{20} = -23°$ in dimethyl formamide. The residue is dissolved in a mixture of 500 cc of dioxane and 100 cc of water, and hydrogenation is effected at 20° and normal pressure in presence of a palladium catalyst. Filtration is effected, the filtrate is evaporated to dryness, the residue is washed with ether and dried. H-Thr-Tyr-Pro-OH, having a M.P. of 180° (decomp.), $[\alpha]_D^{20} = -31°$ in 95% acetic acid, is obtained.

b) *Z-Leu-Gln-OMe*

70 g of Z-Gln-OH are dissolved in 1.5 liters of dioxane, and a solution of diazomethane in ether is allowed to flow in until the solution remains yellow coloured. The solution is subsequently concentrated by evaporation and the white residue is treated with ether. Z-Gln-OMe, having a M.P. of 136°, $[\alpha]_D^{20} = -21°$ in dimethyl formamide, is obtained. 71 g of the ester are dissolved in 1.7 liters of methanol. 5 g of 10% palladium on active charcoal are subsequently mixed by stirring with 59 cc of 4 N hydrochloric acid, and this is added to the solution. The entire material is subjected to hydrogenation at room temperature and normal pressure for 3 hours, whereby about 83% of the theoretic amount of hydrogen are used up. The catalyst is filtered off and the filtrate is completely concentrated by evaporation, whereby H-Gln-OMe . HCl is obtained in the form of a viscous oil.

64 g of Z-Leu-OH are dissolved in 200 cc of acetonitrile, 27.7 g of N-hydroxysuccinimide are added and the solution is cooled to −10°. A solution of 50 g of

dicyclohexyl carbodiimide in 100 cc of acetonitrile is added thereto. After about 30 minutes the dicyclohexyl urea is filtered off. 47.3 g of H-Gln-OMe . HCl, dissolved in dimethyl formamide, are added to the filtrate while simultaneously adding one equivalent of N-methyl morpholine. The reaction mixture is allowed to stand for 4 hours and is concentrated by evaporation. The residue is dissolved in ethyl acetate and the solution is washed with a 5% sodium bicarbonate solution, 1 N hydrochloric acid and water. The organic phase is dried over sodium sulphate and concentrated by evaporation in a vacuum. After recrystallization from methanol and ether Z-Leu-Gln-OMe, having a M.P. of 179°, $[\alpha]_D^{20} = -16°$ in dimethyl formamide, is obtained.

c) *Z-Lys(BOC)-Leu-Gln-OMe*

42 g of Z-Leu-Gln-OMe are dissolved in 1500 cc of methanol. 16 g of 10% palladium on active charcoal are subsequently mixed by stirring with 25.8 cc of 4 N hydrochloric acid, and this is added to the solution. The entire material is subjected to hydrogenation at room temperature and normal pressure for 8 hours, whereby 95% of the theoretic amount of hydrogen are used up. The catalyst is filtered off and the filtrate concentrated by evaporation. H-Leu-Gln-OMe . HCl is obtained in the form of a foam. H-Leu-Gln-OMe . HCl is subsequently dissolved in 300 cc of dimethyl formamide, 34 g of Z-Lys(BOC)-OCP and 8.4 cc of triethyl amine are added. After shaking the reaction mixture is allowed to stand for 16 hours. The reaction mixture is subsequently concentrated by evaporation in a vacuum. The residue is treated with water and filtered off from the water. The residue is subsequently dissolved in a small amount of methanol and precipitation is effected with ether. After filtration and drying Z-Ls(BOC)-Leu-Gln-OMe, $[\alpha]_D^{20} = -21°$ in dimethyl formamide, is obtained.

d) *Z-His-Lys(BOC)-Leu-Gln-NHNH₂*

38 g of Z-Lys(BOC)-Leu-Gln-OMe are dissolved in 300 cc of dimethyl formamide, 15 g of 10% palladium charcoal are added, hydrogenation is effected at normal pressure and room temperature, and the catalyst is filtered off. Z-His-N_3, produced from 25.2 g of Z-His-$NHNH_2$, is subsequently added, the reaction mixture is allowed to stand at 20° for 5 hours, is evaporated, the residue is dissolved in ethyl acetate, the solution is washed with water, dried over sodium sulphate, evaporated, and the residue is washed with ether. Z-His-Lys(BOC)-Leu-Gln-OMe, having a M.P. of 120° (decomp.), $[\alpha]_D^{20} = -19°$ in dimethyl formamide, is obtained.

33 g of Z-His-Lys(BOC)-Leu-Gln-OMe are dissolved in 330 cc of methanol, 7.5 cc of hydrazine hydrate are added, the reaction mixture is allowed to stand at 25° for 2 days and is subsequently evaporated to dryness. The residue is subsequently dissolved in dimethyl formamide, is concentrated by evaporation and this treatment is repeated until no traces of hydrazine can be detected. The residue is washed with ether, water and ether, and drying is effected, whereby Z-His-Lys(BOC)-Leu-Gln-$NHNH_2$, having a M.P. of 199°, $[\alpha]_D^{20} = -22°$ in methanol, is obtained.

e) *Z-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-OH*

40 g of Z-His-Lys(BOC)-Leu-Gln-$NHNH_2$ are dissolved in 400 cc of dimethyl formamide, the solution is cooled to −20°, 75 cc of dioxane/2 N HCl are added and subsequently 6 cc of tert. butyl nitrite are added, the mixture is stirred at −20° for 10 minutes, 30 cc of triethyl amine and 20 g of H-Thr-Tyr-Pro-OH are added and the mixture is allowed to react at 25° for 16 hours. The reaction mixture is subsequently evaporated to dryness, the residue is washed with ether, dilute acetic acid, ether and subsequently with hot ethyl acetate. Drying is subsequently effected in a high vacuum, whereby Z-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-OH, having a M.P. of 215° (decomp.), $[\alpha]_D^{20} = -23°$ in dimethyl formamide, is obtained.

PARTIAL SEQUENCE ABC: *H-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2 . 3 CH_3COOH*

10 g of heptapeptide (partial sequence C) are dissolved in 100 cc of dimethyl formamide, the solution is concentrated by evaporation, the residue is dissolved in 100 cc of dimethyl formamide, 15 g of N-hydroxysuccinimide are added, the solution is cooled to 0°, 5 g of dicyclohexyl carbodiimide are added, the reaction mixture is allowed to react for 3 hours, the precipitated dicyclohexyl urea is filtered off, the filtrate is concentrated to 30 cc and the resulting heptapeptide oxysuccinimide ester Z-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-OSu is precipitated by the addition of ether. After washing out with ether the residue is dissolved in 100 cc of dimethyl formamide, 10 g of nonapeptide amide (partial sequence AB) are added and the mixture

is allowed to react for 16 hours. 500 cc of ethyl acetate are added and filtration is effected. The residue is dissolved in dimethyl formamide, 10 cc of acetic acid are added and precipitation is again effected with ethyl acetate. Filtration, washing with ethyl acetate and ether and drying are effected Z-His-Lys(BOC)-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2 . 2 AcOH, $[\alpha]_D^{20} = -19°$ in dimethyl formamide, is obtained and is directly dissolved in 80% acetic acid. 5 g of palladium charcoal are subsequently added and hydrogenation is effected at normal pressure and room temperature. Filtration is effected, the filtrate is evaporated to dryness at 20° in a high vacuum and the residue is washed with ether. After drying over KOH shavings partial sequence ABC, having a M.P. of 170° (decomp.), $[\alpha]_D^{20} = -53°$ in 1 N acetic acid, is obtained.

PARTIAL SEQUENCE D: *Trt-Gly-Lys(BOC)-Leu-Ser-Gln-Glu(OTB)-Leu-*$NHNH_2$

a) *Z-Glu(OTB)-Leu-OMe*

151 g of Z-Glu(OTB)-OH and 54 g of N-hydroxysuccinimide are dissolved in 700 cc of acetonitrile and the solution is cooled to −20°. 96.5 g of dicyclohexyl carbodiimide, dissolved in 350 cc of acetonitrile, are subsequently added. After allowing the solution to stand for about 30 minutes the resulting dicyclohexyl urea is filtered off. 71.2 g of H-Leu-OMe are added to the filtrate, the mixture is allowed to stand for 4 hours and is concentrated by evaporation in a vacuum. Ethyl acetate/water (2:1) is added to the solid residue. The organic phase is subsequently washed with 5% sodium bicarbonate solution, water and dilute sulphuric acid (pH 3), is washed until neutral, dried over sodium sulphate and concentrated by evaporation. The residue is dissolved in chloroform containing 1% of methanol, and filtration is effected over a silica gel column (10-fold quantity). Z-Glu(OTB)-Leu-OMe, having a M.P. of 31°, $[\alpha]_D^{20} = -12°$ in dimethyl formamide, is obtained.

b) *H-Gln-Glu(OTB)-Leu-OMe*

118 g of Z-Glu(OTB)-Leu-OMe are dissolved in 2000 cc of methanol. 15 g of 10% palladium on active charcoal are subsequently mixed by stirring in 63 cc of 4 N hydrochloric acid, and this is added to the solution. The entire material is subjected to hydrogenation at room temperature and normal pressure for 2 hours, whereby about 85% of the theoretic amount of hydrogen are used up. The catalyst is filtered off and the filtrate is completely concentrated by evaporation, whereby H-Glu(OTB)-Leu-OMe . HCl is obtained in the form of an amorphous foam.

91.7 g of Z-Gln-ONP and 93.8 g of H-Glu(OTB)-Leu-OMe . HCl are dissolved in 500 cc of dimethyl formamide, and 51 cc of N-methyl morpholine are added. The solution is allowed to stand at room temperature for 15 hours and is subsequently completely concentrated by evaporation in a vacuum. The resulting mixture is mixed well with water and is then filtered off from the water. Recrystallization from methanol is subsequently effected, whereby Z-Gln-Glu(OTB)-Leu-OMe, having a M.P. of 210° (decomp.), $[\alpha]_D^{20} = -40°$ in dimethyl formamide, is obtained.

44.4 g of Z-Gln-Glu(OTB)-Leu-OMe are dissolved in 1200 cc of methanol/dimethyl formamide (1:1). 15 g of 10% palladium on active charcoal are subsequently mixed with 50 cc of water, and this is added to the solution. After hydrogenation for about 2 hours an almost quantitative amount of hydrogen is used up. The catalyst is filtered off and the filtrate is concentrated by evaporation in a vacuum. The residue is dissolved in ethyl acetate and washed thrice with a saturated sodium chloride solution. The organic phase is dried over sodium sulphate and the filtrate is concentrated by evaporation to about one third of its original volume. After the addition of petroleum ether the solid tripeptide can be filtered off. H-Gln-Glu(OTB)-Leu-OMe, having a M.P. of 123°, $[\alpha]_D^{20} = -14°$ in dimethyl formamide, is obtained.

c) *Z-Lys(BOC)-Leu-Ser-OMe*

43 g of H-Leu-Ser-OMe . HCl and 80 of Z-Lys(BOC)-OCP are dissolved in 400 cc of dimethyl formamide, 22 cc of triethyl amine are added, the solution is allowed to stand at 25° for 25 hours, the precipitated triethyl amine is filtered off and the filtrate is evaporated to dryness. The residue is dissolved in ethyl acetate, the solution is washed with dilute hydrochloric acid and dilute potassium bicarbonate solution, drying over sodium sulphate, filtration and concentration by evaporation are effected. The residue is recrystallized from ethyl acetate/ether, whereby Z-Lys(BOC)-Leu-Ser-OMe, having a M.P. of 110°, $[\alpha]_D^{20} = -14°$ in dimethyl formamide, is obtained.

d) *Trt-Gly-Lys(BOC)-Leu-Ser-NHNH₂*

158 g of Trt-Gly-OH are dissolved in 2000 cc of dichloromethane and 72 cc of triethyl amine, the solution is cooled to −5°, 50 cc of chloroformic acid ethyl ester are added and the mixture is stirred at −5° for 10 minutes. 300 g of Z-Lys(BOC)-Leu-Ser-OMe are simultaneously dissolved in 3000 cc of dioxane/water (8:2), hydrogenation is effected at room temperature and normal pressure in the presence of palladium charcoal, filtration and evaporation are effected. The residue is dissolved in chloroform and the mixed anhydride obtained above is added to the solution. The reaction solution is allowed to stand at 0° for 1 hour, the chloroform solution is washed with dilute acetic acid and subsequently with dilute potassium bicarbonate solution, is dried and evaporated to dryness. The residue is dissolved in 1250 cc of methanol, 175 cc of hydrazine hydrate are added and the reaction mixture is allowed to stand at 25° for 16 hours. The reaction mixture is concentrated to 500 cc, water is added, filtration is effected, the residue is washed with water and dried over phosphorus pentoxide. Trt-Gly-Lys(BOC)-Leu-Ser-NHNH$_2$, having a M.P. of 204°, $[\alpha]_D^{20}=-6°$ in dimethyl formamide, is obtained.

e) *Trt-Gly-Lys(BOC)-Leu-Ser-Gln-Glu(OTB)-Leu-NHNH₂*

76 g of Trt-Gly-Lys(BOC)-Leu-Ser-NHNH$_2$ are dissolved in 300 cc of dimethyl formamide, the solution is cooled to −20°, 75 cc of dioxane/4 N HCl are added and subsequently 11.6 cc of tert. butyl nitrite are added, the mixture is stirred at −15° for 10 minutes, 70 cc of triethylamine and 49.5 g of H-Gln-Glu(OTB)-Leu-OMe are added. The reaction mixture is subsequently stirred at 0° for 16 hours. The precipitated triethyl amine hydrochloride is filtered off, the filtrate is concentrated to about 100 cc and water is added to the residue. The precipitated residue is filtered off, is then washed thoroughly with water and subsequently dried in a high vacuum at 50°. The residue is boiled with ethyl acetate, washed with ether and dried. The resulting heptapeptide ester (Trt-Gly-Lys(BOC)-Leu-Ser-Gln-Glu(OTB)-Leu-OMe, having a M.P. of 234°, $[\alpha]_D^{20}=-20°$ is dimethyl formamide, is dissolved in 200 cc of dimethyl formamide, 4 cc of hydrazine are added and the mixture is allowed to stand at 25° for 24 hours. After concentrating to about 100 cc 500 cc of water are added, the precipitated material is filtered off, washing is effected with water until a neutral reaction is obtained and drying is effected over phosphorus pentoxide at 50°. Trt-Gly-Lys(BOC)-Leu-Ser-Gln-Glu(OTB)-Leu-NHNH$_2$, having a M.P. of 265°, $[\alpha]_D^{20}=-18°$ in dimethyl formamide/water (8:2), is obtained.

PARTIAL SEQUENCE ABCD: *H-Gly-Lys(BOC)-Leu-Ser-Gln-Glu(OTB)-Leu-His - Lys(BOC) - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr - Gly - Ser-Gly-Thr-Pro-NH₂ . 3 AcOH . 3 H₂O*

3 g of heptapeptide hydrazide (partial sequence D) are dissolved in 30 cc of dimethyl formamide, the solution is cooled to −20°, 2 cc of dioxane/4 N HCl are added and subsequently 0.3 cc of tert. butyl nitrite are added. The reaction mixture is stirred at −20° for 10 minutes, 2 cc of triethyl amine and 3 g of hexadecapeptide amide (partial sequence ABC) are added, the mixture is stirred at 0° for 16 hours and is evaporated to dryness. The residue is washed at 0° with dilute acetic acid and subsequently with water, filtration is effected, the residue is dissolved in methanol/water (9:1), the solution is filtered over silica gel, the residue is evaporated, washed with ethyl acetate, dissolved in 200 cc of 80% acetic acid, the solution is allowed to stand at 25° for 4 hours, is evaporated to dryness at 20°, the residue is washed with ether, ethyl acetate and ether, filtration and drying are effected. Partial sequence ABCD, having a M.P. of 240° (decomp.), $[\alpha]_D^{20}=-38°$ in acetic acid, is obtained.

PARTIAL SEQUENCE E: *H-Thr-Cys(Bzl)-Val-Leu-OH*

a) *H-Cys(Bzl)-Val-Leu-OMe . HBr*

21.0 g of Z-Cys(Bzl)-OCP and 12.3 g of H-Val-Leu-OMe . HCl are dissolved in 120 cc of dimethyl formamide. 5.9 cc of triethyl amine are subsequently added, the reaction mixture is allowed to stand at 25° for 16 hours, ethyl acetate is added, washing with dilute hydrochloric acid, drying over sodium sulphate and evaporation to dryness are effected, and the residue is crystallized from ethyl acetate/diethyl ether. Z-Cys(Bzl)-Val-Leu-OMe, having a M.P. of 160°, $[\alpha]_D^{20}=-28°$ in dimethyl formamide, is obtained and is dissolved in 210 cc of a 40% solution of hydrogen bromide in glacial acetic acid. The solution is allowed to stand at 25° for 1 hour, is evaporated to dryness and the residue is recrystallized from isopropanol/diethyl ether. H-Cys

(Bzl)-Val-Leu-OMe . HBr, having a M.P. of 168°, $[\alpha]_D^{20} = +14°$ in dimethyl formamide, is obtained.

b) *H-Thr-Cyr(Bzl)-Val-Leu-OMe . 1.3 HBr*

20 g of Z-Thr-$NHNH_2$ are dissolved in 350 cc of dimethyl formamide, the solution is cooled to −20°, 100 cc of a solution of 2 N hydrochloric acid in dioxane are added and subsequently 10 cc of tert . butyl nitrite are added. The reaction mixture is allowed to stand at −20° for 10 minutes, whereupon 45 cc of triethyl amine and 25.5 g of H-Cys(Bzl)-Val-Leu-OMe . HBr are added and the resulting mixture is shaken at 0° for 16 hours. The reaction mixture is evaporated to dryness, the residue is dissolved in a mixture of ethyl acetate/water, the organic phase is washed with dilute hydrochloric acid, dried over sodium sulphate, concentrated by evaporation, and the residue is recrystallized from ethyl acetate. Z-Thr-Cys(Bzl)-Val-Leu-OMe, having a M.P. of 208°, $[\alpha]_D^{20} = -27°$ in dimethyl formamide, is obtained.

20 g of the tetrapeptide obtained above are dissolved in 200 cc of a mixture of trifluoroacetic acid/ethyl acetate (1:1), a stream of gaseous hydrogen bromide is passed through the solution at 0° for 1 hour, the solution is subsequently concentrated by evaporation and the residue is recrystallized from methanol/diethyl ether. H-Thr-Cys(Bzl)-Val-Leu-OMe . 1.3 HBr, having a M.P. of 202°, $[\alpha]_D^{20} = -10°$ in dimethyl formamide, is obtained.

c) *H-Thr-Cys(Bzl)-Val-Leu-OH*

372 g of H-Thr-Cys(Bzl)-Val-Leu-OMe . 1.3 HBr are dissolved in 1800 cc of methanol, 900 cc of a 2 N caustic soda solution are added, the mixture is allowed to stand at 25° for 1 hour, 240 cc of glacial acetic acid are added and the mixture is allowed to stand at 0° for 2 hours. The precipitated crystalline mass is filtered off, is washed first with 1 N acetic acid and subsequently with water, and drying is effected at 50° in a high vacuum. H-Thr-Cys(Bzl)-Val-Leu-OH, having a M.P. of 219°, $[\alpha]_D^{20} = -53°$ in 1 N ammonia, is obtained.

PARTIAL SEQUENCE F: *BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-$NHNH_2$*

a) *H-Asn-Leu-Ser-OMe . HCl*

43 g of H-Leu-Ser-OMe . HCl and 53 g of BOC-Asn-ONP are dissolved in 400 cc of dimethyl formamide, 22 cc of triethyl amine are added, the solution is allowed to stand at 25° for 16 hours, is evaporated to dryness and the residue is recrystallized from methanol. BOC-Asn-Leu-Ser-OMe, having a M.P. of 190°, $[\alpha]_D^{20} = -24°$ in dimethyl formamide, is obtained and is dissolved in 500 cc of a 4 N solution of hydrochloric acid in methanol. The solution is allowed to stand at 25° for 1 hour, is evaporated to dryness, the residue is dissolved in methanol and precipitation is effected with diethyl ether. H-Asn-Leu-Ser-OMe . HCl, having a M.P. of 180°, $[\alpha]_D^{20} = -23°$ in dimethyl formamide, is obtained.

b) *H-Ser-Asn-Leu-Ser-OMe . HCl*

39.5 g of BOC-Ser-$NHNH_2$ are dissolved in 500 cc of dimethyl formamide, the solution is cooled to −20°, 200 cc of a 2 N solution of hydrochloric acid in dioxane are added and subsequently 20 cc of tert . butyl nitrite are added. After allowing the reaction mixture to stand at −20° for 10 minutes 90 cc of triethyl amine and 38.0 g of H-Asn-Leu-Ser-OMe . HCl are added, the mixture is stirred at 0° for 16 hours, is evaporated to dryness, and the residue is recrystallized from chloroform/diethyl ether. BOC-Ser-Asn-Leu-Ser-OMe, having a M.P. of 135°, $[\alpha]_D^{20} = -22°$ in dimethyl formamide, is obtained and is dissolved in 420 cc of a 4 N solution of hydrochloric acid in methanol. The solution is allowed to stand at 25° for 1 hour, is evaporated to dryness, and the residue is recrystallized from methanol/ethyl acetate. H-Ser-Asn-Leu-Ser-OMe . HCl, having a M.P. of 155° (decomp.), $[\alpha]_D^{20} = -15°$ in dimethyl formamide, is obtained.

c) *BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-$NHNH_2$*

18.5 g of H-Ser-Asn-Leu-Ser-OMe . HCl and 18 of BOC-Cys(Bzl)-ONP are dissolved in 100 cc of dimethyl formamide, 10 cc of water, 3.5 cc of acetic acid and 5.6 cc of triethyl amine are added, the reaction mixture is allowed to stand at 25° for 16 hours, is evaporated to dryness and the residue is recrystallized from methanol. BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-OMe, having a M.P. of 182°, $[\alpha]_D^{20} = -17°$ in dimethyl formamide, is obtained and is dissolved in 200 cc of dimethyl formamide with slight heating. 200 cc of methanol and 20 cc of hydrazine hydrate are added, the

mixture is allowed to stand at 30° for 16 hours, precipitation is effected with diethyl ether, the precipitate is washed with diethyl ether/methanol (1:1) and the resulting BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-$NHNH_2$, having a M.P. of 224°, $[\alpha]_D^{20} = -13°$ in dimethyl formamide, is dried.

PARTIAL SEQUENCE EF: *BOC-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-OH*

18.4 g of BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-$NHNH_2$ (partial sequence F) are dissolved in 150 cc of dimethyl formamide, the solution is cooled to −20°, and 40 cc of a 2 N solution of hydrochloric acid in dioxane and 15 cc of tert. butyl nitrite are added. After allowing the reaction mixture to stand at −20° for 10 minutes, 28 cc of triethyl amine and 16.2 g of H-Thr-Cys(Bzl)-Val-Leu-OH (partial sequence E) are added and the mixture is stirred at 25° for 16 hours. Filtration is subsequently effected, the solution is concentrated by evaporation and the residue washed thoroughly with 1 N acetic acid. BOC-Cys(Bzl)-Ser-Asn-Leu-Ser-Thr-Cys(Bzl)-Val-Leu-OH, having a M.P. of 217°, $[\alpha]_D^{20} = -17°$ in dimethyl formamide, is obtained.

The resulting product is dissolved in 5000 cc of dried ammonia, sodium metal is added with stirring and while the ammonia boils, until a dark blue colouration is obtained. Ammonium chloride is added for purposes of decolouration. The solution is evaporated to dryness and the residue is washed with 1 N acetic acid and acetone. After drying BOC-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-OH, having a M.P. of 248° (decomp.), $[\alpha]_D^{20} = -41°$ in dimethyl formamide/water (3:1), is obtained.

The resulting nonapeptide is dissolved in 5000 cc of 0.01 N ammonia, 1 N hydrogen peroxide is added with stirring, until a negative nitroprussiate reaction is obtained, 200 cc of glacial acetic acid are added, filtration and lyophilization is effected.

BOC-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-OH,

having a M.P. of 238° (decomp.), $[\alpha]_D^{20} = -18°$ in dimethyl formamide/water (3:1), is obtained.

Having regard to section 9 of the Patents Act 1949, attention is direction to our copending patent application No. 24779/70 (Serial No. 1314051) out of which the present application has been divided.

WHAT WE CLAIM IS:—

1. A process for the production of a compound of general formula I,

H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2. I

comprising joining two peptide units by an amide linkage, the peptide units being such that when joined the amino acid sequence of the compound of formula I is achieved.

2. A process according to Claim 1, wherein one of the peptide units incorporates the group of formula II,

H-Cys-Ser-Asn-Leu-Ser-Thr-Cys- II

wherein an S—S bridge exists between the L-cysteinyl units.

3. A process according to Claim 2, wherein in the production of the peptide unit incorporating the group of formula II, the S—S bridge is formed during the last stage.

4. A process for the production of the compound of formula I, defined in Claim 1, substantially as described herein with reference to the Example.

5. H - Cys - Ser - Asn - Leu - Ser - Thr - Cys - Val - Leu - Gly - Lys - Leu - Ser - Gln - Glu - Leu - His - Lys - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr-Gly-Ser-Gly-Thr-Pro-NH_2, whenever produced by the process of any one of the preceding claims.

6. H - Cys - Ser - Asn - Leu - Ser - Thr - Cys - Val - Leu - Gly - Lys - Leu - Ser - Gln - Glu - Leu - His - Lys - Leu - Gln - Thr - Tyr - Pro - Arg - Thr - Asn - Thr-Gly-Ser-Gly-Thr-Pro-NH_2.

7. A compound according to either of Claims 5 or 6 in pharmaceutically acceptable acid addition salt or heavy metal complex form.

8. A pharmaceutical composition comprising a compound of formula I, defined in Claim 1, in free form or in pharmaceutically acceptable acid addition salt or heavy metal complex form as active agent, in association with a pharmaceutical diluent or carrier.

9. A pharmaceutical composition according to Claim 8, in unit dosage form comprising from 2 to 700 MRC units of active agent.

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