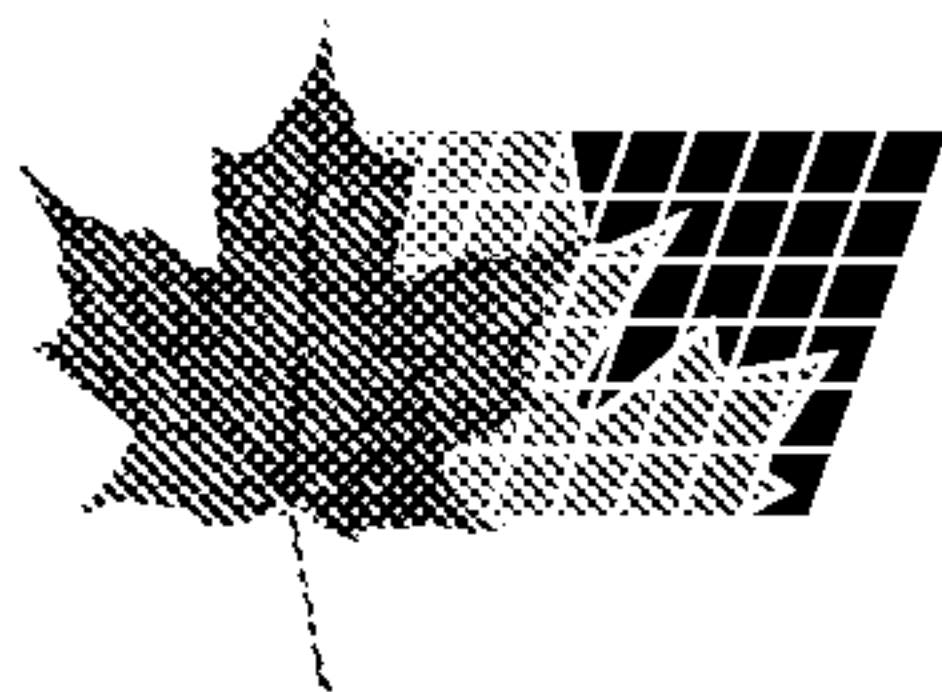
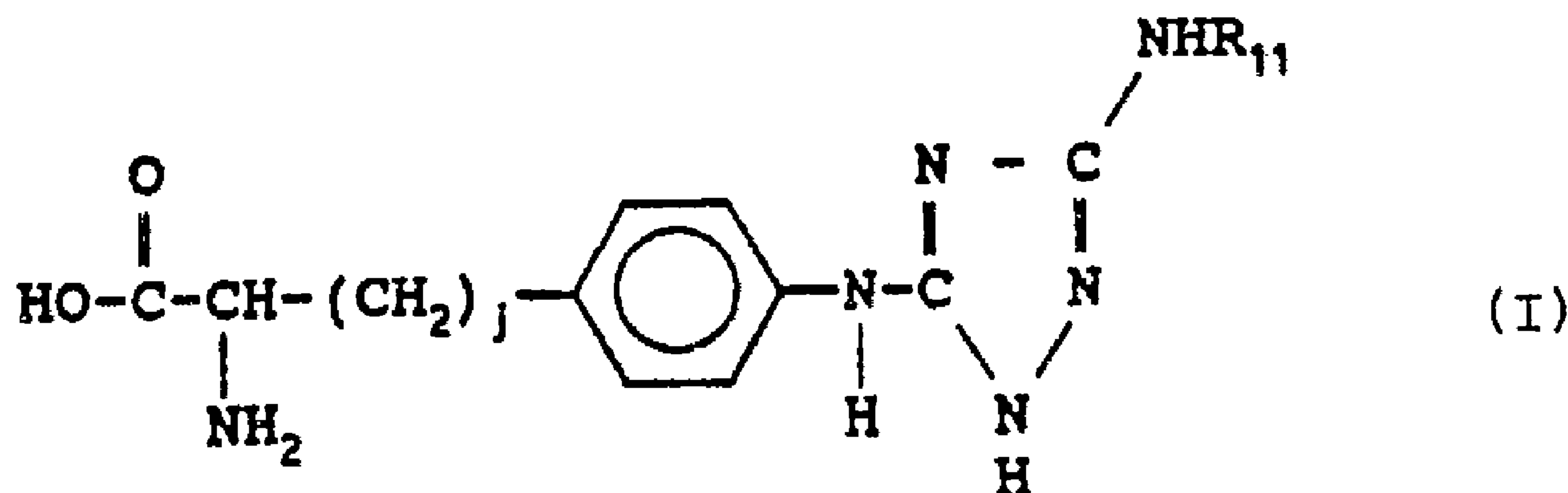




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(54) **ANALOGUES DE LA GN-RH**
(54) **GNRH ANALOGS**



(57) L'invention concerne des peptides qui comprennent des acides aminés non-naturels et qui inhibent la sécrétion de gonadotropines par la glande pituitaire et inhibent la libération de stéroïdes par les gonades. Les peptides sont des analogues de GnRH décapeptide où il existe au moins un reste d'un acide aminé non-naturel U* dans les positions 3, 5 et/ou 6. De tels acides aminés non-naturels sont utiles dans la synthèse des peptides et peuvent être formés après synthèse de la chaîne de peptides. L'acide aminé non-naturel U* peut avoir un groupe cyanoguanidino sur l'atome de carbone oméga de la chaîne latérale, mais possède de préférence la formule U* (I), dans laquelle j est égal à 1 ou 2 et R₁₁ représente H ou un radical acyle ayant de 1 à 6 atomes de carbone.

(57) Peptides which include unnatural amino acids and which inhibit the secretion of gonadotropins by the pituitary gland and inhibit the release of steroids by the gonad. The peptides are analogs of the decapeptide GnRH wherein there is at least one residue of an unnatural amino acid U* in the 3-, 5- and/or 6-positions. Such unnatural amino acids are useful in the synthesis of peptides and can be formed after synthesis of the peptide chain. The unnatural amino acid U* can have a cyanoguanidino group on the omega carbon atom of the side chain, but preferably has formula U* (I) where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 6 carbon atoms.





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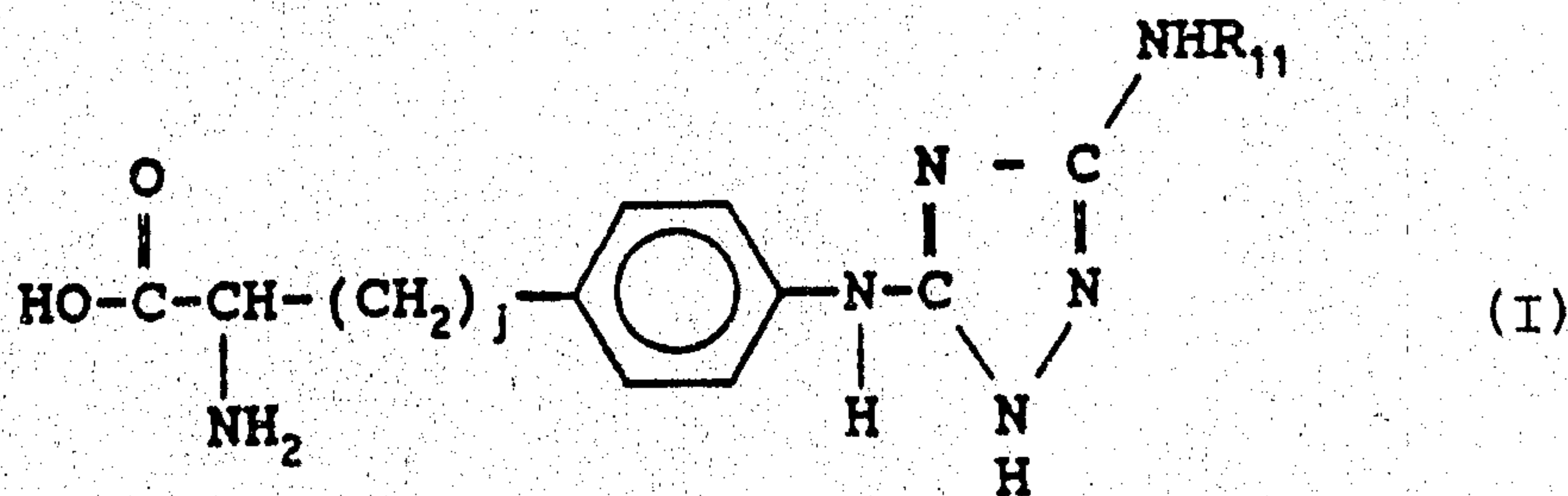
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(54) Title: GnRH ANALOGS



(57) Abstract

Peptides which include unnatural amino acids and which inhibit the secretion of gonadotropins by the pituitary gland and inhibit the release of steroids by the gonads. The peptides are analogs of the decapeptide GnRH wherein there is at least one residue of an unnatural amino acid U* in the 3-, 5- and/or 6-positions. Such unnatural amino acids are useful in the synthesis of peptides and can be formed after synthesis of the peptide chain. The unnatural amino acid U* can have a cyanoguanidino group on the omega carbon atom of the side chain, but preferably has formula U* (I) where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 6 carbon atoms.

GnRH ANALOGS

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This invention relates generally to peptides
5 having unnatural amino acids and to the preparation of
new unnatural amino acids, which may be derived from
amino acids having an amino-substituted aromatic side
chain, such as para-amino Phe. More particularly, it
relates to GnRH analogs having such unnatural amino acids
10 which can be prepared either in such fully assembled
peptides or for incorporation into such peptides as a
part of the usual chain elongation synthesis process.

In one more particular aspect, the present
invention relates to peptides which inhibit gonadal
15 function and the release of the steroidal hormones,
progesterone and testosterone, as well as to methods of
preventing ovulation.

BACKGROUND OF THE INVENTION

20

Follicle stimulating hormone (FSH) and luteinizing
hormone (LH), sometimes referred to as gonadotropins or
gonadotropic hormones, are released by the pituitary
gland. These hormones, in combination, regulate the
25 functioning of the gonads to produce testosterone in the
testes and progesterone and estrogen in the ovaries, and
they also regulate the production and maturation of
gametes.

The release of a hormone by the anterior lobe of
30 the pituitary gland usually requires a prior release of
another class of hormones produced by the hypothalamus.
The hypothalamic hormone GnRH triggers the release of the
gonadotropic hormones, particularly LH; this hormone is
also referred to as LH-RH and as LRF. Mammalian GnRH is

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a well known decapeptide as described in U.S. Patent No. 4,072,668.

Peptides are compounds which contain two or more amino acids in which the carboxyl group of one acid is
5 linked to the amino group of the other acid. In conventional representation of peptides, the amino terminus appears to the left and the carboxyl terminus to the right. The positions of the amino acid residues are identified by numbering the amino acid residues from left
10 to right. In GnRH, the hydroxyl portion of the carboxyl group of glycine at the C-terminus has been replaced with an amino group(NH₂) i.e. the C-terminus is amidated. The abbreviations for the individual amino acid residues above are conventional and are based on the trivial name
15 of the amino acid, e.g. pGlu is pyroglutamic acid, Glu is glutamic acid, His is histidine, Trp is tryptophan, Ser is serine, Tyr is tyrosine, Gly is glycine, Leu is leucine, Nle is norleucine, Orn is ornithine, Arg is arginine, Har is homoarginine, Pro is proline, Sar is
20 sarcosine, Phe is phenylalanine, Ala is alanine, Val is valine, Nva is norvaline, Ile is isoleucine, Thr is threonine, Lys is lysine, Asp is aspartic acid, Asn is asparagine, Gln is glutamine, and Met is methionine. Except for glycine, amino acids of the peptides of the
25 invention are of the L-configuration unless noted otherwise.

There are reasons for desiring to prevent ovulation in female mammals, and the administration of GnRH analogs that are antagonistic to the normal function
30 of GnRH have been used to suppress or delay ovulation. For this reason, analogs of GnRH which are antagonistic to GnRH are being investigated for their potential use as a contraceptive or for regulating conception periods. GnRH antagonists may also be used for the treatment of
35 precocious puberty and endometriosis. Such antagonists have also been found useful to regulate the secretion of

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gonadotropins in male mammals and can be employed to arrest spermatogenesis, e.g. as male contraceptives for treatment of male sex offenders, and for treatment of prostatic hypertrophy. More specifically, GnRH

5 antagonists can be used to treat steroid-dependent tumors, such as prostatic and mammary tumors, and for the control of the timing of ovulation for in vitro fertilization. In the female, they can also be used to treat hirsutism.

10 In one aspect, it is desired to provide improved peptides which are strongly antagonistic to endogenous GnRH and prevent secretion of LH and FSH and the release of steroids by the gonads of mammals and which have good solubility at physiologically acceptable pH, such as pHs
15 of about 5 to about 6.5. Of particular interest are compounds which are more effective in vivo when administered orally.

SUMMARY OF THE INVENTION

20

The present invention provides unnatural amino acids that can be prepared de novo or by modifying a previously prepared peptide, or a protected peptide-resin, containing a desired overall sequence
25 which includes one or more amino acid residues having an amino group which is to be modified. Preferred amino acids of the invention have a side chain which contains either a modified guanidino group or a guanidino equivalent or a derivative that is obtained by further
30 elucidation of a modified guanidino group, as set forth hereinafter.

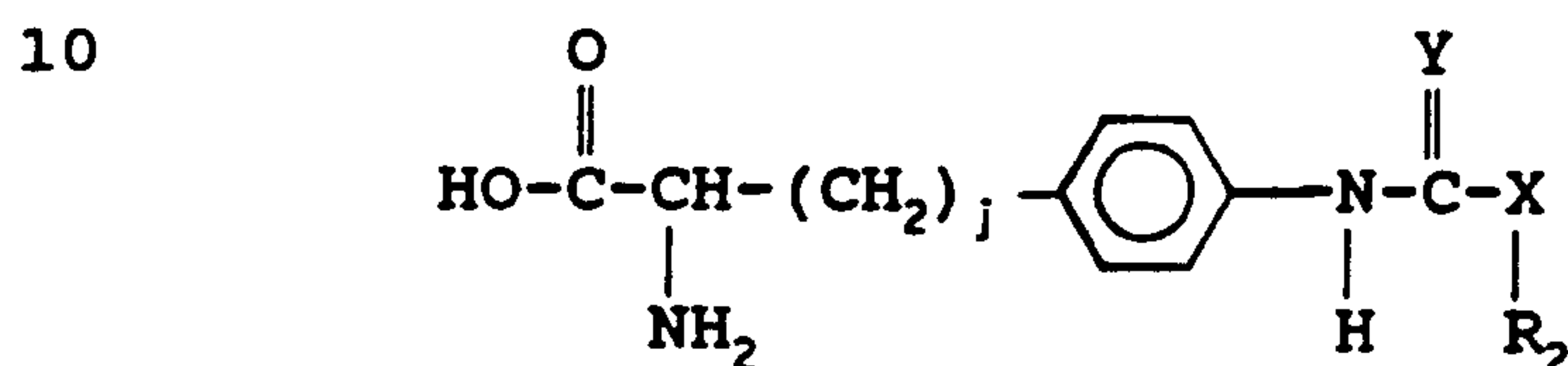
In another particular aspect, the invention provides peptides which inhibit the release of
35 gonadotropins in mammals, including humans, and it also provides methods for inhibiting the release of

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steroids by the gonads of male and female mammals.
As mentioned above, these GnRH antagonists may be used to inhibit the production of gonadotropins and sex hormones under various circumstances, including precocious
5 puberty, hormone dependent neoplasia, dysmenorrhea, endometriosis and steroid-dependent tumors.

The invention provides unnatural amino acids having the following formula U*:



where j is 1, 2 or 3; Y = N-CN, N-CONHR₉, O, S or CH-NO₂, where R₉ is H, Ac, alkyl (preferably C₁ to C₄), naphthyl, pyridyl, pyrimidyl, pyrazinyl, indolyl, quinolinyl or imidazolyl, which alkyl and cyclic groups are
20 unsubstituted or substituted (preferably by chloro, fluoro, bromo, amino, nitro, alkyl (C₁ to C₄) and alkoxy (C₁ to C₄)); X = NH, O, N₃, M₁-(CH_q)_p-M₂ or M₁-(CH₂)_p-M₂(CH₂)_{p'}-M₃, where M₁ is NR₁₀, N, O or CHR₃ wherein R₃ is methyl, ethyl, propyl, phenyl, pyridinyl,
25 pyrimidinyl or purinyl; q=1 or 2; p, p' and p'' are integers between 0 and 6; R₁₀ is H, methyl, ethyl, propyl, phenyl or substituted phenyl (preferably by Cl, F, NO₂ or NH₂); and M₂ and M₃ = M₁, COOH, CONH₂, COOR₃ or CN (preferably X is NH or O); R₁ = H, alkyl (preferably C₁ to
30 C₆ and most preferably C₁ to C₄), modified alkyl (preferably C₁ to C₅, the terminal carbon of which is either substituted with NH₂, OH, Cl, Br or F or is replaced with CF₃ or CF₂CF₃), alkenyl (preferably C₂ to C₄), such as CH₂CH=CHR₃, alkynyl (preferably C₂ to C₄),
35 such as CH₂C≡CR₃, aryl such as benzyl, tolyl, p-amino-benzyl (anilinyl) and pCl-benzyl or a direct bond to X; R₂ = R₁, OH, NH₂, NHR₁, heterocycle (preferably as illustrated hereinafter) or desR₂, with R₂ being desR₂

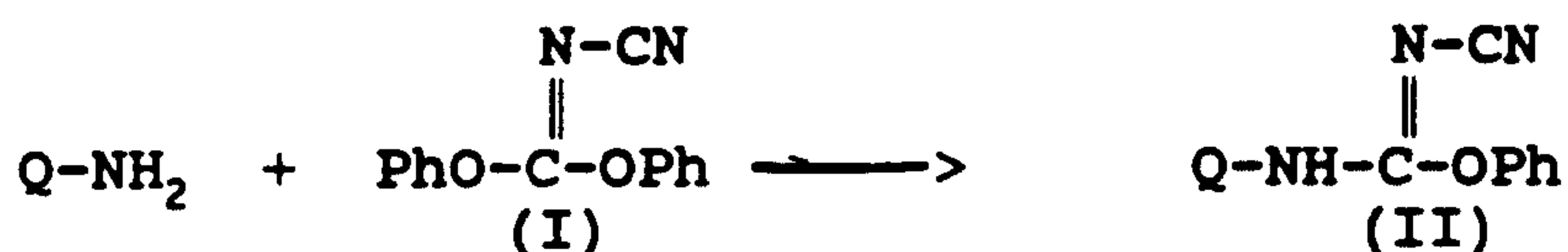
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when $X = N_3$. Optionally R_2 and X can be interconnected, or R_1 and R_2 can be connected to each other via a branched or unbranched methylene bridge of type $-(CH_2)_m-$ or $-(CH_2)_m-M-(CH_2)_{m'}-$. In such an R_1-R_2 moiety, m and m' are
 5 integers from 1 to 6 and preferably from 1 to 3; and $M = NH, O, S$ or CHR_4 , wherein R_4 is lower alkyl or aryl and is preferably methyl, ethyl, propyl, phenyl or pCl-phenyl, with M preferably being O or S . Most preferably, when R_1 and R_2 are interconnected, they form a 5, 6, or 7-member
 10 heterocyclic ring with the "N-C-X" portion of the formula U^* . If desired to form a cyclic peptide, XR_2 can contain a part of another diamino acid within the same peptide.

Modification of the specified primary amino function of a given amino acid or peptide is carried out
 15 by treatment of either the appropriately protected peptide or the amino acid with an appropriate reagent(s). Peptides or amino acids where Y is $N-CN$ (herein referred to as cyanoguanidines) are prepared by reaction of an amino group with diphenyl cyanocarbonimidate (I):

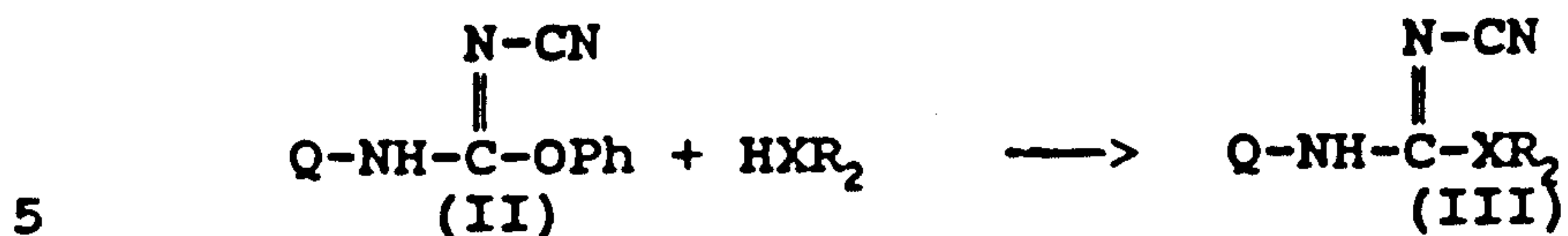
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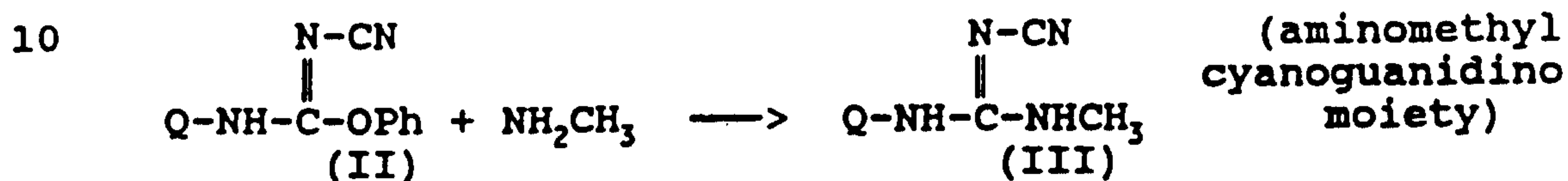
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wherein "Q" is used to broadly represent either the major portion of a peptide or an amino acid having a primary amino group (such as the amino acid which was described
 30 above) as a part of formula U^* .

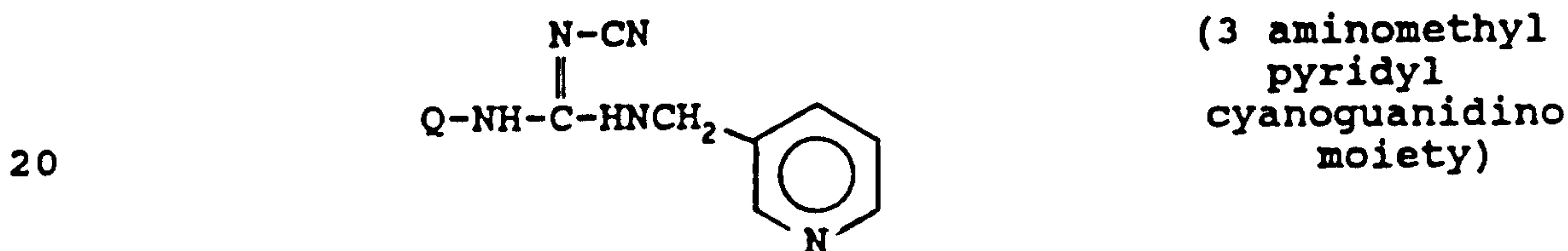
The peptide or amino acid having the N-substituted-N'-cyano-O-phenylisourea moiety (II) can then be either isolated or further functionalized by reaction with a second nucleophile HXR_2 to produce
 35 cyanoguanidine-containing peptides or amino acids having the formula (III):



For example,

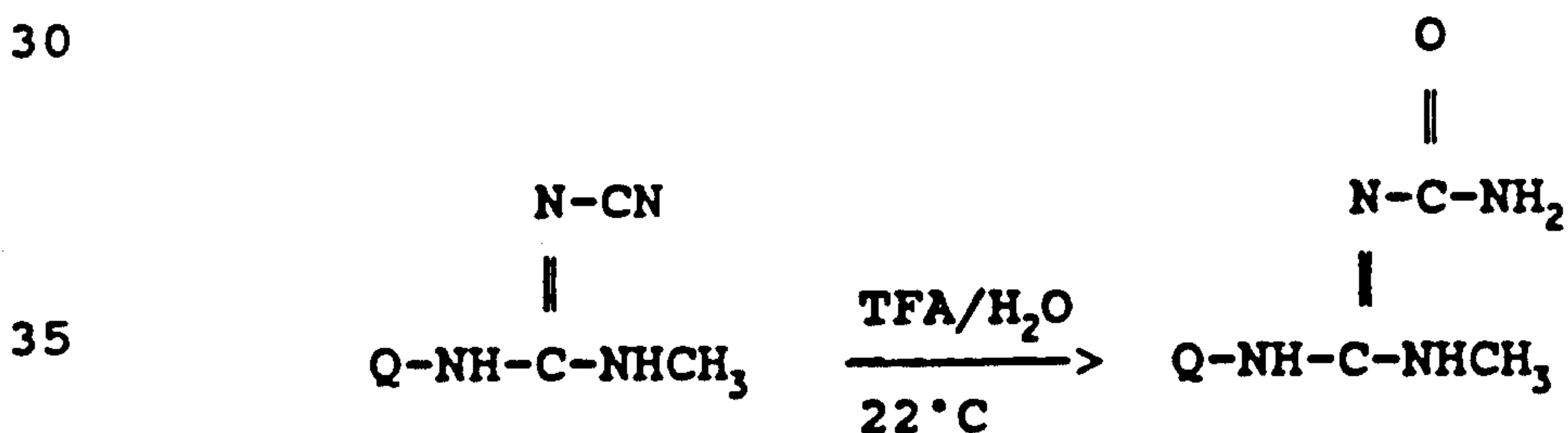


15 For example, where $\text{HXR}_2 = \text{H}_2\text{N-CH}_2\text{-pyridine}$, the result is:



25 This group may also be referred to (IUPAC nomenclature) as N-g-cyano-N-g'-3-methylpyridylguanidine.

Such compounds can be hydrolyzed under acidic conditions to produce compounds which are also biopotent--for example:



40 The hydrolyzed versions, referred to herein as including the N-g'-amido group, can also be synthesized directly by reacting phosgene derivatives with moieties having a guanidino function.

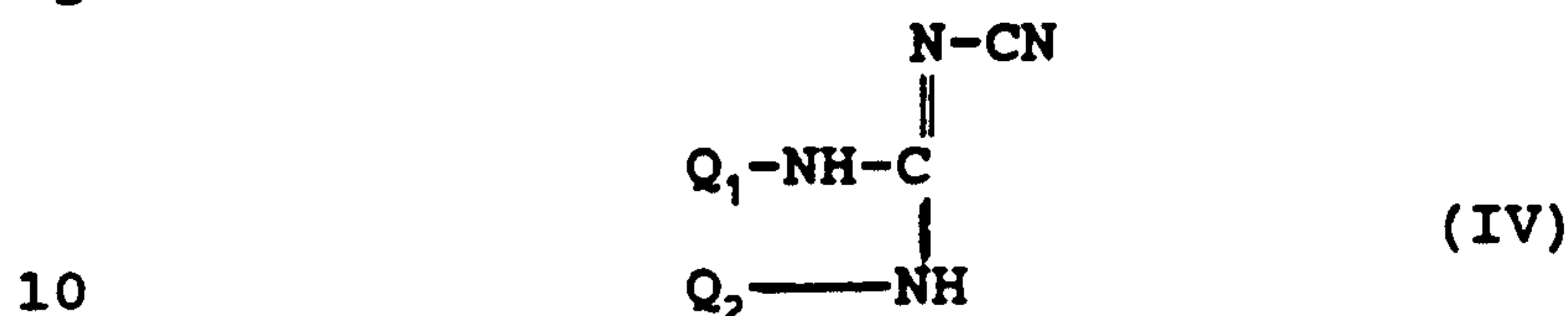
45 If HXR_2 is an amino group of another peptide or protein, one will obtain a peptide-peptide dimer or peptide-protein dimer conjugated via the cyanoguanidine

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moiety. If HXR_2 is the N-terminal primary amino group or the side chain amino group of another amino acid in the same peptide, one will obtain a cyclic peptide (IV) linked via the cyanoguanidine moiety:

5



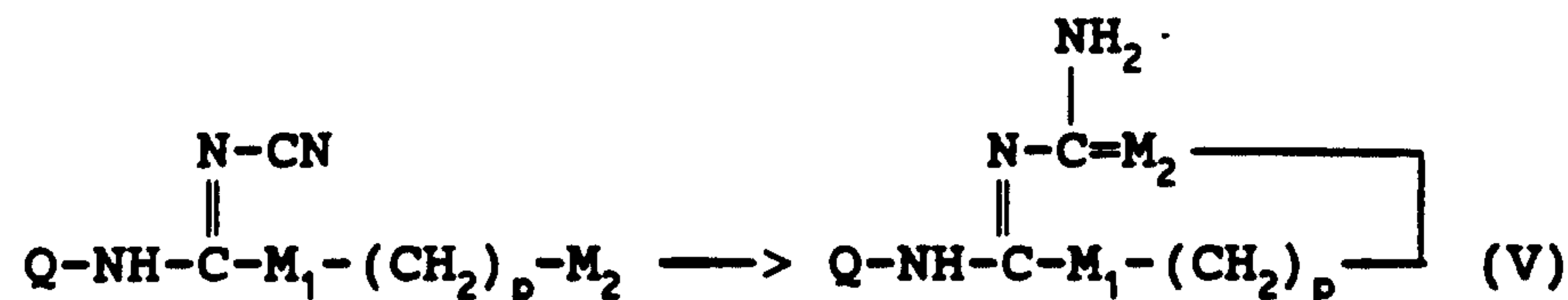
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wherein Q_1 and Q_2 represent the remainders of two amino acid residues in the same peptide. Cyclization via the cyanoguanidine derivative is preferably effected while a part of the peptidoresin, as opposed to subsequently cyclizing the linear peptide.

A special case arises when -XR_2 contains a second nucleophilic site and X has the general form:

20 $\text{M}_1\text{-(CH}_q\text{)}_p\text{-M}_2$ or $\text{M}_1\text{-(CH}_2\text{)}_{p'}\text{-M}_2\text{-(CH}_2\text{)}_{p''}\text{-M}_3$, where M_1 , M_2 and M_3 are individually NH , N , O or CHR_3 , with p , p' , p'' being 0,1,2 or 3 and q being 1 or 2. Examples of such nucleophiles include H_2NNH_2 , CH_3HNNH_2 , $\text{CH}_3\text{HNNHCH}_3$, H_2NOH , and $\text{H}_2\text{N-CH}_2\text{-CH}_2\text{OH}$. In this case, the cyanoguanidine moiety that is formed can be converted into the corresponding heterocycle (V) which forms from the initial intermediate by reaction of the omega amino group with the cyano group such as:

30



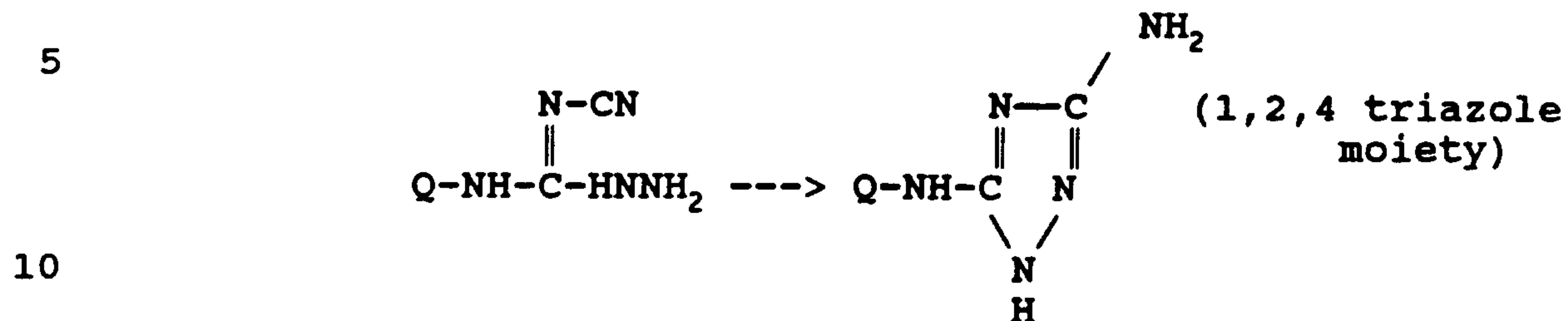
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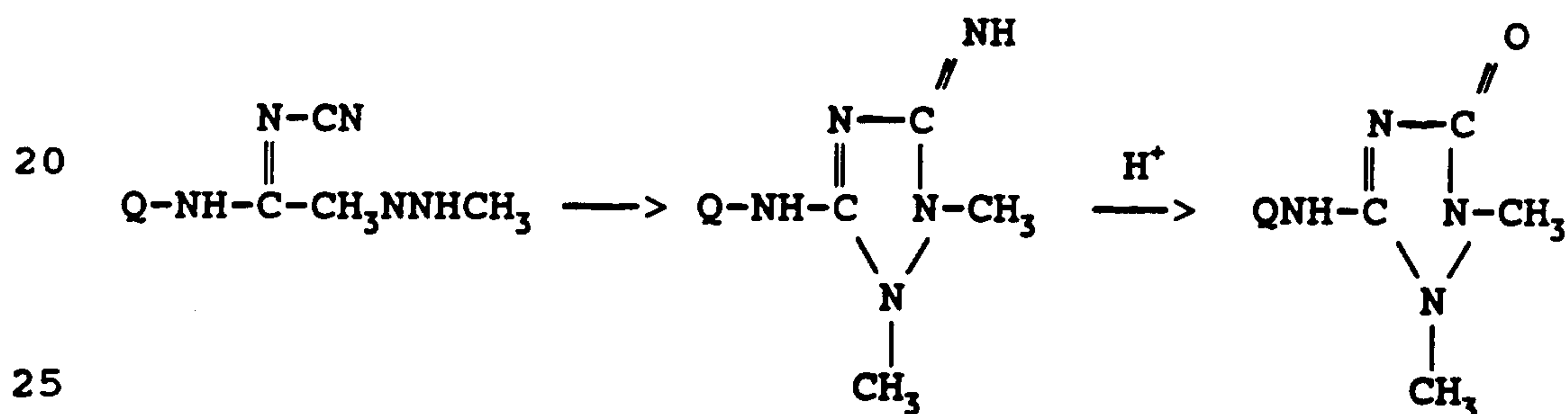
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For example, where $-XR_2 = -HNNH_2$,



Furthermore, where $-XR_2 = -CH_3NNHCH_3$

15



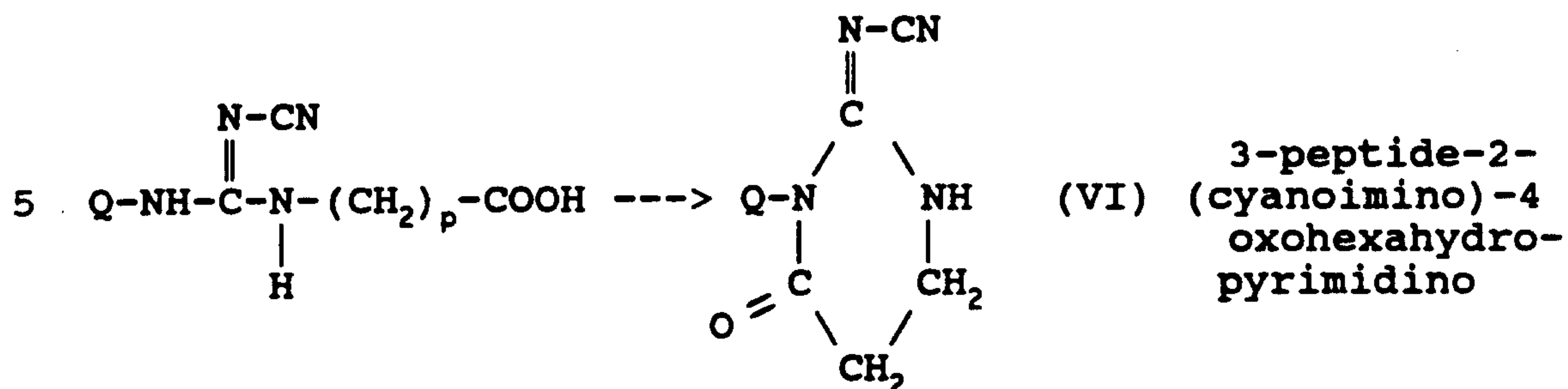
which may then undergo hydrolysis, as indicated above.

Where XR_2 contains a carboxylic acid group or the
 30 equivalent, particularly a carboxylic ester or carboxylic
 amide, a heterocyclic moiety, such as a saturated
 pyrimidine-like moiety (VI), is formed, by reaction of
 the carboxylic group with the secondary amino group (R_1),
 when M_1 is N, and similar 6-membered heterocyclic moieties
 35 are formed when M_1 is O or S. For example, R_2 may be
 $M_1-(CH_2)_p-M_2$ with $M_2 = COOH$, $COOCH_3$ or $CONH_2$ and p being an
 integer between 1 and 4. For instance in such a case
 where an aliphatic carboxylic acid group is present and
 $p=2$:

40

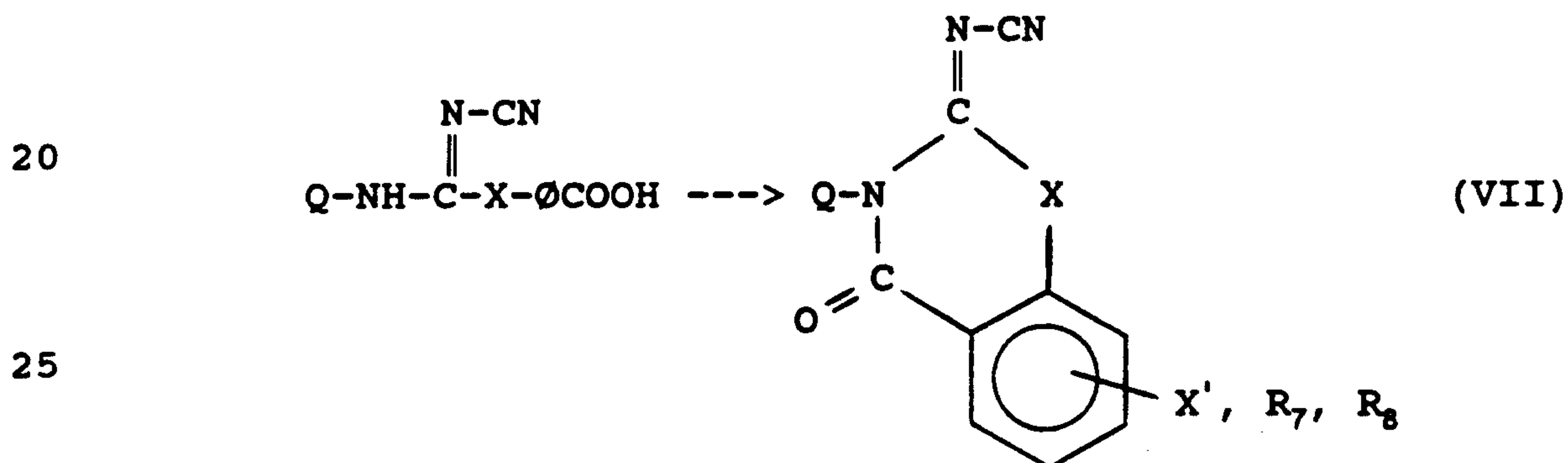
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If R_2 includes an ortho-substituted aromatic carboxylic acid, e.g. benzoic acid ($q=1$ and $p=6$), the corresponding quinazoline-like species (VII) is formed:

15



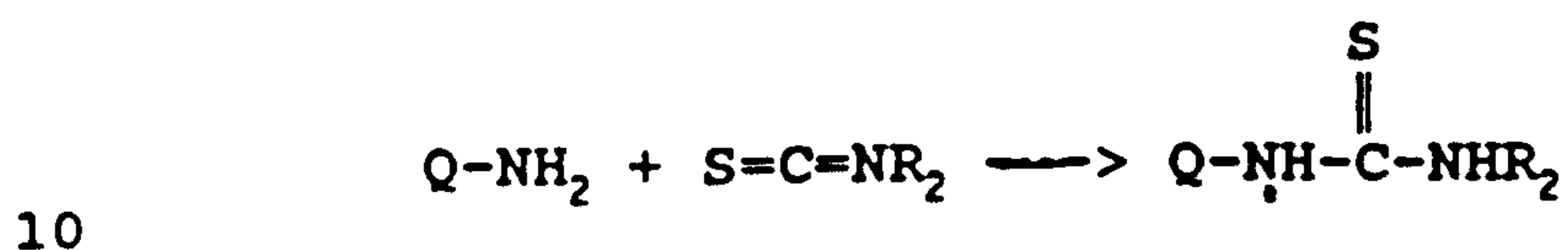
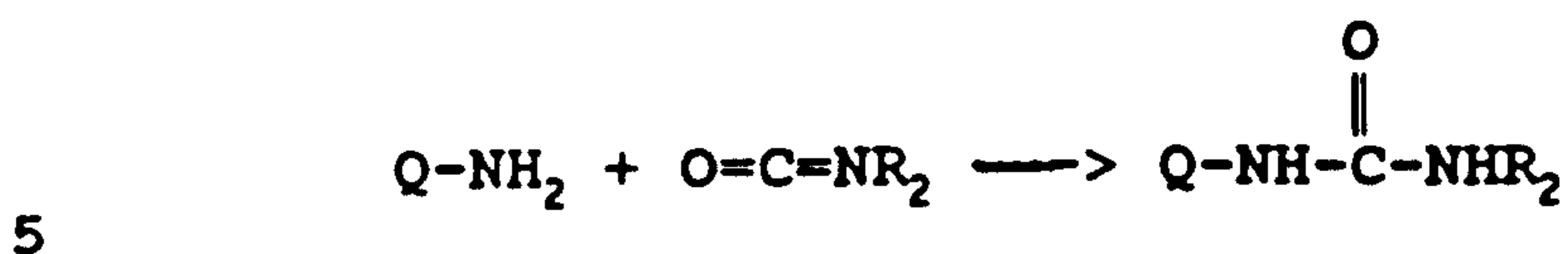
Such benzoic acid may be further substituted, and such substitutions may in any of the other 4 ring positions, as shown, creating the corresponding substituted quinazoline-like moiety which is considered to be equivalent to the unsubstituted. X' may be H, Cl, Br, F, $NHCH_3$ or SCH_3 , and R_7 and R_8 may be H, CH_3 or CH_2CH_3 .

30

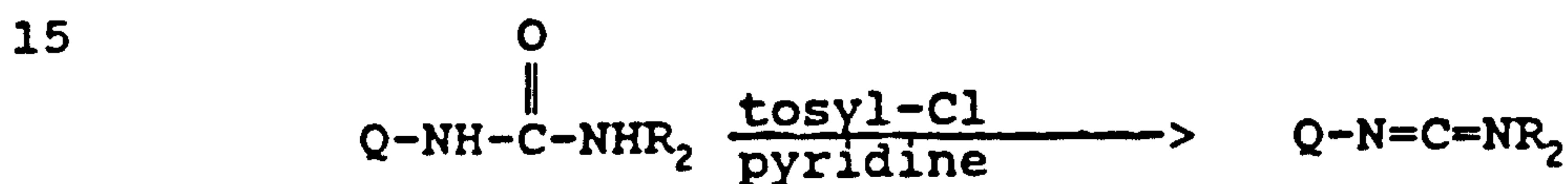
35 The molecules wherein $X = N_3$ and R_2 is $desR_2$ (i.e. deleted) are useful for photolabeling because of the activity of the $-N_3$ group and are formed by reacting the moiety (II) with sodium azide (NaN_3).

Peptides wherein Y is O (herein referred to as ureas) or S (referred to as thioureas) are prepared by the well known procedure in which the desired side chain amino group is treated with an appropriate isocyanate or thioisocyanate to obtain such ureas or thioureas.

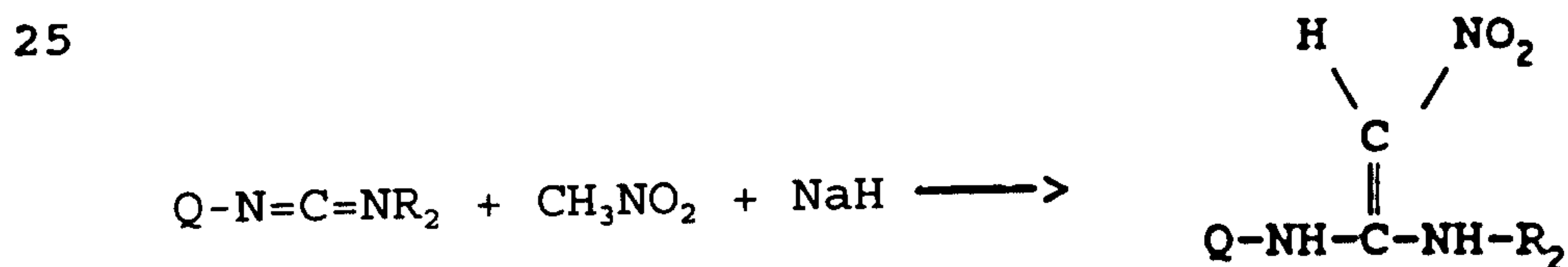
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Peptides or amino acids wherein Y is CH-NO₂ (herein referred to as diaminonitroethylenes) are prepared by conversion of the corresponding urea to a carbodiimide:

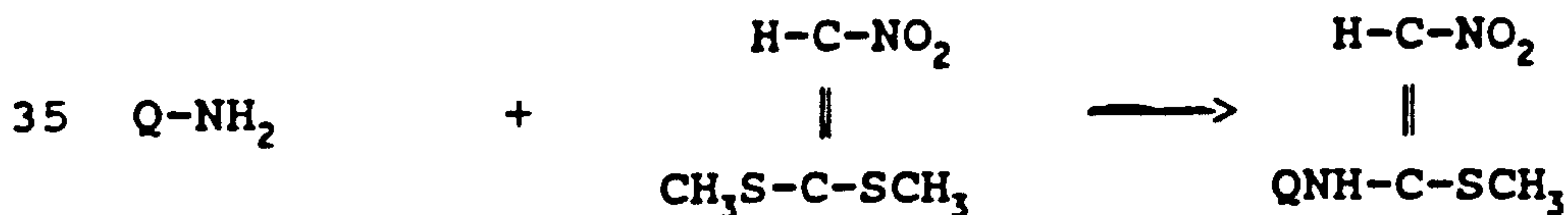


20 followed by treatment with nitromethane anion (prepared by the action of sodium hydride on nitromethane in dry DMF) as disclosed generally in F. Meimas, et al., Synthesis, 509-510 (1985):



30

An alternative synthesis that may be used is as follows:



Generally, in accordance with the present invention, peptides are synthesized which are antagonists

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of GnRH, i.e., they strongly inhibit the secretion of gonadotropins by the pituitary gland of mammals, including humans, and/or inhibit the release of steroids by the gonads. These peptides are analogs of GnRH containing one or more unnatural amino acids of the formula U* in the 3-position, the 5-position and/or the 6-position. Preferably they are present only in the 5- and 6-positions. All the peptides contain at least one D-isomer, which may be the unnatural amino acid. When U* is in the 3- and/or 6-position, it is always in the form of a D-isomer; whereas when U* is in the 5-position, it is always in the form of an L-isomer. The antagonist should have a 1-position substitution, preferably β -(1-or 2-naphthyl)-D-alanine (hereinafter β -D-1NAL or β -D-2NAL) or dehydroPro, a 2-position substitution in the form of a modified D-Phe, preferably 4Cl or 4F, and a 3-position substitution, preferably in the form of substituted or unsubstituted D-Trp, D-3PAL, β -D-NAL or the residue of a D-isomer amino acid U*, most preferably D-3PAL. The 5-position may be occupied by (a) Tyr, (b) a halogenated or methylated Phe or Tyr, (c) Arg, (d) Lys in which the side chain amino group is acylated by 3-carboxypyridine (nicotinic acid) or by 2 or 4-carboxypyridine, i.e. Lys(cpd), preferably Lys(3cpd) which is also referred to as Lys(Nic), (e) His or (f) the residue of an L-isomer amino acid U*, preferably the latter. The antagonists preferably have a D-isomer U* but may have a substituted or acylated D-Lys in the 6-position. If Leu is not in the 7-position, it is preferably NML; however, it may be Nle, Phe, Nva, Met, Tyr, Trp or PAL (of which the Phe or Trp residue may be substituted). The antagonists may also have an optional substitution in the 8-position, which is preferably isopropyl Lys, i.e., ILys or Lys(Ipr) wherein the side chain amino group is substituted by

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isopropyl. If a substitution is made in the 10-position, it is preferably D-Ala. At least one residue of a D-isomer amino acid of the formula U* is most preferably present in each peptide of the invention.

- 5 Modified D-Phe in the 2-position provides increased antagonistic activity as a result of the specific modifications present in the benzene ring. Single substitutions for hydrogen in the ring are preferably made in the para- or 4-position, but might be
10 in either the 2- or 3-position also; the substitutions are selected from chloro, fluoro, bromo, methyl, methoxy and nitro, with 4 chloro being most preferred. Dichloro substitutions are in the 2,4 or 3,4 positions in the ring. The α -carbon atom may also be methylated, e.g.
15 (C^αMe/4Cl)Phe. The 1-position substituent is preferably modified so that its α -amino group contains an acyl group, such as formyl(For), acetyl(Ac), acrylyl(Acr), vinylacetyl(Vac) or benzoyl(Bz), with acetyl and acrylyl being preferred and with acetyl being most preferred.
- 20 PAL and D-PAL represent the L- and D-isomers of pyridylalanine where the β -carbon of Ala is linked to the 2-, 3- or 4-position, preferably to the 3-position, on the pyridine ring. When β -D-NAL is present in the 1-position and R₅ is not Arg, a hydrophilic D-amino acid
25 residue, such as 4NH₂-D-Phe, 4-guanidino-D-Phe, D-His, D-Lys, D-Orn, D-Arg, D-Har(Homoarginine) or D-PAL is preferably present in the 6-position if U* is not present. When dehydroPro is present in the 1-position, D-PAL or a
30 D-isomer of a lipophilic amino acid, such as D-Trp, D-Phe, For-D-Trp, NO₂-D-Trp, D-Leu, D-Ile, D-Nle, D-Tyr, D-Val, D-Ala, dialkyl Arg, dialkyl Har, D-Ser(OtBu), β -D-NAL or (imBzl)D-His is preferably in the 6-position, if U* is not present.

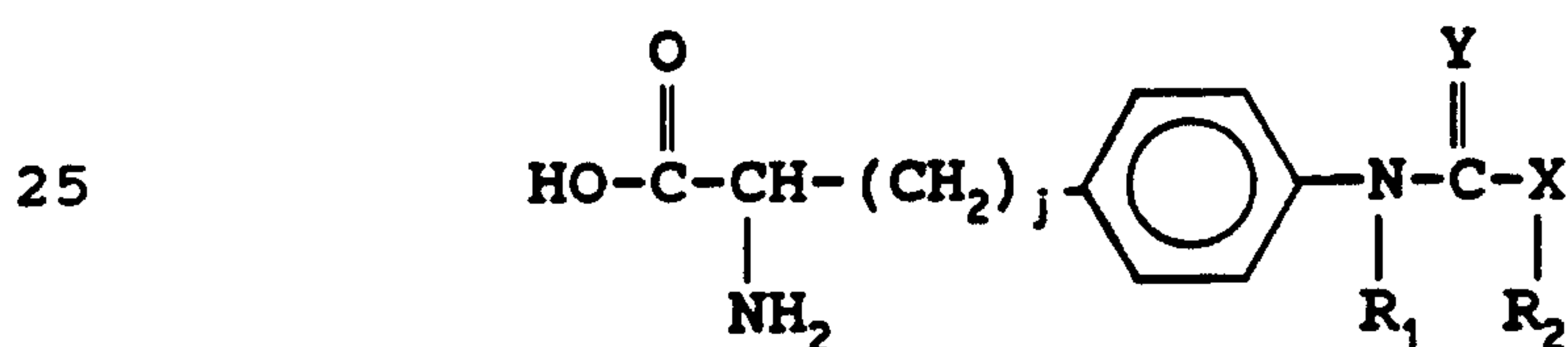
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These GnRH analogs are very soluble at a pH just below physiological pH, i.e. at about 4.5 to about 6, and thus can be formulated and administered in concentrated form, greatly facilitating administration at a pH of about 5 to 7.4 which is presently preferred. These antagonists inhibit ovulation of female mammals when administered at low levels at proestrus and are also effective to cause resorption of fertilized eggs if administered shortly after conception. These antagonists are also effective for the contraceptive treatment of male mammals and the treatment of steroid-dependent tumors. Certain of these antagonists are surprisingly long-acting in their suppression of LH levels following administration, and certain have a particularly low side effect in respect of histamine release.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As previously mentioned, the unnatural amino acids (which can be L- or D-isomers) are represented by the formula U*:



wherein W, X, Y, R₁ and R₂ are as defined previously, and there is at least one such residue (preferably a D-isomer) in each peptide of the invention.

More specifically, the GnRH antagonists of the present invention are represented by the following Formula (F₁):

G-AA₁-(A)D-Phe-AA₃-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀
 wherein G is hydrogen or an acyl group having 7 or less
 carbon atoms; AA₁ is dehydroPro, D-pGlu, (A)D-Phe,
 (B)D-Trp, Pro, or β -D-NAL; A is H, Cl, F, NO₂, CH₃, OCH₃,
 5 C^aMe/4Cl, Cl₂ or Br; B is H, NO₂, NH₂, OCH₃, F, Cl, Br, CH₃,
 NⁱⁿFor or NⁱⁿAc; AA₃ is U*, D-PAL, β -D-NAL or (B)D-Trp; AA₅
 is U*, Tyr, (C)Arg, Lys(cpd), Orn(cpd), Dbu(cpd),
 Dpr(cpd), (A)Phe, (3I)Tyr or His; AA₆ is U*, β -D-NAL,
 (B)D-Trp, (A')D-Phe, (D)D-Orn, (D)D-Lys, (D)D-Dbu,
 10 (D)D-Dpr, D-Har, D-Tyr, (E)D-His, D-PAL, (C)D-Arg or a
 suitable lipophilic D-isomer; A' is A, NH₂, NHCH₃ or guanadino;
 C is H or lower alkyl; D is G, cpd or an aryl group; E is H,
 imBzl or dinitrophenol; AA₇ is Nle, Leu, NML, (A)Phe, Met, Nva,
 Tyr, (B)Trp or PAL; AA₈ is (C')Arg, (C')Har or ILys; C' is H or
 15 di-lower alkyl; AA₁₀ is D-Ala-NH₂, Gly-NH₂, AzaGly-NH₂ or NH(R);
 R is lower alkyl, preferably CH₂CH₃; and U* is as defined
 above. When AA₁ is β -D-NAL and AA₅ is not Arg, then AA₆ is
 preferably U*, 4-NH₂-D-Phe, D-Lys, D-Orn, D-Har, D-His, 4-gua-
 D-Phe, D-PAL or D-Arg.

20 By dehydroPro is meant 3,4 dehydroproline, C₅H₇O₂N.
 By β -D-NAL is meant the D-isomer of alanine which is
 substituted by naphthyl on the β -carbon atom, i.e., also
 3-D-NAL. Preferably β -D-2NAL is employed wherein the
 attachment to naphthalene is at the 2-position on the
 25 ring structure; however, β -D-1NAL may also be used. The
 preferred 1-position residues are β -D-NAL, substituted
 D-Phe and optionally substituted D-Trp. PAL represents
 alanine which is substituted by pyridyl on the β -carbon
 atom; preferably the linkage is to the 3-position on the
 30 pyridine ring. When substituted D-Trp is employed,
 single substitutions for hydrogen are preferably made in
 either the 5- or 6-position, which are selected from
 chloro, fluoro, bromo, methyl, amino, methoxy and nitro,
 with chloro, fluoro and nitro being preferred.
 35 Alternatively, the indole nitrogen may be acylated, e.g.

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with formyl (N^{in} Formyl- or 1For-) or with acetyl (N^{in} Acetyl). D-3PAL, N^{in} For-D-Trp and 6NO₂-D-Trp are the preferred residues for the 3-position although D-Trp is also often used. When U* is not in the 5-position, it is preferably Tyr, Arg or Lys(cpd). By NML is meant N^αCH₃-L-Leu. By Dbu is meant alpha, gamma diamino butyric acid, and by Dpr is meant α,β diamino propionic acid. By Aph is meant 4NH₂Phe. By Hap is meant 4-amino-homophenylalanine (where j is 2); by Hhp is meant 4-amino-homohomophenylalanine (where j is 3). When dehydroPro is present in the 1-position, Tyr or U* is preferably present in the 5-position and a lipophilic residue is in the 6-position. By 4-gua-D-Phe is meant a residue of D-Phe having guanidine substituted in the para-position. By AzaGly-NH₂ is meant NHNHCONH₂.

The guanidino group of an Arg residue in the 5- or 6-position may be substituted by lower alkyl, i.e. 1 to 4 carbon atoms, e.g., propyl(Pr). When D-Lys, D-Dbu, D-Dpr or D-Orn is present in the 6-position, its side-chain-amino group may be acylated by an acyl group which may be aliphatic, heterocyclic or aromatic, e.g. nicotinic acid, or may be substituted by an aryl group having not more than 1 phenyl ring. When U* is not present in the 6-position, it is preferably D-PAL or D-Lys(cpd) and most preferably D-3PAL. The 7-position residue is preferably Leu, NML, Nle or Phe and most preferably Leu or NML. The 8-position residue is preferably either Arg or ILys, but most preferably ILys.

One preferred subgenus of GnRH antagonists has the following formula:

30

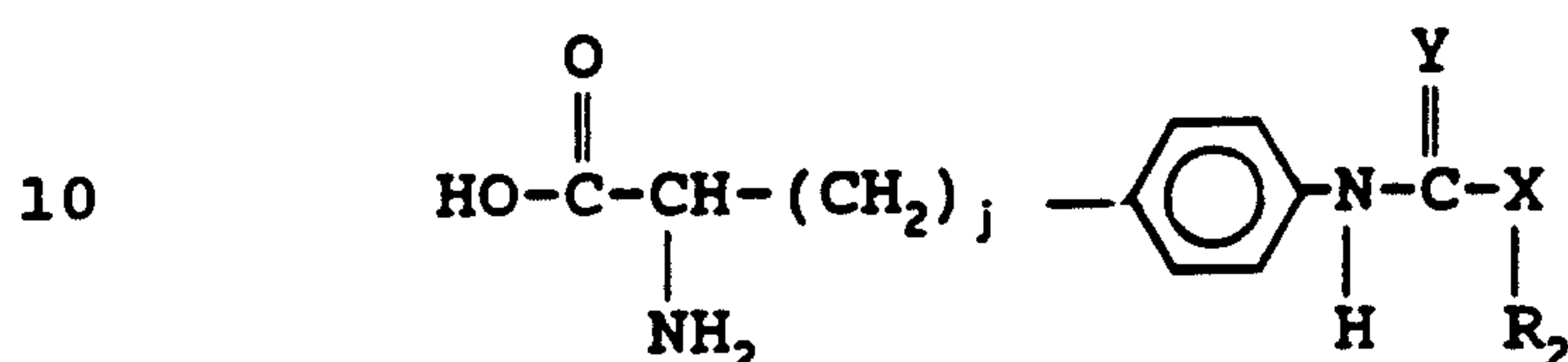
Ac-AA₁-(A)D-Phe-AA₃-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀ wherein AA₁ is β-D-NAL, (A)D-Phe, (B)D-Trp or dehydro Pro; A is H, 4Cl, 4F, 4NO₂, 4CH₃, 4OCH₃, C^αMe/4Cl, 2,4Cl₂ or 4Br; B is H, 6NO₂, 6NH₂, 6OCH₃, 6F, 6Cl, 6Br, 6CH₃, N^{in} For or N^{in} Ac;

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AA₃ is U*, D-PAL, β-D-NAL or (B)D-Trp; AA₅ is U*, Lys(cpd) or Tyr; AA₆ is U*, β-D-NAL, 4NH₂D-Phe, (B)D-Trp, D-Lys(cpd), D-PAL or D-Arg; AA₇ is Nle, Leu, NML or Phe; AA₈ is ILys, or Arg; AA₁₀ is D-Ala-NH₂, Gly-NH₂, NHNHCONH₂ or NH(R); R is lower alkyl; and U* is either

(a)

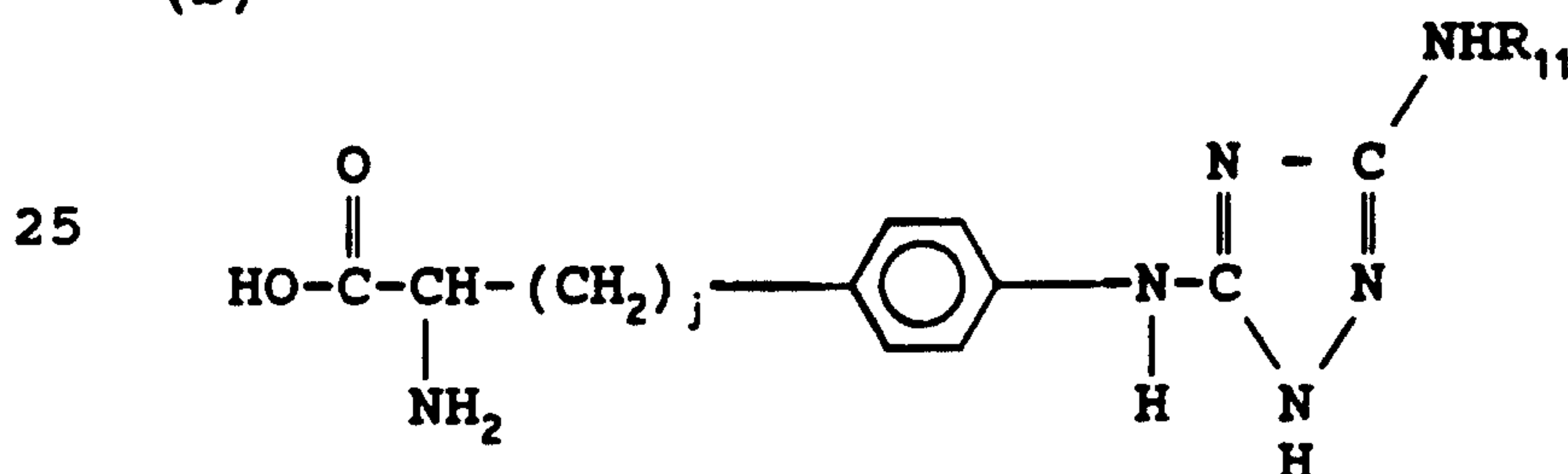


15

where j is 1, 2 or 3; X is NH or O; Y is N-CN or N-CONHR₉, where R₉ is H or lower alkyl; R₂ is lower alkyl, cyclohexyl, phenyl, pyridyl, methyl pyridyl or histaminy; or

20

(b)



30

where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 6 carbon atoms; provided, however, that at least one of AA₃, AA₅ and AA₆ is U*; preferably AA₅ and AA₆ are U*.

35

Another preferred subgenus of GnRH antagonists has the following formula:

Ac-AA₁-(A)D-Phe-U*-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀ wherein AA₁ is (A)D-Phe or β-D-NAL; A is 4Cl, 4F or 4NO₂; B is H, 6NO₂ or NⁱⁿFor; AA₅ is Lys(cpd) or Tyr; AA₆ is β-D-NAL, 4NH₂D-Phe, (B)D-Trp, D-Lys(cpd), D-PAL or D-Arg; AA₇ is Nle,

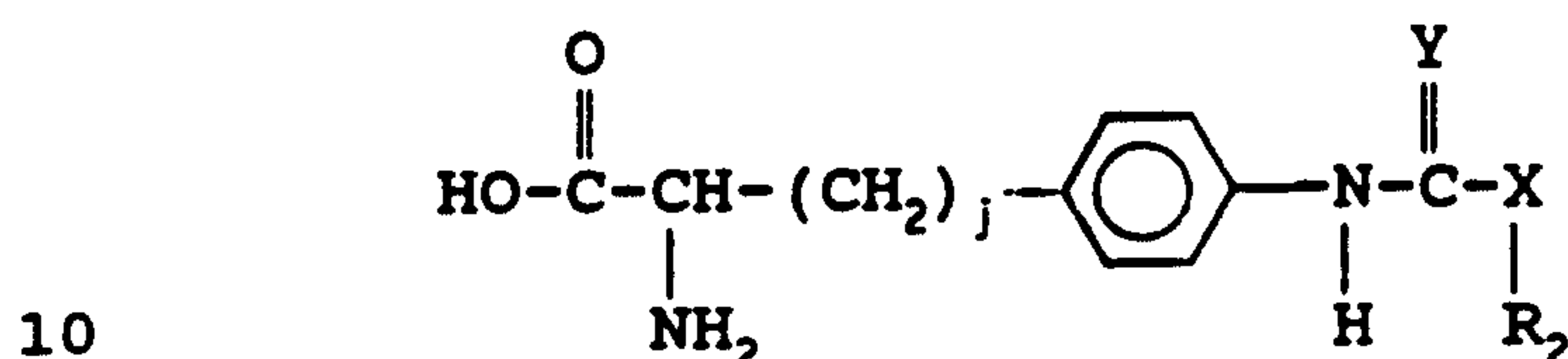
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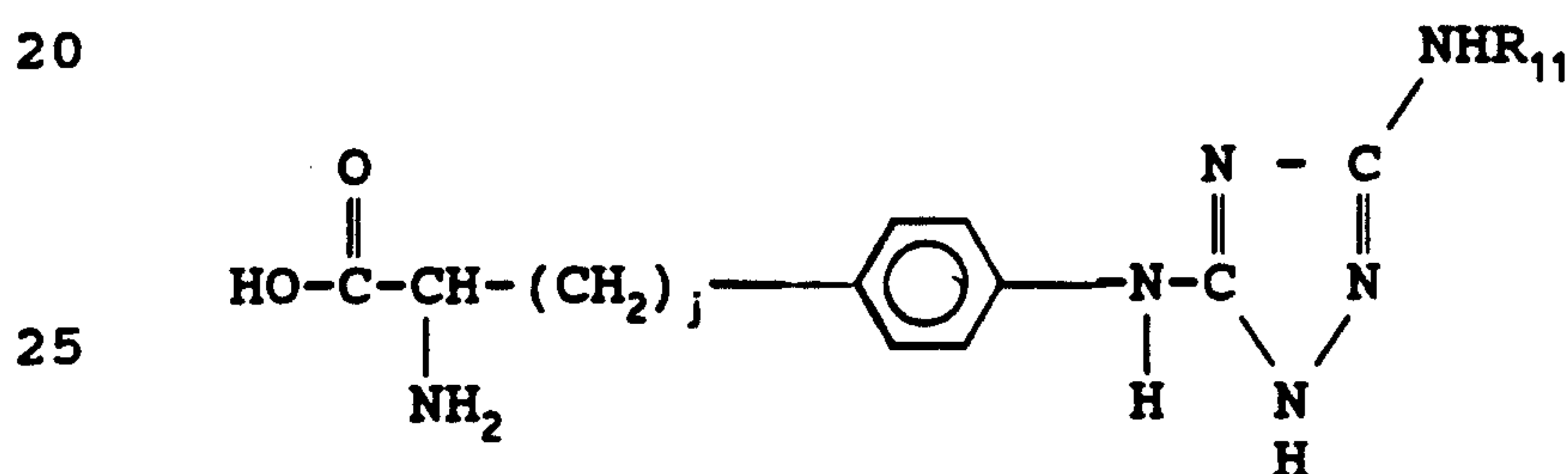
Leu, NML or Phe; AA₈ is ILys or Arg; AA₁₀ is D-Ala-NH₂, Gly-NH₂, NHNHCONH₂ or NH(R); R is lower alkyl; and U* is either

5 (a)



where j is 1, 2 or 3 (preferably 1); X is NH or O; Y is
 15 N-CN or N-CONHR₉, where R₉ is H or lower alkyl (C₁-C₃), preferably H; R₂ is lower alkyl (C₁-C₆), cyclohexyl, phenyl, pyridyl, methyl pyridyl or histaminy; or

(b)



30

where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 3 carbon atoms, preferably acetyl.

Yet another preferred subgenus of GnRH antagonists has the following formula:

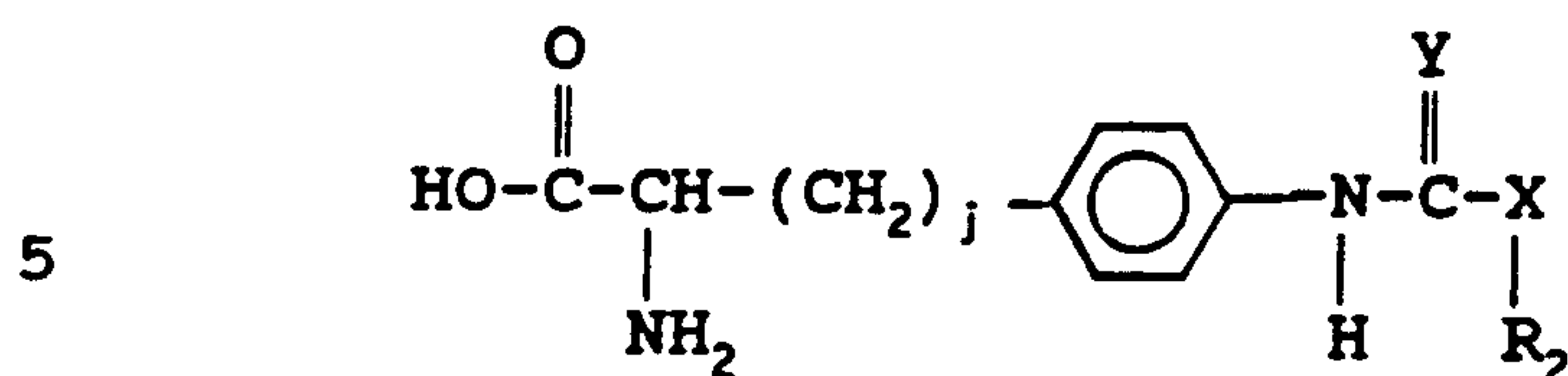
35

Ac-β-D-2NAL-(A)D-Phe-D-3PAL-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀
 wherein A is 4Cl or 4F; AA₅ is U*, Lys(cpd) or Tyr; AA₆ is U*, β-D-NAL, D-Lys(cpd), D-3PAL or D-Arg; AA₇ is Leu or NML; AA₈ is ILys or Arg; AA₁₀ is D-Ala-NH₂ or Gly-NH₂; and
 40 U* is either

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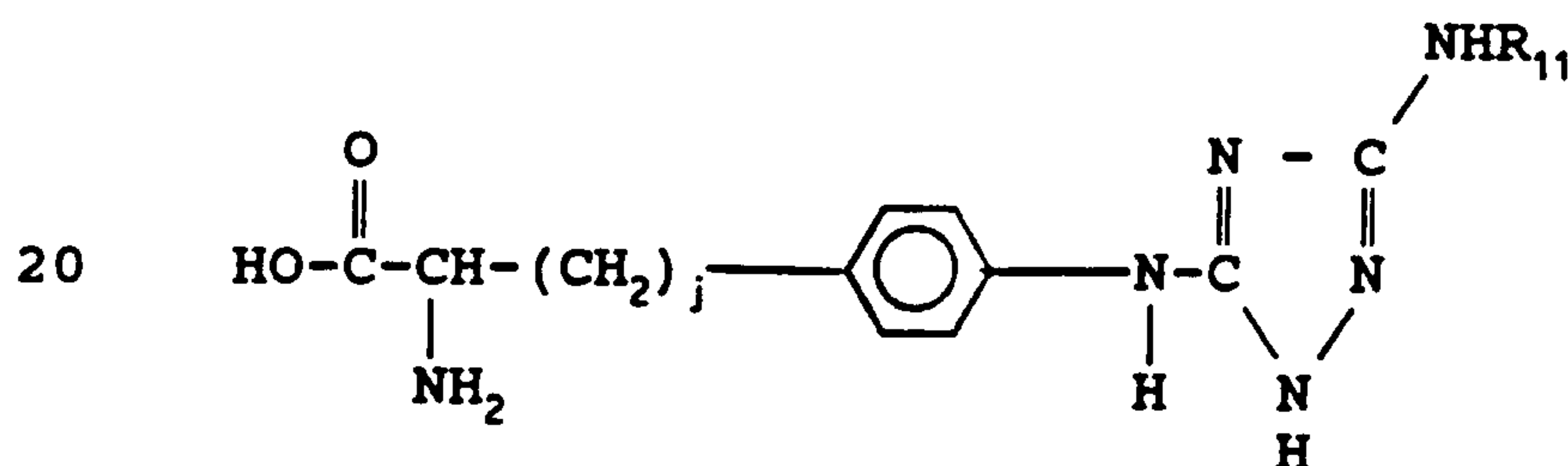
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(a)



10 where j is 1, 2 or 3 (preferably 1); X is NH or O; Y is N-CN or N-CONHR₉, where R₉ is H or lower alkyl (C₁-C₃), preferably H; R₂ is lower alkyl (C₁-C₆), cyclohexyl, phenyl, pyridyl or methyl pyridyl; or

15 (b)



25

where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 3 carbon atoms, preferably acetyl; provided that at least one and preferably both of AA₅ and AA₆ are U*.

The peptides of the present invention can be
 30 synthesized by classical solution synthesis, but are preferably synthesized by a solid phase technique. A chloromethylated resin or a hydroxymethylated resin may be used; however, a methylbenzhydrylamine (MBHA) resin, a benzhydrylamine (BHA) resin or some other suitable resin
 35 known in the art which directly provides a C-terminal amide or substituted amide upon cleavage is preferably employed when such a C-terminus is desired. For example, peptides having a substituted amide at the C-terminus are preferably synthesized using an N-alkylamino methyl resin

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as taught in United States Patent No. 4,569,967, issued February 11, 1986. Solid phase synthesis is conducted in a manner to stepwise add amino acids in the chain in the manner set forth in detail in the U.S. Patent No.

5 4,211,693. Side-chain protecting groups, as are well known in the art, are preferably included as a part of any amino acid which has a particularly reactive side chain and optionally in the case of others, such as Trp, which amino acids are to be coupled in the chain being
10 built upon the resin. Such synthesis provides the fully protected intermediate peptidoresin.

Chemical intermediates made generally in accordance with the invention may be represented by the formula: $X^1-AA_1-AA_2(X^5)-U_3-Ser(X^3)-U_5-U_6-AA_7(X^2 \text{ or } X^7)-AA_8(X^5 \text{ or } X^6)-Pro-X^8$ wherein: U_3 is either U' or $AA_3(X^2)$; U_5 is either U' or $AA_5(X^4 \text{ or } X^5)$; U_6 is either U' or $AA_6(X^4 \text{ or } X^5 \text{ or } X^6)$; U' is either $Aph(X^8)$, $Hap(X^8)$ or $Hhp(X^8)$; X^1 is an α -amino protecting group of the type known to be useful in the art in the stepwise synthesis of polypeptides and
20 when G in the desired peptide composition is a particular acyl group, that group may be used as the protecting group. Among the classes of α -amino protecting groups covered by X^1 are (1) acyl-type protecting groups, such as formyl(For), trifluoroacetyl, phthalyl, p-toluene-
25 sulfonyl(Tos), benzoyl(Bz), benzenesulfonyl, dithiasuccinoyl(Dts) o-nitrophenylsulfenyl(Nps), tritylsulfenyl, o-nitrophenoxycetyl, acrylyl(Acr), chloroacetyl, acetyl(Ac) and γ -chlorobutyryl; (2) aromatic urethan-type protecting groups, e.g.,
30 benzyloxycarbonyl(Z), fluorenylmethyloxycarbonyl(Fmoc) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxy-carbonyl(ClZ), p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl and p-methoxybenzyloxycarbonyl; (3) aliphatic urethan

protecting groups, such as tertbutyloxycarbonyl(Boc), diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl and allyloxycarbonyl; (4) cycloalkyl urethan-type protecting groups, such as

5 cyclopentyloxycarbonyl, adamantyloxycarbonyl and cyclohexyloxycarbonyl; (5) thiourethan-type protecting groups, such as phenylthiocarbonyl; (6) alkyl-type protecting groups, such as allyl(Aly), triphenylmethyl(trityl) and benzyl(Bzl); (7)

10 trialkylsilane groups, such as trimethylsilane. The preferred α -amino protecting group is Boc when X is hydrogen.

X^2 is hydrogen or a protecting group for the indole nitrogen of Trp, such as Bz, Ac or For. In many

15 syntheses there is no need to protect Trp, and such protection is not used if acylated D-Trp is present elsewhere in the peptide.

X^3 is a protecting group for the hydroxyl side chain of Ser or Thr, e.g. Ac, Bz, trityl, DCB or benzyl

20 ether(Bzl) and is preferably Bzl.

X^4 is hydrogen or a protecting group for the phenolic hydroxyl group of Tyr selected from the group consisting of tetrahydropyranyl, tert-butyl, trityl, benzyl, Z, 2-bromobenzyloxycarbonyl(2BrZ) and

25 2,6-dichlorobenzyl(DCB). 2BrZ is preferred.

X^5 is a protecting group for a side chain guanidino group, such as that in Arg or Har, or for the imidazole group of His, such as nitro, Tos, trityl, adamantyloxy-carbonyl, Z and 2,4-dinitrophenol(Dnp), or X^5 may be

30 hydrogen, which means there is no protection on the side chain group atoms. Tos is generally preferred.

X^6 is a protecting group for an amino side chain group, primary or secondary amino, such as Z or ClZ; X^6 is a subclass of X^6 comprising such protecting groups that

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can be removed without removing other side chain protecting groups so as to allow the omega-amino group to thereafter take part in the reactions to build the unnatural amino-acid residue. Preferably a base-labile group, such as Fmoc, methylsulfonyl ethoxycarbonyl (Msc) or trifluoroacetyl (Tfa), is used; however, it may also be possible to use a hydrazine-labile group such as phthaloyl,



or a thiolabile group such as Nps or Dts.

20 X^7 is hydrogen or a protecting group for Met, such as oxygen; Met is generally left unprotected.

X^8 may be Gly-NH-[resin support], D-Ala-NH-[resin support] or N(A)-[resin support]; X^8 may also be an amide either of Gly or of D-Ala or a substituted amide attached directly to Pro or NHNHCONH_2 .

The criterion for selecting side chain protecting groups for X^2 - X^7 is that the protecting group should be stable to the reagent under the reaction conditions selected for removing the α -amino protecting group (preferably Boc) at each step of the synthesis. Protecting groups generally should not be split off under coupling conditions but should be removable upon completion of the synthesis of the desired amino acid sequence under reaction conditions that will not alter the peptide chain.

35 When the X^8 group is Gly-NH-[resin support] or D-Ala-NH-[resin support], an amide bond connects Gly or

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D-Ala to a BHA resin or to a MBHA resin. When the X^8 group is N(A)-[resin support], a substituted amide bond connects Pro to an N-alkylaminomethyl (NAAM) resin. When X^8 is AzaGly-NH₂, the peptide is preferably made by
 5 classical solution synthesis, as disclosed in U.S. Patent No. 4,234,571.

When G is acetyl, for example, in the final formula, it may be possible to employ it as the X^1 protecting group for the α -amino group of β -D-NAL or
 10 whatever amino acid is used in the 1-position by adding it before coupling this last amino acid to the peptide chain. However, a reaction is preferably carried out with the peptide on the resin (after deblocking the α -amino group while the side-chain groups remain
 15 protected), e.g. by reacting with acetic acid in the presence of diisopropyl or dicyclohexyl carbodiimide (DIC or DCC) or preferably with acetic anhydride or by another suitable reaction as known in the art.

Thus, the invention also provides a method for
 20 making a peptide, said peptide having the formula:

G-AA₁-AA₂-AA₃-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀, wherein at least one of AA₃, AA₅ and AA₆ is U* and the symbols are as set forth hereinbefore, which method comprises (a) forming an intermediate peptide having the
 25 formula: X^1 -AA₁-AA₂(X^5)-U₃-Ser(X^3)-U₅-U₆-AA₇(X^2 or X^7)-AA₈(X^5 or X^6)-Pro- X^8 wherein: U₃ is either U' or AA₃(X^2); U₅ is either U' or AA₅(X^4 or X^5); U₆ is either U' or AA₆(X^4 or X^5 or X^6); U' is either Aph(X^8), Hap(X^8) or Hhp(X^8); X^1 is hydrogen or an α -amino protecting group; X^2 is hydrogen or
 30 a protecting group for an indole nitrogen; X^3 is a protecting group for a hydroxyl group of Ser or Thr; X^4 is hydrogen or a protecting group for a phenolic hydroxyl group of Tyr; X^5 is either hydrogen or a protecting group for a guanidino or imidazole side chain; X^6 is a

protecting group for a primary amino side chain of which X^6 is a subgroup that is removable without removing other protecting groups; X^7 is hydrogen or a protecting group for Met; X^8 is Gly-NH-[resin support], D-Ala-NH-[resin support], N(A)-[resin support], an amide either of Gly or of D-Ala or a substituted amide attached directly to Pro or NHNHCONH₂; provided however that at least one of U_3 , U_5 and U_6 is either Aph(X^8), Hap(X^8), or Hhp(X^8); (b) removing at least one X^8 to deprotect a side chain primary amino group of at least one amino acid residue of said intermediate peptide; (c) reacting said deprotected side chain primary amino group to build said residue into one having the formula U^* ; and (d) splitting off any remaining groups X^1 to X^7 and/or cleaving from any resin support included in X^8 .

Purification of the peptide is effected by ion exchange chromatography on a CMC column, followed by partition chromatography using the elution system: n-butanol;0.1N acetic acid (1:1 volume ratio) on a column packed with Sephadex G-25, or by using HPLC, as known in the art and specifically set forth in J. Rivier, et al. J. Chromatography, 288 (1984) 303-328.

The antagonists of the invention are effective at levels of less than 100 micrograms per kilogram of body weight, when administered subcutaneously at about noon on the day of proestrus, to prevent ovulation in female rats. For prolonged suppression of ovulation, it may be necessary to use dosage levels in the range of from about 0.1 to about 2.5 milligrams per kilogram of body weight. These analogs are particularly soluble at physiologically acceptable pHs and thus can be prepared as relatively concentrated solutions for administration. The antagonists are also effective to arrest spermatogenesis when administered to male mammals on a regular basis and

*Trade-mark

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can thus be used as contraceptives. Since these compounds will reduce testosterone levels (an undesired consequence in the normal, sexually active male), it may be reasonable to administer replacement dosages of testosterone along with the GnRH antagonist. These antagonists can also be used to regulate the production of gonadotropins and sex steroids for other purposes as indicated hereinbefore.

In the following formulas, the U* residues are defined in terms of the original amino acid residue having a side chain amino group plus the modification in question which is set forth in the accompanying parentheses. Preferably, the original residue Aph or Hap or Hhp, or the D-isomer form thereof, is incorporated in the main peptide chain and is modified while a part of the peptide chain that is still attached to the resin to form the desired residue of the amino acid U*. However, as indicated hereinbefore, the suitably protected unnatural amino acid U* can be added as a part of the usual chain elongation process.

With respect to ultimate modified side chain omega-amino group, the following abbreviations are used:

act=acetyl aminotriazole
bcg=aminobutyl cyanoguanidino
25 bzcg=aminobenzyl cyanoguanidino
bur=N-g-amido, N-g'-butylguanidino
chcg=aminocyclohexyl cyanoguanidino
ecg=aminoethyl cyanoguanidino
icg=aminoisopropyl cyanoguanidino
30 hcg=aminohexyl cyanoguanidino
hicg=histaminyl cyanoguanidino (ethylimidazole)
mcg=aminomethyl cyanoguanidino
ncg=aminoethyl(1 or 2)naphthyl cyanoguanidino
mncg=aminomethyl(1 or 2)naphthyl cyanoguanidino

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Ocg=O-phenyl cyanoguanidino
 pcg=aminopropyl cyanoguanidino
 Sbcg=thiobutyl cyanoguanidino
 tcg=3-amino 1,2,4 triazole
 5 trcg=indole ethylamino cyanoguanidino(tryptamino
 cyanoguanidino)
 mpcg= aminomethyl pyridyl cyanoguanidino (number
 indicates position of aminomethyl group on pyridyl
 ring)

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10

EXAMPLE 1

Peptides as indicated in TABLE 1 having the
 formula: Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-AA₅-AA₆-
 Leu-AA₈-Pro-D-Ala-NH₂ are prepared by the solid-phase
 15 procedure described generally hereinbefore.

TABLE 1

	AA ₅	AA ₆	AA ₈
103A	Aph(tcg)	D-Aph(tcg)	ILys
20 104A	Aph(bcg)	D-Aph(bcg)	ILys
105B	Hap(tcg)	D-Lys(Nic)	ILys
106C	Hhp(tcg)	D-Hhp(tcg)	ILys
107A	Aph(2ncg)	D-Aph(2ncg)	ILys
108A	Hhp(bcg)	D-Hhp(bcg)	ILys
25 109A	Hap(tcg)	D-Hap(tcg)	Arg
110A	Aph(icg)	D-Aph(icg)	ILys

As a working example, a solid phase synthesis is
 set forth hereinafter of Peptide No. 103A above, which is
 30 referred to as [Ac- β -D-2NAL¹, (4Cl)D-Phe², D-3PAL³,
 Aph(tcg)⁵, D-Aph(tcg)⁶, ILys⁸, D-Ala¹⁰]-GnRH. This peptide
 has the following formula:

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[Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-Aph(3-amino 1,2,4 triazole)-D-Aph(3-amino 1,2,4 triazole)-Leu-Lys(isopropyl)-Pro-D-Ala-NH₂.

An MBHA resin is used, and Boc-protected D-Ala is
5 coupled to the resin over a 2-hour period in CH₂Cl₂ using a 3-fold excess of Boc derivative and DCC as an activating reagent. The D-Ala residue attaches to the MBHA residue by an amide bond.

Following the coupling of each amino acid residue,
10 washing, deblocking and coupling of the next amino acid residue is carried out in accordance with the following schedule using an automated machine and beginning with about 5 grams of resin:

	STEP	REAGENTS AND OPERATIONS	MIX TIMES MIN.
15	1	CH ₂ Cl ₂ wash-80 ml. (2 times)	3
	2	Methanol(MeOH) wash-30 ml. (2 times)	3
	3	CH ₂ Cl ₂ wash-80 ml. (3 times)	3
	4	50 percent TFA plus 5 percent 1,2-ethanedithiol in CH ₂ Cl ₂ -70 ml. (2 times)	10
20	5	Isopropyl alcohol + 1% ethanedithiol wash-80 ml. (2 times)	3
	6	TEA 12.5 percent in CH ₂ Cl ₂ -70 ml. (2 times)	5
	7	MeOH wash-40 ml. (2 times)	2
25	8	CH ₂ Cl ₂ wash-80 ml. (3 times)	3
	9	Boc-amino acid (10 mmoles) in 30 ml. of either dimethylformamide(DMF) or CH ₂ Cl ₂ , depending upon the solubility of the particular protected amino acid, (1 time)	
30		plus DIC or DCC (10 mmoles) in CH ₂ Cl ₂	30-300
	10	MeOH wash-40 ml. (2 times)	3
	11	Triethylamine(TEA) 12.5 percent in CH ₂ Cl ₂ -70 ml. (1 time)	3

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After step 3, an aliquot may be taken for a ninhydrin test as well known in the art: if the test is negative, proceed to step 4 for removal of BOC-group prior to coupling of the next amino acid; if the test is positive or slightly positive, repeat steps 9 through 11.

The above schedule is used for coupling of each of the amino acids of the peptide of the invention after the first amino acid has been attached. N^αBoc protection is used for each of the remaining amino acids throughout the synthesis. N^αBoc-β-D-2NAL is prepared by a method known in the art, e.g. as described in detail in U.S. Patent No. 4,234,571, issued November 18, 1980 or commercially available from SyntheTech, Oregon, U.S.A. The side chain primary amino groups of Aph in the 5-position and of D-Aph in the 6-position are protected by Fmoc. Bzl(benzyl ether) is used as a side chain protecting group for the hydroxyl group of Ser. Boc-Lys(Ipr,Z) is used for the 8-position. After deblocking the α-amino group at the N-terminus using trifluoroacetic acid (TFA), acetylation is achieved using a large excess of acetic anhydride in dichloromethane.

Following completion of the assembly of the peptide and acetylation of the N-terminus, the following intermediate is present: Ac-β-D-2NAL-(4Cl)D-Phe-D-3PAL-Ser(Bzl)-Aph(Fmoc)-D-Aph(Fmoc)-Leu-Lys(Ipr,Z)-Pro-D-Ala-NH-[MBHA resin support]. The unnatural amino acids in the 5- and 6-positions are formed by simultaneously carrying out the following reactions with the deprotected side chains of the Aph residues. The Fmoc protecting group is removed from both by treatment of the peptidoresin with 20 percent piperidine in DMF for 5 minutes, then washing with DMF, then treatment with more piperidine/DMF for 20 minutes. After washing the resin with DMF, CH₃OH, CH₂Cl₂, and finally DMF, the newly freed

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amino group is treated with a large excess (>10 fold) of diphenyl cyanocarbonimidate (PCI) in DMF. Thereafter, the peptide is then subjected to the standard wash and then treated with hydrazine, dissolved in DMF, for 24 hours at
5 about 22°C. to complete the formation of the cyanoguanidino moiety; this step is preferably repeated. The cyanoguanidino moieties that are formed spontaneously convert to the corresponding heterocycle, i.e. 3-amino, 1,2,4 triazole.

10 The cleavage of the peptide from the resin and deprotection of the Ser and the Lys side chains takes place very readily at 0°C. with HF. Anisole is added as a scavenger prior to HF treatment. After the removal of HF under vacuum, the resin is extracted with 50% acetic
15 acid, and the washings are lyophilized to provide a crude peptide powder.

Purification of the peptide is then effected by high performance liquid chromatography (HPLC), as known in the art and specifically set forth in J. Rivier, et
20 al. J. Chromatography, 288 (1984) 303-328.

The peptide is judged to be homogeneous using capillary zone electrophoresis (CZE), as well as by using reversed-phase high pressure liquid chromatography and an aqueous triethylammonium phosphate solution plus
25 acetonitrile. Amino acid analysis of the resultant, purified peptide is consistent with the formula for the prepared structure, showing substantially integer-values for each amino acid in the chain; mass spectral analysis is also consistent. The optical rotation is measured on
30 a photoelectric polarimeter as

$$[\alpha]_D^{20} = -33 \pm 1.0 (c=1, 50\% \text{ acetic acid}).$$

35 The other peptides in Table 1 are similarly synthesized and purified. The peptides are assayed

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in vivo which determines their effectiveness to prevent ovulation in female rats. In this test, a specified number of mature female Sprague-Dawley rats, e.g. five to ten, each having a body weight from 225 to 250 grams, are
5 injected with a specified microgram dosage of peptide in either saline, bacteriostatic water, polyethylene glycol, corn oil or mixtures of the above with ethanol at about noon on the day of proestrus. Proestrus is the afternoon of ovulation. A separate female rat group is used as a
10 control to which the peptide is not administered. Each of the control female rats ovulates on the evening of proestrus; of the rats treated, the number of them which ovulate is recorded. In vivo testing of peptide 103A shows that at a dosage of 2.5 micrograms, 0 out of 8 rats
15 treated ovulate, and at a dosage of 1 microgram, 0 out of 7 rats ovulate. The peptide shows some activity at even 1/2 microgram where only 8 out of 18 rats ovulate. Peptide 104A is tested at 5 micrograms and shows that only 6 out of 11 rats ovulate.

20 In addition to the working synthesis set forth above, Peptide 103A is also synthesized with glycineamide at the C-terminus instead of D-Ala-NH₂; biological testing in vivo shows it is only slightly less biologically potent than Peptide 103A.

25 Peptide 103A was also synthesized with the N-ethylamide at the C-terminus instead of D-Ala-NH₂; biological testing in vivo showed it exhibited just slightly less biological potency than for Peptide 103A. In addition, Peptide 104A is also synthesized with the
30 glycineamide at the C-terminus instead of D-Ala-NH₂; biological testing in vivo shows somewhat less biological potency than Peptide 104A.

All peptides listed in Table 1 are considered effective to block GnRH-induced LH secretion in vitro at

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some reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female mammals at low dosages.

In addition to the above-mentioned in vivo testing, to determine effectiveness to prevent ovulation in female rats, groups of about 6 adult castrate male Sprague-Dawley rats, each having a body weight from 225 to 250 grams, are each injected IV with a 50 microgram dosage of either Peptide No. 103A or a standard antagonist referred to as the Nal-Glu antagonist (see J. Clin. Endo. Metab., 71, 4, 881-888 (1990)) in either corn oil or a phosphate buffer in the presence of BSA (bovine serum albumin). A separate rat group is used as a control, to which only the carrier is administered. Each of the control rats and the rats being treated is monitored for LH levels in the bloodstream at about 3 hours, 24 hours, 36 hours and 48 hours following this single bolus injection. Peptide No. 103A is surprisingly effective to suppress the level of circulating LH in the bloodstream of male rats, at levels very substantially below that of the control and, after 12 hours, below that of the rats treated with the Nal-Glu antagonist. This very long-acting duration of biological effect was truly surprising.

25

EXAMPLE 2

Peptides as indicated in TABLE 2 having the formula:

Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-AA₅-AA₆-AA₇-ILys-Pro-AA₁₀

5 are prepared by the solid-phase procedure referred to above.

TABLE 2

	AA ₅	AA ₆	AA ₇	AA ₁₀
111A	Aph(tcg)	D-Aph(tcg)	NML	D-Ala-NH ₂
112A	" (bcg)	" (bcg)	"	"
113B	" (bcg)	" (bcg)	Leu	Gly-NH ₂
114B	" (tcg)	" (tcg)	"	NHCH ₂ CH ₃
115A	" (tcg)	" (tcg)	NML	"
116A	" (tcg)	" (tcg)	Leu	Gly-NH ₂
117B	" (2mpcg)	" (2mpcg)	NML	D-Ala-NH ₂
118A	" (4mpcg)	" (4mpcg)	"	"
119A	" (icg)	" (icg)	"	"
120B	" (ecg)	" (ecg)	"	"
121A	" (3mpcg)	β -D-2NAL	"	"
122B	" (tcg)	"	Leu	"
123A	Lys(Nic)	D-Aph(tcg)	"	"
124A	Hap(bcg)	β -D-2NAL	"	"
125C	Hhp(bcg)	D-3PAL	"	"
126A	Tyr	D-Aph(tcg)	"	"

All peptides listed in Table 2 are considered effective to block GnRH-induced LH secretion in vitro at some reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female mammals at low dosages.

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EXAMPLE 3

Peptides as indicated in TABLE 3 having the formula: Ac- β -D-2NAL-(4Cl)D-Phe-AA₃-Ser-AA₅-AA₆-Leu-AA₈-Pro-D-Ala-NH₂ are prepared by the solid-phase procedure referred to above.

TABLE 3

	AA ₃	AA ₅	AA ₆	AA ₈
	127A D-Aph(tcg)	Tyr	D-3PAL	ILys
	128A " (icg)	"	"	"
5	129A " (bcg)	"	"	"
	130A " (tcg)	"	"	Arg
	131A " (bcg)	Arg	β -D-2NAL	"
	132A " (2mpcg)	"	"	"
	133A " (tcg)	"	"	"
10	134A " (bcg)	Tyr	D-Arg	ILys
	135A " (tcg)	"	"	"
	136A D-Hap(tcg)	"	D-3PAL	Arg
	137A " (bcg)	"	β -D-2NAL	ILys
	138A D-Hhp(bcg)	"	D-3PAL	"
15	139A " (2mpcg)	"	"	"
	140A D-Aph(2mpcg)	Arg	β -D-2NAL	Arg
	141A D-Hhp(bcg)	Tyr	D-Arg	"
	142A D-Hap(bcg)	Hap(bcg)	D-Hap(bcg)	ILys
	143A D-Aph(bcg)	Tyr	D-Aph(bcg)	"
20	144A D-3PAL	Aph(bur)	β -D-2NAL	Arg
	145A D-Aph(bur)	" "	"	"
	146A " (2mpcg)	Tyr	"	ILys
	147A " (tcg)	Tyr	D-Aph(tcg)	"
	148A " (tcg)	Aph(tcg)	D-3PAL	"

25 All peptides listed in Table 3 are considered effective to block GnRH-induced LH secretion in vitro at some reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female mammals at low dosages.

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EXAMPLE 4

Peptides as indicated in TABLE 4 having the formula: Ac-dehydroPro-(A)D-Phe-AA₃-Ser-AA₅-D-Aph(tcg)-Leu-ILys-Pro-D-Ala-NH₂ are prepared by the solid-phase
 5 procedure referred to above.

TABLE 4

		A	AA ₃	AA ₅
10	149	4Cl	β -D-2NAL	Tyr
	150	"	"	"
	151	4F	(1For) D-Trp	(2F) Phe
	152	"	"	Tyr
	153	"	"	(2NO ₂) Phe
15	154	"	(1Ac) D-Trp	(2CH ₃) Phe
	155	4Br	"	Tyr
	156	"	"	(2Br) Phe
	157	H	D-Trp	(2Cl) Phe
	158	4NO ₂	(5CH ₃) D-Trp	(3CH ₃) Phe
20	159	"	(5F) D-Trp	His
	160	2,4Cl ₂	(5Cl) D-Trp	(3F) Phe
	161	"	(6NO ₂) D-Trp	(3Br) Phe
	162	C ^α Me/4Cl	(5OCH ₃) D-Trp	(3I) Tyr
	163	3,4Cl ₂	(5NH ₂) D-Trp	(3Cl) Phe

25

All peptides listed in Table 4 are considered effective to block GnRH-induced LH secretion in vitro at some reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female
 30 mammals at low dosages.

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EXAMPLE 5

Peptides as indicated in TABLE 5 having the formula:

G-β-D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-Aph(tcg)-AA₆-Leu-

5 ILys-Pro-AA₁₀ are prepared by the solid-phase procedure referred to above.

TABLE 5

		G	AA ₆	AA ₁₀
10				
	164	Acr	D-Arg	D-Ala-NH ₂
	165	Ac	D-Arg	"
	166	Acr	D-Aph(tcg)	"
	167	For	D-Tyr	Gly-NH ₂
15	168	Bz	(Et)D-Arg	"
	169	Ac	D-Lys	"
	170	Vac	D-Har	"
	171	Acr	(4gua)D-Phe	AzaGly-NH ₂
	172	Ac	D-Orn	D-Ala-NH ₂
20	173	Acr	D-His	"
	174	Ac	(Bu)D-Arg	"
	175	"	(Bz)D-Orn	"
	176	Vac	(4NH ₂)D-Phe	"
	177	Bz	(Ac)D-Lys	AzaGly-NH ₂

25

All peptides listed in Table 5 are considered effective to block GnRH-induced LH secretion in vitro at some reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female
30 mammals at low dosages.

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EXAMPLE 6

Peptides as indicated in TABLE 6 having the formula: Ac-AA₁-(4Cl)D-Phe-AA₃-Ser-AA₅-AA₆-Leu-I-Lys-Pro-D-Ala-NH₂ are prepared by the solid-phase
 5 procedure referred to above.

TABLE 6

		AA ₁	AA ₃	AA ₅	AA ₆
10	178	β-D-2NAL	D-3PAL	Aph(bcg)	β-D-2NAL
	179	"	(6NO ₂) D-Trp	" "	(Dnp) D-His
	180	"	D-Trp	" (ecg)	(4gua) D-Phe
	181	dehydroPro	β-D-2NAL	Hap "	(6NO ₂) D-Trp
	182	"	β-D-1NAL	" (mcg)	D-Val
15	183	β-D-2NAL	(1For) D-Trp	" (tcg)	(Pr) D-Arg
	184	"	"	Aph(bcg)	(5NH ₂) D-Trp
	185	dehydroPro	D-Trp	" (2mpcg)	D-Tyr
	186	"	D-2PAL	" (4mpcg)	D-Nle
	187	"	(1Ac) D-Trp	" (hcg)	(4F) D-Phe
20	188	Pro	D-3PAL	Hhp(pcg)	β-D-1NAL
	189	(1For) D-Trp	"	" (chcg)	(4NHCH ₃) D-Phe
	190	β-D-2NAL	"	Aph(hcg)	(Ac) D-Orn
	191	"	"	" (Ocg)	(4NH ₂) D-Phe
	192	β-D-1NAL	(6Br) D-Trp	" (tcg)	(1For) D-Trp
25	193	(6CH ₃) D-Trp	D-4PAL	" (bzcg)	D-4PAL

The peptides listed in Table 6 are considered effective to block GnRH-induced LH secretion in vitro at a reasonable concentration. All of the peptides are
 30 considered to be effective to prevent ovulation of female mammals at low dosages.

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EXAMPLE 7

Peptides as indicated in TABLE 7 having the formula: Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-AA₅-AA₆-Leu-AA₈-Pro-D-Ala-NH₂ are prepared by the solid-phase
5 procedure referred to above.

TABLE 7

		AA ₅	AA ₆	AA ₈
10	194	Arg	D-Aph(bcg)	Arg
	195	"	" (Ocg)	"
	196	Aph(bcg)	(4gua) D-Phe	(Et ₂) Arg
	197	" (2ncg)	D-Aph(2ncg)	ILys
	198	" (bzcg)	" (bzcg)	Har
15	199	" (act)	" (act)	ILys
	200	Hap(hicg)	" (hicg)	"
	201	" (trcg)	D-Hap(trcg)	"
	202	Hhp(bcg)	" (bcg)	"
	203	" "	D-3PAL	(EtPr) Har
20	204	" "	D-Lys(Nic)	(Me ₂) Arg
	205	Aph(tcg)	D-Aph(tcg)	(MeBu) Arg
	206	" (Sbcg)	" (Sbcg)	ILys

The peptides listed in Table 7 are considered
25 effective to block GnRH-induced LH secretion in vitro at a reasonable concentration. All of the peptides are considered to be effective to prevent ovulation of female mammals at low dosages.

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Results of in vivo testing of selected of these antagonists are shown in the following Table A with dosages being given in micrograms per rat:

		TABLE A		
	Peptide No.	Dosage	Rats Ovulating	In Vitro histamine release: ED ₅₀ ± SEM(μg/ml)
5				
10	103A.	2.5	0/8	50 ± 6.7
		1.0	1/17	
		0.5	8/18	
	104A.	5.0	6/11	
15		2.5	2/3	
	111A	1.0	0/8	
		0.5	6/10	
	114B.	2.5	0/8	
		1.0	8/9	
20	115A.	10	0/5	
	116A.	1.0	9/16	
	117B.	5.0	0/6	
	127A.	15	1/5	
	128A.	15	0/9	
25		5	2/4	
	129A.	15	4/6	14 ± 2.1

The above Table A also reports the testing of various of these GnRH antagonists in an in vitro histamine release assay. All of these analogs are considered to be substantially less potent in releasing histamine than [Ac-D-2NAL¹, (4F)D-Phe², D-Trp³, D-Arg⁶]-GnRH for which the ED₅₀ was 0.17 ± 0.01 μg/ml--a very substantial advantage for these antagonists.

35

EXAMPLE 8

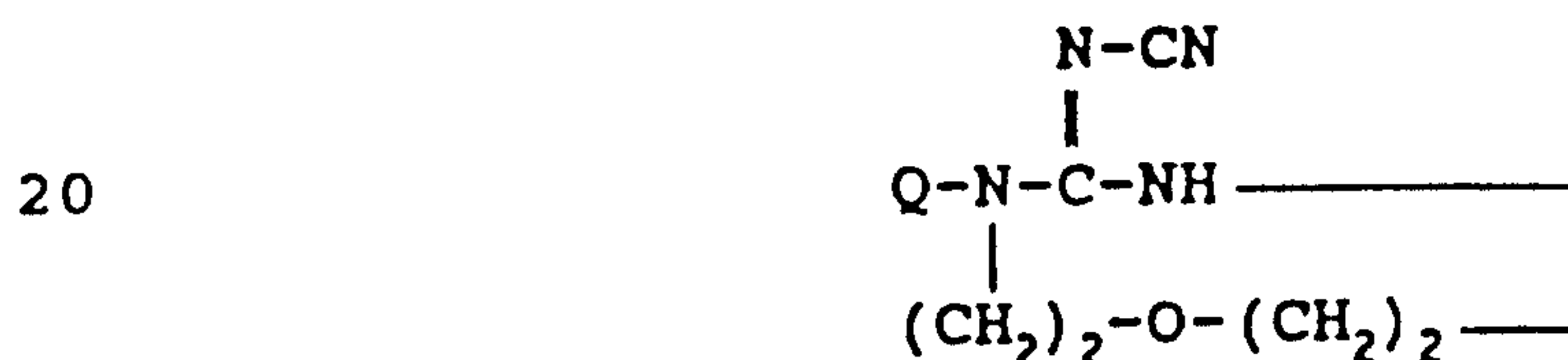
A peptide intermediate having the formula:
Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser(Bzl)-Tyr(2BrZ)-
5 D-Aph(Fmoc)-Leu-Lys(Ipr)-Pro-D-Ala-NH-[resin support] is
prepared by the solid phase procedure referred to above.
Following the removal of the Fmoc protection, the peptide
intermediate is reacted as generally described in Example
1 using naphthyl isocyanate instead of PCI to form the
10 naphthylurea moiety with the side chain amino group of the
D-Aph residue in the 6-position. Following cleavage and
HPLC purification as previously described, the GnRH
antagonist is tested. The peptide is considered to be
effective to prevent ovulation of female mammals at low
15 dosages.

EXAMPLE 9

A peptide intermediate having the formula:
Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser(Bzl)-Tyr(2BrZ)-
D-Aph(Fmoc)-Leu-Lys(Ipr)-Pro-D-Ala-NH-[resin support] is
20 prepared by the solid phase procedure referred to above.
Following the removal of the Fmoc protection, the peptide
intermediate is reacted as generally described in Example
1 using naphthyl isothiocyanate instead of PCI to form
the naphthylthiourea moiety with the side chain amino
25 group of the residue in the 6-position. Following
cleavage and HPLC purification as previously described,
the GnRH antagonist is tested. The peptide is considered
to be effective to prevent ovulation of female mammals at
low dosages.

EXAMPLE 10

A peptide intermediate having the formula:
 Ac- β -D-2NAL-(4Cl)D-Phe-D-3PAL-Ser(Bzl)-Tyr(2BrZ)-
 D-Aph(Fmoc)-Leu-Lys(Ipr)-Pro-D-Ala-NH-[resin support] is
 5 prepared by the solid phase procedure referred to above.
 Following the removal of the Fmoc protection, the peptide
 intermediate is first reacted using 2-bromoethyl,2'(Boc-
 amino)ethyl ether dissolved in DMF for 1 hour or until
 the ninhydrin test is negative to link the carbon atom to
 10 the side-chain amino group by the removal of the halogen
 to form: Q-NH-(CH₂)₂-O-(CH₂)₂-NH(Boc). This compound is
 then reacted as generally described in Example 1 using
 PCI to form the cyanoguanidino moiety with the side chain
 secondary amino group of the residue in the 6-position.
 15 Next the Boc protection group is removed, and the primary
 amino group reacts with the -OPh group to give the
 compound:



Following cleavage and HPLC purification as previously
 25 described, the GnRH antagonist is tested. The peptide is
 considered to be effective to prevent ovulation of female
 mammals at low dosages.

Following purification of the peptides, various of
 30 them are further characterized by dissolving in 0.1% TFA
 (10 μ g/10 μ L) and subjection to high performance liquid
 chromatography on C₁₈ silica (Vydac^{*} 0.45 x 25 cm) using a
 flow rate of 2.0 ml/min under isocratic conditions using
 a solution of 0.1% TFA in water plus the volume % (v/v)
 35 of acetonitrile indicated. The following Table B shows
 when the specific peptides elute from the C₁₈ silica

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having a particle size of about 5μ and a pore size of $300\text{\AA}$ when subjected to the isocratic flow described hereinbefore, with the time given in minutes. The optical rotation measurements are taken at $c=1$, 50% acetic acid, at room temperature.

TABLE B

	<u>Peptide No.</u>	<u>%CH₃CN</u>	<u>Time of Elution</u>	<u>[α]_D</u>
	103A	30.6	4.48	-33°
10	104A	43.2	4.02	-23°
	111A	38.4	3.75	
	113B	42.0	6.02	
	114B	38.4	4.00	
	115A	37.7	4.44	
15	116A	31.2	4.01	
	117B	31.8	4.76	
	127A	33.6	4.63	-30°
	128A	39.6	4.19	-30.3°
	129A	40.2	4.20	-42.0°

20

The peptides of the invention are often administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts, or of metal complexes, e.g., with zinc, barium, calcium, magnesium, aluminum or the like (which are considered as addition salts for purposes of this application), or of combinations of the two. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, nitrate, oxalate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginate, malate, ascorbate, tartrate and the like. The acetate salt is preferred. For example, an aqueous solution of the peptide can be repeatedly treated with 1N

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acetic acid and then lyophilized to yield the acetic acid salt thereof. If the active ingredient is to be administered in tablet form, the tablet may contain a pharmaceutically-acceptable diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

The pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier. Usually, the dosage will be from about 10 micrograms to about 2.5 milligrams of the peptide per kilogram of the body weight of the host when given intravenously; although oral dosages will be higher, it is anticipated that the nature of these compounds will permit effective oral administration. Overall, treatment of subjects with these peptides is generally carried out in the same manner as the clinical treatment using other antagonists of GnRH using a suitable carrier in which the peptide is soluble. Because of the favorable solubility, administration in saline is feasible.

It may also be desirable to deliver the GnRH analog over prolonged periods of time, for example, for periods of one week to one year from a single administration, and slow release, depot or implant dosage forms may be utilized.

These peptides can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, percutaneously, e.g. intranasally or intravaginally to

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achieve fertility inhibition and/or control and also in applications calling for reversible suppression of gonadal activity, such as for the management of precocious puberty or during radiation- or chemotherapy.

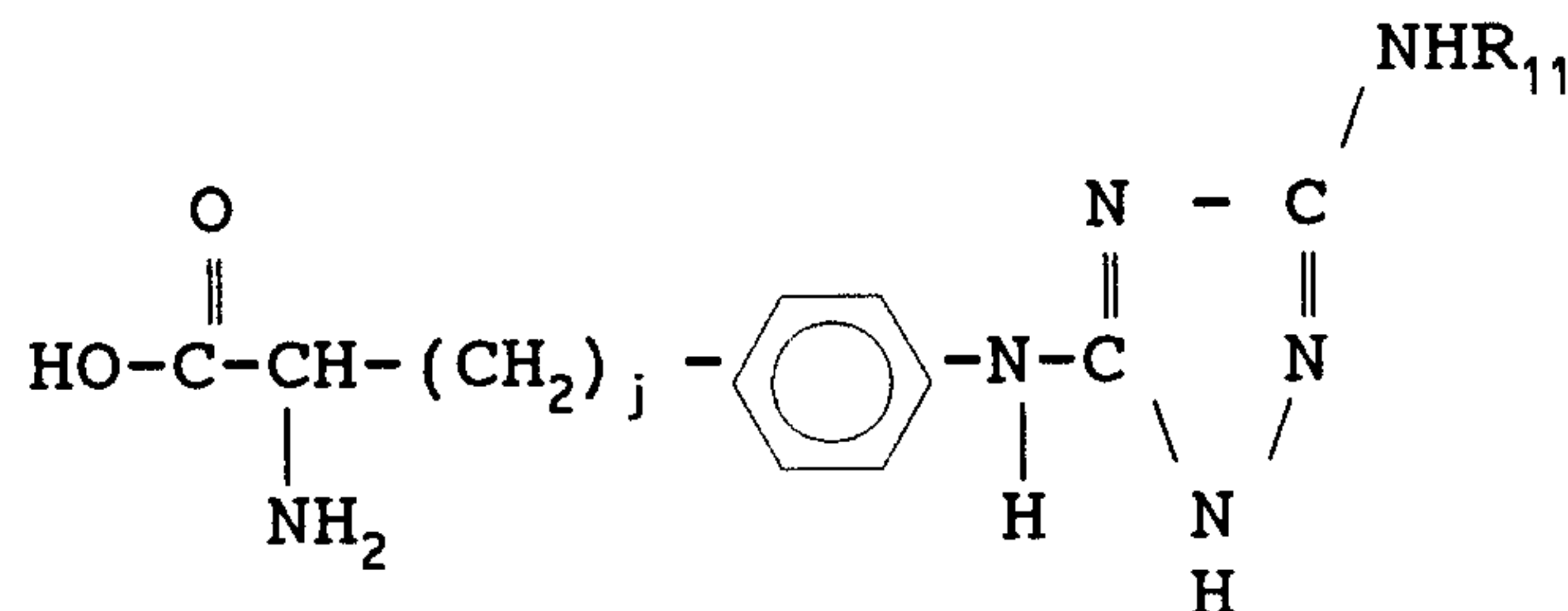
5 They are also useful for treatment of steroid-dependent tumors. Effective dosages will vary with the form of administration and the particular species of mammal being treated. An example of one typical dosage form is a bacteriostatic water solution containing the peptide
10 which solution is administered parenterally to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight per day. Oral administration of the peptide may be given in either solid form or liquid form.

Although the invention has been described with
15 regard to its preferred embodiments, it should be understood that changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims which are appended
20 hereto. For example, other substitutions known in the art which do not significantly detract from the effectiveness of the peptides may be employed in the peptides of the invention. D-2PAL and D-4PAL are considered to be equivalents of D-3PAL. Other equivalent
25 acylating groups can be used instead of acetyl at the N-terminus. Substituted Phe, such as (4F)Phe, can be used instead of Phe in the 7-position. Both butyl Lys and diethyl Lys are considered to be equivalents of ILys; however, ILys is preferred. Har is considered the
30 equivalent of Arg in the 8-position. Other hydrophobic amino acid residues can also be employed in the 1-position, preferably in D-isomer form, and are considered equivalents of those specified.

The embodiments of the invention in which an exclusive property of privilege is claimed are defined as follows:

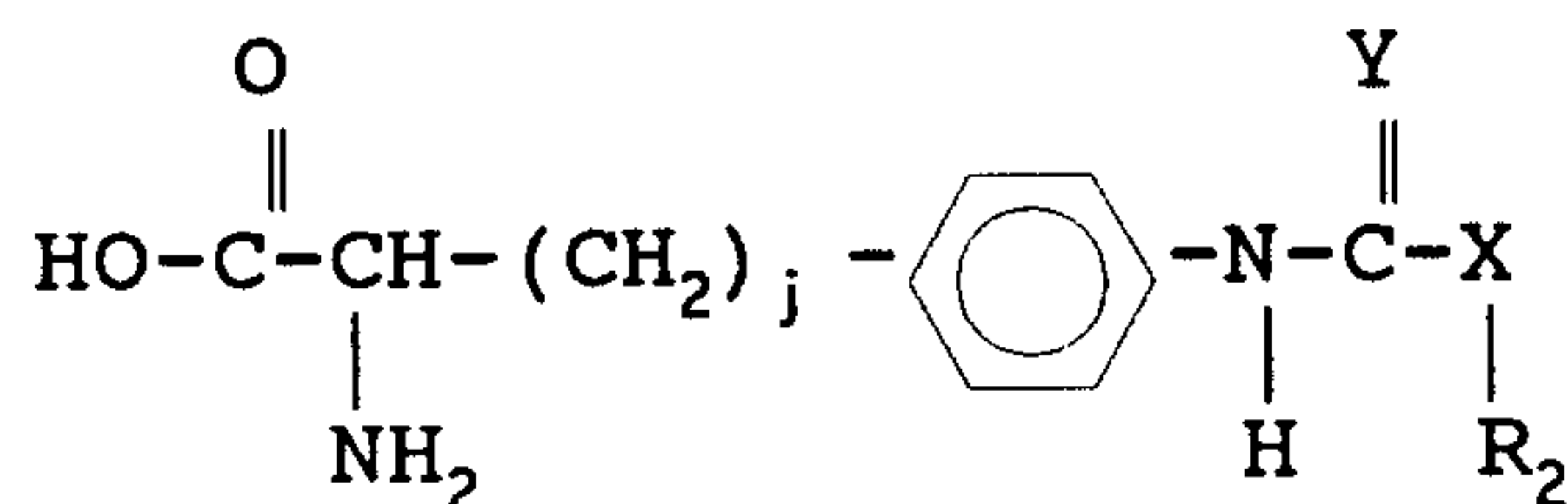
1. A GnRH antagonist peptide or a nontoxic salt thereof, said peptide having the formula: Ac-AA₁-(A)D-Phe-AA₃-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀ wherein AA₁ is β-D-NAL, (A)D-Phe or (B)D-Trp; A is 4Cl, 4F, 4NO₂, 4CH₃, 4OCH₃, C^αMe/4Cl, 2,4Cl₂, 4Br or H; B is H, 6NO₂, 6NH₂, 6OCH₃, 6F, 6Cl, 6Br, 6CH₃, NⁱⁿFormyl or NⁱⁿAcetyl; AA₃ is D-PAL, U*, β-D-NAL or (B)D-Trp; AA₅ is U*, Lys(carboxypyridine) or Tyr; AA₆ is U*, β-D-NAL, 4NH₂D-Phe, (B)D-Trp, D-Lys(carboxypyridine), D-PAL or D-Arg, AA₇ is Leu, N^αCH₃Leu, Nle or Phe; AA₈ is isopropylLys or Arg; AA₁₀ is D-Ala-NH₂, Gly-NH₂, NHNHCONH₂ or NH(R); R is lower alkyl; and U* is either

(a)



where j is 1 or 2 and R₁₁ is H or an acyl radical having 1 to 6 carbon atoms;

or (b)



where j is 1, 2 or 3; X is NH or O; Y is N-CN or N-CONHR₉, where R₉ is H or lower alkyl; R₂ is lower alkyl,

cyclohexyl, phenyl, pyridyl, methyl pyridyl or histaminyll;

provided, however, that at least one of AA₃, AA₅ and AA₆ is U*, with AA₃ and AA₆ always being a D-isomer.

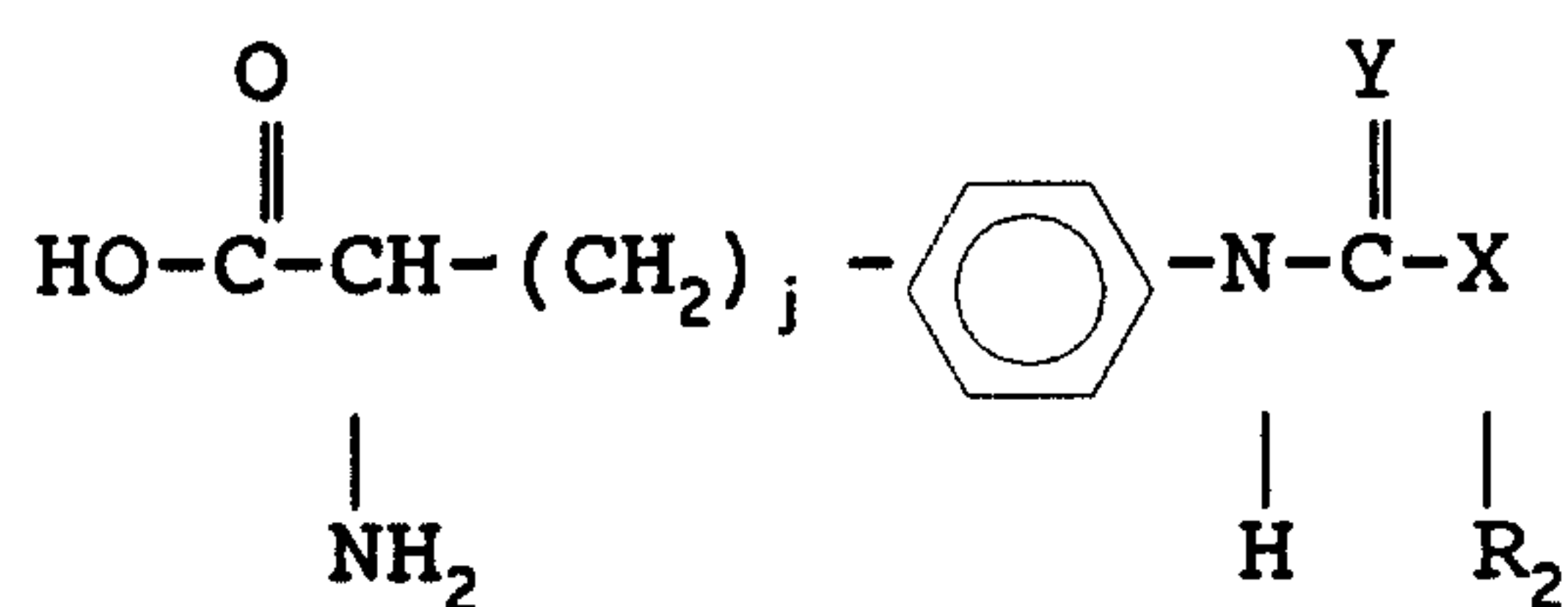
2. A GnRH antagonist peptide according to Claim 1 wherein j is 1, X is NH, Y is N-CN or N-CONH₂ and R₁₁ is H or acetyl.

3. A GnRH antagonist peptide according to Claim 1 having the formula:

Ac-β-D-2NAL-(4Cl)D-Phe-D-3PAL-Ser-4NH₂Phe(3-amino 1,2,4 triazole)-D-4NH₂Phe(3-amino 1,2,4 triazole)-Leu-Lys(isopropyl)-Pro-D-Ala-NH₂.

4. A peptide or a nontoxic salt thereof, said peptide having the formula:

G-AA₁-(A)D-Phe-AA₃-Ser-AA₅-AA₆-AA₇-AA₈-Pro-AA₁₀ wherein G is hydrogen or an acyl group having 7 or less carbon atoms; AA₁ is dehydroPro, D-pGlu, (A)D-Phe, (B)D-Trp, Pro, or β-D-NAL; A is H, Cl, F, NO₂, CH₃, OCH₃, C^αMe/4Cl, Cl₂ or Br; B is H, NO₂, NH₂, OCH₃, F, Cl, Br, CH₃, NⁱⁿFormyl or NⁱⁿAcetyl; AA₃ is U*, D-PAL, β-D-NAL or (B)D-Trp; AA₅ is U*, Lys(carboxypyridine), Orn(carboxypyridine), Dbu(carboxypyridine), Dpr(carboxypyridine), Tyr, (C)Arg, (A)Phe, (3I)Tyr or His; C is H or lower alkyl; AA₆ is U*, β-D-NAL, (B)D-Trp, (A')D-Phe, (D)D-Orn, (D)D-Lys, (D)Dbu, (D)D-Dpr, D-homoArg, D-Tyr, (E)D-His, D-PAL, (C)D-Arg, D-Leu, D-Ile, D-Nle, D-Val, D-Ala, or D-Ser(OtBu); A' is A, NH₂, NHCH₃ or guanadino; D is G, carboxypyridine or an aryl group; E is H, imBzl or dinitrophenol; AA₇ is Nle, Leu, N^αCH₃Leu, (A)Phe, Met, Nva, Tyr, (B)Trp or PAL; AA₈ is isopropylLys, (C')Arg or (C')homoArg; C' is H or di-lower alkyl; AA₁₀ is D-Ala-NH₂, Gly-NH₂, NHNHCONH₂ or NH(R); R is lower alkyl; and U* is



where $j = 1, 2$ or 3 ; Y is $N\text{-CN}$, $N\text{-CONHR}_9$, O , S or CH-NO_2 where R_9 is H , Ac , alkyl , naphthyl , pyridyl , pyrimidyl , pyrazinyl , indolyl , quinoliny or imidazolyl , which alkyl and cyclic groups are unsubstituted or substituted ; X is NH , O , N_3 , $\text{M}_1(\text{CH}_q)_p\text{M}_2$ or $\text{M}_1-(\text{CH}_2)_{p'}-\text{M}_2(\text{CH}_2)_{p''}-\text{M}_3$, where M_1 is NR_{10} , N , O or CHR_3 wherein R_3 is methyl , ethyl , propyl , phenyl , pyridinyl , pyrimidinyl or purinyl ; q is 1 or 2 ; p , p' and p'' are integers between 0 and 6 ; R_{10} is H , methyl , ethyl , propyl , phenyl or $\text{substituted phenyl}$; and M_2 and M_3 are M_1 , COOH , CONH_2 , COOR_3 or CN ; R_2 is R_1 , OH , NH_2 , NHR_1 , heterocycle or desR_2 , with R_2 being desR_2 when X is N_3 ; R_1 is alkyl , $(\text{CH}_2)_n\text{X}''$, alkenyl , alkynyl or aryl ; n' is $1, 2, 3$ or 4 ; X'' is CH_2NH_2 , CH_2OH , CH_2Cl , CH_2Br , CH_2F , CF_3 or CF_2CF_3 ; provided however, that Y and R_2 are optionally interconnected; and provided still further that at least one of AA_3 , AA_5 and AA_6 is U^* , with U^* being a D-isomer whenever present as AA_3 or AA_6 .

5. A peptide in accordance with Claim 4 wherein AA_6 is U^* .

6. A peptide in accordance with Claim 4 wherein AA_5 is U^* .

7. A peptide in accordance with Claim 4 wherein both AA_5 and AA_6 are U^* .

8. A peptide in accordance with Claim 4 wherein AA_3 is U^* .

9. A peptide in accordance with Claim 4 wherein AA_1 is $\beta\text{-D-2NAL}$, (A) is 4Cl or 4F and AA_3 is D-3PAL .

10. A peptide in accordance with Claim 9 wherein AA_6 is D-Trp , D-PAL , $\beta\text{-D-NAL}$, $(\text{imBzl})\text{D-His}$ or $(6\text{NO}_2)\text{D-Trp}$ and AA_5 is U^* .

11. A peptide in accordance with Claim 4 wherein U* is 4NH₂Phe(3amino-1,2,4 triazole).

12. A peptide in accordance with Claim 4 wherein U* is 4NH₂Phe(aminobutyl cyanoguanidino).

13. A peptide in accordance with any one of Claims 4-12 wherein AA₈ is Arg, G is Ac and (D) and (E) are both H.

14. A peptide in accordance with any one of Claims 4-10 wherein U* contains a cyanoguanidine moiety and wherein j is 1.

15. A peptide in accordance with any one of Claims 4-12 wherein AA₈ is isopropylLys.

16. A peptide in accordance with either Claim 4 or 9 wherein AA₅ is 4NH₂Phe(3amino-1,2,4 triazole), AA₆ is D-4NH₂Phe(3amino-1,2,4 triazole) and AA₈ is isopropylLys.

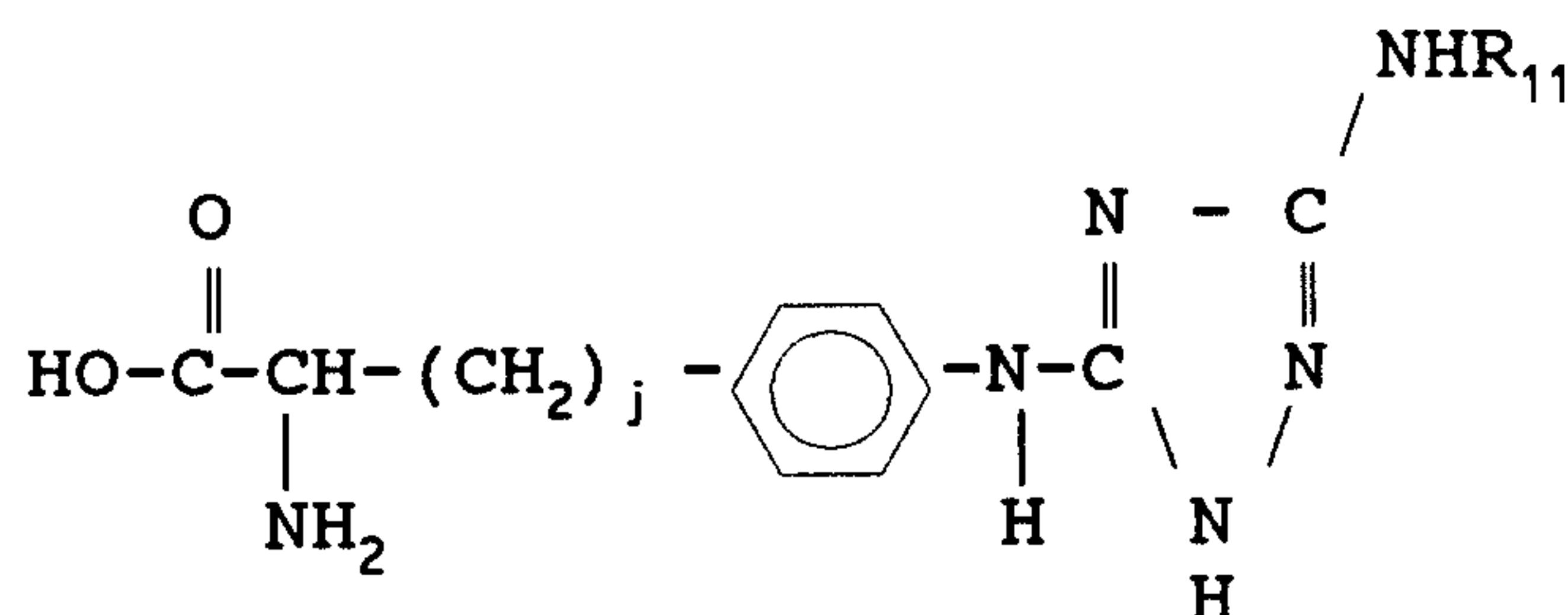
17. A peptide in accordance with either Claim 4 or 9 wherein AA₅ is Lys(3-carboxypyridine), AA₆ is U* and AA₈ is isopropylLys.

18. A peptide intermediate for making a peptide according to Claim 1, which intermediate has the formula: Ac-AA₁-(4Cl)D-Phe-AA₃-Ser(X³)-4NH₂Phe(X^a)-D-4NH₂Phe(X^a)-Leu-Lys(isopropyl, X⁶)-Pro-D-Ala-NH-[resin support], wherein AA₁ is β-D-NAL, (A)D-Phe or (B)D-Trp; B is H, 6NO₂ or NⁱⁿFor; AA₃ is D-PAL, β-D-NAL or (B)D-Trp, X³ is a protecting group for a hydroxyl group of Ser or Thr; X^a is a protecting group for a primary amino group that is base-labile, hydrazine-labile or thio-labile; and X⁶ is Z benzyloxycarbonyl or p-chlorobenzyloxy-carbonyl.

19. A peptide intermediate according to Claim 18 having the formula: Ac-β-D-2NAL-(4Cl)D-Phe-D-3PAL-Ser(X³)-4NH₂Phe(X^a)-D-4NH₂Phe(X^a)-Leu-Lys(isopropyl, benzyloxycarbonyl)-Pro-D-Ala-NH-[resin support].

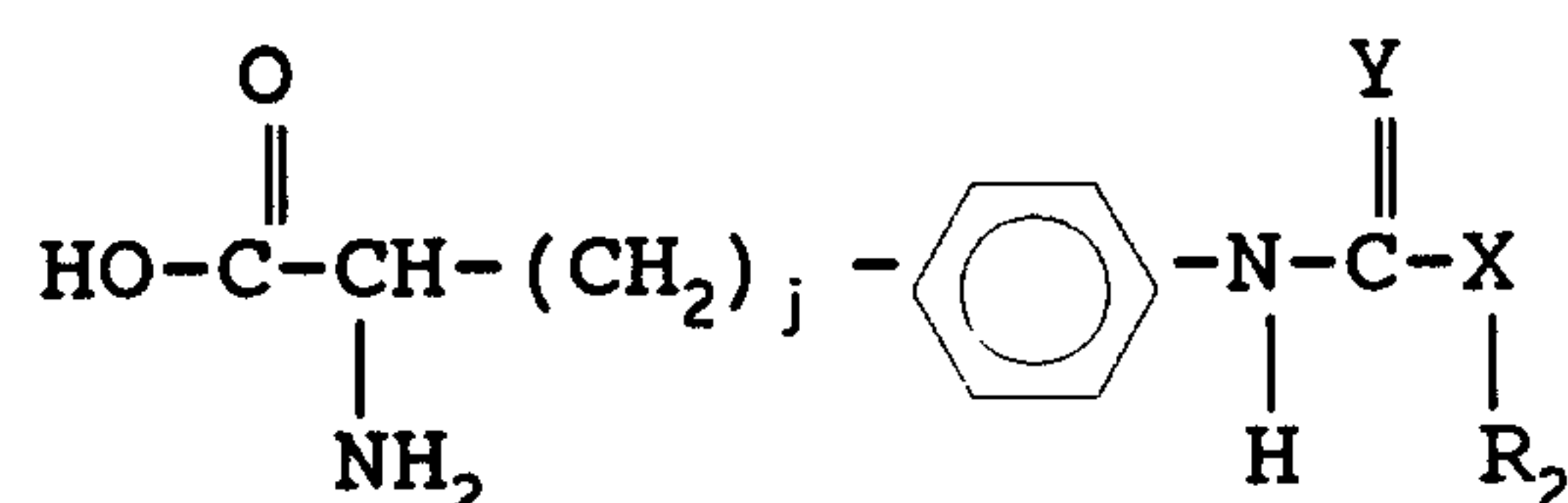
20. A method for making a peptide which includes a residue U^* of an unnatural amino acid, which peptide has the formula: $G-AA_1-(A)D-Phe-AA_3-Ser-AA_5-AA_6-AA_7-AA_8-Pro-AA_{10}$ wherein G is hydrogen or an acyl group having 7 or less carbon atoms; AA_1 is dehydroPro, D-pGlu, (A)D-Phe, (B)D-Trp, Pro, or β -D-NAL; A is H, Cl, F, NO_2 , CH_3 , OCH_3 , $C^{\alpha}Me/4Cl$, Cl_2 or Br; B is H, NO_2 , NH_2 , OCH_3 , F, Cl, Br, CH_3 , $N^{in}Formyl$ or $N^{in}Acetyl$; AA_3 is U^* , D-PAL, β -D-NAL or (B)D-Trp; AA_5 is U^* , Lys(carboxypyridine), Orn(carboxypyridine), Dbu(carboxypyridine), Dpr(carboxypyridine), Tyr, (C)Arg, (A)Phe, (3I)Tyr or His; C is H or lower alkyl; AA_6 is U^* , β -D-NAL, (B)D-Trp, (A')D-Phe, (D)D-Orn, (D)D-Lys, (D)Dbu, (D)D-Dpr, D-homoArg, D-Tyr, (E)D-His, D-PAL, (C)D-Arg, D-Leu, D-Ile, D-Nle, D-Val, D-Ala, or D-Ser(OtBu); A' is A, NH_2 , $NHCH_3$ or gua; D is G, cpd or an aryl group; E is H, imBzl or dinitrophenol; AA_7 is Nle, Leu, $N^{\alpha}CH_3Leu$, (A)Phe, Met, Nva, Tyr, (B)Trp or PAL; AA_8 is isopropylLys, (C')Arg or (C')homoArg; C' is H or di-lower alkyl; AA_{10} is D-Ala- NH_2 , Gly- NH_2 , $NHNHCONH_2$ or $NH(R)$; R is lower alkyl; U^* is either

(a)



where j is 1 or 2 and R_{11} is H or an acyl radical having 1 to 6 carbon atoms;

or (b)



where j is 1, 2 or 3; X is NH or O ; Y is N-CN or N-CONHR_9 , where R_9 is H or lower alkyl; R_2 is lower alkyl, cyclohexyl, phenyl, pyridyl, methyl pyridyl or histaminy; provided, however, that at least one of AA_3 , AA_5 , and AA_6 is U^* , which method comprises (a) forming an intermediate peptide having the formula:

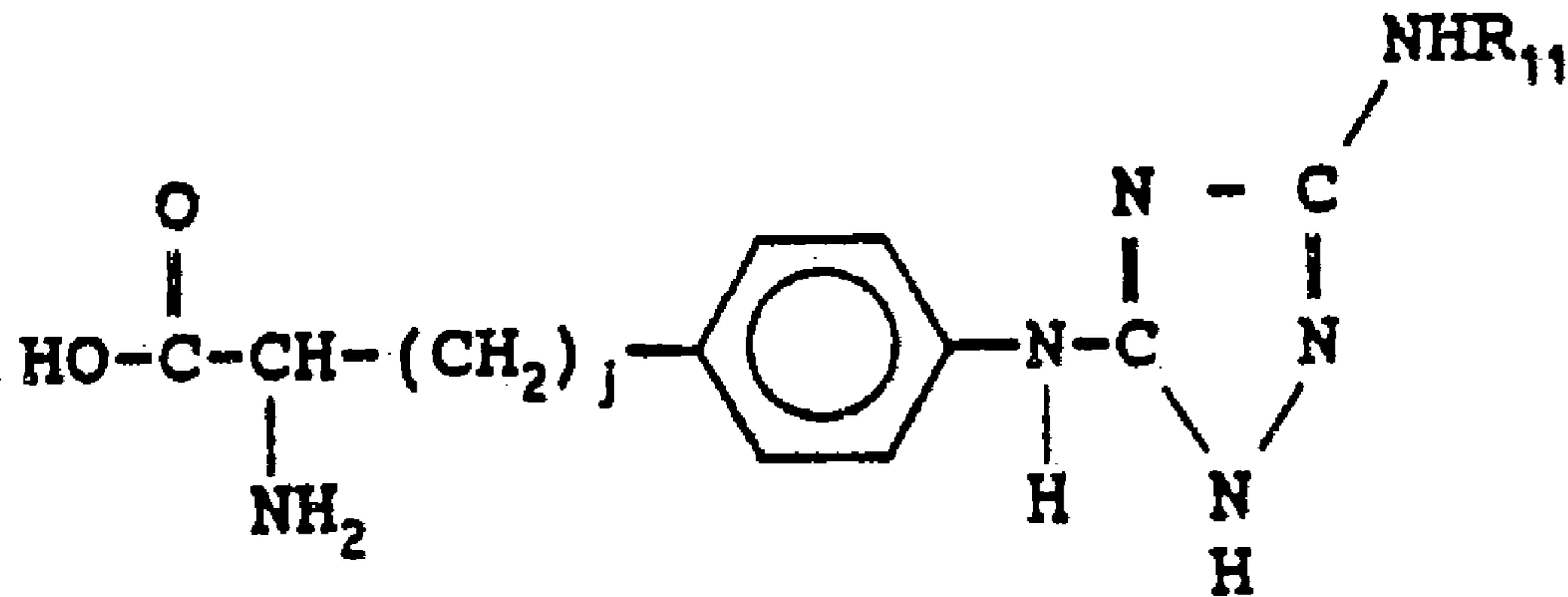
$\text{X}^1-\text{AA}_1-(\text{A})\text{D-Phe-U}_3-\text{Ser}(\text{X}^3)-\text{U}_5-\text{U}_6-\text{AA}_7(\text{X}^2 \text{ or } \text{X}^7)-\text{AA}_8(\text{X}^5 \text{ or } \text{X}^6)-\text{Pro-X}^8$ wherein: U_3 is either U' or $\text{AA}_3(\text{X}^2)$; U_5 is either U' or $\text{AA}_5(\text{X}^4 \text{ or } \text{X}^5)$; U_6 is either U' or $\text{AA}_6(\text{X}^4 \text{ or } \text{X}^5 \text{ or } \text{X}^6)$; U' is $4\text{NH}_2\text{Phe}(\text{X}^a)$, $4\text{NH}_2\text{homPhe}(\text{X}^a)$ or $4\text{NH}_2\text{homohomPhe}(\text{X}^a)$; X^1 is hydrogen or an α -amino protecting group; X^2 is hydrogen or a protecting group for an indole nitrogen; X^3 is a protecting group for a hydroxyl group of Ser or Thr; X^4 is hydrogen or a protecting group for a phenolic hydroxyl group of Tyr; X^5 is either hydrogen or a protecting group for a guanidino or imidazole group; X^6 is a protecting group for a primary amino group; X^a is a protecting group for a primary amino group that is base-labile, hydrazine-labile or thio-labile; X^7 is hydrogen or a protecting group for Met; X^8 is Gly-NH-[resin support], D-Ala-NH-[resin support], N(A)-[resin support], an amide either of Gly or of D-Ala or a substituted amide attached directly to Pro; provided however that at least one of U_3 , U_5 and U_6 is $4\text{NH}_2\text{Phe}(\text{X}^a)$, $4\text{NH}_2\text{homPhe}(\text{X}^a)$, or $4\text{NH}_2\text{homohomPhe}(\text{X}^a)$, with U_3 and U_6 always being a D-isomer; (b) removing at least one X^a to deprotect a side chain primary amino group of at least

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one amino acid residue of said intermediate peptide; (c) reacting said deprotected side chain primary amino group to build said residue into one having the formula U^* ; and (d) splitting off any remaining groups X^1 to X^7 and/or cleaving from any resin support included in X^8 .

21. A method in accordance with Claim 20 wherein either U_5 is U' or U_6 is U' or both are U' .



(I)