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(54) Title: PROCESS FOR PRODUCTION OF DEGARELIX

(57) Abstract: The invention relates to methods for the preparation of high purity Degarelix and Degeralix prepared by such methods in a high purity of at least 98.5% (by HPLC).

PROCESS FOR PRODUCTION OF DEGARELIX

5 Cross Reference to Related Applications

The present application claims the benefit of the following United States Provisional Patent Application Nos.: 61/264,491, filed November 25, 2009; and 61/407,175, filed October 27, 2010. The contents of these applications are incorporated herein by reference.

10

Field of the Invention

The present invention encompasses processes for the preparation and purification of Degarelix.

15 Background of the Invention

Gonadotropin-releasing hormone (GnRH) antagonists are used in protocols for ovulation induction and are recognized as potential drugs for the management of sex steroid-dependent pathophysiologies, such as hormoneresponsive prostate cancers and, in females, the management or treatment of breast and gynecological cancers,
20 endometriosis, precocious puberty, uterine myoma, ovarian hyperandrogenism, and premenstrual syndrome.

Most of these disorders can be treated with long-acting preparations of superagonists, which desensitize the gonadotrophs after approximately two weeks of treatment. An optimized antagonist will likely become the preferred choice over the
25 agonist in the clinic because it avoids the initial up-regulation of the gonadotropin-gonadal axis, which leads to rapid and predictable recovery, achieves more profound inhibition of gonadotropins, and can be used as a diagnostic test of gonadotropin-dependent gonadal dysfunction.

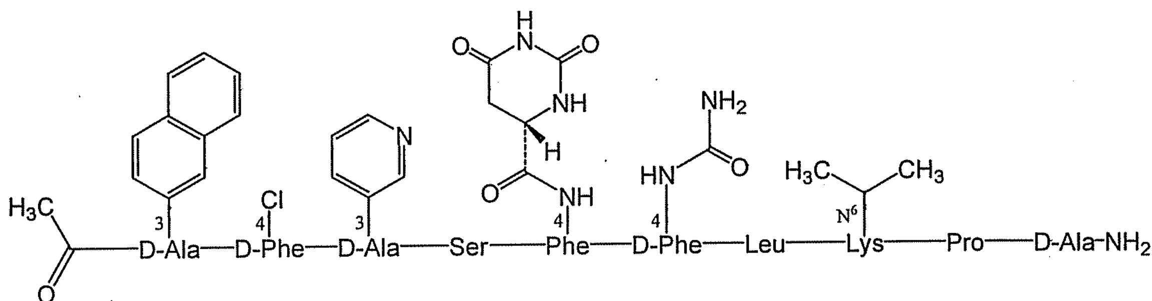
Degarelix, a third generation GnRH receptor antagonist (a GnRH blocker), has
30 been developed as a new therapy for prostate cancer patients in need of androgen ablation therapy. The aim of the Degarelix development has been to achieve testosterone suppression in the same range as for GnRH agonist therapy without any increase in testosterone levels after the initial dose.

Degarelix binds to GnRH receptors in the anterior pituitary gland resulting in a
35 decreased secretion of LH and FSH, and subsequently decreased production of

testosterone by the Leydig cells in the testes. Testosterone suppression is achieved almost immediately after subcutaneous (s.c.) administration of Degarelix. The degree and duration of testosterone suppression are related to plasma concentrations of Degarelix. The formation of a depot following s.c. administration gives rise to sustained plasma concentrations of Degarelix, resulting in prolonged GnRH receptor-mediated suppression of testosterone levels.

Degarelix (INN), which is a third generation gonadotropin releasing hormone (GnRH) antagonist (blocker), is a synthetic linear decapeptide containing seven unnatural amino acids, five of which are D-amino acids.

Degarelix is a decapeptide and is chemically designated as D-Alaninamide, *N*-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4*S*)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L-phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L-leucyl-*N*6-(1-methylethyl)-L-lysyl-L-prolyl-. Its common name is: [Ac-D-2Nal^{1}, D-4Cpa^{2}, D-3Pal^{3}, 4Aph(L-Hor)^{5}, D-4Aph(Cbm)^{6}, Lys(iPr)^{8}, D-Ala^{10}] GnRH where: 2Nal is 2-Naphthylalanine, 4Cpa is 4-Chlorophenylalanine, 3Pal is 3-Pyridylalanine, Hor is hydroorotyl, Lys(iPr) is *N*6-Isopropyllysine, 4Aph is 4-Aminophenylalanine, and Cbm is the carbamoyl group. The primary sequence of Degarelix is:



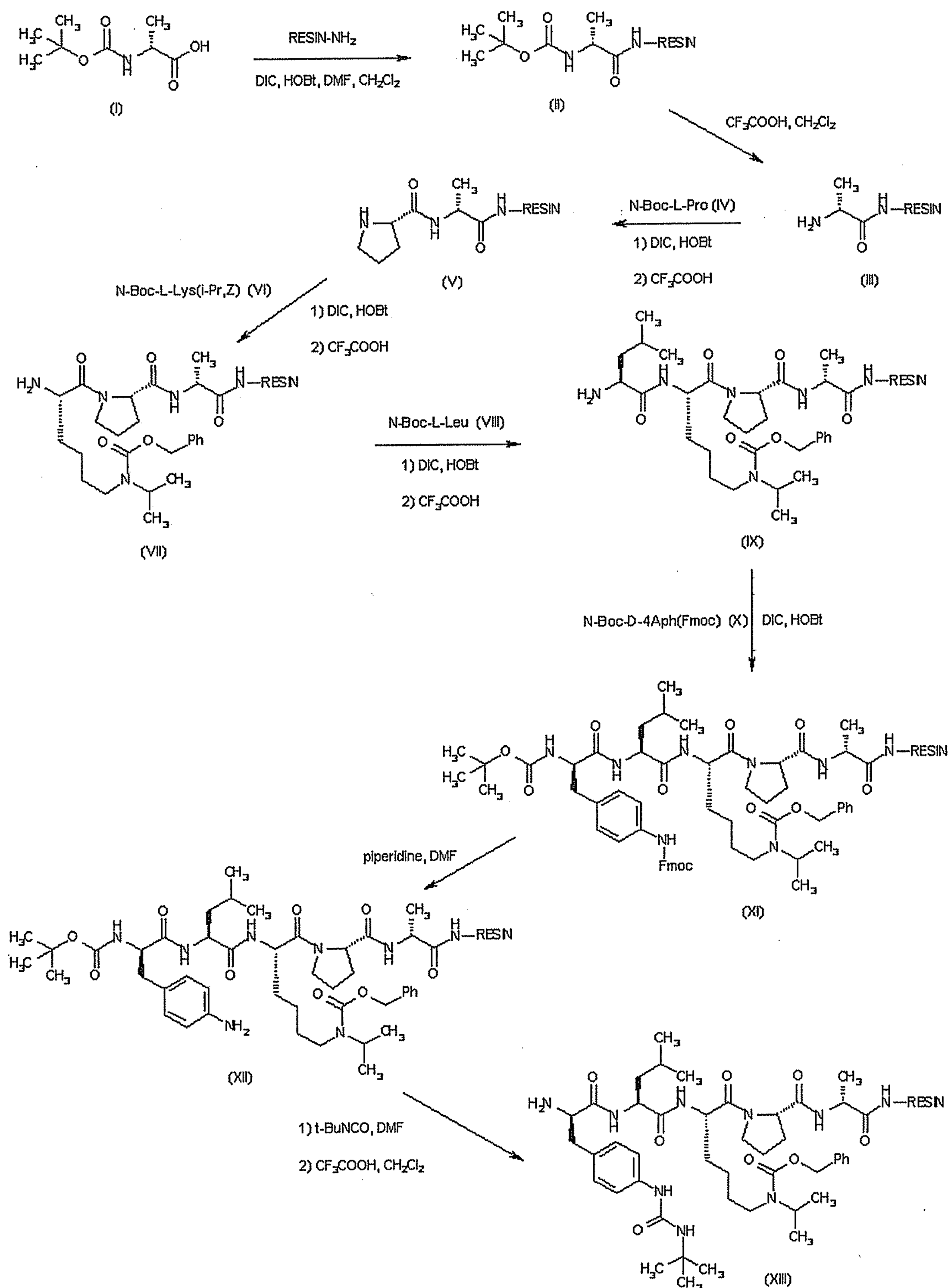
The synthesis of Degarelix has been described by solid phase synthesis using Boc chemistry (2001:880 SYNTHLINE):

N-Boc-D-alanine (I) was coupled to the MBHA resin using diisopropyl carbodiimide and 1-hydroxybenzotriazole to afford resin (II). Subsequent cleavage of the Boc protecting group by means of trifluoroacetic acid (TFA) provided the D-alanine-bound resin (III). Sequential coupling and deprotection cycles were carried out with the following protected amino acids: N-Boc-L-proline (IV), N-alpha-Boc-N6-isopropyl-N6-carbobenzoxy-L-lysine (VI) and N-Boc-L-leucine (VIII) to afford the respective peptide resins (V), (VII)

and (IX). N-alpha-Boc-D-4-(Fmoc-amino)phenylalanine (X) was coupled to (IX), yielding resin (XI). Cleavage of the side-chain Fmoc protecting group with piperidine in DMF gave the aniline derivative (XII). This portion of the synthesis route is shown below in Scheme 1.

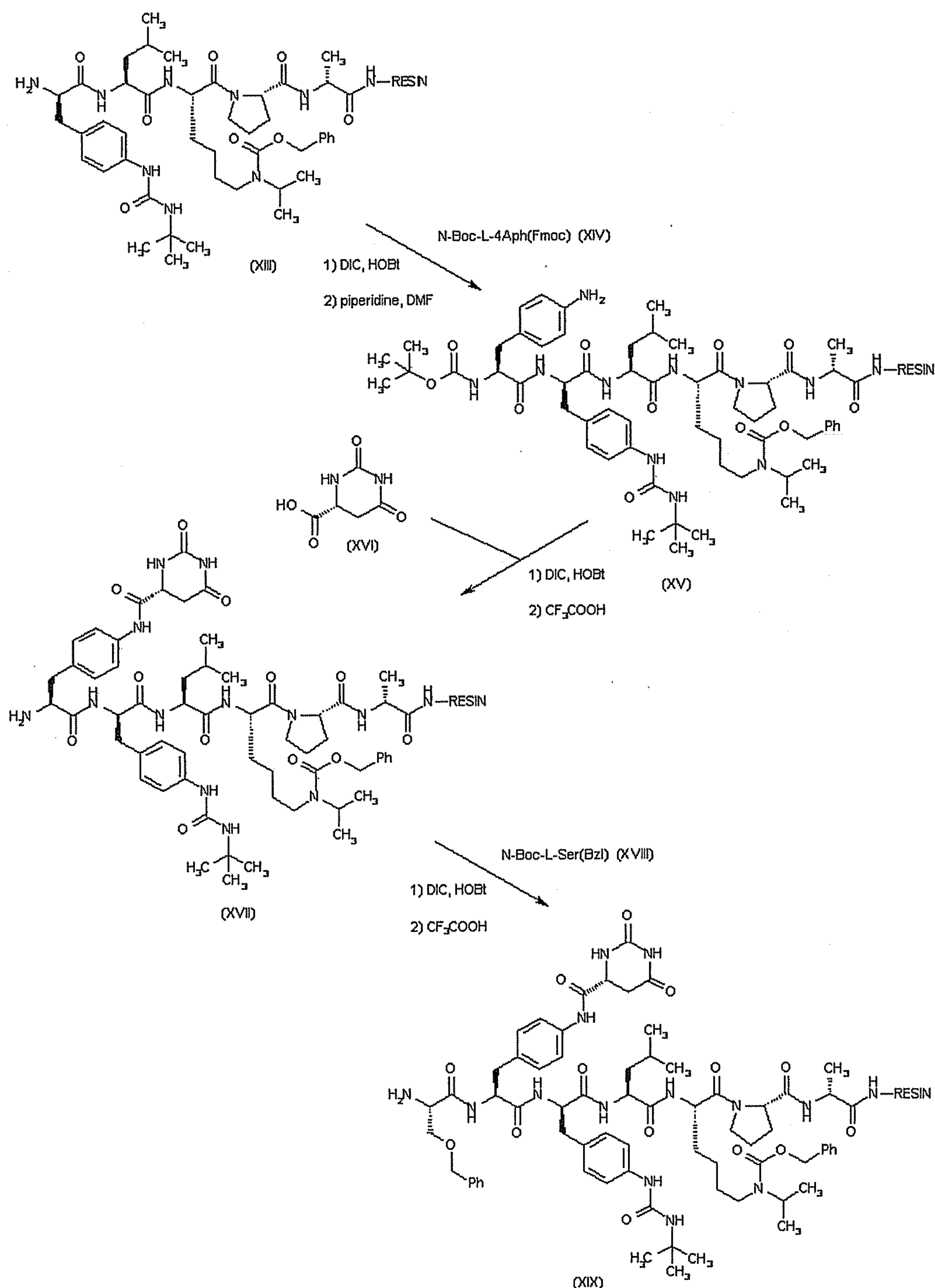
5

Scheme 1



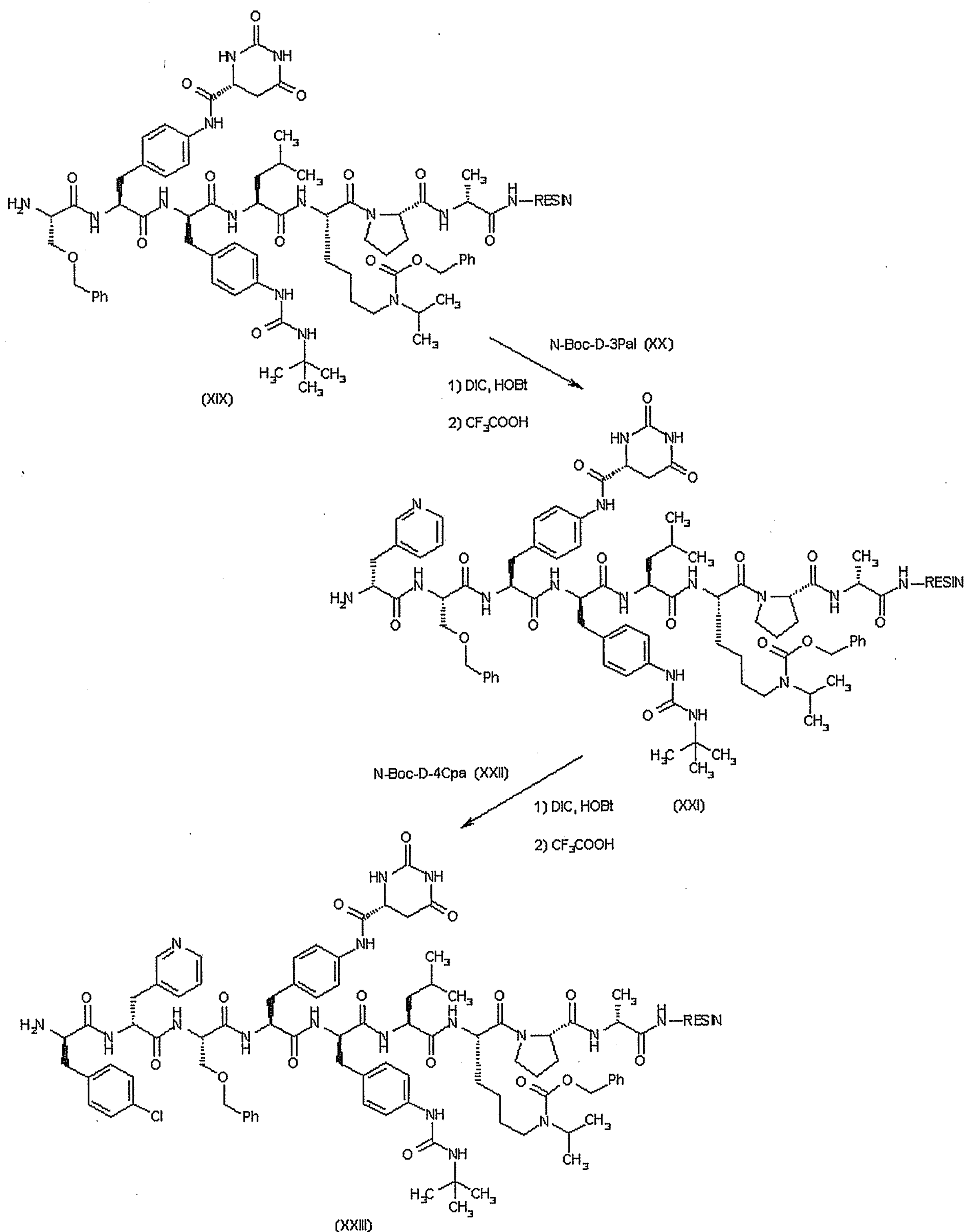
After conversion to the corresponding urea by treatment with tert-butyl isocyanate, the Boc group was cleaved with TFA to produce resin (XIII). Further coupling with N-alpha-Boc-L-4-(Fmoc-amino)phenylalanine (XIV), followed by Fmoc deprotection with piperidine, furnished (XV). The aniline derivative (XV) was acylated with L-hydroorotic
5 acid (XVI) to yield, after Boc group cleavage, resin (XVII). Coupling of (XVII) with N-Boc-L-serine(O-benzyl) (XVIII) and subsequent deprotection gave (XIX), as shown in Scheme 2, below:

Scheme 2



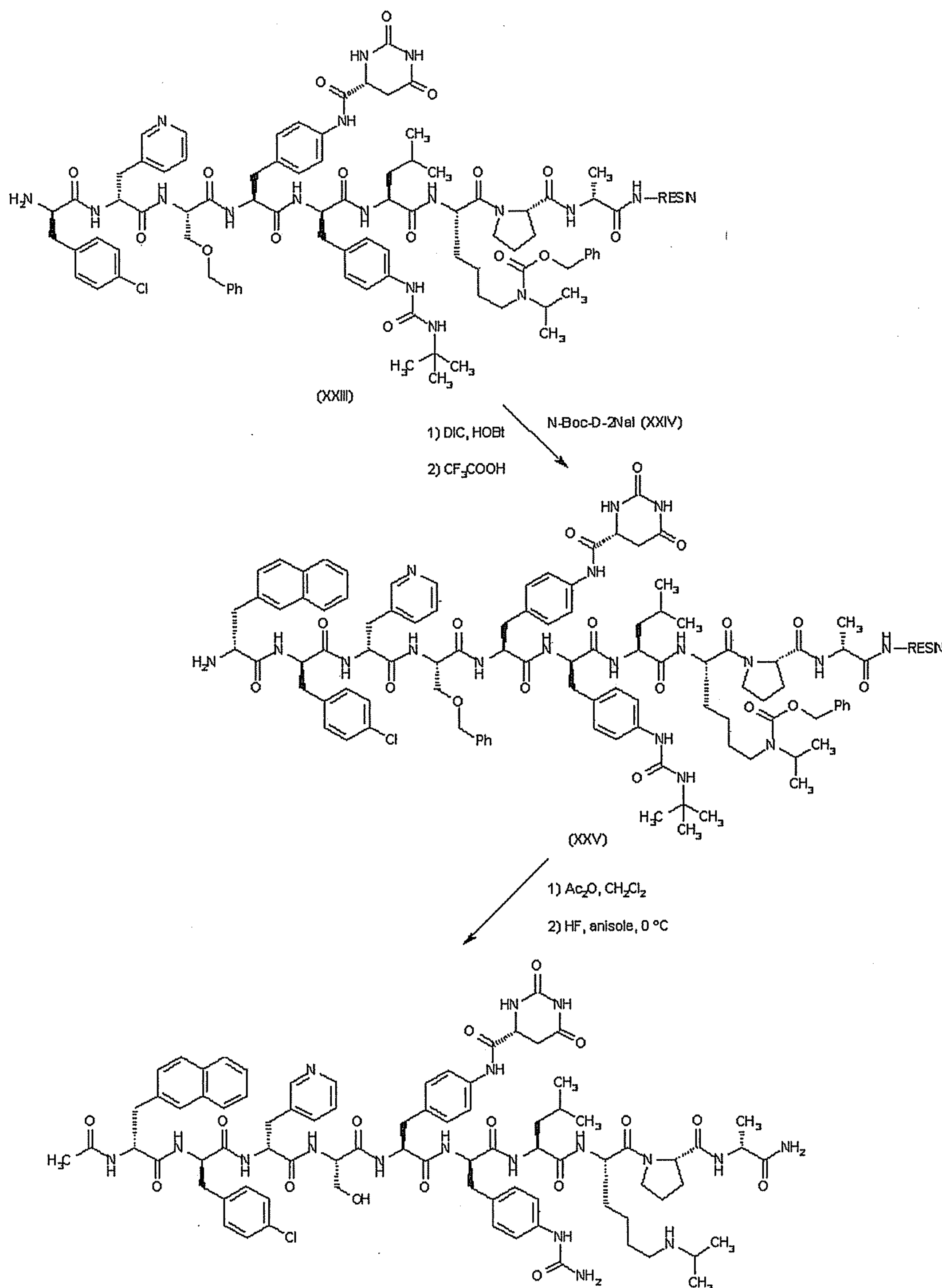
Peptide (XIX) was sequentially coupled with N-alpha-Boc-D-(3-pyridyl)alanine (XX) and N-Boc-D-(4-chlorophenyl)alanine (XXII) to furnish, after the corresponding deprotection cycles with TFA, the resins (XXI) and (XXIII), respectively, as shown in Scheme 3, below:

5 Scheme 3



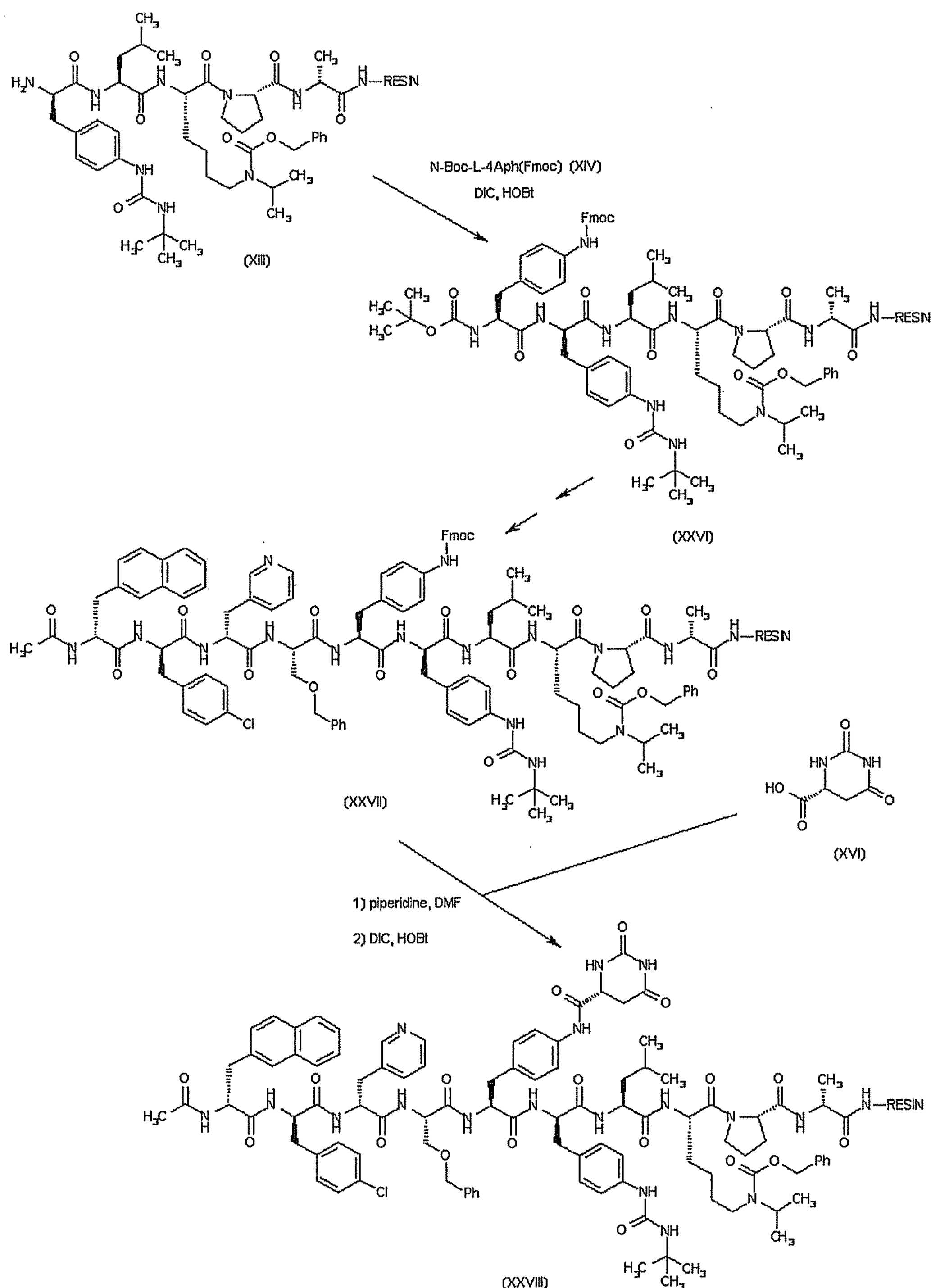
The coupling of resin (XXIII) with N-Boc-D-(2-naphthyl)alanine (XXIV) as before gave, after the corresponding deprotection cycle with TFA, resin (XXV). The peptide resin (XXV) was acetylated with Ac2O and finally deprotected and cleaved from the resin by treatment with HF to provide the target peptide, as shown in Scheme 4 below:

5 Scheme 4



Alternatively, after coupling of the peptide resin (XIII) with alpha-Boc-L-4-(Fmoc-amino)-phenylalanine (XIV), the Fmoc protecting group was not removed, yielding resin (XXVI). Subsequent coupling cycles with amino acids (XVIII), (XX), (XXII) and (XXIV) as above finally produced resin (XXVII). The Fmoc group was then deprotected
5 by treatment with piperidine, and the resulting aniline was acylated with L-hydroorotic acid (XVI) to provide resin (XXVIII), as shown in Scheme 5 below:

Scheme 5



Resin (XXVIII) was finally cleaved and deprotected by treatment with HF, as shown in Scheme 6 below:

(XXVIII)

HF, anisole, 0° C

US 6214798 B1, which describes a similar synthetic approach, suggests that classical
5 peptide solution synthesis would be preferable for producing large quantities of product. The purity of the final material obtained in US 6214798 B1 is estimated, therein, to be about 98%, yet *J. Med. Chem.* **2005,** *48,* 4851-4860 describing the same preparation yields a purity as measured by capillary zone electrophoresis (CZE) of 98%, which corresponds to 96% according to HPLC analysis.

10 Fmoc chemistry generally employs milder reaction conditions than the Boc chemistry, and thus can sometimes be less likely to cause the formation of by-products..

In addition, Fmoc chemistry does not employ hydrofluoric acid or other very strong acid (such as TFMSA or HBr/AcOH) is needed. Therefore these processes are much safer and environmentally friendly; factors that are important when engineering
15 such a synthesis process on the large production scale.

In stepwise SPPS at least two types of protection for reactive functionalities other than those involved in peptide bond formation are necessary: temporary protection of the

alpha-amino group, removable after formation of each peptide bond; and, side chain protection removable after assembly of the complete peptide chain.

When using Fmoc chemistry, a tBu protecting group is commonly used for the protection of the hydroxyl groups on Ser, Tyr and Thr residues. The tBu group is easily removed together with other side-chain protecting groups at the end of the peptide synthesis by using TFA.

WO2010121835 ('WO'835') describes the preparation of Degarelix using an Fmoc strategy and using tBu as the protecting group for the Ser residue. WO'835 also teaches that, unusually severe cleavage conditions such as long reaction time and 100% TFA as cleavage cocktail were required to successfully complete the final deprotection.. Such severe conditions are commonly known to result in increased side reactions, increased degradation of the peptide, and accordingly production of a lower quality of the resulting product.

One alternative to avoid using these severe deprotection conditions could be the use of different acid labile protecting group for Ser, such as Trt. However, it is well known in the art that the Trt group is very bulky and hydrophobic, and is frequently used to increase the "steric hindrance" effect. See, e.g. *Chem. Lett.* 27, 1999 and *J. Pept. Sci.* 2010; 16: 364–374, which describes that using the Trt protecting group in an Fmoc peptide synthesis could prevent formation of the target peptide due to "steric hindrance." The Degarelix sequence is inherently very hydrophobic. Therefore coupling of a large hydrophobic residue to the growing peptide chain is expected to cause slowing of the reaction and thus increased liability for the formation of impurities associated with deletions in the sequence and racemization. Synthesis efficiency is an important consideration when determining which general coupling reaction to use in an SPPS process. This efficacy can be limited by a number of different variables. One major variable that must be considered is the steric hindrance. This steric hindrance is determined by the nature of the peptide side chains and of their protecting groups. It is an important variable because it will allow the rapid acylation of the amino function and have a limiting effect on possible side reactions. See, *e.g.*, ChemPep@, Fmoc Solid Phase Peptide Synthesis; Coupling reaction). Moreover, for the Degarelix synthesis, Fmoc-Ser(Trt)-OH would be added to extremely bulky 4Aph(L-Hor) [4-[[[(4*S*)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L-phenylalanyl] residue. Accordingly, the use of this protecting residue would likely be quite difficult due to "steric hindrance". These "steric effects" are known to be of great importance when two molecules or two groups

are in a close approach resulting in van der Waals forces repulsive effect. The repulsive potential can become quite large if the nonbonded distances are sufficiently short. This van der Walls repulsion can thus serve to slow a reaction, a situation that is typically referred to as "steric hindrance" (Stereochemistry of Organic Compounds, E.L. Eliel et al, John Willey & Sons, 1994, 720 – 721).

There is a need in the art for an industrially applicable process for obtaining highly pure Degarelix.

Summary of the Invention

In one embodiment, the present invention provides a solid-phase peptide synthesis (SPPS) of Degarelix, using Fmoc chemistry.

In another embodiment, the present invention provides a combined SPPS and solution synthesis for obtaining Degarelix.

Detailed Description of the Invention

For the purpose of clarity and as an aid in the understanding of the invention, as disclosed and claimed herein, the following terms and abbreviations are defined below:

ACN	acetonitrile
BOC	tert-butoxycarbonyl
DCC	N,N-dicyclohexylcarbodiimide
DIPEA	diisopropylethylamine
DMF	dimethylformamide
ES/MS	Electrospray Mass Spectroscopy
Fmoc	9-fluorenylmethoxycarbonyl
TBTU	2-[1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
HOBt	N-hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
SPPS	solid phase peptide synthesis
TFA	trifluoroacetic acid
t-Bu	tert-butyl
TIS	Triisopropylsilane
DIC	*N,N'*-Diisopropylcarbodiimide
EDT	Ethanedithiol

Trt trityl

6 fragment Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH

3 fragment Ac-D-Nal-D-4Cpa-D-3Pal-OH

5 1 fragment D-Ala-NH_2

9 fragment Ac-D-2Nal-D-4Cpa-D-3-Pal-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH

As used herein, the term "TFA cocktail containing various scavengers" refers to TFA containing scavenger reagents including, but not limited to ethanedithiol (EDT), TIS
10 (triisopropylsilane) and water.

As used herein, the term "Normal/regular cleavage conditions" refer to cleavage which is performed under an acidic conditions comprising a relative ratio of acidic material to scavenger to water. For example the ratio can be from about 85% to about 99% acidic material, from about 0.1% to about 15% scavenger, and from about 0.1% to
15 about 15% water (by weight). A preferred acidic composition comprises about 95% TFA, about 2.5% EDT, and about 2.5% water. The cleavage can be done for a period of about 1 h, at room temperature.

The present invention is directed to several processes for production of Degarelix.

We report here the use of mild Fmoc chemistry instead of previously reported Boc
20 chemistry for preparing Degarelix.

It was found that, in the synthesis of Degarelix, a tBu protecting group on the Ser residue could not be removed using normal cleavage conditions. This resulted in a partially protected peptide sequence and thus a low purity of the final peptide product and severe loss of the product yield. It was also found that an attempt to drive the deprotection
25 reaction to completion by increasing the temperature resulted in removing the protecting group, but also simultaneous partial degradation of the peptide, once again resulting in a lower purity and lower yield. Surprisingly, it was discovered that protection of Ser residue by Trt protecting group made it possible to achieve complete deprotection under regular cleavage conditions and afforded a high purity peptide product.

30 The Degarelix sequence contains several unnatural amino acids and thus requires the availability of the suitably protected starting materials to be used in the production process. One of these amino acids is D-4Aph(Cbm) [4-[(aminocarbonyl)amino]-D-phenylalanine]. This residue is commercially available as Fmoc-D-4Aph(Cbm) or as Fmoc-D-4Aph(Cbm/tBu) (non protected on the side chain and protected by tBu on the

side chain). When using a Boc synthesis approach, the tBu group is observed to be stable under repeated deprotection cycles with TFA and is removed under final HF cleavage/deprotection conditions, i.e., under conditions that are not suitable for an Fmoc synthetic strategy. Unexpectedly though, it was found that the tBu protected residue could be used in the Degarelix synthesis using Fmoc chemistry if heating is applied at the final deprotection stage to effect complete removal of the tBu group.

As used herein, the term "sequential synthesis" refers to repeated steps of adding amino acids, according to peptide sequence.

Using sequential strategy, acidic treatment, such as TFA based cleavage cocktail, will detach the peptide from the solid support and remove all protecting groups leaving an unprotected Degarelix molecule. An evaluation of the chromatographic profile at this stage shows clearly a main peak corresponding to a high purity product .

Thus, the product may be easily purified further by methods such as HPLC or other known methods to obtain a purified Degarelix in high purity. By these means, a high purity product is easily obtained in high yield without need for several recycling cycles that require large volumes of solvents, long operation time, and results in a lower purity and lower yield of the final product.

In one embodiment, the present invention provides a solid-phase peptide synthesis (SPPS) of Degarelix, using Fmoc chemistry, preferably wherein Ser is protected by Trt. This synthesis is preferably a "sequential synthesis".

The solid-phase peptide synthesis of the present invention comprises: a) preparing a protected peptide attached to a Rink amide type resin, in a sequential synthesis wherein the prepared peptide contains the complete amino acid sequence of Degarelix, of which all or most multi-functional amino acids are protected with acid labile protecting groups; b) reacting the protected peptide resin with an acidic composition to produce an unprotected peptide; c) isolating the peptide by precipitation, crystallization, extraction or chromatography; d) purifying the unprotected Degarelix by chromatography, e) replace its counter-ion with acetate, and f) drying to obtain a final material as dry powder of Degarelix acetate.

In one embodiment the acidic composition consists of a cleavage cocktail based on TFA and various scavengers.

In yet a more specific embodiment, sequential synthesis of Degarelix of the present invention comprises:

a) Loading of the first Fmoc-protected amino acid, Fmoc-D-Ala-OH, on the resin, and washing and masking all active sites on the resin;

b) Repetitive cycles of the following:

i. Deprotecting to remove the Fmoc group;

5 ii. Washing with at least one solvent to remove all soluble compounds from the resin;

iii. Coupling of a suitable Fmoc-protected amino acid (according to the required sequence) to the terminal amino group residue attached to the resin using a suitable coupling reagent to form an Fmoc-protected peptide fragment attached

10 to the resin; and

iv. Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Deblocking the last amino acid Fmoc-D-Nal and acetylating the N-terminus;

15 d) Washing and drying the peptide-resin;

e) Cleaving of the protected peptide intermediate from the resin using a TFA cocktail containing various scavengers;

f) Precipitating the crude Degarelix from the cleavage solution;

g) Purifying the crude Degarelix by a suitable separation method including

20 counter-ion exchange to acetate to form a Degarelix acetate solution; and

h) Drying the Degarelix acetate solution to obtain the Degarelix product as Degarelix acetate.

The resin could be, but is not limited to, Rink amide resin, Rink amide AM resin, Rink amide MBHA resin, and the permanently stable protecting group should be

25 compatible with Fmoc strategy. In a preferred embodiment, the resin is Rink amide AM resin.

The final peptide can be purified by suitable methods to obtain a high purity peptide. Preferably, purification can be carried out using RP-HPLC.

All synthetic steps of the above described process are performed under mild

30 conditions providing products containing a low content of by-products and producing a final product in high yield and high purity. The amino acids introduced in Step (iii) of the sequential synthesis described above are commercially available as protected amino acids that are stable to any reactions and modifications of the side chains during the synthesis that could result in derivatives of the constituent amino acids. One specific embodiment

comprises obtaining intermediate XVII from intermediate XII by introducing, at step (iii), 4-(2,6-dioxohexahydropyrimidin-4(S)-ylcarboxamido)-L-phenylalanine to the terminal amino group residue attached to the resin.

One embodiment of the invention encompasses Degarelix having a purity of at least about 98.5% (by HPLC), more preferably at least about 99%.

Preferably in any of the embodiments disclosed above, the ser is protected with Trt.

Preferably, in any of the above embodiments disclosed above, the 4Aph(Cbm) can be unprotected on its side chain group.

The process of the present invention further encompasses preparing a peptide from amino residues by employing Fmoc as a protecting group for the amino residues, Trt for Ser, Boc for iPr-Lys and tBu for 4Aph(Cbm) [4Aph(Cbm) could be used also unprotected on its side chain group].

The above processes proceed via novel synthetic intermediates, including intermediates of the following formulae:

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-D-Ala-resin.

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-D-Ala- resin.

The present invention encompasses these intermediates, as well as their use in a process for the manufacture of Degarelix.

In another embodiment, the present invention provides a combined SPPS and solution synthesis for obtaining Degarelix. In one specific embodiment, fragments of the peptide are synthesized in a sequential synthesis, and further reacted in a solution to obtain Degarelix. Optionally, these processes can include synthesis via 9 + 1 fragments and synthesis via 3 + 6 +1 fragments.

The combined SPPS and solution synthesis for obtaining Degarelix going via the 9 + 1 fragment protocol comprise a) providing a protected peptide attached to a super acid-labile resin, wherein the peptide contains the almost complete amino acid sequence of Degarelix except the C-terminal D-Ala-NH_2, wherein all or most multi-functional amino acids are protected with a strong acid labile protecting group; b) Cleaving the protected peptide intermediate from the resin using mild acidic composition; c) isolating the protected peptide by precipitation, crystallization, extraction or chromatography; d)

treating the protected peptide obtained in step (c) with a coupling agent in the presence of D-Ala-NH_2 to produce a protected Degarelix; e) reacting the protected Degarelix with strong acidic composition, f) isolating the unprotected Degarelix, and g) purifying the unprotected Degarelix by chromatography. Optionally, the process further comprises
5 neutralizing excess acid after step (b).

The obtained Degarelix preferably has purity of a least above 98.5% (by HPLC), more preferably, at least above 99%.

In a more specific embodiment, the combined SPPS and solution synthesis for obtaining Degarelix acetate, going via a 9 + 1 fragment protocol comprises:

10 a) Loading of the first Fmoc-protected amino acid, Fmoc-Pro-OH, on the CTC resin, and washing and masking all active sites on the resin.

b) Repetitive cycles of the following:

i) Deprotecting to remove the Fmoc group;

ii) Washing with at least one solvent to remove all soluble compounds
15 from the resin;

iii) Coupling of a suitable Fmoc-protected amino acid (to obtain a protected 9 amino acid peptide sequence) with a suitable coupling reagent to the terminal amino group residue attached to the resin to form an Fmoc-protected peptide fragment attached to the resin; and

20 iv) Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Deblocking the last amino acid Fmoc-D-Nal and acetylating the N-terminus;

d) Washing and drying of the peptide-resin;

25 e) Cleaving the protected peptide intermediate from the resin using a mild acidic composition comprising a dilute TFA solution in DCM to form a protected peptide;

f) Optionally, coarse purification of the protected peptide by a suitable chromatographic or other method; for example by precipitation, crystallization or
30 extraction.

g) Optionally, precipitating the protected 9 amino acid fragment from the cleavage solution;

h) Coupling the protected 9 amino acid with D-Ala-NH_2 dissolved in a suitable solvent;

i) Deprotecting all protecting groups of the protected peptide with a TFA cocktail containing various scavengers;

j) Precipitating the crude Degarelix from the deprotection solution;

k) Purifying the crude Degarelix by a suitable separation method including counter-ion exchange to replace the TFA counterion with acetate to form Degarelix acetate solution; and

l) Drying the Degarelix acetate solution to obtain Degarelix product as Degarelix acetate.

Suitable resins for use in the process include, but are not limited to, super-acid labile resins such as chlorotrityl resins. A preferred super acid labile resin is 2-Cl-Trt-Cl resin.

In preferred embodiments, the mild and strong acidic solutions consist of different concentrations of TFA. The mild acidic solution may be, for example, 1% TFA in DCM The strong acidic solution may be, for example, 95% TFA 5% water

The above processes proceed via novel synthetic intermediates, including intermediates of the following formulae:

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro--CTC resin

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro--CTC resin

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-OH

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-OH

The present invention encompasses these intermediates, as well as their use in a process for the manufacture of Degarelix.

The combined SPPS and solution synthesis for obtaining Degarelix going via the 3 + 6 +1 fragment protocol comprise: a) providing a protected peptide fragment attached to a super acid-labile resin, wherein the peptide contains six amino acid sequence of Degarelix (Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH); b) Cleaving the protected peptide intermediate from the resin using mild acidic

composition; c) isolating the protected peptide fragment by precipitation, crystallization, extraction or chromatography; d) treating the protected peptide fragment obtained in step (c) with a coupling agent in the presence of D-Ala-NH_2 to produce a protected C-terminal Degarelix fragment containing 7 amino acids (Fmoc-Ser(Trt)-4Aph(L-Hor)-D-
5 4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-D-Ala-NH_2); e) isolating the protected peptide fragment by precipitation, crystallization, extraction or chromatography; f) reacting the protected C-terminal fragment with N-terminal fragment containing three amino acids (Ac-D-Nal-D-4Cpa-D-3Pal) to obtain protected Degarelix; g) isolating the protected peptide by precipitation, crystallization, extraction or chromatography; h) reacting the
10 protected peptide with strong acidic composition, i) isolating the unprotected Degarelix, and j) purifying the unprotected Degarelix by chromatography.

The N-terminal fragment containing three amino acids (Ac-D-Nal-D-4Cpa-D-3Pal) can be purchased or can be obtained in any method known in the art, such as described in (2001:880 SYNTHLINE). The preparation of this fragment can also be
15 according to the above described solid-phase peptide synthesis (SPPS), using Fmoc chemistry.

The obtained Degarelix preferably has purity of a least above 98.5% (by HPLC), more preferably, at least above 99%.

In yet a more specific embodiment, the combined SPPS and solution synthesis for
20 obtaining Degarelix, going via a 3 + 6 +1 fragment protocol comprises:

a) Loading of the first Fmoc-protected amino acid, Fmoc-Pro-OH, on the CTC resin, and washing and masking all active sites on the resin;

b) Repetitive cycles of the following:

i) Deprotecting to remove the Fmoc group;
25 ii) Washing with at least one solvent to remove all soluble compounds from the resin;

iii) Coupling of a suitable Fmoc-protected amino acids (to obtain a protected 6 amino acid peptide sequence of the following: Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH) with a suitable coupling reagent to the
30 terminal amino group residue attached to the resin to form a Fmoc-protected peptide fragment attached to the resin; and

iv) Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Washing and drying of the peptide-resin;

d) Cleaving the protected peptide intermediate from the resin using a mild acidic composition comprising a TFA solution in DCM to form a protected peptide;

e) Optionally, coarse purification of the protected peptide by suitable chromatographic or other method;

5 f) Optionally, precipitating the protected 6 amino acid fragment from the cleavage solution;

g) Coupling the 6 amino acid fragment with D-Ala-NH_2 dissolved in a suitable solvent;

h) Deblock of the Fmoc group;

10 i) Optionally, coarse purification of the protected peptide by suitable chromatographic or other method;

j) Coupling of acetyl-D-Nal-D-4Cpa-D-3Pal-OH with the protected fragment obtained in paragraph h or i;

k) Optionally, coarse purification of the protected peptide by suitable

15 chromatographic or other method;

l) Deprotecting all protecting groups of the protected peptide by TFA cocktail containing various scavengers;

m) Precipitating of the crude Degarelix from the deprotection solution;

n) Purificatiion of crude Degarelix by a suitable separation method including

20 counter-ion exchange to replace the TFA counterion with acetate to form Degarelix acetate solution; and

o) Drying of the Degarelix acetate solution to obtain Degarelix product as Degarelix acetate.

Suitable resins for use in the process include, but are not limited to, super-acid

25 labile resins such as chlorotrityl resins. A preferred super acid labile resin is 2-Cl-Trt-Cl resin.

In preferred embodiments, the mild and strong acidic solutions consist of different concentration of TFA. The mild acidic solution may be, for example, 1% TFA in DCM The strong acidic solution may be, for example, 95% TFA 5% water

30 The above processes proceed via novel synthetic intermediates, including intermediates of the following formulae:

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro—CTC resin;

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro—CTC resin;

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-OH;

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-OH;

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-D-Ala-NH_2;

Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-D-Ala-NH_2;

5 Ac-D-2Nal-D-4Cpa-D-3Pal-OH;

Ac-D-2Nal-D-4Cpa-D-3Pal—CTC resin.

The present invention encompasses these intermediates, as well as their use in a process for the manufacture of Degarelix.

Those skilled in the art would understand, that for all the processes described in 10 the present invention:

Suitable coupling agents include, but are not limited to, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or DIC (diisopropylcarbodiimide).

Suitable solvents for use in the washing steps of the process include, but are not 15 limited to, dimethylformamide (DMF), dichloromethane (DCM), methanol (MeOH), or isopropanol (IPA).

The N-terminal amino acid residue protecting group is removed by any known method, such as reaction with piperidine solution in DMF. Although one of ordinary skill in the art may substitute the reagents with other suitable reagents depending on the nature 20 of the protecting group. In the case of Fmoc, beside piperidine, other reagents could be used such as DBU, diethyl amine, piperazine, dimethylethylamine and etc.

Cleavage of the protecting groups from the peptide may be affected by addition of a strong acidic composition. The acidic composition is preferably based on an acidic material such as TFA, and contains scavenger reagents including, but not limited to, 25 ethanedithiol (EDT), TIS (triisopropylsilane) and water. The relative ratio of acidic material to scavenger to water may be from about 85% to about 99% acidic material, from about 0.1% to about 15% scavenger, and from about 0.1% to about 15% water by weight. A preferred acidic composition comprises about 95% TFA, about 2.5% EDT, and about 2.5% water.

30 The crude peptide product may be purified by any known method. Preferably, the peptide is purified using HPLC on a reverse phase (RP) column.

At the end of the purification process or as a part of the purification process the counter ion of the peptide may be exchanged by a suitable ion such as, but are not limited to, acetate ion. The counter-ion exchange can be done by any suitable method such as HPLC or ion exchange.

5 Suitable HPLC method can be done for example by loading a solution of the peptide to the head of the column; washing the column by actate buffer to replace and remove TFA or other acids, after completion of washings the peptide is eluted from the column by addition of strong solvent such as acetonitrile to the acetate buffer; The ion-exchange can be done by attaching the peptide to the ion exchange column as a salt of the 10 functional acidic residues of the ion-exchange resin, washing the column to remove TFA or other acids, releasing the peptide by gradient salt concentration increase.

The resulting purified product is dried and may be lyophilized.

While the present invention is described with respect to particular examples and preferred embodiments, it is understood that the present invention is not limited to these 15 examples and embodiments. The present invention as claimed therefore includes variations from the particular examples and preferred embodiments described herein, as will be apparent to one of skill in the art. Each reference, including all patents, patent applications, and publications, both conventional paper publications and those that are published electronically, that are cited in this application is incorporated herein by 20 reference in its entirety.

Examples

Example 1: Preparation of Degarelix via sequential strategy

Synthesis of the protected peptide was carried out by a stepwise Fmoc SPPS (solid 25 phase peptide synthesis) procedure starting from coupling of Fmoc-D-Ala-OH to Rink amide AM resin (100 g). The resin was first deprotected by removing the Fmoc group by washing with 25% piperidine/DMF solution. After washing with DMF, the first amino acid was loaded by a regular coupling procedure using TBTU/HOBt in the presence of DIPEA and reacted for 2 h. After washing of the resin with DMF, the Fmoc protecting 30 group was removed by treatment with 20% piperidine in DMF. After washing of residual reagents with DMF, the second amino acid (Fmoc-Pro-OH) was introduced to start the second coupling step. Fmoc protected amino acids were activated *in situ* using TBTU/HOBt (N-hydroxybenzotriazole) or DIC/HOBt, and subsequently coupled to the resin for 50 minutes. Diisopropylethylamine was used during coupling as an organic base

(only for TBTU couplings). Completion of the coupling was indicated by a Ninhydrin test. After washing of the resin, the Fmoc protecting group on the α-amine was removed with 20% piperidine in DMF for 20 min. These steps were repeated each time with the next amino acid according to peptide sequence. After deblocking of the last amino acid
5 (D-Nal), the peptide was acetylated on its N-terminus by reaction with acetic anhydride. All amino acids used were Fmoc-N^{α} protected. Trifunctional amino acids were side chain protected as follows: Ser(Trt) and Lys(iPr-Boc). D-4Aph(Cbm) was used as non-protected on its side chain group. At the end of the synthesis the peptide-resin was washed with DMF, followed by MeOH, and dried under vacuum to obtain the dry
10 peptide-resin.

The peptide, prepared as described above, was cleaved from the resin using a 95% TFA, 2.5% TIS, 2.5% EDT solution for 2 hours at room temperature. The crude product was precipitated by the addition of 10 volumes of ether, filtering and drying in vacuum to obtain crude product.

15 The crude peptide was dissolved in an aqueous solution of acetonitrile. The resulting solution was loaded on a C_{18} RP-HPLC column and purified to obtain fractions containing Degarelix trifluoroacetate at a purity of >99.0%. After treatment to replace trifluoroacetate with acetate, the fractions were collected and lyophilized to obtain final dry peptide (Degarelix acetate) >99.0% pure (by HPLC) and each impurity <0.50%.

20

Example 2: Comparative cleavage experiments and LC/MS analytics for synthesis of Degarelix using Fmoc-Ser(tBu) or Fmoc-D-4Aph(Cbm/tBu)

Using HPLC and LC/MS analytics, it was shown that protected Degarelix-resin obtained using Fmoc-Ser(tBu) or Fmoc-D-4Aph(Cbm/tBu) under regular cleavage conditions (at
25 room temperature) resulted in partially protected peptide (containing tBu protecting group), whereas using Fmoc-Ser(Trt) or Fmoc-D-4Aph(Cbm) the product obtained is Degarelix.

Cleavage of the protected Degarelix resin obtained using Fmoc-Ser(tBu) or Fmoc-D-4Aph(Cbm/tBu) using the same cleavage cocktail, but at 45° C, resulted in complete
30 deprotection of the tBu group but with formation of degradation impurities.

Example 3: Preparation of Degarelix via (9 + 1) fragment condensation strategy

Synthesis of the protected peptide fragment (1 to 9) is carried out by a regular stepwise Fmoc SPPS (solid phase peptide synthesis) procedure starting from 2-Cl-Trt resin. The first amino acid (Fmoc-Pro-OH) is loaded onto the resin in a preliminary step
5 to provide loading of about 0.7 mmol/g. After resin washing and deblock of the Fmoc group, a second amino acid (Fmoc-Lys(iPr-Boc)-OH) is introduced to start the first coupling step. Fmoc protected amino acid is activated in situ using TBTU/HOBt and subsequently coupled to the resin for 50 minutes. Diisopropylethylamine or Collidine are used during coupling as an organic base. Completion of the coupling is indicated by
10 ninhydrine test. After washing of the resin, the Fmoc protecting group on the α-amine is removed with 20% piperidine in DMF for 20 min. These steps are repeated each time with another amino acid according to peptide sequence. At the end of the synthesis of the peptide sequence the Fmoc group is removed and the N-terminus is acetylated by reaction with acetic anhydride. All amino acids used are Fmoc-N^{α} protected. Trifunctional amino
15 acids are side chain protected as follows: Ser(Trt), D-4Aph(Cbm-tBu), Lys(iPr-Boc). Three equivalents of the activated amino acids are employed in the coupling reactions. At the end of the synthesis the peptide-resin is washed with DMF, followed by DCM.

The peptide, prepared as described above, is cleaved from the resin using a 1% TFA solution in DCM by three repeated washings (15 min each). The acidic peptide
20 solution is neutralized by DIPEA. The solvent is evaporated under reduced pressure and the protected peptide is precipitated by the addition of 10 volumes of water, filtered and dried under vacuum. It is identified by MS as Ac-D-2Nal-D-4Cpa-D-3-Pal-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH.

Protected peptide fragment is reacted with D-Ala-NH_2 dissolved in DMF. The activation
25 of carboxyl group of the peptide is done in-situ by addition of TBTU/HOBt into reaction mixture. Diisopropylethyl amine is used as organic base. Completion of the reaction is monitored by HPLC analysis. At the end of reaction the DMF solution is added slowly to water and crude protected peptide is precipitated as off-white solid. It is separated by filtration, dried and identified by MS as Ac-D-2Nal-D-4Cpa-D-3-Pal-Ser(Trt)-4Aph(L-
30 Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-D-Ala-NH_2.

The protecting groups are removed using a 95% TFA, 2.5% TIS, 2.5% EDT solution for 1 hour at about 45 °C. The crude product is precipitated by the addition of 10 volumes of ether, filtered and dried in vacuum.

The crude peptide is dissolved in aqueous solution of acetonitrile. The resulting solution is loaded on a C_{18} RP-HPLC column and purified to obtain fractions containing Degarelix trifluoroacetate at a purity of >99.0%. After treatment to replace trifluoroacetate by acetate, the fractions are collected and lyophilized to obtain final dry peptide >99.0% pure
5 (by HPLC).

Example 4: Preparation of Degarelix via (3+ 6 + 1) fragment condensation strategy

Synthesis of the protected peptide fragment (4 to 9) is carried out by a regular stepwise Fmoc SPPS (solid phase peptide synthesis) procedure starting from 2-Cl-Trt
10 resin. The first amino acid (Fmoc-Pro-OH) is loaded onto the resin in a preliminary step to provide loading of about 0.7 mmol/g. After resin washing and deblock of the Fmoc group, a second amino acid (Fmoc-Lys(iPr-Boc)-OH) is introduced to start the first coupling step. Fmoc protected amino acid is activated in situ using TBTU/HOBt and subsequently coupled to the resin for 50 minutes. Diisopropylethylamine or Collidine are
15 used during coupling as an organic base. Completion of the coupling is indicated by ninhydrine test. After washing of the resin, the Fmoc protecting group on the α-amine is removed with 20% piperidine in DMF for 20 min. These steps are repeated each time with another amino acid according to peptide sequence. At the end of the synthesis of the peptide sequence the Fmoc group is not removed from the N-terminus. All amino acids
20 used are Fmoc-N^{α} protected. Trifunctional amino acids are side chain protected as follows: Ser(Trt), D-4Aph(Cbm-tBu), Lys(iPr-Boc). Three equivalents of the activated amino acids are employed in the coupling reactions. At the end of the synthesis the peptide-resin is washed with DMF, followed by DCM.

The peptide, prepared as described above, is cleaved from the resin using a 1%
25 TFA solution in DCM by three repeated washings (15 min each). The acidic peptide solution is neutralized by DIPEA. The solvent is evaporated under reduced pressure and the protected peptide is precipitated by the addition of 10 volumes of water, filtered and dried under vacuum. It is identified by MS as Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH.
30 Protected peptide fragment is reacted with D-Ala-NH_2 dissolved in DMF. The activation of carboxyl group of the peptide is done in-situ by addition of TBTU/HOBt into reaction mixture. Diisopropylethyl amine is used as organic base. Completion of the reaction is monitored by HPLC analysis. At the end of reaction the DMF solution is added slowly to

water and crude protected peptide is precipitated as off-white solid. It is separated by filtration, dried and identified by MS as Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-D-Ala-NH_2.

The peptide fragment is dissolved in DMF containing 20% piperidine. After 2 minutes the
5 solvent is concentrated under reduced pressure and the peptide is precipitated by addition of water. The peptide is collected by filtration and dried.

Protected peptide fragment is reacted with Ac-D-Nal-D-4Cpa-D-3Pal-OH dissolved in DMF. The activation of carboxyl group of the peptide is done in-situ by addition of TBTU/HOBt into reaction mixture. Diisopropylethyl amine is used as organic base.
10 Completion of the reaction is monitored by HPLC analysis. At the end of reaction the DMF solution is added slowly to water and crude protected peptide is precipitated as off-white solid. It is separated by filtration, dried and identified by MS as Ac-D-Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-D-Ala-NH_2.

The protecting groups are removed using a 95% TFA, 2.5% TIS, 2.5% EDT
15 solution for 1 hour at about 45 °C. The crude product is precipitated by the addition of 10 volumes of ether, filtered and dried in vacuum.

The crude peptide is dissolved in aqueous solution of acetonitrile. The resulting solution is loaded on a C_{18} RP-HPLC column and purified to obtain fractions containing Degarelix trifluoroacetate at a purity of >99.0%. After treatment to replace trifluoroacetate by
20 acetate, the fractions are collected and lyophilized to obtain final dry peptide >99.0% pure (by HPLC).

What is claimed is:

1. A protected peptide bound to a synthesis resin, selected from Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-D-Ala-resin and Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-D-Ala-resin.

2. The compound of claim 1, which is Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-D-Ala-resin.

3. The compound of claim 1, which is Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-D-Ala-resin.

4. The compound of claim 1, wherein the resin is selected from Rink amide resin, Rink amide AM resin and Rink amide MBHA resin.

5. A protect peptide selected form Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro--CTC resin, Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro--CTC resin, Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-OH and Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-OH.

6. A protect peptide selected form Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-OH; Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-OH; Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro-D-Ala-NH_2 and Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro-D-Ala-NH_2.

7. A protected peptide bound to a synthesis resin, selected from Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm/tBu)-Leu-ILys(Boc)-Pro—CTC resin; Fmoc-Ser(Trt)-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-ILys(Boc)-Pro—CTC resin and Ac-D-2Nal-D-4Cpa-D-3Pal—CTC resin.

8. A method for the preparation of Degarelix comprising sequential synthesis on a solid-phase using Fmoc chemistry.

9. The method according to claim 8, wherein Ser is protected with Trt.

10. The method according to claim 8 wherein, the preparation of Degarelix comprises: a) preparing a protected peptide attached to a Rink amide type resin, in a sequential synthesis wherein the prepared peptide contains the complete amino acid sequence of Degarelix, of which all or most multi-functional amino acids are protected with acid labile protecting groups; b) reacting the protected peptide resin with an acidic composition to produce an unprotected peptide; c) isolating the peptide by precipitation, crystallization, extraction or chromatography; d) purifying the unprotected Degarelix by chromatography, e) replace its counter-ion with acetate, and f) drying to obtain a final material as dry powder as Degarelix acetate.

11. The method according to claim 8 wherein, the sequential synthesis of Degarelix comprises: a) Loading of the first Fmoc-protected amino acid, Fmoc-D-Ala-OH, on the resin, and washing and masking all active sites on the resin;

b) Repetitive cycles of the following:

i. Deprotecting to remove the Fmoc group;

ii. Washing with at least one solvent to remove all soluble compounds from the resin;

iii. Coupling of a suitable Fmoc-protected amino acid (according to the required sequence) to the terminal amino group residue attached to the resin using a suitable coupling reagent to form an Fmoc-protected peptide fragment attached to the resin; and

iv. Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Deblocking the last amino acid Fmoc-D-Nal and acetylating the N-terminus;

d) Washing and drying the peptide-resin;

e) Cleaving of the protected peptide intermediate from the resin using a TFA cocktail containing various scavengers;

f) Precipitating the crude Degarelix from the cleavage solution;

g) Purifying the crude Degarelix by a suitable separation method including counter-ion exchange to acetate to form a Degarelix acetate solution; and

h) Drying the Degarelix acetate solution to obtain the Degarelix product as Degarelix acetate.

12. The method according to claim 8 wherein, the resin is selected from Rink amide resin, Rink amide AM resin and Rink amide MBHA resin.
13. A method for the preparation of Degarelix using fragments synthesis of Degarelix via the 9 + 1 fragment protocol , wherein Ser is protected by Trt comprising: a) providing a protected peptide attached to a super acid-labile resin, wherein the peptide contains the almost complete amino acid sequence of Degarelix except the C-terminal D-Ala-NH_2, wherein all or most multi-functional amino acids are protected with a strong acid labile protecting group; b) Cleaving the protected peptide intermediate from the resin using mild acidic composition; c) isolating the protected peptide by precipitation, crystallization, extraction or chromatography; d) treating the protected peptide obtained in step (c) with a coupling agent in the presence of D-Ala-NH_2 to produce a protected Degarelix; e) reacting the protected Degarelix with strong acidic composition, f) isolating the unprotected Degarelix, and g) purifying the unprotected Degarelix by chromatography.
14. The method according to claim 13 wherein, the fragments synthesis of Degarelix via the 9 + 1 fragment protocol comprises: a) Loading of the first Fmoc-protected amino acid, Fmoc-Pro-OH, on the CTC resin, and washing and masking all active sites on the resin.

b) Repetitive cycles of the following:

i) Deprotecting to remove the Fmoc group;

ii) Washing with at least one solvent to remove all soluble compounds from the resin;

iii) Coupling of a suitable Fmoc-protected amino acid (to obtain a protected 9 amino acid peptide sequence) with a suitable coupling reagent to the terminal amino group residue attached to the resin to form an Fmoc-protected peptide fragment attached to the resin; and

iv) Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Deblocking the last amino acid Fmoc-D-Nal and acetylating the N-terminus;

d) Washing and drying of the peptide-resin;

e) Cleaving the protected peptide intermediate from the resin using a mild acidic composition comprises a dilute TFA solution in DCM to form a protected peptide;

f) Optionally, coarse purification of the protected peptide by a suitable chromatographic or other method;

g) Optionally, precipitating the protected 9 amino acid fragment from the cleavage solution;

5 h) Coupling the protected 9 amino acid fragment with D-Ala-NH_2 dissolved in a suitable solvent;

i) Deprotecting all protecting groups of the protected peptide with a TFA cocktail containing various scavengers;

j) Precipitating the crude Degarelix from the deprotection solution;

10 k) Purifying the crude Degarelix by a suitable separation method including counter-ion exchange to replace the TFA counterion with acetate to form Degarelix acetate solution; and

l) Drying the Degarelix acetate solution to obtain Degarelix product as Degarelix acetate.

15 15. A method for the preparation of Degarelix using fragments synthesis of Degarelix via the 3 + 6 +1 fragment protocol wherein Ser is protected by Trt comprising: a) providing a protected peptide fragment attached to a super acid-labile resin, wherein the peptide contains six amino acid sequence of Degarelix (Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH); b)
20 Cleaving the protected peptide intermediate from the resin using mild acidic composition; c) isolating the protected peptide fragment by precipitation, crystallization, extraction or chromatography; d) treating the protected peptide fragment obtained in step (c) with a coupling agent in the presence of D-Ala-NH_2 to produce a protected C-terminal Degarelix fragment containing 7 amino acids
25 (Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-D-Ala-NH_2); e) isolating the protected peptide fragment by precipitation, crystallization, extraction or chromatography; f) reacting the protected C-terminal fragment with N-terminal fragment containing three amino acids (Ac-D-Nal-D-4Cpa-D-3Pal) to obtain protected Degarelix; g) isolating the protected peptide by precipitation,
30 crystallization, extraction or chromatography; h) reacting the protected peptide with strong acidic composition, i) isolating the unprotected Degarelix, and j) purifying the unprotected Degarelix by chromatography.

16. The method of claim 15 wherein, the fragments synthesis of Degarelix via a 3 + 6 +1 fragment protocol comprises:

a) Loading of the first Fmoc-protected amino acid, Fmoc-Pro-OH, on the CTC resin, and washing and masking all active sites on the resin;

b) Repetitive cycles of the following:

i) Deprotecting to remove the Fmoc group;

5 ii) Washing with at least one solvent to remove all soluble compounds from the resin;

iii) Coupling of a suitable Fmoc-protected amino acids (to obtain a protected 6 amino acid peptide sequence of the following: Fmoc-Ser(Trt)-4Aph(L-Hor)-D-4Aph(Cbm-tBu)-Leu-Lys(iPr-Boc)-Pro-OH) with a suitable coupling reagent to

10 the terminal amino group residue attached to the resin to form a Fmoc-protected peptide fragment attached to the resin; and

iv) Washing the product of step (iii) with at least one solvent to remove all soluble compounds from the resin;

c) Washing and drying of the peptide-resin;

15 d) Cleaving the protected peptide intermediate from the resin using a mild acidic composition comprises a TFA solution in DCM to form a protected peptide;

e) Optionally, coarse purification of the protected peptide by suitable chromatographic or other method;

f) Optionally, precipitating the protected 6 amino acid fragment from the cleavage

20 solution;

g) Coupling the 6 amino acid fragment with D-Ala-NH_2 dissolved in a suitable solvent;

h) Deblock of the Fmoc group;

i) Optionally, coarse purification of the protected peptide by suitable

25 chromatographic or other method;

j) Coupling of acetyl-D-Nal-D-4Cpa-D-3Pal-OH with the protected fragment obtained in paragraph 8 or 9;

k) Optionally, coarse purification of the protected peptide by suitable chromatographic or other method;

30 l) Deprotecting all protecting groups of the protected peptide by TFA cocktail containing various scavengers;

m) Precipitating of the crude Degarelix from the deprotection solution;

n) Purificating of crude Degarelix by a suitable separation method including counter-ion exchange to replace the TFA counterion with acetate to form Degarelix acetate solution; and

o) Drying of the Degarelix acetate solution to obtain Degarelix product as Degarelix acetate.

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17. The method according to claims 8, 13 or 15 wherein, the resin is super-acid labile resins such as chlorotrityl resins.

18. The method according to claim 8, 13 or 15 wherein, Degarelix having a purity of at least about 98.5% (by HPLC).

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/058004

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/23
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, BEILSTEIN Data, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2010/121835 A1 (CARLBIOTECH LTD AS [DK]; ZHANG HAIXIANG [FR]; FOMSGAARD JENS [DK]; STA) 28 October 2010 (2010-10-28) cited in the application the whole document page 4, lines 9-10; claims -----	8,10-12, 18
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Y	the whole document line 63 - column 7; example 1 -----	9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2011	25/02/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cervigni, S

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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