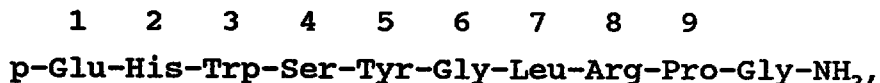


The present invention relates to novel peptides which inhibit the release of gonadotropins by the pituitary gland in mammals without inducing oedematous reactions. In particular, the present invention relates to analogues of the luteinizing hormone releasing hormone (LHRH), which has the following structure:



and salts thereof, and to pharmaceutical compounds and methods of use pertaining to these analogues.

For more than 15 years, researchers have been looking for selectively potent antagonists of the LHRH decapeptide [M. Karten and J.E. Rivier, *Endocrine Reviews*, 7, 44-66 (1986)]. The great interest in such antagonists is based on their usefulness in the fields of endocrinology, gynaecology, contraception and cancer. A large number of compounds have been prepared as potential LHRH antagonists. The most interesting antagonists found to date are compounds whose structure is a modification of the LHRH structure.

The first series of potent antagonists was obtained by introducing aromatic amino acid residues in the 1-, 2-, 3- and 6-positions or 2-, 3- and 6-positions. The compounds are described as LHRH antagonists obtained by the replacement of the original amino acid residues with others in the position indicated by the superscript numbers. The known antagonists include:

[Ac-D-Phe(4-Cl)<sup>1,2</sup>, D-Trp<sup>3,6</sup>]LHRH (D.H. Coy et al., in: Gross, E. and Meienhofer, J. (eds), *Peptides, Proceedings of the 6th American Peptide Symposium*, pp. 775-779, Pierce Chem. Co., Rockville, Il., 1979);

[Ac-Pro<sup>1</sup>,D-Phe(4-Cl)<sup>2</sup>,D-Nal(2)<sup>3,6</sup>]LHRH (US patent no. 4,419,347); and

[Ac-<sup>3</sup>-Pro<sup>1</sup>,D-Phe(4-Cl)<sup>2</sup>,D-Trp<sup>3,6</sup>]LHRH  
(J.L. Pineda et al., J. Clin. Endocrinol. Metab. 56, 420, 1983).

The water solubility of the antagonists was later increased by introducing basic amino acids, such as D-Arg, in the 6-position, examples being

[Ac-D-Phe(4-Cl)<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]LHRH (ORG-30276) (D.H. Coy et al., Endocrinology, 100, 1445, 1982); and  
[Ac-D-Nal(2)<sup>1</sup>,D-Phe(4-F)<sup>2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>]LHRH (ORF 18260) (J.E. Rivier et al., in: Vickery B.H., Nestor, Jr. J.J., Hafez, E.S.E. (eds), LHRH and Its Analogs, pp. 11-22, MTP Press, Lancaster, UK, 1984).

Such water-soluble LHRH antagonists with a basic amino acid residue in the 6-position are also the subject of European patent application 81 877, the basic amino acids being D-Lys, D-Arg, D-Orn, D-homo-Arg or D-His. Also, Zeitschrift für Naturforschung, volume 426, 1987, pages 101 - 106, describes LHRH antagonists with a basic amino acid residue in the 6-position and preferably a basic amino acid residue derived from D-3-Pal in the 3-position.

These analogues not only have the expected improved water solubility but also showed an improved antagonistic activity. However, these extremely potent, hydrophilic analogues with D-Arg and other basic side chains in the 6-position cause transient oedema of the face and extremities when administered subcutaneously to rats in doses of 1.25 or 1.5 mg/kg (F. Schmidt et al., Contraception, 29, 283, 1984; J.E. Morgan et al., Int. Archs. Allergy Appl. Immun. 80, 70 (1986)). Since the occurrence of oedematogenic effects after

administration of these antagonists to rats cast doubts on their safety for use in humans and thus delayed the introduction of these drugs into clinical use, it is desirable to provide antagonistic peptides which are free of these side effects.

The present invention relates to LHRH antagonists which have an improved water solubility and a high antagonistic potency of the basic peptides and are free of oedematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins by the pituitary gland in mammals, including humans.

The compounds of the present invention have formula I:



wherein

X is an acyl group consisting of linear or branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms, or a carbamoyl group,

R<sup>1</sup> is D- or L-Pro, D- or L-Δ<sup>3</sup>-Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),

R<sup>2</sup> is D-Phe or D-Phe(4-H1),

R<sup>3</sup> is D-Trp, D-Phe, D-Pal(3), D-Nal(1) or D-Nal(2),

R<sup>6</sup> is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

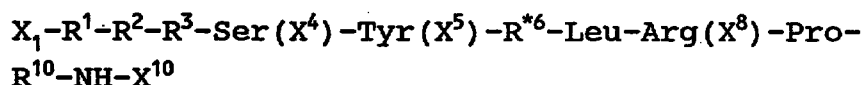
R<sup>10</sup> is Gly or D-Ala,

H1 being fluorine, chlorine or bromine and Q being C<sub>1</sub>-C<sub>3</sub>-alkyl,

and pharmaceutically acceptable acid addition salts thereof.

The compounds of formula I are synthesized by the known methods, for example purely by the solid-phase technique, partially by the solid-phase technique or by the classical coupling reactions in solution. The compounds of formula I are preferably prepared by a known solid-phase technique.

Such a method provides intermediate peptides or intermediate peptide resins of formula II:



II

wherein  $X_1$  is an acyl group based on linear and branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms (especially acetyl), t-Boc, hydrogen or carbamoyl,

$X^4$  is a protecting group for the Ser hydroxyl group (for example benzyl, 2,6-dichlorobenzyl),

$X^5$  is a protecting group for the Tyr phenolic hydroxyl group (for example benzyl, 2,6-dichlorobenzyl, 2-chloro(2-bromo)-benzyloxycarbonyl, benzyloxycarbonyl),

$X^8$  is a protecting group for the Arg guanidino group ( $NO_2$ , Tos),

$X^{10}$  is a carrier resin containing benzhydryl or methylbenzhydryl groups,

$R^1$  is D- or L-Pro, D- or L- $\Delta^3$ -Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),

$R^2$  is D-Phe or D-Phe(4-H1),

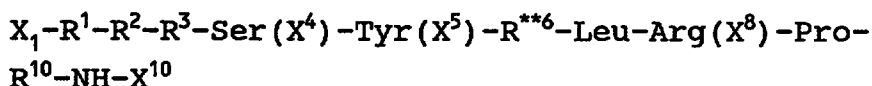
$R^3$  is D-Trp, D-Phe, D-Pal(3), D-Nal(1) or D-Nal(2),

$R^{*6}$  is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

$R^{10}$  is Gly or D-Ala,

H1 being fluorine, chlorine or bromine.

One procedure consists for example in reacting a peptide of formula II wherein  $R^{*6}$  is D-Lys or D-Orn and  $X^6$  is hydrogen with a cyanate or a cyanate-providing compound (cyanate source) to give a peptide of formula III:



III

wherein  $X_1$ ,  $R^1$ ,  $R^2$ ,  $R^3$ ,  $X^4$ ,  $X^5$ ,  $X^8$ ,  $R^{10}$  and  $X^{10}$  are as defined and  $R^{*6}$  is Cit, Hci, Cit(Q) or Hci(Q). This reaction is preferably carried out when  $X_1$  is acyl and all the other radicals X are hydrogen.

Suitable cyanates or cyanate-providing compounds are: alkali metal cyanates (for example potassium cyanate) and N-lower alkyl isocyanates (N-ethyl isocyanate). The peptides of formula II are preferably synthesized by the known solid-phase technique. The reaction with cyanates or cyanate-providing compounds is carried out in solvents, preferably aprotic solvents such as aqueous DMF, at temperatures of between 0 and 50°C, preferably of between 0 and 25°C.

The progress of this reaction, i.e. the conversion of Orn/Lys peptides to the corresponding Cit/Hci peptides, can easily be followed by HPLC in MeCN/aqueous TFA systems by virtue of a characteristic increase of  $2.6 \pm 0.3$  minutes in the retention time of Cit/Hci and for example Cit(ethyl)/Hci(ethyl) peptides compared with the corresponding Orn/Lys peptides.

Peptides of formula I wherein X is an acyl group are obtained directly by cleavage and deprotection of the intermediate peptide resins of formula II wherein  $X_1$  is an acyl group and  $R^{*6}$  is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q). Peptides of formula I wherein X is a carbamoyl group are obtained from peptide resins of formula II wherein  $X_1$  is hydrogen or Boc by cleavage and deprotection followed by carbamoylation.

A gonadotropin-antagonizing pharmaceutical composition is provided by mixing the compound of formula I with a pharmaceutically acceptable excipient, including microspheres for delayed release.

There is also provided a method of alleviating complications arising from the physiological availability, from the

pituitary gland in mammals, of amounts of gonadotropins in excess of the desired amount, which involves administering to a mammal a gonadotropin-antagonizing dose of the compound of formula I.

The nomenclature used to define the peptides conforms to that specified by the IUPAC-IUB Commission on Biochemical Nomenclature (European J. Biochem., 1984, 138, 9-37), wherein, in accordance with the customary representation, the amino groups at the N terminus are shown on the left and the carboxyl group at the C terminus is shown on the right. The expression "natural amino acids" is generally used to mean naturally occurring amino acids found in proteins, which comprise Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met, Phe, Tyr, Pro, Trp and His. The abbreviations for the individual amino acid residues are based on the trivial name of the amino acid and are Ala, alanine; Arg, arginine; Cit, citrulline; Gly, glycine; Hci, homocitrulline; Leu, leucine; Lys, lysine; Pal(3), 3-(pyrid-3-yl)alanine; Nal(2), 3-(naphth-2-yl)alanine; Nal(1), 3-(naphth-1-yl)alanine; Orn, ornithine; Phe, phenylalanine; Phe(4-Cl), 4-chlorophenylalanine; Phe(4-F), 4-fluorophenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; and Tyr, tyrosine. All the amino acids described here are derived from the L series, unless stated otherwise; for example, D-Trp is D-tryptophan and D-Nal(2) is 3-(naphth-2-yl)-D-alanine. D-Cit(Q) or D-Hci(Q) indicates that one H atom of the amino group of the ureido moiety of the citrulline (homocitrulline) is substituted by the alkyl group Q.

Other abbreviations used are:

|                   |                      |
|-------------------|----------------------|
| AcOH              | acetic acid          |
| AcOEt             | ethyl acetate        |
| Ac <sub>2</sub> O | acetic anhydride     |
| Boc-              | tert-butoxycarbonyl- |

|         |                                     |
|---------|-------------------------------------|
| DIC     | diisopropylcarbodiimide             |
| DIEA    | N,N-diisopropylethylamine           |
| DMF     | dimethylformamide                   |
| HOBt    | 1-hydroxybenzenetriazole hydrate    |
| HPLC    | high pressure liquid chromatography |
| MeOH    | methyl alcohol                      |
| TEA     | triethylamine                       |
| DCC     | dicyclohexylcarbodiimide            |
| MeCN    | acetonitrile                        |
| IpOH    | isopropanol                         |
| Z(2-Cl) | 2-chlorobenzyloxycarbonyl           |
| DCB     | 2,6-dichlorobenzyl                  |
| Tos     | p-toluenesulphonyl                  |
| TFA     | trifluoroacetic acid                |
| Z       | benzyloxycarbonyl                   |

Particularly preferred LHRH analogues of formula I are those wherein:

X is acetyl or carbamoyl,  
 $R^1$  is D-Phe(4-Cl), D-Nal(2), Pro or D-Phe,  
 $R^2$  is D-Phe(4-Cl) or D-Phe(4-F),  
 $R^3$  is D-Trp or D-Pal(3),  
 $R^6$  is D-Cit, D-Hci, D-Cit(ethyl) or D-Hci(ethyl) and  
 $R^{10}$  is D-Ala.

The peptides according to the invention are prepared by fragment condensation of corresponding peptide fragments, as conventionally used in peptide chemistry, or by stepwise synthesis as conventionally used in peptide chemistry, or by converting the free amino group of D-Orn or D-Lys, in a peptide of formula I wherein  $R^6$  is D-Orn or D-Lys, to the ureido group by reaction with an alkali metal cyanate or a cyanate-providing compound or a  $C_1$ - $C_3$ -alkyl isocyanate, and optionally the peptides obtained are acylated and/or

converted to the amides and optionally converted to physiologically acceptable acid salts.

The stepwise synthesis is carried out for example by a procedure in which firstly the carboxy-terminal amino acid glycine or D-alanine or the amides thereof, whose amino group in the  $\alpha$ -position is protected, is covalently bonded to a synthetic carrier conventionally used for this purpose, the  $\alpha$ -amino protecting group is cleaved, the next protected amino acid is bonded via its carboxyl group to the free amino group obtained in this way, the  $\alpha$ -amino protecting group of this second amino acid is then cleaved in turn, the next amino acid is bonded thereto and the remaining amino acids of the peptide to be synthesized are linked stepwise in this way in the correct sequence and, after all the amino acids have been linked, the finished peptide according to Claim 1 is cleaved from the carrier and optionally other side-group protecting groups present are cleaved.

For the fragment coupling reaction, it is preferable to use the azide coupling reaction, which proceeds without racemization, or the dicyclohexylcarbodiimide/1-hydroxybenzotriazole or DCC/3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine method. It is also possible to use activated esters of fragments. (DCC = dicyclohexylcarbodiimide)

Activated esters of benzyloxycarbonylamino acids, for example N-hydroxysuccinimide esters or 2,4,5-trichlorophenyl esters and 4-nitrophenyl esters, are particularly suitable for the stepwise condensation of amino acids. The aminolysis of the last two active esters can be catalyzed very well by N-hydroxyl compounds possessing approximately the acidity of acetic acid, for example 1-hydroxybenzotriazole.

Possible intermediate amino protecting groups are groups which can be cleaved by hydrogenation, for example the

benzyloxycarbonyl radical (= Z radical), or groups which can be cleaved with weak acid, for example the 2-(p-diphenyl)-isopropoxycarbonyl or 2-(3,5-dimethoxyphenyl)-isopropoxycarbonyl radical.

The stepwise condensation reaction is carried out in customary manner by synthesis from the corresponding amino acids, which are optionally protected in conventional manner. It is also possible to use automatic peptide synthesizers, for example the Beckman model 990 peptide synthesizer, with the protected amino acids commercially available in conventional manner.

This synthesis is carried out for example in the manner conventionally used for this purpose, from the corresponding protected amino acids, by a procedure in which firstly the carboxy-terminal amino acid of the peptide to be synthesized, whose amino group in the  $\alpha$ -position is protected, is covalently bonded to a synthetic carrier conventionally used for this purpose, the  $\alpha$ -amino protecting group is cleaved, the next protected amino acid is bonded via its carboxyl group to the free amino group obtained in this way, the  $\alpha$ -amino protecting group of this second amino acid is then cleaved in turn, the next amino acid is bonded thereto and the remaining amino acids of the peptide to be synthesized are linked stepwise in this way in the correct sequence and, after all the amino acids have been linked, the finished peptide is cleaved from the carrier and optionally other side-group protecting groups present are cleaved.

The reaction for linking the amino acids take place in the temperature range of 10 - 40°C, preferably 20 - 30°C, optionally in an inert solvent or suspending agent conventionally used for this purpose (for example dichloromethane), it optionally being possible to add up to 20% of dimethylformamide in order to improve the solubility.

Suitable synthetic carrier materials are synthetic polymers, for example swellable polystyrene resin in bead form (such as a chloromethylated copolymer of polystyrene and 1% of divinylbenzene). Examples of suitable protecting groups for the amino groups in the  $\alpha$ -position are the tertiary butoxycarbonyl group, carbobenzoxy group or carbobenzthio group (optionally with a p-bromo- or p-nitro-benzyl radical in each case), trifluoroacetyl group, phthalyl radical, o-nitrophenoxycetyl group, trityl group, p-toluenesulphonyl group, benzyl group, benzyl radicals substituted in the benzene ring (p-bromo- or p-nitro-benzyl radical) and  $\alpha$ -phenylethyl radical. Reference is also made in this connection to the book by Jesse P. Greenstein and Milton Winitz, *Chemistry of Amino Acids*, New York 1961, John Wiley and Sons, Inc., volume 2, for example page 883 *et seq.*, and *The Peptides*, volume 2, ed. E. Gross and J. Meienhofer, Academic Press, New York, Tables III and IV. In principle, these protecting groups are also suitable for protecting other functional side groups (OH groups,  $\text{NH}_2$  groups) of the relevant amino acids.

Hydroxyl groups present (serine, threonine) are preferably protected by benzyl groups and similar groups. Other amino groups not in the  $\alpha$ -position (for example amino groups in the w-position; guanidino group of arginine) are preferably protected by nitro groups.

The individual amino acids are linked together by the methods conventionally used for this purpose, the following being particularly suitable:

- symmetrical anhydride method (in the presence of dicyclohexylcarbodiimide),
- carbodiimide method and
- carbodiimide/hydroxybenzotriazole method (see *The Peptides*, volume 2, ed. E. Gross and J. Meienhofer).

The carbodiimide method is preferably used for linking arginine and the carbodiimide/hydroxybenzotriazole method is preferably used for linking asparagine and glutamine (see *The Peptides*, volume 2, ed. E. Gross and J. Meienhofer). The symmetrical or mixed anhydride method is generally used for the other amino acids.

Peptides according to Claim 1 wherein  $R^6$  is D-Orn or D-Lys (starting materials for the reaction with cyanate) can be prepared for example by the stepwise peptide synthesis indicated or by fragment condensation.

When peptides according to the invention are prepared wherein  $R^6$  is Cit(Q) or Hci(Q), the peptide synthesis is generally carried out using the corresponding Cit or Hci substituted on the terminal ureido group by the alkyl group Q.

The conversion of the peptides I to their acid addition salts can be effected by reacting said peptides with acids in a manner known *per se*. Conversely, the free peptides can be liberated by reacting their acid addition salts with bases.

Thus the preparative methods are for example exclusively solid-phase techniques, partially solid-phase techniques, fragment condensation or classical solution-phase synthesis. (See M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984.)

For example, the methods of exclusively solid-phase synthesis are described in the textbook "Solid Phase Peptide Synthesis", J.M. Stewart and J.D. Young, Pierce Chem. Company, Rockford, Ill., 1984 (2nd edition), and by G. Barany and R.B. Merrifield, "The Peptides", ch. 1, pp. 1-285, 1979, Academic Press, Inc.

Classical solution synthesis is described in detail in the treatise "Methoden der Organischen Chemie (Methods of Organic Chemistry) (Houben-Weyl), Synthese von Peptiden (Peptide Synthesis)", E. Wunsch (editor) (1974), Georg Thieme Verlag, Stuttgart, Germany.

One common feature of such syntheses is that the reactive side-chain functional groups of the various amino acid residues are protected by suitable protecting groups which will prevent a chemical reaction from occurring at this site until the group is ultimately removed. In general, another common feature of such syntheses is the protection of an alpha-amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, after which the alpha-amino protecting groups are selectively removed in order to allow the subsequent reaction to take place at this site. Accordingly, yet another common feature of such syntheses is to prepare, as a synthesis step, an intermediate which comprises each of the amino acid residues and is located in its desired sequence in the peptide chain, the side-chain protecting groups being attached to the appropriate residues.

The methods of the present invention can be carried out using a large number of support phases. These support phases can be for example resins such as benzhydrylamine resins (appropriately 2% crosslinked), p-methylbenzhydrylamine resins (appropriately 2% crosslinked) or the like.

In formula II:

$R^1$ ,  $R^2$  and  $R^3$  are described in greater detail above.

$X_1$  is hydrogen or an acyl group derived from linear or branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms, or an alpha-amino protecting group. The alpha-amino protecting groups provided by  $X_1$  are

those which are well known from the state of the art for the stepwise synthesis of polypeptides. Fluorenylmethoxycarbonyl (Fmoc) or t-butoxycarbonyl (Boc) may be mentioned among the classes of alpha-amino protecting groups which can be used as  $X_1$ .

$X^4$  can be an appropriate protecting group for the hydroxyl group of Ser, for example benzyl (Bzl) and 2,6-dichlorobenzyl (DCB). The preferred protecting group is Bzl.

$X^5$  can be an appropriate protecting group for the phenolic hydroxyl group of Tyr, for example Bzl, 2-Br-Z and 2,6-dichlorobenzyl (DCB). The preferred protecting group is DCB.

$X^6$  is an appropriate protecting group for the side-chain amino group of Lys or Orn. Examples of appropriate side-chain amino protecting groups are benzyloxycarbonyl (Z) and 2-chlorobenzyloxycarbonyl [Z(2-Cl)].

$X^8$  is an appropriate protecting group for the guanidino group of Arg, for example nitro, Tos, methyl(t-butylbenz)sulphonyl or 4-methoxy-2,3,6-trimethylbenzsulphonyl; the preferred group is Tos.

$X^{10}$  is a benzhydryl or methylbenzhydryl group protecting the amide group, which is incorporated in a carrier resin conventionally used for this purpose (for example 98% styrene/2% divinylbenzene copolymer containing benzhydrylamine or methylbenzhydrylamine groups).

The choice of side-chain amino protecting group is not critical, although it is general practice to select one which is not removed in the course of removal of the alpha-amino protecting groups during the synthesis.

The peptides of formula I are prepared from intermediate peptide resins of formula II by methods known from the state of the art.

The solid-phase synthesis of the intermediate peptide resins of formula II is carried out essentially as described by Merrifield, J. Am. Chem. Soc., 85, p. 2149 (1963). Solid-phase synthesis begins at the C-terminal end of the peptide by coupling a protected  $\alpha$ -amino acid with an appropriate resin. Such a starting material can be prepared by attaching  $\alpha$ -amino-protected Gly or D-Ala to a benzhydrylamine resin by means of an amide bond. Such carrier resins are generally available commercially and are generally used when the desired polypeptide to be synthesized has an  $\alpha$ -carboxamide at the C terminus.

In one embodiment of the synthesis, the primary amino group of Gly or D-Ala is protected by a t-butoxycarbonylating reagent and the coupling reaction is carried out using any one of the known dialkylcarbodiimide coupling methods.

The choice of an appropriate coupling reagent is known to those skilled in the art. A particularly appropriate coupling reagent is N,N'-diisopropylcarbodiimide (DIC).

Activating reagents and their use in peptide coupling are described in M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984.

Each protected amino acid or amino acid sequence is introduced into the solid-phase reactor in approximately two-fold excess and the coupling reaction can be carried out in a medium of DMF:CH<sub>2</sub>Cl<sub>2</sub> (1:1) or in CH<sub>2</sub>Cl<sub>2</sub> alone. In cases where incomplete coupling occurs, the coupling process is repeated before the alpha-amino protecting group is removed prior to coupling with the next amino acid. The success of the

coupling reaction at each stage of the synthesis is preferably checked by the ninhydrin reaction, as described by E. Kaiser et al., Anal. Biochem., **34**, 595 (1970).

After the desired amino acid sequence of the peptide resins of formula II has been completed, the terminal Boc group is removed and, if desired, N-terminal acylation is carried out using an appropriate acyl anhydride or acid chloride in 50-fold excess in a halogenated hydrocarbon solvent, appropriately acetic anhydride in dichloromethane for 30 minutes. The intermediate peptide can be removed from the carrier resin by treatment with a reagent, for example liquid hydrogen fluoride, which not only cleaves the peptide from the resin but also cleaves all other side-chain protecting groups  $X^4$ ,  $X^5$ ,  $X^8$ ,  $X^{10}$  and, if present,  $X^6$ .

If hydrogen fluoride is used as the cleaving reagent, anisole or m-cresol or, if desired, methyl ethyl sulphide is present as a scavenger in the reaction tube.

The conversion of peptides II wherein  $R^6$  is D-Lys or D-Orn and  $X^6$  is hydrogen to peptides I by means of cyanate is described on the preceding pages.

When acylation is omitted, treatment of peptide resins of formula II with hydrogen fluoride yields decapeptides which have free omega-amino and/or alpha-amino groups and have formula II wherein  $X_1$ ,  $X^4$ ,  $X^5$ ,  $X^8$ ,  $X^{10}$  and, if present,  $X^6$  are hydrogen. These free peptides are converted to peptides of formula I wherein X is a carbamoyl group by treatment with cyanate, appropriately an alkali metal cyanate and preferably potassium cyanate. The last-mentioned reaction, i.e. the conversion of  $H_2N$  to  $H_2N-CO-NH$  at the amino terminus of the peptides and the conversion of Orn/Lys residues to Cit/Hci residues, can easily be observed by HPLC using MeCN/aqueous TFA systems by virtue of a characteristic increase of 2-3

minutes in the retention times of the carbamoylated peptides, i.e. compounds containing the  $H_2N-CO-NH$  group, compared with their related compounds containing the  $H_2N$  group.

As a preferable alternative, peptides I wherein X = acyl or carbamoyl are obtained directly from intermediate peptide resins of formula II wherein  $X_1$  is acyl or carbamoyl and  $R^{*6}$  is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) by cleavage and the removal of protecting groups.

Although an exclusively solid-phase synthesis and a partially solid-phase synthesis of the compounds of formula I have been described here, the compounds can also be prepared by means of classical solution-phase methods.

The synthetic peptides prepared as described in the Examples are compared with two of the most potent LHRH antagonists recently described, namely [Ac-D-Phe(4-Cl)<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]LHRH (ORG-30276) (Coy et al., Endocrinology, 100, 1445, 1982) and [Ac-D-Nal(2)<sup>1</sup>, D-Phe(4-F)<sup>2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>]LHRH (ORF 18260) (Rivier et al., in: Vickery, B.H., Nestor, Jr., J.J., Hafez, E.S.E. (eds), "LHRH and Its Analogs", pp. 11-22, MTP Press, Lancaster, UK, 1984), and it was found that they exhibited similarly high inhibitory effects both *in vitro* and *in vivo*, but that, in contrast to the control peptides, they showed no oedematous effects *in vivo*.

The hormonal activities *in vitro* were compared with superfused cell systems of the rat pituitary gland (S. Vigh and A.V. Schally, Peptides, 5th suppl. 1: 241- 247, 1984), in which the activity of LHRH (and other releasing hormones) can be accurately established since the amount of LH (or other pituitary hormones) secreted into the effluent medium is not only proportional to the hormone-releasing potency of the peptides used, but also easily measurable with the aid of well-described radioimmunoassays.

To determine the potency of an LHRH antagonist, mixtures containing the LHRH in a constant concentration (usually 1 nM) and the antagonist in varying concentrations are used for the superfusion in order to determine the molecular ratio of antagonist to LHRH at which the action of the LHRH is completely blocked. These ratios are approximately 5 for both peptides of the present invention and the reference peptides when the cell system of the rat pituitary gland is preincubated with antagonists for 9 minutes.

In an *in vivo* antiovation test (A. Corbin and C.W. Beattie, *Endocr. Res. Commun.* 2, 1-23, 1975; D.H. Coy et al., *Endocrinology*, 100, 1445, 1982), it was again found that the peptides according to the invention have approximately the same activity as the control antagonist, i.e. 87.5 - 100% inhibition of ovulation can be observed for each peptide with a subcutaneously administered dose of 1-3 µg/rat.

In the oedema test of Schmidt et al. (*Contraception*, 29, 283-289, 1984), however, a marked difference could be found between the control peptides and the peptides according to the invention. When administered subcutaneously to rats in doses of 1.25 or 1.5 mg/kg, the control peptides induce oedema of the face and extremities. No such reaction could be observed when the peptides according to the invention were administered subcutaneously in a dose of 1.5 mg/kg.

In the tests performed, the rats were divided up into three groups of five rats per group and per compound tested. A comparison was made with a known compound from the state of the art having the designation ORG 30276 (N-Ac-D-p-Cl-Phe<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>).

The groups were injected subcutaneously once a day on two consecutive days with LHRH antagonists in a dose of

1.5 mg/kg. A control group was injected with diluent only. The rats were observed for 5 hours per day. The responses of the rats were classified as follows: NR no apparent response;

PR partial response: oedema in the nasal and paranasal areas;  
FR full response: facial oedema with oedematous extremities.

The results are collated in Table 1 below.

Table 1

| LHRH antagonist | 1st day |    |    | 2nd day |    |    |
|-----------------|---------|----|----|---------|----|----|
|                 | NR      | PR | FR | NR      | PR | FR |
| ORG 30276       | 3       | 7  | 0  | 0       | 0  | 10 |
| Control         | 9       | 0  | 0  | 9       | 0  | 0  |
| Example I       | 8       | 0  | 0  | 8       | 0* | 0  |
| Example III     | 8       | 0  | 0  | 8       | 0  | 0  |
| Example IV      | 9       | 0  | 0  | 9       | 0  | 0  |
| Example V       | 9       | 0  | 0  | 8       | 1* | 0  |
| Example XX      | 9       | 0  | 0  | 8       | 1* | 0  |
| Example XXI     | 9       | 0  | 0  | 9       | 0  | 0  |
| Example XXVI    | 8       | 0  | 0  | 8       | 0  | 0  |
| Example XXVII   | 9       | 0  | 0  | 9       | 0  | 0  |

\* very slight facial oedema

All the peptides shown are fully active in inhibiting LHRH secretion at fairly reasonable concentrations *in vitro*, although most are somewhat less potent than the present *in vitro* standards; however, these peptides are much more potent *in vivo*.

This was shown in a test on the release of histamine *in vitro* from peritoneal mast cells, which was carried out in

accordance with the method of Morgan et al. (Int. Archs. Allergy appl. Immun. 80, 70, 1986).

#### Release of histamine in vitro

In this test, rats were anaesthetized with ether and peritoneal exudate cells were harvested by washing with 12 ml of mast cell medium (MCM) (150 mM NaCl; 3 mM KCl; 3.0 mM  $\text{Na}_2\text{HPO}_4$ ; 3.5 mM  $\text{KH}_2\text{PO}_4$ ; 0.98 mM CaCl; 5.6 mM dextrose; 0.1% bovine serum albumin; 0.1% gelatin and 10 U/ml heparin) [9]. Cells from 4 or 5 rats were pooled, centrifuged at 120 g and resuspended with MCM to a concentration of  $0.5 \times 10^6$  ml and 1 ml aliquots were placed in 12 x 75 mm polyethylene tubes. The tubes were equilibrated for 15 min at 37°C and incubated alone (background release of histamine), with 48/80 (positive control) (Sigma Chemicals, St. Louis, Mo.) or with appropriate concentrations (1 ng to 10  $\mu\text{g/ml}$ ) of LHRH antagonists for 60 min. The reaction was terminated by cooling the tubes to 4°C. The tubes were centrifuged; the supernatants were removed and stored at -20°C until they were tested for histamine. The tests were performed in duplicate. The total cell histamine was determined by boiling for 10 min. The histamine secreted in response to the antagonist was expressed as a percentage of the total amount secreted. This concentration, which was secreted in approximately 50% of the total mast cell histamine ( $\text{HRD}_{50}$   $\mu\text{g/ml}$ ), was determined for each antagonist. The results have been collated in Figure 1.

All the peptides were found to be active in inhibiting ovulation in female mammals at very low doses. The peptides of the present invention were often administered in the form of pharmaceutically acceptable, non-toxic salts, for example acid addition salts. Examples of such acid addition salts are hydrochlorides, hydrobromides, sulphate, phosphate,

fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginate, pamoate, malate, ascorbate, tartrate and the like. If the active ingredient is administered in tablet form, the tablet can contain a pharmaceutically acceptable diluent, including a binder such as tragacanth, maize starch or gelatin; a disintegrating agent such as alginic acid; and a lubricant such as magnesium stearate.

If it is desired to administer the active ingredient in liquid form, a sweetener and/or flavouring can be used as part of the pharmaceutically acceptable diluent, and intravenous administration is effected in isotonic saline, phosphate buffer solutions or the like.

The pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically acceptable excipient. Conventionally, the dose will be from approximately 1 to 100 micrograms of peptide per kilogram of body weight of the host when administered intravenously; the oral doses will be higher. In general, the treatment of subjects with these peptides is carried out in the same manner as clinical treatment with other LHRH antagonists.

These peptides can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, intranasally or intravaginally to achieve fertility inhibition and/or control and also in applications requiring a reversible suppression of gonadal activity, for example in the treatment of precocious puberty or during radiation therapy or chemotherapy. Effective doses vary according to the form of administration and the particular species of mammal to be treated. An example of a typical dosage form is an isotonic saline solution containing peptide, which is administered in a dose of approximately 0.1 to 2.5 mg/kg of body weight.

Oral administration of the peptide can be effected in either solid or liquid form.

Although the invention has been described with regard to the preferred embodiment, it should be self-evident that changes and modifications obvious to those skilled in the art can be made without departing from the scope of the invention as defined by the attached Claims. Substitutions from the state of the art which do not significantly deviate from the effectiveness can also be used in the invention.

SB-030, SB-075 and SB-088 are decapeptides, as described in greater detail below, which have particularly advantageous activities:

Ac-D-Nal(2)-D-Phe(4-Cl)-R<sup>3</sup>-Ser-Tyr-R<sup>6</sup>-Leu-Arg-Pro-D- Ala-NH<sub>2</sub>

SB-030, R<sup>3</sup> = D-Trp, R<sup>6</sup> = D-Cit;

SB-075, R<sup>3</sup> = D-Pal, R<sup>6</sup> = D-Cit;

SB-088, R<sup>3</sup> = D-Pal, R<sup>6</sup> = D-Hci.

Of the amino acids contained in the constituents, D-Pal, D-Cit and D-Hci are not commercially available. D-Pal requires a complete synthesis, which begins with diethyl acetamido-malonate and 3-picoly1 chloride and is carried out in known manner. D-Cit and D-Hci can easily be prepared from commercially available diamino acids, for example D-Orn or D-Lys.

Each peptide has a high LHRH-inhibiting activity, as shown below, and is completely free of oedematogenic side effects.

Table 1. LHRH-inhibiting activities of SB-030, SB-075  
and SB-088

| Peptide | % inhibition of LHRH (3 nM)<br>with equimolar peptide |               | Antiovation test |              |
|---------|-------------------------------------------------------|---------------|------------------|--------------|
|         | at 0 min                                              | after 2 hours | dose ( $\mu$ g)  | % inhibition |
| SB-030  | 83                                                    | 58            | 1.5              | 50           |
|         |                                                       |               | 3.0              | 100          |
| SB-088  | 86                                                    | 60            | 1.5              | 66           |
|         |                                                       |               | 2.0              | 83           |
|         |                                                       |               | 3.0              | 100          |
| SB-075  | 89                                                    | 54            | 1.5              | 75           |
|         |                                                       |               | 2.0              | 100          |

Abbreviations: The amino acids and protecting groups are abbreviated as recommended by IUPAC IUB JCBN [Eur. J. Biochem. 138, 9-37 (1984)]. Other abbreviations are: Boc<sub>2</sub>O, di-t-butyl dicarbonate; DCM, dichloromethane; TLC/TLE, thin layer chromatography/thin layer electrophoresis.

Compared with SB-075 and SB-088, SB-030 has the advantage of comprising only one unusual amino acid, namely D-Cit. On the other hand, both SB-075 and SB-088 have a higher potency and a better water solubility than SB-030.

The observations made and the tests performed during the synthesis of SB-030, SB-075 and SB-088 can be summarized as follows:

The solid-phase synthesis of SB-030, SB-075 and SB-088 on a benzhydrylamine resin using the Boc protocol is described.

This procedure is totally adequate for building up these peptides because it comprises stepwise chain lengthening via the carboxyl terminus so as to avoid significant racemization during the formation of the amide bond, and also easy isolation of the intermediates, which can prove particularly difficult during the synthesis of fragments of hydrophilic character (i.e. fragments 9-10 to 5-10).

The usual stepwise synthesis in a solution is of course also an appropriate method of preparing these peptides, but is certainly more labour-intensive and more time-consuming.

## STARTING MATERIALS

### Solid carriers

A benzhydrylamine resin containing 1.1 meq  $\text{NH}_2/\text{g}$  (Advanced ChemTech, Louisville, KY) was used. Resins with a lower  $\text{NH}_2$  content, e.g. 0.4-0.6 meq/g, can also be used but are naturally less economical.

### Amino acid derivatives

The Boc-amino acids listed below were purchased from Bachem and used without further purification:

Boc-D-Ala, Boc-Arg(Tos), Boc-Leu $\cdot\text{H}_2\text{O}$ ,  
Boc-D-Nal, Boc-D-Phe(4-Cl), Boc-Pro,  
Boc-Ser(Bzl), Boc-D-Trp and Boc-Tyr(DCB).

Boc-D-Cit, Boc-D-Hci and Boc-D-Pal, which are not commercially available, were prepared according to the literature (1-4) with very slight modifications.

## Boc-D-Cit

## Step 1, D-Cit

D-Orn (Sigma, 98%) was converted to D-Cit as described (1) except that the reaction mixture of  $(\text{D-Orn})_2\text{Cu}$  and KOCN was stirred for 2 to 3 hours in order to prevent a crust of  $(\text{D-Cit}, \text{D-Orn})\text{Cu}$  from depositing on the vessel wall.

The purity of the D-Cit was tested by means of TLE (thin layer electrophoresis) at a pH of 6.5 in a pyridine/acetic acid/water buffer (100:4:900) and at 30 V/cm for 45 min.

## Step 2, Boc-D-Cit

6.6 g (30 mmol) of  $\text{Boc}_2\text{O}$  in 15 ml of acetonitrile were added to a solution of 4.4 g (25 mmol) of D-Cit in 25 ml of water and 12.6 ml (90 mmol) of triethylamine. The mixture was stirred for 2 to 3 hours and then subjected to TLC (thin layer chromatography) using ethyl acetate/pyridine/acetic acid/water (60:10: 3:6) as the solvent system: Boc-D-Cit, D-Cit and Boc-D-Orn(Boc), which was formed from the D-Orn impurity, have RFs of 0.4, 0.0 and 0.9 respectively. The mixture was diluted with 25 ml of water and extracted with diethyl ether (2 x 20 ml) and ethyl acetate (2 x 20 ml) to remove the excess dicarbonate and Boc-D-Orn(Boc) impurity. The aqueous phase was acidified with 1 M  $\text{KHSO}_4$  and, after the addition of approximately 10 g of  $\text{Na}_2\text{SO}_4$ , extracted with ethyl acetate (3 x 30 ml). The combined ethyl acetate solutions (carefully separated from the aqueous layer) were evaporated under vacuum. The residue was diluted in dry dioxane and evaporated three times under vacuum. The light oil formed was diluted in 25 ml of DMF and used for coupling as mmol/ ml of this stock solution of Boc-D-Cit.

The synthesis of Boc-Cit using Boc-azide as the acylating reagent, and the preparation of the crystalline dicyclohexylamine salt, have been described (2).

## Boc-D-Hci

## Step 1, D-Hci

D-Hci was prepared from D-Lys·HCl (Sigma) analogously to D-Cit (see above and reference 1).

## Step 2, Boc-D-Hci

6.6 g of ditert-butyl dicarbonate diluted in 15 ml of acetonitrile were added to a solution of 25 mmol (4.73 g) of D-Hci in 25 ml of water and 12.6 ml (90 mmol) of triethylamine and the reaction mixture was stirred for 2 to 3 hours. The completeness of the reaction was tested by means of TLC as in the case of Boc-D-Cit; the RFs of Boc-D-Hci, D-Lys and Boc-D-Lys(Boc) were 0.3, 0.0 and 0.8 respectively. The reaction mixture was extracted with ether. The aqueous phase was acidified with 1 M KHSO<sub>4</sub> and, after the addition of 10 g of Na<sub>2</sub>SO<sub>4</sub>, extracted with a 1:1 mixture of ethyl acetate and n-butanol (4 x 30 ml). The combined organic solutions were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The residue was triturated with diethyl ether, filtered off, washed with ether, then suspended in ethyl acetate and stirred for 2 hours at 20°C and for a further 2 hours at 0°C. The resulting crystalline material was filtered off, washed with cold ethyl acetate and dried to give approximately 3.9 g of Boc-D-Hci melting at 141-142°C (dec.).

## Boc-D-Pal(3)

Step 1, Diethyl acetamido(pyrid-3-ylmethyl)malonate (3)  
113.0 g (60.3%) of diethyl acetamido(pyrid-3-ylmethyl)malonate were obtained from 132.0 g of diethyl acetamidomalonate by the method of J.E. Rivier *et al.* (3). The crude material melted at 95°C. A small sample, recrystallized from water, melted at 104-106°C (literature 95°C).

Step 2, N-Acetyl-3-(pyrid-3-yl)-DL-alanine ethyl ester (4) 61.6 g (200 mmol) of diethyl acetamido(pyrid-3-ylmethyl)-malonate were diluted in 210 ml of ethanolic NaOH in a stoppered flask and left to stand overnight at room temperature. The next day, the mixture was brought to the reflux point and kept at this temperature for 30 min. It was then cooled to room temperature and filtered to remove the insoluble solids. The insoluble substances were washed with various small portions of ethanol and then discarded. The combined filtrates were evaporated to dryness and the dry residue was crystallized from water using 3-5 ml of H<sub>2</sub>O per gram of solid. 38.0 g (160 mmol, 80%) of the material were recovered. Melting point 124-126°C (literature 118-121°C).

Step 3, N-Acetyl-3-(pyrid-3-yl)-D-alanine ethyl ester (3) 45.0 g (190 mmol) of the above-described DL mixture were diluted in 225 ml of hot 4.5% HCl. This solution was then cooled rapidly to 25°C to form a suspension of fine crystals. The pH was brought to 7.2 using 2 N KOH, 150 mg of alpha-chymotrypsin were then added and the pH was then kept at 6.8-7.2 by the dropwise addition of 2 N KOH. After the pH had ceased to change for 15 min, a further 50 mg of alpha-chymotrypsin were added to ensure completion of the hydrolysis. No further change in the pH was found after this fresh addition of the enzyme, and the final pH was 7.2. This solution is extracted with 4 x 200 ml of ethyl acetate. The ethyl acetate extracts are combined and dried over Na<sub>2</sub>SO<sub>4</sub>, the desiccant is filtered off and the solvent is removed under vacuum. The residue is recrystallized from toluene (13-15 ml/g) to recover 13.0 g (55 mmol, 57%) melting at 79-81°C (literature 122°C).

Step 4, 3-(Pyrid-3-yl)-D-alanine, D-Pal(3) (4) 13.0 g (55 mmol) of N-acetyl-3-(pyrid-3-yl)-D-alanine ethyl ester are refluxed overnight with 100 ml of 6 N HCl. The solvent is removed under vacuum. 15 mg of ethanol were added and removed under vacuum. This ethanol treatment is repeated

twice. The residue is recrystallized from 90% ethanol to give 13.0 g (54 mmol, 98%) of the amine dihydrochloride. Melting point 237-239°C (dec.) (literature 253-256°C).

Step 5, N-Boc-3-(pyrid-3-yl)-D-alanine, Boc-D-Pal(3) (3) 13.0 g (54 mmol) of the above-described D-Pal(3) were diluted in 50 ml of H<sub>2</sub>O. 50 ml of tetrahydrofuran and 30 ml (214 mmol) of triethylamine were added. 4 x 5 g portions of Boc<sub>2</sub>O (90.7 mmol) were added to this mixture at hourly intervals, with stirring. After stirring for a total of 6 hours, the tetrahydrofuran is stripped off under vacuum. Excess dicarbonate is removed with hexane. The aqueous layer is brought to a pH of 4 with 6 N HCl and then extracted with 4 x 100 ml portions of ethyl acetate. The ethyl acetate extracts are combined and back-extracted with 2 x 5 ml portions of saturated sodium chloride solution. The organic phase is dried over Na<sub>2</sub>SO<sub>4</sub> and filtered and the solvent is stripped off under vacuum. The residue is recrystallized from toluene to give 8.4 g (31 mmol, 57%) of Boc-D-Pal(3) melting at 141-142°C (dec.) (literature 135-136°C).

#### PREPARATION OF THE PEPTIDE RESIN

A reaction vessel for manual synthesis, of approximately 60 ml capacity, was filled with 1 g (1.1 mmol) of benzhydrylamine·HCl resin. This was left to swell for 2 hours in 20 ml of DCM and then filtered. 15 ml of 10% DIEA/DCM were subsequently added to the resin and the mixture was shaken for 2 minutes and filtered. The latter treatment was repeated twice. The Boc-amino acids, as preformed HOBt esters, were successively coupled on this resin as illustrated in Table 2. Steps 1 - 12 form a coupling cycle for one amino acid residue. Ten consecutive cycles would give a decapeptide amide resin terminating with a free D-Nal(2) residue. This peptide resin

was acetylated as described in steps 2e and 2f and then removed from the reaction vessel, washed with DCM and n-hexane and dried under vacuum to give 2.9-3.0 g of the desired acetyl-decapeptide amide resin<sup>2</sup> having the following structures:

1. Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser(Bzl)-Tyr(DCB)-D-Cit-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN, for SB-030;
2. Ac-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser(Bzl)-Tyr(DCB)-D-Cit-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN, for SB-075;
3. Ac-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser(Bzl)-Tyr(DCB)-D-Hci-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN, for SB-088.

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<sup>2</sup> SB-088 was synthesized in a smaller amount (0.1866 mmol); peptide resin no. 3 and the crude HF salt of the free decapeptide obtained amounted to 410 mg and 200 mg respectively.

Table 2. Synthesis programme

| STEP | REAGENT AND PROCEDURE                                                                                                                                    | MIXING TIMES<br>IN MIN |
|------|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| 1    | prepared HOBt ester <sup>3</sup> , couple                                                                                                                | 90                     |
| 2    | ninhydrin test (5) <sup>4</sup> ; if the beads are medium or dark blue, repeat steps 1 + 2; if the colouration is light blue, proceed with steps 2a - 2f |                        |
| 2a   | MeOH (10 ml), wash                                                                                                                                       | 1                      |
| 2b   | 10% DIEA/DCM (10 ml), neutralize                                                                                                                         | 2                      |
| 2c   | MeOH (10 ml), wash                                                                                                                                       | 1                      |
| 2d   | DCM (10 ml), wash (three times)                                                                                                                          | 2                      |
| 2e   | acetylimidazole <sup>4</sup> , acetylate                                                                                                                 | 30                     |
| 2f   | ninhydrin test; if positive, repeat steps 2d - 2f                                                                                                        |                        |
| 3    | MeOH (10 ml), wash (twice)                                                                                                                               | 1                      |
| 4    | DCM (10 ml), wash (three times)                                                                                                                          | 2                      |
| 5    | 50% TFA/DM (10 ml), deblock                                                                                                                              | 1                      |
| 6    | 50% TFA/DM (10 ml), deblock                                                                                                                              | 30                     |
| 7    | IpOH (10 ml), wash                                                                                                                                       | 1                      |
| 8    | 10% DIEA/DCM (10 ml), neutralize                                                                                                                         | 3                      |
| 9    | MeOH (10 ml), wash                                                                                                                                       | 1                      |
| 10   | 10% DIEA/DCM (10 ml), neutralize                                                                                                                         | 3                      |
| 11   | MeOH (10 ml), wash                                                                                                                                       | 1                      |
| 12   | DCM (10 ml), wash                                                                                                                                        | 2                      |

<sup>3</sup> With the exception of Boc-D-Cit, Boc-amino acid HOBt esters were prepared as follows:

3 mmol of Boc-amino acid [*i.e.* (in mg) D-Ala: 568, D-Pro: 646, Arg(Tos): 1286, Leu: 748, Tyr(DCB): 1321, Ser(Bzl): 886, D-Hci: 865, D-Trp: 913 or D-Pal(3): 799, D-Phe(4-Cl): 899,

D-Nal(2): 946] and 460 mg (3 mmol) of HOBt·H<sub>2</sub>O were dissolved in 1 ml of DMF, diluted with 2 ml of DCM, cooled to 0°C and reacted with 0.6 ml (3.8 mmol) of DIC at 0°C for 10 min; the mixture was then added to the resin, rinsing with 4 x 1 ml of DCM. In the case of Boc-D-Cit, HOBt·H<sub>2</sub>O was dissolved in 3 ml of stock solution of Boc-D-Cit, reacted with DIC as above and then mixed with the resin, rinsing with 1 x 1 ml of DMF and 3 x 1 ml of DCM.

<sup>4</sup> In the case of Cit-containing peptide resins, the test is negative if the beads are white (or pale yellow) and the solution is lilac-coloured. Complete coupling of the Boc-Arg(Tos) with the Pro residue on the resin can be verified using an isatin reagent (2.5 g of isatin, 20 g of phenol, 30 g of benzyl alcohol and 2.5 g of Boc-Phe); the test is negative if the beads are white (or light yellow but not blue) after washing 3 to 4 times with acetone.

<sup>4</sup> 0.34 g of imidazole dissolved in 2 ml of DCM was reacted with 0.5 ml of acetic anhydride at 0°C for 2 min and then added to the resin, rinsing with 3 x 2 ml of DCM.

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## PREPARATION OF THE FREE DECAPEPTIDE AMIDES

### Cleavage and deprotection

Each peptide resin was treated with 3 ml of m-cresol and approximately 30 ml of hydrogen fluoride (HF) at 0°C for 45 min. After removal of the HF under high vacuum, the remaining peptide resin was triturated with dry diethyl ether, filtered off and washed with diethyl ether and the peptide was then extracted as follows:

1. 50% acetic acid and DMF (2-2 x 20 ml) for SB-030

2. 50% acetic acid (3 x 15 ml) for SB-075 and SB-088 and separated from the resin by filtration. The peptide solution was evaporated under vacuum and the residue was triturated with diethyl ether, filtered off, washed with diethyl ether and dried to give 1.2-1.3 g of HF salt containing crude decapeptides<sup>2</sup> with a purity of 70-85%.

### Purification

The crude peptide was purified using a Beckman Prep-350 preparative HPLC system. The separations were performed on a 41.5 x 250 mm Dynamax column packed with spherical C<sub>18</sub> silica gel (300 Å pore size, 12 µm particle size) (RAININ Inc. Co. Woburn, MA).

Crude SB-030 was applied to the column in 30% formic acid solution while crude SB-075 and SB-088 were dissolved in 30% acetic acid. Differential pressure elution was used. Solvent A contained 0.1% aqueous TFA and solvent B was 0.1% TFA in 70% aqueous acetonitrile. The pressure flow rate was 35 ml/min. The column elution was monitored by means of a variable wavelength UV detector (Beckman type 163) at 220 nm.

The amounts of the pure decapeptide amides and the yields generally obtained were as follows:

1. 600 mg (34%) of SB-030,
  2. 960 mg (52%) of SB-075 and
  3. 140 mg (45%) of SB-088 (see footnote 2),
- with a purity of >95%.

### Analytical HPLC

The HPLC analysis was carried out with a Hewlett-Packard model 1090 liquid chromatograph. The peptide samples were chromatographed with solvents A and B on a 4.6 x 250 mm W-Porex 5  $\mu$ m C<sub>18</sub> column (Phenomex, Rancho Palos Verdes, CA) with a flow rate of 1.2 ml/min using differential pressure elution (chromatograms of the crude samples attached).

### Amino acid analysis

The peptide samples were hydrolyzed at 110°C for 20 hours in evacuated sealed tubes using 4 M methanesulphonic acid containing 0.2% of 3-(2-aminoethyl)indole, and the amino acid composition of the hydrolyzate was determined in a Beckman 6300 amino acid analyzer.

The attached Table shows the affinity of the LHRH analogues for the pituitary gland or receptors of the human breast cancer membrane, as determined using radiolabelled LHRH or radiolabelled D-Trp<sup>6</sup>-LHRH.

The measurements with radiolabelled LHRH (A) show that SB-075 has the highest affinity for the pituitary receptors, i.e. the greatest capacity to displace LHRH, which is one of the main features of LHRH antagonists. On the other hand, SB-088 seems to exhibit a particularly high affinity for receptors of the human breast cancer membrane in both tests (A + B).

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## EXAMPLE I

The peptide of the formula Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is synthesized from the intermediate peptide Ac-D-Nal(2)-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>.

This intermediate peptide was built up stepwise on a benzhydrylamine resin containing approx. 0.6 meq NH<sub>2</sub>/g, using a Beckman 990 synthesizer, starting with Boc-D-Ala according to the method described below.

Coupling is carried out in accordance with schedule A as follows:

SCHEDULE A

| Reagent                                                  | Mixing time (minutes) |
|----------------------------------------------------------|-----------------------|
| 1. Boc-amino acid (2-3 mmol/g resin) + eq. amount of DIC | 60 - 90               |
| 2. MeOH (twice)                                          | 1                     |
| 3. CH <sub>2</sub> Cl <sub>2</sub> (twice)               | 1                     |

Deblocking is carried out in accordance with schedule B as follows:

SCHEDULE B

| Reagent                                                                   | Mixing time (min) |
|---------------------------------------------------------------------------|-------------------|
| 4. 50% TFA/1% ethanedithiol<br>in CH <sub>2</sub> Cl <sub>2</sub> (twice) | 15 + 15           |
| 5. IpOH/1% ethanedithiol                                                  | 1                 |
| 6. 10% TEA in CH <sub>2</sub> Cl <sub>2</sub>                             | 2                 |
| 7. MeOH                                                                   | 1                 |
| 8. 10% TEA in CH <sub>2</sub> Cl <sub>2</sub>                             | 2                 |
| 9. MeOH (twice)                                                           | 1 + 1             |
| 10. CH <sub>2</sub> Cl <sub>2</sub> (twice)                               | 1 + 1             |

Briefly, Boc is used to protect the alpha-amino groups. Tos is used to protect the guanidino group of Arg. DCB is used as the protecting group for the phenolic hydroxyl group of Tyr, and the OH group of Ser is protected by Bzl. A two- to three-fold excess of the protected amino acid is used for two hours, based on the NH<sub>2</sub> content of the benzhydrylamine resin plus one equivalent of DIC in CH<sub>2</sub>Cl<sub>2</sub> or 10-50% DMF/CH<sub>2</sub>Cl<sub>2</sub>, depending on the solubility of the Boc-amino acid.

N-terminal acetylation is carried out with a 50-fold excess of acetic anhydride in CH<sub>2</sub>Cl<sub>2</sub> for 0.5 hour. The protected intermediate peptide obtained in this way has the following composition:

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser(X<sup>4</sup>)-Tyr(X<sup>5</sup>)-D-Lys(X<sup>6</sup>)-Leu-Arg(X<sup>8</sup>)-Pro-D-Ala-NH-X<sup>10</sup>

wherein X<sup>4</sup> is Bzl, X<sup>5</sup> is DCB, X<sup>6</sup> is Z(2-Cl), X<sup>8</sup> is Tos and X<sup>10</sup> is a benzhydryl group incorporated in the resin.

To cleave and deprotect the protected peptide resin, it is treated with 1.4 ml of m-cresol and 15 ml of hydrogen fluoride per gram of peptide resin for 0.5 hour at 0°C and

for 0.5 hour at room temperature. After elimination of the hydrogen fluoride under high vacuum, the peptide resin is washed with diethyl ether and the peptide is then extracted with DMF and separated from the resin by filtration. The DMF solution is then concentrated to a small volume under high vacuum and triturated with diethyl ether. The resulting crude product is then purified by preparative HPLC as described above. This gives the pure free intermediate peptide of the formula indicated above wherein  $X^4$ ,  $X^5$ ,  $X^6$ ,  $X^8$  and  $X^{10}$  are hydrogen.

The free intermediate peptide containing D-Lys( $X^6$ ) is then reacted at room temperature with potassium cyanate in 80% aqueous DMF (81 mg KCNO/ml; time: 24 hours). After removal of the solvent under high vacuum, the reaction product is purified by preparative HPLC to give the desired peptide containing D-Hci.

The resulting peptide containing D-Hci is essentially pure (95%). HPLC analysis is carried out in a Hewlett-Packard 1090A gradient liquid chromatography system on a PHENOMENEX (W-Porex 5C18) column and elution is carried out with the following solvents: A: 0.1% TFA, B: 0.1% TFA in 70%  $CH_3CN$ , with a gradient of 30-60%, in 30 minutes. The desired peptide has an HPLC retention time of 28.2 minutes.

The peptides are purified on a Beckman Prep-350 gradient liquid chromatograph using a 41.4 x 250 mm cartridge containing a preparative reversed DYNEMAX C18 phase (300 Å, 12  $\mu m$ ) with the following solvents: A: 0.1% TFA, B: 0.1% TFA in 70%  $CH_3CN$ , with a pressure gradient of 45-60%, in 30 minutes. The pure peptide, obtained in the form of the TFA salt, can be converted to the acetate form, if desired, by passage through an AG3X column (Bio-Rad) in the acetate form, followed by lyophilization.

## EXAMPLE II

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(ethyl)-Leu- Arg-  
Pro-D-Ala-NH<sub>2</sub>

This is prepared from the corresponding intermediate peptide containing the amino acid D-Lys instead of D-Hci(ethyl) in the 6-position, analogously to Example I, by reaction with N-ethyl isocyanate (0.1 mg in 10 ml per 1 g of intermediate peptide) in DMF at 0-10°C for 10 hours. The retention time is 30.8 minutes.

## EXAMPLE III

The peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is synthesized as described in Example I. The starting peptide contains Boc-D-Orn(Z) in the 6-position instead of the Boc-D-Lys[Z(2-Cl)] used in Example I. This starting peptide is prepared analogously to Example I. Retention time: 27.8 minutes. Retention time of the starting peptide: 25.5 minutes.

## EXAMPLE IV

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(ethyl)-Leu- Arg-  
Pro-D-Ala-NH<sub>2</sub>

This peptide is obtained from Ac-D-Nal(2)- Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> and N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of intermediate peptide) at 0-10°C, analogously to Example III. Reaction time: 10 hours. HPLC retention time: 30.4 minutes.

## EXAMPLE V

The peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is synthesized as described in Example I except that Boc-D-Phe(4-Cl) is incorporated instead of Boc-D-Nal(2) in the 1-position of the starting peptide, Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> then being obtained as the direct starting peptide.

Retention time: 26.6 minutes; retention time of the starting peptide with Ac in the 1-position: 24.0 minutes.

## EXAMPLE VI

Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe(4-Cl)-Phe-(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example V), which is reacted with N-ethyl isocyanate (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C in DMF.

Reaction time: 10 hours.

HPLC retention time: 29.2 minutes.

## EXAMPLE VII

The peptide Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is synthesized as described in Example I except that Boc-D-Phe(4-Cl) is incorporated instead of Boc-D-Nal(2) in the 1-position and Boc-D-Orn(Z) instead of Boc-D-Lys[Z(2-Cl)] in the 6-position, giving Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> as the direct starting peptide (retention time: 24.0 minutes), which is then reacted with KCNO analogously to Example I.

HPLC retention time: 26.3 minutes.

## EXAMPLE VIII

Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe(4-Cl)-D-Phe-(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example VII), which is reacted with N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C for 10 hours.

HPLC retention time: 28.6 minutes.

## EXAMPLE IX

The peptide Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-Gly-NH<sub>2</sub> is synthesized analogously to Example I. The peptide obtained has an HPLC retention time of 27.4 minutes.

## EXAMPLE X

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(ethyl)-Leu-Arg-Pro-Gly-NH<sub>2</sub>

The starting peptide is Ac-D-Nal(2)-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH<sub>2</sub> (see Example IX), which is reacted with N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C for 10 hours.

HPLC retention time: 30.0 minutes.

## EXAMPLE XI

Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (retention time: 16.8 minutes), which is reacted with potassium cyanate analogously to Example I. The starting peptide is obtained analogously to Example I except that Boc-Pro is incorporated instead of Boc-D-Nal(2) in the 1-position.

HPLC retention time: 19.3 minutes.

## EXAMPLE XII

Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Pro-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example XI), which is reacted with N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C for 10 hours.

HPLC retention time: 22 minutes.

## EXAMPLE XIII

Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (retention time: 16.85 minutes), which is reacted with potassium cyanate analogously to Example I. The starting peptide is likewise obtained analogously to Example I except that Boc-Pro is incorporated instead of Boc-D-Nal(2) in the 1-position and Boc-D-Orn(2) instead of Boc-D-Lys[Z(2-Cl)] in the 6-position.

HPLC retention time: 18.8 minutes.

## EXAMPLE XIV

Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example XIII), which is reacted with N-ethyl isocyanate analogously to Example VI.

HPLC retention time: 24.9 minutes.

## EXAMPLE XV

Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (retention time: 20.8 minutes), which is reacted with potassium cyanate analogously to Example I. The starting peptide is obtained analogously to Example I except that the amino acid residue Boc-D-Phe is incorporated instead of Boc-D-Nal(2) in the 1-position.

HPLC retention time: 23.4 minutes.

## EXAMPLE XVI

Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example XV), which is reacted with N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C for 10 hours.

HPLC retention time: 26.0 minutes.

## EXAMPLE XVII

Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (retention time: 21.0 minutes), which is reacted with potassium cyanate analogously to Example I. The starting peptide is prepared analogously to Example I except that Boc-D-Phe is incorporated instead of Boc-D-Nal(2) in the 1-position and Boc-D-Orn(Z) instead of Boc-D-Lys[Z(2-Cl)] in the 6-position. HPLC retention time: 23.1 minutes.

## EXAMPLE XVIII

Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit(ethyl)-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

The starting peptide is Ac-D-Phe-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> (see Example XVII), which is reacted with N-ethyl isocyanate in DMF (0.1 mg in 10 ml per gram of starting peptide) at 0-10°C for 10 hours. HPLC retention time: 25.4 minutes.

## EXAMPLE XIX

Example of the general preparation of a peptide according to the invention of the formula Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

This peptide is .... stepwise by means of a benzhydrylamine resin containing approximately 1.0 mol equivalent of amino groups per gram, using a Beckman 990 synthesizer, starting with Boc-D-Ala. Coupling is carried out in accordance with schedule A given in Example I.

Deblocking is carried out in accordance with schedule B given in Example I. The other conditions correspond to the relevant data in Example I.

The protected intermediate peptide obtained in this way has the following composition:

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser(X<sup>4</sup>)-Tyr(X<sup>5</sup>)-D-Hci-Leu-Arg(X<sup>8</sup>)-Pro-D-Ala-NHX<sup>10</sup>

wherein X<sup>4</sup> = Bzl, X<sup>5</sup> = Dzb, X<sup>8</sup> = Tos and X<sup>10</sup> is a benzhydryl group incorporated in the resin.

The protected peptide obtained in this way is cleaved according to Example I with 1.4 ml of m-cresol and 15 ml of hydrogen fluoride per gram of peptide resin (0.5 hour at 0°C and 0.5 hour at room temperature). All other measures are as indicated in Example I. The crude product obtained is purified by preparative HPLC. HPLC analysis is performed according to Example I in a Hewlett-Packard 1090A gradient liquid chromatography system using a Phenomenex column, eluting with the following solvents:

solvent A: 0.1% TFA, solvent B: 0.1% TFA in 70% CH<sub>3</sub>CN, with a gradient of 35 - 75%, in 30 minutes. The resulting dipeptide, which contains D-Hci in the 6-position, has an

HPLC retention time of 22.9 minutes. Further purification is effected on a Beckman Prep-350 gradient liquid chromatograph according to Example I.

#### EXAMPLE XX

The peptide  $\text{H}_2\text{N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH}_2$  is obtained according to Example XIX except that  $\text{H}_2\text{N-CO-D-Nal(2)}$  is incorporated instead of Boc-D-Nal(2) in the 1-position and N-terminal acetylation is not carried out.

HPLC retention time: 24.0 minutes.

#### EXAMPLE XXI

The peptide  $\text{Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH}_2$  is synthesized as described in Example XIX except that Boc-D-Cit is incorporated instead of Boc-D-Hci in the 6-position. The desired peptide has an HPLC retention time of 22.5 minutes.

#### EXAMPLE XXII

$\text{Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH}_2$

This is prepared according to Example XIX except that Boc-D-Phe(4-Cl) is incorporated instead of Boc-D-Nal(2) in the 1-position.

HPLC retention time: 24.0 minutes.

## EXAMPLE XXIII

Ac-D-Phe(4-Cl)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

This is prepared analogously to Example XIX except that Boc-D-Phe(4-Cl) is incorporated instead of Boc-D-Nal(2) in the 1-position and Boc-D-Cit instead of Boc-D-Hci in the 6-position.

Retention time of the peptide obtained: 20.8 minutes.

## EXAMPLE XXIV

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-Gly-NH<sub>2</sub>

This is prepared according to Example XIX except that Boc-Gly is incorporated instead of Boc-D-Ala in the 10-position.

The peptide obtained has an HPLC retention time of 22.4 minutes.

## EXAMPLE XXV

Ac-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

This is prepared according to Example XIX except that Boc-D-Pal(3) is incorporated instead of Boc-D-Trp in the 3-position.

The peptide obtained has an HPLC retention time of 13.6 minutes.

## EXAMPLE XXVI

Ac-D-Nal-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>

This is prepared according to Example XIX except that Boc-D-Cit is incorporated instead of D-Hci in the 6-position and Boc-D-Pal(3) instead of Boc-D-Trp in the 3-position.

The peptide obtained has an HPLC retention time of 13.3 minutes.

## EXAMPLE XXVII

The peptide H<sub>2</sub>N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is synthesized as described in Example XIX except that Boc-D-Cit is incorporated instead of Boc-D-Hci in the 6-position, Boc-D-Pal(3) is incorporated instead of Boc-D-Trp in the 3-position and N-terminal acetylation is omitted.

This gives the intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>. The free peptide obtained in this way is then reacted with potassium cyanate in 80% aqueous DMF (81 mg KOCN/300 mg peptide/ml) at room temperature for 24 hours. After evaporation under high vacuum, the reaction mixture is purified by preparative HPLC. The resulting peptide has an HPLC retention time of 14.4 minutes.

## EXAMPLE XXVIII

H<sub>2</sub>N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> is prepared analogously to Example XIX except that Boc-D-Pal(3) is incorporated instead of Boc-D-Trp in the 3-position and N-terminal acetylation is omitted. This gives the intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Pal(3)-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>. The free peptide obtained in this way is then reacted with potassium cyanate in aqueous DMF (81 mg KOCN/300 mg peptide/ml) at room temperature for 24 hours. After evaporation under high vacuum, the reaction mixture is purified by preparative HPLC. The resulting peptide has an HPLC retention time of 14.7 minutes.

## EXAMPLE XXIX

The synthesis of the peptide H<sub>2</sub>N-CO-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub> begins with preparation of the intermediate peptide H-D-Nal(2)-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>. The intermediate peptide is synthesized as described in Example XIX except that Boc-D-Orn(Z) is incorporated instead of Boc-D-Hci in the 6-position and N-terminal acetylation is omitted. The free peptide containing D-Orn in the 6-position is then reacted with potassium cyanate in 80% aqueous DMF (162 mg KOCN/300 mg peptide/ml) at room temperature for 24 hours. After evaporation under high vacuum, the reaction mixture is purified by preparative HPLC. The resulting peptide has an HPLC retention time of 23.6 minutes.

## EXAMPLE XXX

The synthesis of the peptide  $\text{H}_2\text{N}-\text{CO}-\text{D-Nal}(2)-\text{D-Phe}(4-\text{Cl})-\text{D-Trp-Ser-Tyr-D-Hci-Leu-Arg-Pro-D-Ala-NH}_2$  begins with preparation of the intermediate peptide  $\text{H-D-Nal}(2)-\text{D-Phe}(4-\text{Cl})-\text{D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH}_2$ . The intermediate peptide is synthesized as described in Example XIX except that  $\text{Boc-D-Lys}[Z(2-\text{Cl})]$  is incorporated instead of  $\text{Boc-D-Hci}$  in the 6-position and N-terminal acetylation is omitted. The free peptide containing D-Lys in the 6-position is then reacted with potassium cyanate in 80% aqueous DMF (164 mg KOCN/ 300 mg peptide/ml) at room temperature for 24 hours. After evaporation under high vacuum, the reaction mixture is purified by preparative HPLC. The resulting peptide has an HPLC retention time of 14.0 minutes.

## EXAMPLE XXXI

Tablet formulation for buccal (e.g. sublingual) administration

|                    |         |
|--------------------|---------|
| 1. LHRH antagonist | 10.0 mg |
| Compressible sugar | 86.0 mg |
| Calcium stearate   | 4.0 mg  |
| 2. LHRH antagonist | 10.0 mg |
| Compressible sugar | 88.5 mg |
| Magnesium stearate | 1.5 mg  |
| 3. LHRH antagonist | 5.0 mg  |
| Mannitol           | 83.5 mg |
| Magnesium starch   | 1.5 mg  |

|                        |         |
|------------------------|---------|
| 4. LHRH antagonist     | 10.0 mg |
| Pregelatinized starch  | 10.0 mg |
| Lactose                | 74.5 mg |
| Pregelatinized starch  | 15.0 mg |
| Magnesium stearate USP | 1.5 mg  |

Method A. The LHRH antagonist is dissolved in a sufficient amount of water to form wet granules when it is mixed with the sugar part of the excipient. After complete mixing, the granules are dried in the drip tray of a fluidized bed dryer. The dry granules are then illuminated to show up any large aggregates and comminute them, and are then mixed with the other components.

The granules are then compressed to a specific tablet weight in a standard tableting machine.

Method B. In this method of preparation, all the formulations contain 0.01% gelatin.

The gelatin is first dissolved in the aqueous granulation solvent, after which the LHRH antagonist is added. The other steps are as described for method A.

#### EXAMPLE XXXII

##### Long-acting formulation for intramuscular injection

##### Long-acting sesame oil gel for intramuscular injection

LHRH antagonist 10.0 mg  
Aluminium monostearate 20.0 mg  
Sesame oil ad 10 ml

The aluminium monostearate is heated together with the sesame oil at 125°C, with stirring, until a clear yellow solution is

obtained. This mixture is then sterilized in a sterilizing apparatus and cooled. The LHRH antagonist is then added under aseptic conditions by means of trituration. Particularly preferred LHRH antagonists are salts of low solubility, e.g. zinc salts, zinc tannate salts, pamoate salts and the like. These have an exceptionally long duration of activity.

#### EXAMPLE XXXIII

Long-acting biodegradable polymer microcapsules for intramuscular injection

LHRH antagonists 1%

25/75 glycolide/lactide copolymer (copolymer of glycolic acid and lactic acid) (intrinsic viscosity 0.5) 99%

Microcapsules (0-150 Angstrom) of the formulation given above, suspended in:

Dextrose 5.0%

Carboxymethylcellulose sodium 0.5%

Benzyl alcohol 0.9%

Tween 80 0.1%

Water, purified, ad (amount necessary for) 100.0%

25 mg of microcapsules are suspended in 1.0 ml of the vehicle.

#### EXAMPLE XXXIV

Aqueous solution of an intramuscular injection

LHRH antagonist 500 mg

Gelatin (non-antigenic) 5 mg

Water for injection ad 100 ml

The gelatin and the LHRH antagonist are dissolved in the water for injection. The solution is then filtered under sterile conditions.

#### EXAMPLE XXXV

Formulation for rectal administration

Suppository vehicle for rectal administration

LHRH antagonist 5.0 mg

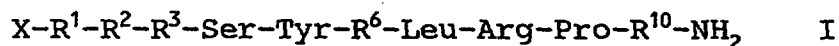
Witepsol H15 20.0 mg

The LHRH antagonist is mixed with the molten Witepsol H15 and poured into a 2 gram mould.



## Claims

1. A peptide of the formula



wherein

X is an acyl group consisting of linear or branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms, or a carbamoyl group,

$R^1$  is D- or L-Pro, D- or L- $\Delta^3$ -Pro, D-Phe, D-Phe(4-H1), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),

$R^2$  is D-Phe or D-Phe(4-H1),

$R^3$  is D-Trp, D-Phe, D-Pal(3), D-Nal(1) or D-Nal(2),

$R^6$  is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

$R^{10}$  is Gly or D-Ala,

H1 being fluorine, chlorine or bromine and Q being  $C_1$ - $C_3$ -alkyl, and pharmaceutically acceptable acid addition salts thereof.

2. Peptides according to Claim 1, characterized in that

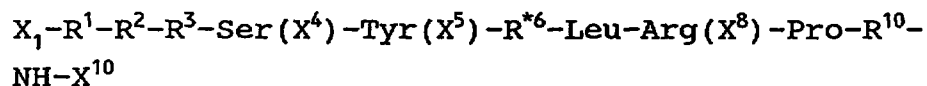
X is an acetyl or carbamoyl group,

$R^1$  is D-Nal(2), D- or L-Pro, D-Phe or D-Phe(4-Cl),

$R^2$  is D-Phe(4-Cl) or D-Phe(4-F) and

$R^3$  is D-Trp or D-Pal(3).

3. Peptides of the formula



wherein

$X_1$  is an acyl group consisting of linear or branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms, or t-Boc, hydrogen or carbamoyl,



X<sup>4</sup> is hydrogen or a protecting group for the Ser hydroxyl group,  
X<sup>5</sup> is hydrogen or a protecting group for the Tyr phenolic hydroxyl group,  
X<sup>8</sup> is hydrogen or a protecting group for the Arg guanidino group,  
X<sup>10</sup> is hydrogen or a carrier resin having benzhydryl or methylbenzhydryl groups,  
R<sup>1</sup> is D- or L-Pro, D- or L- $\Delta^3$ -Pro, D-Phe, D-Phe(4-Hl), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),  
R<sup>2</sup> is D-Phe or D-Phe(4-Hl),  
R<sup>3</sup> is D-Trp, D-Phe, D-Pal(3), D-Nal(1) or D-Nal(2),  
R<sup>\*6</sup> is D-Cit or D-Hci and  
R<sup>10</sup> is Gly or D-Ala,  
Hl being fluorine, chlorine or bromine.

4. Peptides according to one of Claims 1 to 3, characterized in that  
X is acetyl, Boc or hydrogen,  
X<sup>4</sup> is benzyl,  
X<sup>5</sup> is 2,6-dichlorobenzyl,  
X<sup>6</sup> is benzyloxycarbonyl or 2-chlorobenzyloxycarbonyl,  
X<sup>8</sup> is p-toluenesulphonyl or nitro,  
X<sup>10</sup> is a carrier resin containing benzhydryl or methylbenzhydryl groups,  
R<sup>1</sup> is D-Nal(2) or D-Phe(4-Cl),  
R<sup>2</sup> is D-Phe(4-Cl),  
R<sup>3</sup> is D-Trp or D-Pal(3) and  
R<sup>10</sup> is D-Ala.

5. A peptide according to one or more of the preceding claims, characterized in that X or X<sub>1</sub> is acetyl.



6. A peptide according to one or more of the preceding claims, characterized in that X or X<sub>1</sub> is carbamoyl.
7. A peptide according to one or more of the preceding claims, characterized in that R<sup>6</sup> or R<sup>\*6</sup> is D-Cit or D-Hci.
8. A method of preparing peptides of the formula



wherein

X is an acyl group consisting of linear or branched chains of aliphatic or alicyclic carboxylic acids having 1 to 7 carbon atoms, or a carbamoyl group,

R<sup>1</sup> is D- or L-Pro, D- or L-Δ<sup>3</sup>-Pro, D-Phe, D-Phe(4-Hl), D-Ser, D-Thr, D-Ala, D-Nal(1) or D-Nal(2),

R<sup>2</sup> is D-Phe or D-Phe(4-Hl),

R<sup>3</sup> is D-Trp, D-Phe, D-Pal(3), D-Nal(1) or D-Nal(2),

R<sup>6</sup> is D-Cit, D-Hci, D-Cit(Q) or D-Hci(Q) and

R<sup>10</sup> is Gly or D-Ala,

Hl being fluorine, chlorine or bromine and Q being C<sub>1</sub>-C<sub>3</sub>-alkyl,

and pharmaceutically acceptable acid addition salts thereof, characterized in that they are prepared by fragment condensation of corresponding peptide fragments, as conventionally used in peptide chemistry, or by stepwise synthesis as conventionally used in peptide chemistry, or in that, in a peptide of formula I wherein R<sup>6</sup> is D-Orn or D-Lys, the free amino group of D-Orn or D-Lys is converted to the ureido group by reaction with an alkali metal cyanate or a cyanate-providing compound or a C<sub>1</sub>-C<sub>3</sub>-alkyl isocyanate, and, optionally, the peptides obtained are acylated and/or converted to the amides and, optionally, converted to physiologically acceptable acid salts.



9. A method according to Claim 8, characterized in that the stepwise synthesis is carried out by a procedure in which firstly the carboxy-terminal amino acid glycine or D-alanine or the amides thereof, whose amino group in the  $\alpha$ -position is protected, is covalently bonded to a synthetic carrier conventionally used for this purpose, the  $\alpha$ -amino protecting group is cleaved, the next protected amino acid is bonded via its carboxyl group to the free amino group obtained in this way, the  $\alpha$ -amino protecting group of this second amino acid is then cleaved in turn, the next amino acid is bonded thereto and the remaining amino acids of the peptide to be synthesized are linked stepwise in this way in the correct sequence and, after all the amino acids have been linked, the finished peptide according to Claim 1 is cleaved from the carrier and, optionally, other side-group protecting groups present are cleaved.

10. Compounds according to Claim 1 or Claim 2 for use as therapeutic active ingredients.

11. A drug containing a compound of general formula I according to Claim 1 or Claim 2, together with conventional excipients and/or diluents or adjuncts.

12. A method of preparing a drug, characterized in that a compound I according to Claim 1 or Claim 2 is processed to pharmaceutical formulations with customary pharmaceutical excipients and/or diluents or other adjuncts, or is converted to a form usable in therapy.

13. A method according to Claim 11 or Claim 12, characterized in that the amount of active ingredient according to one or more of the preceding Claims 1 to 8 is between 0.1 and 2.5 mg/kg of body weight per day.



14. Use of compounds according to Claim 1 or Claim 2 for the preparation of drugs.