Title: PROCESS FOR THE MANUFACTURE OF DEGARELIX AND ITS INTERMEDIATES

Abstract: The present invention relates to a liquid (or solution)-phase manufacturing process for preparing the decapeptide Degarelix, its protected precursor, and other useful intermediates. The invention further relates to polypeptides useful in the solution-phase manufacturing process and to the purification of Degarelix itself. Degarelix can be obtained by subjecting a Degarelix precursor according to formula (II): \( P_1-A\text{-Acetyl}-P_2-\text{NH}_2 \) to a treatment with a cleaving agent in an organic solvent, wherein \( P_1 \) is an amino protecting groups; preferably acetyl; \( P_4 \) is hydrogen or an hydroxy! protecting group, preferably a hydroxyl protecting group; \( P_6 \) is hydrogen or an amino protecting groups; preferably an amino protecting groups; and \( P_4 \) is an amino protecting group.
Process for the manufacture of Degarelix and its intermediates

[Technical field]

The present invention relates to a liquid (or solution)-phase manufacturing process for preparing the decapeptide Degarelix, its protected precursor, and other useful intermediates. The invention further relates to polypeptides useful in the solution-phase manufacturing process and to the purification of Degarelix itself.

[Background of the Invention]

Prostate cancer is a leading cause of morbidity and mortality for men in the industrialised world. Degarelix, also known as FE200486, is a third generation gonadotropin releasing hormone (GnRH) receptor antagonist (a GnRH blocker) that has been developed and recently approved for prostate cancer patients in need of androgen ablation therapy (Döhn et al., Drugs 2006, vol. 9, No. 8, pp. 565-571; WO 09846634). Degarelix acts by immediate and competitive blockade of GnRH receptors in the pituitary and, like other GnRH antagonists, does not cause an initial stimulation of luteinizing hormone production via the hypothalamic-pituitary-gonadal axis, and therefore does not cause testosterone surge or clinical flare (Van Poppel, Cancer Management and Research, 2010:2 39-52; Van Poppel et al., Urology, 2008, 71(6), 1001-1006); James, E.F. et al., Drugs, 2009, 69(14), 1967-1976).

Degarelix is a synthetic linear decapeptide containing seven unnatural amino acids, five of which are D-amino acids. It has ten chiral centers in the backbone of the decapeptide. The amino acid residue at position 5 in the sequence has an additional chiral center in the side-chain substitution giving eleven chiral centers in total. Its CAS registry number is 214766-78-6 (of free base) and it is commercially available under the Trademark Firmagon™. The drug substance is chemically designated as D-Aianinamide, N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4S)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L-phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L-
leucy-[N6-(1-methylethyl)-L-lysyl-L-prolyl- and is represented by the chemical structure below (in the following also referred to as Formula I):

The structure of Degarelix can also be represented as:

\[
\text{Ac-D-2Nai-D-4Cpa-D-3Pal- Ser-4Aph(L-Hor)-D-4Aph(Cbm)-Leu-Lys(iPr)-Pro-D-Ala-NH}_2
\]

where Ac is acetyl, 2Nai is 2-naphthylalanine, 4Cpa is 4-chlorophenylalanine, 3Pal is 3-pyridyialanine, Ser is serine, 4Aph is 4-aminophenylalanine, Hor is hydroorotyl, Cbm is carbamoyl, Leu is leucine, Lys(iPr) is N6-isopropyllysine, Pro is proline and Ala is alanine.

For the purposes of describing this invention, each amino acid in Degarelix will be given the shorthand notation as follows:

AAi is D-2Nai, AA2 is D-4Cpa, AA3 is D-3Pal, AA4 is Ser, AA5 is 4Aph(L-Hor), AA6 is D-Aph(Cbm), AA7 is Leu, AA8 is Lys(iPr), AA9 is Pro and AA10 is D-Ala.

Thus, as an example, Degarelix can be represented as Ac-AAi-AAi-NH2, the tetrapeptide Ac-D-2Nai-D-4Cpa-D-3Pal-Ser can be represented as Ac-AA2-AA4 and the hexapeptide 4Aph(L-Hor)-D-4Aph(Cbm)-Leu-Lys(iPr)-Pro-D-Ala-NH2 as AA5-AA10-NH2.

Basically, Boc-protected D-Ala is first coupled to MBHA resin in dimethylformamide (DMF)/CH$_2$Cl$_2$ using diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) as activating or coupling agents. Once D-Ala is coupled to the resin, synthesis proceeds by washing, deblocking and then coupling the next amino acid residue until the decapeptide has been completed. The side chain primary amino groups of 4Aph in the 5-position and of D-4Aph in the 6-position are protected by Fmoc when they are added and modified with L-Hor and Cbm respectively before the next amino acid in the chain is added. This requires the additional steps of first removing the side-chain protection with piperdine, reacting the newly freed amino group on the peptidoresin with tert-butyl isocyanate or L-hydroorotic acid, ensuring that the reaction is complete with a ninhydrin test and then washing the peptidoresin before adding the next amino acid residue (see also Sorbera et al., Drugs of the Future 2006, Vol. 31, No. 9, pp 755-766).

While Boc-SPPS methodology has afforded sufficient quantities of Degareix until now, the growing demand for this polypeptide means that ever larger quantities are required. Boc-SPPS, which requires HF cleavage, is not suited to large scale industrial synthesis. Indeed, WO 98/46634 mentions that SPPS is only suitable for limited quantities of up to 1 kg while classical peptide solution synthesis, or liquid phase peptide synthesis (LPPS), is preferred for larger quantities of product. WO 98/46634 does not specify how such synthesis should be performed. While the existence of a liquid phase peptide synthesis of Degareix has been reported [EMEA Report: Assessment Report for Firmagon™ (Degareix): Doc. Ref. EMEA/CHMP/635761/2008], as of now no details of such a process have been publicly disclosed.

WO 97/34923 and WO 99/26964 are documents concerned with liquid phase processes for the preparation of biologically active peptides. WO 99/26964 is particularly concerned with the liquid phase synthesis of decapeptides having activity as GnRH antagonists. WO 99/26964 lists a number of inherent limitations of the SPPS methodology for producing GnRH antagonists including the limited capacity of the resin, the large excess of reagents and amino acids required, as well as the need to protect all reactive side chains such as the hydroxy group in Ser, the aromatic amino groups in Aph and D-Aph, the ε-i-propylamino group in Lys(i-Pr).
WO 99/26964 proposes a liquid phase process which involves first preparing the central peptide fragments of the 5 and 6 positions of a decapeptide with the side chains fully elaborated and then assembling the peptide through a "4-2-4", "3-3-4" or "3-4-3" fragment assembly pattern. For example, in the preparation of the GnRH antagonist Azaline B, a tetrapeptide is coupled with a hexapeptide to form the desired decapeptide. When the same fragment assembly pattern is attempted for Degarelix, racemisation of the Ser amino acid (AA₄) occurs resulting in about 20% impurity of L-Ser. This impurity carries over into the final decapeptide and is difficult to remove. Furthermore, when preparing the tetrapeptide AA₁-AA₄ by adding the Ser unit to the tripeptide AA₁-AA₃ following the procedure described in WO 99/26964, tetrabutylammonium ions from the hydrolysis of the benzyl ester group could not be removed completely during the subsequent operations and were carried through to the final product. It was further found that in the Degarelix synthesis, the L-hydroorotyl group rearranges to its hydantoinacetyl analogue when L-dihydroorotic acid is coupled with 4Amp to prepare AA₅. These and other problems with the solution-phase synthesis of Degarelix have now been overcome and a new solution-phase polypeptide synthesis of this decapeptide is disclosed herein for the first time.

[Summary of the Invention]

The problems of SSPS methods for preparing Degarelix and the drawbacks of LLPS methods as described in WO 97/34923 and WO 99/26964 have now been overcome and are the subject of this invention.

In general, this invention relates to a liquid-phase synthesis of the decapeptide Degarelix.

In one aspect, the invention relates to a liquid-phase process for preparing Degarelix having the formula Ac-AA₁-AA₁₀-NH₂ or a pharmaceutically acceptable salt or solvate thereof, comprising the step of subjecting a Degarelix precursor according to the following formula is or a salt or solvate thereof to a treatment with a cleaving agent:
The compound of Formula II thus corresponds to

\[(P_1)AA_1^2- AA_2^3- AA_4(P_4)- AA_5^4- AA_6(P_6)- AA_7^7- AA_8(P_8)- AA_9^9- AA_{10}^9- \text{NH}_2 \]  \hspace{1cm} (II)

where \( AA_1^2 \) to \( AA_{10}^9 \) are the same as for formula (I).

\( P_1 \) is an amino protecting group or acetyl;

\( P_4 \) is hydrogen or a hydroxy protecting group, preferably a hydroxy protecting group;

\( P_6 \) is hydrogen or an amino protecting groups; preferably an amino protecting groups; and

\( P_8 \) is an amino protecting group.

Preferably, the protecting group \( P_i \), if present, is orthogonal to \( P_8 \), i.e. both protecting groups can be cleaved independently.

In a preferred embodiment, \( P_i \) is acetyl, and each of \( P_4 \) and \( P_8 \) are protecting groups that can be cleaved in a single step, and \( P_6 \) is hydrogen or a protecting group that can be cleaved together with the protecting groups \( P_4 \) and \( P_8 \). The cleavage of the protecting groups is preferably carried out by treating the precursor of formula (II) with trifluoroacetic acid (TFA).

It is particularly preferred that \( P_4 \), \( P_5 \), and \( P_8 \) are protecting groups selected from tert-butyl (tBu) and t-butyloxycarbonyl (Boc), and most preferred is a precursor of formula (II) wherein

\( P_4 \) is tBu, \( P_6 \) is hydrogen or tBu, and \( P_8 \) is Boc.

The present invention also relates to a process for the liquid-phase manufacture of the intermediate represented by formula (II) or pharmaceutically acceptable salt or solvate thereof, comprising the step of coupling a first polypeptide represented by formula (III).
or a salt thereof, with a second polypeptide represented by formula (IV)

\[ \text{(IV)} \]

or salt thereof, in a liquid reagent medium in the presence of a peptide coupling reagent, optionally together with an organic amine base, to form a decapeptide represented by formula (I). In this case AA i to AA i 0, P 4, P 6 and P 8 are the same as for formula (II).

The salts include acid salts such as hydrochlorides and basic salts such as alkali metal salts, alkaline earth metal salts, and ammonium salts.

According to the invention, the second polypeptide represented by formula (IV) may be prepared by eliminating the protective group P N from the following compound (IVa):

\[ \text{(IVa)} \]

P N is preferably an N-terminal amino protecting group and more preferably a protecting group that can be eliminated by hydrogenation such as benzylloxycarbonyl.

The compound of formula (IVa) may be obtained by coupling a polypeptide represented by formula (V) with a polypeptide represented by formula (VI)

\[ \text{(V)} \]

\[ \text{(VI)} \]

or salts of these compounds, where AA 4 to AA 10, P N, P 4, P 6 and P 8 are the same as for formula (IVa).

The invention also relates to the polypeptides represented by formulae (II) to (VI) which are useful in the liquid-phase manufacturing process of the invention.
Figure 1 shows a flow diagram for the synthesis of a derivatives of AA\(^1\) and AA\(_2\) for the peptide synthesis.

Figure 2 shows a flow diagram for the synthesis of a derivative of AA\(_2\)-AA\(_3\) for the peptide synthesis.

Figure 3 shows a flow diagram for the synthesis of Ac-AA\(^1\)-AA\(_3\).

Figure 4 shows a flow diagram for the synthesis of a derivative of AA\(_5\)-AA\(_7\).

Figure 5 shows a flow diagram for the synthesis of a derivative of AA\(_5\)-AA\(_7\).

Figure 6 shows a flow diagram for the synthesis of Z-AA\(_4\)-AA\(_7\).

Figure 7 shows a flow diagram for the synthesis of AA\(_9\)-AA\(_{20}\)-

Figures 8 and 9 show a flow diagram for the synthesis of Z-AAg- AA\(_{10}\).

Figure 10 shows a flow diagram for the synthesis of Z-AA\(_4\)-AA\(_{10}\).

Figure 11 shows a flow diagram for the synthesis of the precursor of formula \(\text{II}\).

Figure 12 shows a flow diagram for the synthesis of Degarelix from the precursor of formula \(\text{II}\) and the purification and lyophilisation.

[Detailed Description of the Invention]

The present invention will now be described in more detail.

**Deprotection step**

In a first aspect, the present invention relates to a liquid-phase process for preparing Degarelix having the formula Ac~AA\(_i\)-AA\(_{10}\)-NH\(_2\) or a pharmaceutically acceptable salt or
solvate thereof. The process comprises the step of cleaving the protecting groups \( (P_4), (P_6), \)
and \( (P_8) \), if present, from the Degarelix precursor according to formula II \((\{P\}AA_1\ldots AA_7\ldots AA_{10})\), or a salt or solvate thereof, in an organic
solution comprising the precursor and a cleaving agent dissolved therein.

AA\textit{i} to AA\textit{j} in formula II have the same meaning as in formula (I), and \( P_1 \) is an amino
protecting group or acetyl, preferably acetyl.

\( P_8 \) is an amino protecting group. Preferably, \( P_8 \) is any side chain protecting group known in
the art such as those described in E. Gross & J. Meienhofer, The Peptides: Analysis, Structure,
1981). Suitable examples include 9-fluorenyl methyl oxycarbonyl (Fmoc), benzyl oxycarbonyl
(Cbz or Z), and substituted Cbz, such as, e.g., 2-bromo-benzoylcarbonyl (2-Br-Z), 2-chloro-
benzoxycarbonyl (2-Cl-Z), p-chlorobenzoxycarbonyl, p-6-nitrobenzoxycarbonyl, p-
benzofuranylcarbonyl, and p-methoxybenzoxycarbonyl, O-chlorobenzoxycarbonyl,
2,4-dichlorobenzyloxycarbonyl, 2,6-dichlorobenzyloxycarbonyl, and the like; aliphatic
urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), t-amyl oxycarbonyl,
isopropyl oxycarbonyl, 2-(p-biphenyl)-isopropyloxycarbonyl, and the like; cycloalkyl
urethane-type protecting groups, such as cyclopentyl oxycarbonyl, adamantan oxycarbonyl,
and cyclohexyl oxycarbonyl; aliroyloxycarbonyl (Alloc) acetyl (Ac), benzoyl (Bz), trifluoracetyl
(Tfa), toluenesulfonyl (Tos), benzyl (Bn), triphenyl methyl (Trt), o-nitrophenyl-sulfonyl (Nps),
t-butyl-dimethyl ethylsiloxycarbonyl, [2-(3,5-di methoxyp henyl)-p-ropyl-2-oxycarbonyl] (Ddz),
2,2,2-trichloroethoxycarbonyl (Trocl), biphenylisopropyl oxycarbonyl (Bpoc), and o-
nitrobenzoxycarbonyl. Preferred protecting groups are Fmoc, Boc and Alloc with Boc being
most preferred.

\( P_4 \) is hydrogen or a hydroxyl protecting group, preferably a hydroxyl protecting group. The
hydroxyl group of Ser (\( P_4 \)) is preferably a C\textsubscript{4}-C\textsubscript{6} alkyl (e.g. t-butyl, cyclohexyl), acetyl (Ac), trityl,
benzyl, a benzyl ether such as p-methoxybenzyl, or other substituted benzyi such as p-
nitrobenzyl, p-chlorobenzyl, o-chlorobenzyl, and 2,6-dichlorobenzyl, tetrahydropryranyl,
tri(Ci-C\textsubscript{6})alkylsityl, 2-m ethoxyethoxymethyl (MEM), 4-dimethylcarbamoylbenzyl and O-
phenoxyacetyl ethers.
Particularly preferred are t-butyl, benzyl and 9-fluorenylmethyl ethers, t-butyl being most preferred.

P is hydrogen or an amino protecting groups, preferably an amino protecting groups. Preferred protecting groups include C4-C6 alkyl (e.g. t-butyl, cyclohexyl), acetyl (Ac), trityl, benzyl, a benzyl ether such as p-methoxybenzyl, or other substituted benzylls such as p-nitrobenzyl, p-chlorobenzyl, o-chlorobenzyl, and 2,6-dichlorobenzyl, tetrahydropyranyl, tri(C1-C6)alkyisilyl, 2-methoxyethoxymethyl (MEM), 4-dimethylcarbamoylbenzyl and O-phenoxyacetyl ethers. Particularly preferred are t-butyl, benzyl and 9-fluorenyl methyl ethers, t-butyl being most preferred.

The cleaving agent used to remove the protecting groups depends on the nature of the protecting group and are well known in the art.

Preferred cleaving agents for the Ser hydroxy! protecting group P4 are:

- trifluoracetic acid (TFA), HCl, or methanesulfonic acid, particularly for t-butyl ether as a protecting group
- H2/Pd-C, HF, or trifluoromethane-sulfonic acid, particularly for benzyl ether as a protecting group, and
- SiCl4/anisol, particularly for 2-(methyisulfinyl)benzylether as a protecting group

Preferred cleaving agents for the amino protecting group P9 are:

- trifluoracetic acid (TFA), HCl, or methanesulfonic acid, particularly for t-butyl carbamates as protecting group
- H2/Pd-C, HF, or trifluoromethane-sulfonic acid, particularly for benzyl carbamates as protecting group, and
- Piperidine, D8U and DEA, particularly for Fmoc as protecting group

Preferred solvents include DCM, DMF, NMP, dioxane, EtOH, Neat HF, and TFA.
Particularly preferred are the different cleavage conditions indicated in the following table 1:

**Table 1: Cleavage conditions**

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Protected group</th>
<th>Cleavage reagent</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abbreviation</strong></td>
<td><strong>Name</strong></td>
<td><strong>group</strong></td>
<td></td>
</tr>
<tr>
<td>t-Bu</td>
<td>t-Butyl ethers and esters</td>
<td>-OH and -CO₂H</td>
<td>TFA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanesulfonic acid</td>
</tr>
<tr>
<td>Bzl</td>
<td>Benzyl ethers and esters</td>
<td>-OH and -CO₂H</td>
<td>H₂/Pd-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>MsOb</td>
<td>4-(Methylsulfonyl)-benzyl ether</td>
<td>-OH</td>
<td>SiCl₄/anisol</td>
</tr>
<tr>
<td>Chz or Z</td>
<td>Benzyloxy carbonyl</td>
<td>-NH₂</td>
<td>H₂/Pd-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxy-carbonyl</td>
<td>-NH₂</td>
<td>TFA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methanesulfonic acid</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-Fluorenylmethoxy carbonyl</td>
<td>-NH₂</td>
<td>Piperidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DBU (1,8- diazabicyclo[5.4.0]-undec-7-ene)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DEA (diethylamine)</td>
</tr>
<tr>
<td>Trt</td>
<td>Trityl (Trt)</td>
<td>-OH</td>
<td>1% TFA-DCM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-NH₂</td>
<td></td>
</tr>
<tr>
<td>TBDMS</td>
<td>Tert-butyl-dimethyl silyl</td>
<td>-OH</td>
<td>TFA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ACOH-THF-H₂O (3:1:1), 18h</td>
</tr>
<tr>
<td>Cyclohexyl (CHX or CH₃)</td>
<td>Cyclohexyl</td>
<td>-OH</td>
<td>HF or TFSMA</td>
</tr>
</tbody>
</table>


Typically, the precursor of formula I is dissolved in a cleaving agent, preferably TFA, with or without an additional solvent, at room temperature (20 to 25 °C) for 20 to 30 hours,
preferably 24 hours. When the protecting group has been removed (preferably the conversion is > 95% yield, most preferably > 99%), the crude Degarelix is then preferably poured into a buffered water-ethanol mixture to provide a buffered solution of crude degarelix for subsequent purification. The preferred pH is preferably in the range of 2 to 4, more preferably in the range of 2.5 to 3.5, and most preferably approximately 3.

Specific embodiments of the deprotection step are shown in Figure 12 and Example 4 (see Step 12).

**Purification and lyophilization**

The solution of crude degarelix is preferably purified using chromatographic techniques such as preparative reverse phase chromatography (RPC).

The resulting product is then preferably lyophilized.

**3 + 7 coupling**

In a further aspect the present invention also relates to a process for the liquid-phase manufacture of the intermediate represented by formula (II) or pharmaceutically acceptable salt or solvate thereof, comprising the step of coupling a first polypeptide represented by formula (III)

\[(P_1)AA_1-AA_2-AA_3\] (III)

or a salt thereof, with a second polypeptide represented by formula (IV)

\[AA_4(p_4)-AA_5-AA_6(P_6)-AA_7-AA_8(P_8)-AA_9-AA_{10-10}NH_2\] (IV)

or a salt thereof, in a liquid reagent medium in the presence of a peptide coupling reagent and optionally an organic amine base, to form a decapeptide represented by formula (II). In this case AA_1 to AA_30, P_1, P_6, P_8 and P_9 are the same as for formula (II). The salts include acid
salts such as hydrochlorides and basic salts such as alkali metal salts, alkaline earth metal salts, and ammonium salts.

The coupling reaction is performed in an organic solution where the two peptides and a peptide coupling reagent and optionally an organic amine base are dissolved therein. A peptide coupling additive and/or an organic amine may also be present.

The organic solvent, peptide coupling reagent, peptide coupling additive and organic amine base may be any of those known in the art of LPPS.

Typical organic solvents are THF, NMP (N-methyl pyrrolidone), DCM, DMF, DMSO, and mixtures thereof. The most preferred solvent is DMSO.

Typical peptide coupling reagents are one or more of o-(7-azabenzotriazoi-l-yl)l, 1,3,3-tetramethyluronium hexafluorophosphate (HATU), o-(benzotriazoi-l-yl)-l,l,3,3-tetramethyluronium hexafluorophosphate (HBTU), o-(benzotriazoi-l-yl)-l,l,3,3-tetramethyluronium tetrafluoroborate (TBTU), benzotriazole-l-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), benzotriazole-l-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP), N,N-bis-(2-oxo-3-oxazolidinyl)phosphonic dichloride (BOP-Ci), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), iso-butylichoroformate (IBCF), 1,3 dicyclohexycarbodiimide (DCC), 1,3-di(isopropyl-carbodiimide (DIC), l-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCD), N-ethoxycarbonyl-2-ethoxy-l,2-dihydroquinoline (EEDQ), isopropylchloroformate (IPCF), 2-(5-norbornen-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate (TNTU), propane phosphonic acid anhydride (PPAA) and 2-succinimido-l,l,3,3-tetramethyluronium tetrafluoroborate (TSTU).

A preferred coupling agent is DCC. DCC is preferably used without an organic amine. In a particularly preferred embodiment, DCC is used in combination with DMSO. DCC is preferably used in an amount of 1.3 to 2, most preferably 1.4 to 1.6 equivalents with respect to the tripeptide.
Another preferred coupling agent is DIC, which is preferably used in combination with 6-chloro-HOBt, optionally together with copper salts.

Typical peptide coupling additives are 1-hydroxy-1H-benzotriazole (HOBt), 6-chloro-HOBt, and 1-hydroxy-7-azabenzotriazole (HOAt).

Typical organic amine bases are NMM, DiPEA, TEA, and colidine.

It is particularly preferred to carry out the coupling reaction using DMSO as solvent and DCC as coupling agent.

Specific embodiments of the coupling step are shown in Figure 11 and Example 4 (see Step 11).

**Synthesis of the heptapeptide**

The compound of formula IV, i.e. AA₄(P₄)–AA₅(P₅)–AA₆(P₆)–AA₇(P₇)–AA₈(P₈)–AA₉(P₉)–NH₂, is preferably obtained by eliminating the protective group Pₐ of the following compound IVa:

\[(Pₐ)AA₄(P₄)·AA₅(P₅)·AA₆(P₆)·AA₇(P₇)·AA₈(P₈)·AA₉(P₉)·NH₂\] (IVa)

While AA₄ to AA₉, P₄, P₅, and P₇ have the same meaning as above, Pₐ is an N-terminal amino protecting group. In a preferred embodiment, Pₐ is a protecting group that can be eliminated by hydrogenation, e.g. using hydrogen and a palladium catalyst (such as Pd/C). The most preferred protecting group Pₐ is benzylxycarbonyl (Z).

Specific embodiments of the elimination step of Pₐ are shown in Figure 11 and Example 4 (see Step 11).

The compound of formula (IVa) may be obtained by coupling a polypeptide represented by formula (V) with a polypeptide represented by formula (VI).
or salts of one or both of these compounds, where AA₄ to AA₆, P₄, P₆ and P₈ are the same as for formula (IVa). The coupling reaction is performed in an organic solution where the two peptides and a peptide coupling reagent are dissolved therein. A peptide coupling additive and/or an organic amine may also be present. The same peptide coupling reagents, organic solvents, peptide coupling additives and organic amines as described above may be used. In a preferred embodiment, DCC is used as a coupling reagent, optionally with ethyl acetate as solvent.

**Synthesis of the tetrapeptide of Formula V**

In a preferred embodiment, the tetrapeptide of formula V, i.e. \((Pₙ)ΑΑ₄(P₄)-ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇\), is prepared by a process comprising the following steps:

(a) providing \((Pₙ₂)ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇(P_c)\), wherein \(P_c\) has the same meaning as above in formula IVa, \((Pₙ₂)\) is an N-terminal amino protecting group or hydrogen, and \(P_c\) is a C-terminal carboxyl protecting group that can be cleaved by hydrogenation;

(b) removing the amino protecting group \((Pₙ₂)\), if present;

(c) hydrogenating \(H-ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇(P_c)\) to obtain \(H-ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇\); and

(d) reacting \(H-ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇\) with an activated ester of \((Pₙ)ΑΑ₄(P₄)\) to provide

\[(Pₙ)ΑΑ₄(P₄)-ΑΑ₅-ΑΑ₆(P₆)-ΑΑ₇\], wherein \((P₄)\) is a hydroxyl protecting group or hydrogen, \(P₆\) is hydrogen or an amino protecting group, and \(P₉\) is a protecting group that can preferably be eliminated by hydrogenation, e.g. using hydrogen and a palladium catalyst (such as Pd/C).

The most preferred protecting group \(P₉\) is benzyloxycarbonyl (Z).

The preferred activated ester of \((Pₙ)ΑΑ₄(P₄)\) is a 4-nitrophenyl ester (ONp).
Hence, preferably, the benzyl ester is removed in the AA5-AA7 intermediate to provide both N- and C- unprotected AA5-AA7. This tripeptide is then reacted with preactivated serine (e.g. Z-Ser(tBu)-ONp).

**Degarelix manufacture**

The present invention thus relates to the manufacture of Degarelix, the above-discussed deprotection step being an essential step of this manufacture. In a preferred embodiment, this deprotection step follows the 3+7 coupling discussed above. In an even more preferred embodiment, the 3+7 coupling follows the synthesis of the heptapeptide discussed above, i.e. the process comprises the following steps:

a) Synthesis of AA4(P4)-AA5-AA6(P6). AA7-AA8(P8)-AA9-AA10-NH2, or a salt or solvate thereof;

b) Coupling of AA4(P4)-AA5-AA6(P6).AA7-AA8(P8)-AA9-AA10-NH2 and (Pi)AAi-AA2-AA3 to provide (P1)AA1-AA2-AA3-AA4(P4).AA5-AA6(P6).AA7-AA8(P8)-AA9-AA10NH2, or a salt or solvate thereof;

c) Deprotection of (Pi)AA1-AA2-AA3-AA4(P4).AA5-AA6(P6).AA7-AA8(P8)-AA9-AA10-NH2, or a salt or solvate thereof to provide degarelix, or a solvate or salt thereof.

AAi to AA10 and P1, P4, P6, and Pg have the same meanings as defined above.

In a particularly preferred embodiment,

- P1 is acetyl;
- P4 is a protecting group that is cleavable with TFA, preferably tBu;
- P5 is a hydrogen or a protecting group that is cleavable with TFA, preferably tBu;
- Pg is a protecting group that is cleavable with TFA, preferably Boc.

In the most preferred embodiment, the heptapeptide is produced using the heptapeptide synthesis described above. Moreover, the deprotection step is preferably followed by the purification and lyophilisation methods described above.
in the synthesis of degarelix or its precursors, and particularly in all steps containing a peptide with the hydroorotyl moiety, the pH is preferably kept below 9, preferably below 8.5, even more preferred being below 8. It is preferred to use a weak base such as NaHCO3 for pH adjustment. It is particularly preferred that all extractions after the coupling steps are carried out within a pH range of 2 to 9, preferably 2.5 to 8 (see steps 6, 7, 10, and 11 in the experimental section). It is additionally preferred to add and C4-5 aliphatic alcohol such as n-butanol or 2-butanol prior to the extraction or washing step.

**Intermediates**

The invention also relates to the polypeptides represented by formulae (I) to (V) which are useful in the liquid-phase manufacturing process of the invention.

Preferred embodiments of formula (I)

Table 2 in the synthesis of degarelix or its precursors, and particularly in all steps containing a
peptide with the hydroorotyl moiety, the pH is preferably kept below 9, preferably below 8.5, even more preferred being below 8. It is preferred to use a weak base such as NaHCO3 for pH adjustment. It is particularly preferred that all extractions after the coupling steps are carried out within a pH range of 2 to 9, preferably 2.5 to 8 (see steps 6, 7, 10, and 11 in the experimental section). It is additionally preferred to add and C4-5 aliphatic alcohol such as n-butanol or 2-butanol prior to the extraction or washing step.

**Intermediates**

The invention also relates to the polypeptides represented by formulae (I) to (V) which are useful in the liquid-phase manufacturing process of the invention.

Preferred embodiments of formula (I)

![Chemical Structure]

(Pi)AA1-AA2-AA3-AA4(P4) - AA5-AA6(P6)-AArAA8(P8)-AAao-NH2 (II)

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>P1</th>
<th>P4</th>
<th>P6</th>
<th>P8</th>
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</thead>
<tbody>
<tr>
<td>IIa</td>
<td>Ac</td>
<td>tBu</td>
<td>tBu</td>
<td>Fmoc</td>
</tr>
<tr>
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<td>tBu</td>
<td>Boc</td>
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</tr>
<tr>
<td>lle</td>
<td>Ac</td>
<td>H</td>
<td>H</td>
<td>Alloc</td>
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<tr>
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<td>Ac</td>
<td>H</td>
<td>H</td>
<td>Boc</td>
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<td>lie</td>
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<tr>
<td>H</td>
<td>Boc</td>
<td>tBu</td>
<td>tBu</td>
<td>Fmoc</td>
</tr>
<tr>
<td>llg</td>
<td>Fmoc</td>
<td>tBu</td>
<td>tBu</td>
<td>Boc</td>
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<td>lli</td>
<td>Fmoc</td>
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<td>H</td>
<td>H</td>
<td>Fmoc</td>
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</table>

Preferred embodiments include salts of these compounds.
Preferred embodiments of formula (III)

\[(P_N)\text{AA}_1\text{AA}_2\text{AA}_3\]  \((I_{11})\)

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>(P_N)</th>
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<tbody>
<tr>
<td>liia</td>
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<tr>
<td>1mb</td>
<td>Boc</td>
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</tbody>
</table>

Preferred embodiments include salts of these compounds.
Preferred embodiments of formula (IV)/(IVA)

\[(P_N)\text{AA}_4(P_4)\text{-AA}_5\text{-AA}_6(P_6)\text{-AA}_7\text{-AA}_8(P_8)\text{-AA}_9(P_9)\text{-AA}_{10}NH_2\]  \((\text{IV})\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(P_4)</th>
<th>(P_6)</th>
<th>(P_8)</th>
<th>(P_N)</th>
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<tbody>
<tr>
<td>lVla</td>
<td>tBu</td>
<td>tBu</td>
<td>Fmoc</td>
<td>H</td>
</tr>
<tr>
<td>lVb</td>
<td>tBu</td>
<td>tBu</td>
<td>Boc</td>
<td>H</td>
</tr>
<tr>
<td>lVc</td>
<td>H</td>
<td>H</td>
<td>Alloc</td>
<td>H</td>
</tr>
<tr>
<td>lVd</td>
<td>H</td>
<td>H</td>
<td>Boc</td>
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<td>Z</td>
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<td>lVi</td>
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<td>tBu</td>
<td>Boc</td>
<td>Z</td>
</tr>
<tr>
<td>lVj</td>
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</tr>
<tr>
<td>lVk</td>
<td>H</td>
<td>H</td>
<td>Boc</td>
<td>Z</td>
</tr>
<tr>
<td>lVl</td>
<td>tBu</td>
<td>H</td>
<td>Boc</td>
<td>Z</td>
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<td>lVm</td>
<td>H</td>
<td>tBu</td>
<td>Boc</td>
<td>Z</td>
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<tr>
<td>lVn</td>
<td>H</td>
<td>H</td>
<td>Fmoc</td>
<td>Z</td>
</tr>
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</table>
Preferred embodiments include salts of these compounds.

Preferred embodiments of formula (V)

\[(P_N)AA_4(P_4)^{-}AA_5-AA_6(P_6)^{-}AA_7-\theta \]

Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>(P_4)</th>
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<th>(P_N)</th>
</tr>
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<tbody>
<tr>
<td>Va</td>
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<td>tBu</td>
<td>Z</td>
</tr>
<tr>
<td>Vb</td>
<td>tBu</td>
<td>H</td>
<td>Z</td>
</tr>
<tr>
<td>Vc</td>
<td>H</td>
<td>tBu</td>
<td>Z</td>
</tr>
<tr>
<td>Vd</td>
<td>H</td>
<td>H</td>
<td>Z</td>
</tr>
</tbody>
</table>

Preferred embodiments include salts and solvates of these compounds.
Preferred embodiments of formula (VI)

\[
AA_8(P_8)\text{-}AA9\text{-}AAio\text{-}NH_2 
\]

Table 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>P_8</th>
</tr>
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<tbody>
<tr>
<td>Via</td>
<td>Boc</td>
</tr>
<tr>
<td>Vlb</td>
<td>Alloc</td>
</tr>
<tr>
<td>Vic</td>
<td>Fmoc</td>
</tr>
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</table>

When a protecting group is not present, the functional group is the deprotected group (e.g. >IMH).

Preferred embodiments include salts of these compounds.
**Experimental Section**

**Materials Used in the Experimental Section**
The materials used in the experimental section are listed below.

**Chemicals:**
- Aqueous ammonia $\text{NH}_3$ (aq)
- Acetonitrile $\text{C}_2\text{H}_3\text{N}$
- $n$-Butanol $\text{C}_4\text{H}_{10}0$
- 2-Butanol $\text{C}_4\text{H}_{10}0$
- Isopropanol (isopropanol) $\text{C}_3\text{H}_60$
- Butyl acetate $\text{C}_9\text{H}_{12}0\text{O}_2$
- Ethanol, 99.9% $\text{C}_2\text{H}_60$
- Methanol $\text{CH}_40$
- Heptane $\text{C}_7\text{H}_{16}$
- Purified water $\text{H}_20$
- Ethyl acetate $\text{C}_3\text{H}_60\text{O}_2$
- Acetic acid $\text{C}_2\text{H}_4\text{G}_2$
- Ammonium acetate $\text{C}_2\text{H}_7\text{N}_0\text{O}_2$
- Acetyl imidazole $\text{C}_5\text{H}_6\text{N}_20$
- Triethylamine $\text{C}_6\text{H}_{15}N$
- N-Methylmorpholine $\text{C}_5\text{H}_1\text{NO}$
- N-Methylpyrrolidone $\text{C}_5\text{H}_7\text{NO}$
- N,N'-Dicyclohexylcarbodiimide $\text{C}_{13}\text{H}_{22}\text{N}_2$
- Dicyclohexylamine $\text{C}_{12}\text{H}_{23}N$
- N,N'-Diisopropylcarbodiimide $\text{C}_7\text{H}_{14}N_2$
- N,N'-Dimethylene diamine $\text{C}_4\text{H}_{12}N$
- N,N'-Dimethylformamide $\text{C}_3\text{H}_7\text{NO}$
- Dimethyl sulphoxide $\text{C}_4\text{H}_6\text{OS}$
- I-Hydroxybenzotriazole $\text{C}_6\text{H}_5\text{N}_30$
- p-Nitrophenol $\text{C}_6\text{H}_7\text{N}_0\text{O}_3$
- I-V-Hydroxysuccinimide $\text{C}_4\text{H}_5\text{N}_0\text{O}_3$
- Isobutyl chloroformate $\text{C}_8\text{H}_9\text{NO}_2$
- Sodium chloride $\text{NaCl}$
- Sodium hydroxide, aqueous NaOH (aq)
Hydrochloric acid, aqueous HCl (aq)
Phosphoric acid H₃PO₄
Sodium hydrogen sulphate NaHSO₄
Sodium hydrogen carbonate NaHCO₃
Methanesulphonic acid CH₄SO₃
Trifluoroacetic acid C₂H₃F₃O₂
Palladium on charcoal, 5% Pd-C
Hydrogen H₂
Toluene C₇H₈

Starting materials:

N-t-Butyloxycarbonyl-D-4-chlorophenylalanine Boc-D-4Cpa-OH C₁₄H₂₈N₀₄
N-t-Butyloxycarbonyl-D-2-naphthylalanine Boc-D-2Na l-OH C₁₈H₂₁N₀₄
D-3-Pyridylalanine hydrochloride H-D-3Pa l-OH x 2HCl C₈H₁₂Cl₂N₂O₂
N-a-t-Butyloxycarbonyl-N-4-(t-butylcarbamoyl)-D-4-aminophenylalanine Boc-D-4Aph(tBuCbm)-OH C₁₉H₂₉N₃O₅
N-a-t-Butyloxycarbonyl-N-4-(L-hydroxyprolyl)-D-4-aminophenylalanine Boc-4Aph(L-Hor)-OH C₁₉H₂₈N₄O₇
Leucine benzyl ester p-tosyliate H-Leu-OBzi x TOS C₁₀H₁₁N₀₅
N-Benzoyloxycarbonyl-O-t-butyloxycarbonyl-L-leucine Boc-Pro-OH C₁₃H₁₇NO₄
D-Alanine hydrochloride H-D-Ala-N₂H₂ x HCl C₅H₈ClN₀₂
N-a-Benzoyloxycarbonyl-N-ε-t-butyloxycarbonyl-N-dicyclohexylamine salt Z-Lys(Pr,Boc)-OH x DCHA C₃₄H₅₇N₅O₆
Example 1: Synthesis of Intermediate Ac(l-3)ONa: Ac-D-2Nal-D-4Cpa-D-3Pal-ONa\[7]\n
Activation of Boc-D-4Cpa-OH and isolation
Step 1 (Reaction step)

8oc-D-4Cpa-OH (299.75 g) is dissolved in iPrOH (3.53 kg), the mixture is stirred and HOSu (0.184 kg) and DIC (0.164 kg) are added, and stirred at 0°C for 1 hour. The precipitate is filtered off and washed with iPrOH. The solid is dried under reduced pressure to yield Boc-D-4Cpa-OSu\[1]\.

Activation of Boc-D-2Nal-OH and isolation
Step 2 (Reaction step)

Boc-D-2Nal-OH (315.38 g) is dissolved in iPrOH (5.35 kg) and IBC (157.07 g) and NMM (116.7 g) are added. A mixture of water (42 mL), iPrOH (1.1 kg) and HOSu (230.14 g) is added after cooling to -10°C together with additional NMM (10.1L), and the mixture stirred 30 min. Water (0.82 L) is added and the precipitate is filtered off, and washed with iPrOH and dried under reduced pressure to yield Boc-D-2Nal-OSu\[2]\.

Synthesis of Boc(2-3)OH: Boc-D-4Cpa-D-3Pal-OH
Step 3 (Reaction step)

H-D-3Pal-OH x 2 HCl (0.251 kg) and Boc-D-4Cpa-OSu \[1\] (0.397 kg) from step 1 are dissolved in DMF (3.33 L) and NMM (318.8 g) is added. The mixture is stirred at 20°C for 6 hours. Water (17 L) is added and pH is adjusted by adding HCl to pH 4.25. The precipitate is filtered off, and dispersed in water. The obtained slurry is then filtered and washed with water. The solid is dried under reduced pressure to yield Boc-D-4Cpa-D-3Pal-OH\[3]\.

Synthesis of Intermediate Ac(l-3)ONa: Ac-D-2Nal-D-4Cpa-D-3Pal-ONa\[7\] (Compound of formula Ilia)
Step 4 (Reaction step)

Boc-D-4Cpa-D-3Pal-OH \[3\] (447.93 g) from step 3 is dissolved in a mixture of AcOEt (3.4 L) and AcOH (675 mL), the mixture is cooled at 5°C where after MSA (672.77 g) is added. The reaction continues at 10°C for 2 hours and to the solution is added TEA (1214.28 g) to yield H-D-4Cpa-D-3Pai-OH\[4\].

Boc-D-2Nal-OSu \[2\] (412.44 g) from step 2 is added to H-D-4Cpa-D-3Pai-OH \[4\], stirred for 24 hours at 20°C. 25% aqueous NH\textsubscript{3} (0.154L) and n-butanol (4.5L) are added, and the mixture is stirred at 45°C for 1 hour.

The solution is washed with:
- Water
- Water at pH 9.5 (pH is adjusted while stirring with aq. NaOH)
- Water
AcOH (4.5 L) is added to the organic phase and the solution is concentrated to an oil under reduced pressure. The oil is re-dissolved in AcOH (4.5 L) and re-concentrated under reduced pressure to yield Boc-D-2Nal-D-4Cpa-D-3Pal-OH[5] as an oil.

Boc-D-2Nal-D-4Cpa-D-3Pal-OH [5] is dissolved in water (0.09 L) and AcOH (1.8 L). MSA (672.77 g) is added and the mixture is stirred at below 35°C for 2 hours. The solution is neutralised with TEA (779.16 g). The solution is concentrated under reduced pressure to an oil. The oil is re-dissolved in toluene (2.5 L) and re-concentrated under reduced pressure to an oil. The last step is repeated to yield H-D-2Nal-D-4Cpa-D-3Pal-OH[6].

H-D-2Nal-D-4Cpa-D-3Pal-OH [6] is dissolved in toluene (2.0 L) and a solution of acetyl imidazole (132.14 g) in toluene (0.25 L) is added. The solution is stirred at 20°C for 2 hours, and water (0.1 L) is added.

n-Butanol (4.5 L) is added and the organic mixture is washed at 35°C with:

- 5% aqueous NaCl
- Methanol and water at acidic pH 5.5 (pH is adjusted while stirring with aq. NaOH)
- Methanol and water at pH 11 (pH is adjusted while stirring with aqueous NaOH)
- Methanol and 10% aqueous NaCl

To the stirred organic phase from the extractions, heptane (15 L) is added at 20°C for 1 hour, and the resulting suspension is left with stirring at 20°C for 1 hour. The precipitate is isolated by filtration, and suspended in heptane (3.5 L). The suspension is filtered again. The last washing step with heptane and the filtration is repeated. The solid is then dried under reduced pressure to yield Ac-D-2Nal-D-4Cpa-D-3Pal-ONa[7].

**Specifications for key intermediates**


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<tr>
<th>Property</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Quality control</td>
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</tr>
<tr>
<td>Description</td>
<td>&quot;White to slightly yellow powder (visual inspection)&quot;</td>
</tr>
<tr>
<td>Identification (1)</td>
<td>&quot;587.2--0.4Da. (MS)&quot;</td>
</tr>
<tr>
<td>Identification (2)</td>
<td>&quot;2Naf 0.9-1.1, 4Cpa 0.9-1.1, 3Pal 0.9-1.1 (AAA)&quot;</td>
</tr>
<tr>
<td>Chiral purity</td>
<td>L-2Nal ≤1.3%, L-4Cpa ≤0.7%, L-3Pal &lt;2.0% (GC-MS)</td>
</tr>
<tr>
<td>Purity</td>
<td>&gt;90% (HPLC, Area ¾)</td>
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</table>

**Example 2: Synthesis of Intermediate Z(4-7)OH x DCHA: Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH x PCHAfi5I**

**Synthesis of Intermediate Boc(6-7)OBzl: Boc-D-4Aph(tBuCbm)-Leu-OBzl**

**Step 5 (Reaction step)**

Boc-D-4Aph(tBuCbm)-OH (379.45 g) is dissolved in NMP (0.76 L) and AcOEt (4.4 kg). After cooling at -4°C, 18C (150.2 g) and NMM (101.1 g) are added, and the solution is stirred at ~7°C for 0.5 hour to yield Boc-D-4Aph(tBuCbm)-OAc[8].

H-Leu-OBzl x TOS (491.88 g) is dissolved in NMP (1.5 L) and AcOEt (2.7 kg) is added, followed by NMM (126.4 g). This solution is subsequently transferred to Boc-D-4Aph(tBuCbm)-OAct [8], and stirred at -10°C for 1 hour. Then, water (0.5 L) is added. The reaction mixture is washed at 20°C with:

- Water at pH 8.5 (pH is adjusted white stirring with aq. NaOH)
- Water at pH 2.0 (pH is adjusted while stirring with aq. HCl)
- Water
The organic phase is concentrated under reduced pressure to an oil. The oil is re-dissolved in AcOEt (0.6 kg) and re-concentrated under reduced pressure to an oil. The remaining oil is dissolved in AcOEt (0.6 kg). Heptane (15.5 L) is added while stirring at 20°C. The precipitate is isolated by filtration, and washed with heptane and subsequently dried under reduced pressure at to yield Boc-D-4Aph(tBuCbm)-Leu-OBzl[9].

Synthesis of Boc-(5-7)-OBzl : Boc-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OBzl

Step 6 (Reaction step)

Boc-D-4Aph(tBuCbm)-Leu-OBzl [9] (582.7 g) from step 5 is dissolved in AcOEt (3.15 kg). MSA (481 g) is added, and stirred below 15°C for 5 hours, and TEA (406 g) is added. DMF (0.333 kg) is added followed by TEA (101 g) and NMM (51 g) to yield H-D-4Aph(tBuCbm)-Leu-OBzl[10].

Boc-4Aph(L-Hor)-OH (462.46 g) is dissolved in DMF (2.09 kg) and AcOEt (1.44 kg). iBC (150.24 g) and NMM (111.27 g) are added, and stirred at -10°C for 0.5 h to yield Boc-4Aph(L-Hor)-OActfllJ.

H-D-4Aph(tBuCbm)-Leu-OBzl [10] is added to Boc-4Aph(L-Hor)-OAct [11] and stirred at -10°C for 1.5 hours. Then, AcOEt (5.4 kg) and n-butanol (6.0 L) are added. The organic phase is washed at 20°C with:

- 5% aqueous NaHCO₃ at about pH 8 (pH is adjusted while stirring with aq NaHCO₃)
- 10% aqueous. NaCl at pH 2.5 (pH is adjusted while stirring with aq.H₃PO₄)

DMF (0.9 L) is added to the organic phase, which is then concentrated under reduced pressure to an oil. The solution is poured into water (14 L) while stirring. The precipitate is isolated on a filter, and washed with water. The solid is dried under reduced pressure to yield Boc-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OBzl[12].

Synthesis of Intermediate Z(4-7)OH x DCHA: Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH x DCHA (Compound of formula Va)

Step 7 (Reaction step)

Boc-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OBzl [12] (885.02 g) from step 6 is added to a mixture of MSA (961.1 g) and AcOEt (7.2 kg) and 2-butanol (2 L) is added, and the resulting mixture stirred at 0°C for 6 hours. MSA is then neutralised with TEA (909.0 g).

5% Pd/C (88.5 g) dispersed in 2-butanol (1 L) is added and the mixture is hydrogenated under pressure at 20°C for 3 hours. Then, the Pd/C is filtered off, and washed with 2-butanol to yield the solution containing H-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH [13].

Z-Ser(tBu)-OH (413.5 g) is dissolved in MeCN (2.5 L) and the solution is cooled to -5°C. HONp (195 g) is added followed by DCC (278.5 g), and the mixture stirred at 20°C for 24 hours. The mixture is then filtered, and washed with MeCN to yield Z-Ser(tBu)-ONp [14].

NMM (354.2 g), DMF (4.75 kg) and Z-Ser(tBu)-ONp [14] is added to the solution of H-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH [13] and the mixture is left with stirring at 20°C for 3 days.

The resulting mixture is washed with:

- 10% aqueous NaCl at pH 2.5 (pH is adjusted while stirring with aqueous HCl)
- Water at acidic pH (pH 2.5) (pH is adjusted while stirring with aqueous HCl)
7.5% aqueous NaHC03
• 5% aqueous NaCl at (pH 2.5) (pH is adjusted while stirring with aqueous HCl)
• 10% aqueous NaCl

To the final organic phase DCHA (181 g) is added and the organic phase is concentrated under reduced pressure to an oil. The oil is re-dissolved in iPrOH (3.14 kg) and re-concentrated under reduced pressure to an oil. The remaining oil is re-dissolved in iPrOH (3.14 kg) and while stirring the solution is poured into AcOEt (31.5 kg). Stirring is continued at 20°C for 1 hour until precipitation and the precipitate is then isolated by filtration, and washed with AcOEt. The solid is dried under reduced pressure at 30°C for 30 hours to yield Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH x DCHA[15]. Purity of intermediate Z(4-7)OH x DCHA[15] is >80% (HPLC).

Step 7 Intermediate Z(4-7)GH [15]

Quality control
Acceptance criteria
While to yellow powder (visual inspection)

Identification (I)
"972.5±0.4 Da (MS)"

Identification (2)
"Ser 0.9-1.1, 4Aph 1.8-2.2, Leu 0.9-1.1 (AAA)"

Clinral purity
D-Ser <2.0%, D-4Aph 47-53%, D-Leu <0.7% (GC-MS)

Purity (1)
[4Aph 5(Hydantoinacetyl)] Z(4-7)OH DCHA <0.5% (HPLC, Area %)

Purity (2)
>80% (HPLC, Area %)

Example 3: Synthesis of Intermediate \( H(8-10)NH_2 \cdot H-Lvs(iPr, Bod-Pro-P-Aia) \cdot NH_2 \) [21]

Synthesis of Boc(9-10)NH_2: Boc-Pro-D-Ala-NH_2

Step 8 (Reaction step)

Boc-Pro-OH (226.02 g) is dissolved in iPrOH (1.73 kg). The reaction mixture is cooled to -5°C. IBC (143.4 g) and NMM (106.2 g) are added and the mixture is stirred at 5°C for 0.5 hour to yield Boc-Pro-OAc[16].

H-D-Ala-NH_2 x HCl (124.57 g) is suspended in a mixture of iPrOH (1.57 kg) and NMM (106.2 g). The suspension is added to Boc-Pro-OAc [16]. The reaction mixture is left with stirring at 10°C for 3 hours. Then DMEGA (10.6 ml) is added. The mixture is filtered, and the filtrate is concentrated under reduced pressure to an oil. The oil is re-dissolved and re-concentrated with AcOEt (1.125 kg).

The residual oil is dissolved in a mixture of AcOEt (1.8 kg) and n-butanol (0.6 L). The organic phase is washed with:
• 15% aqueous NaCl at pH 2.5 (pH is adjusted while stirring with aqueous HCl)
• 15% aqueous NaCl at pH 9.5 (pH is adjusted while stirring with aqueous NaOH)

The organic phase is concentrated under reduced pressure, re-dissolved in AcOEt (1.08 kg) and re-concentrated to an oil.

A mixture of AcOEt (0.33 kg) and heptane (0.75 L) is added at 20°C and stirred for 16 hours. The resulting precipitate is filtered and washed with a mixture of AcOEt and heptane on the filter. The solid is then dried under reduced pressure to yield Boc-Pro-D-Ala-NH_2[17].
Synthesis of Intermediate H(8-10)NH₂: H-Lvs(iPr,Boc)-Pro-D-Ala-NH₂ (Compound of formula Via)

Step 9 (Reaction step)

Boc-Pro-D-Ala-NH₂  [17] (313.89 g) from Step 8 is dissolved in a mixture of MSA (528.61 g) and iPrOH (0.785 kg) and the solution is stirred at 45°C for 1 hour. The mixture then is neutralised with TEA (607.14 g) to yield H-Pro-D-Ala-NH₂[18], Z-Lys(iPr,Boc)-OH x DCHA (603.83 g) is suspended in AcOEt (1.17 kg) and washed with:
- 12 % aqueous NaHSO₄
- Water
- 15 % aqueous NaOH

The organic phase of Z-Lys(iPr,Boc)-OH [19] from the extractions is added to H-Pro-D-Ala-NH₂ [18]. HOBT (183.79 g) and DCC (227.0 g) dissolved in AcOEt (0.135 kg) are added, and the mixture stirred at 20°C for 0.5 hours. Then, water (0.2 L) is added. The mixture is filtered and washed with AcOEt. The combined filtrates are concentrated under reduced pressure to an oil. The oil is dissolved in AcOEt (0.9 kg), filtered and the solution is washed with:
- Water at pH 2.5 (pH is adjusted while stirring with aqueous HCl)
- Water at pH 9 (pH is adjusted while stirring with aqueous NaOH)
- 10 % aqueous NaCl at pH 7 (pH is adjusted while stirring with aqueous HCl or aqueous NaOH)

The organic phase is concentrated under reduced pressure to yield Z-Lys(iPr,Boc)-Pro-D-Ala-NH₂[20].

Z-Lys(iPr,Boc)-Pro-D-Ala-NH₂[20] is dissolved in ethanol (0.04 kg) and water (0.5 L), and 5 % Pd/C (50 g) is added. The slurry is acidified to pH 2.5 by addition of 6 M HCl and hydrogenated at 20°C. After completed reaction the catalyst is removed by filtration and pH is raised to pH 7.0 by addition of 32 % NaOH. The ethanol is subsequently removed by evaporation under reduced pressure. n-Butanol (1 L) is added to the resulting aqueous phase and the pH is adjusted to alkaline pH 9 with aqueous NaOH and the extraction starts. This step is repeated. The combined organic phases are concentrated under reduced pressure to an oil.

The oil is dissolved in AcOBu (0.5 L), concentrated under reduced pressure at 20°C and redissolved in AcOBu (0.5 L). Then, heptane (2 L) is added at 50°C for 1 hour. The suspension is left with stirring at 0°C for 16 hours. The precipitate is isolated by filtration and washed with heptane. Finally, the solid is dried under reduced pressure at to yield H-Lys(iPr,Boc)-Pro-D-Ala-NH₂[21]. Purity of intermediate H(8-10)NH₂[21] is >95% (HPLC).

Step 9 intermediate H(8-10)NH₂ [21]

<table>
<thead>
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<th>Quality control</th>
<th>Acceptance criteria</th>
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<tbody>
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<td>Description</td>
<td>White to slightly yellow powder (visual inspection)</td>
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<td>Identification (1)</td>
<td>456.3±0.4Da (MS)</td>
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<tr>
<td>Identification (2)</td>
<td>&quot;Lys(iPr) 0.9-1.1, Pro 0.9-1.1, Ala 0.9-1.1 (AAA)&quot;</td>
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<tr>
<td>Chiral purity</td>
<td>D-Lys(iPr) &lt;0.3%, D-Pro &lt;0.3%, L-Ala &lt;0.5% (GC-MS)</td>
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<tr>
<td>Purity</td>
<td>&gt;95% (HPLC, Area %)</td>
</tr>
</tbody>
</table>
Example 4: Segment Condensations to Final Intermediate (Compound of Formula II)

Intermediate Z(4-10)NH₂: Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂[22] (Compound of formula IVg)
Step 10 (Reaction step)

Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH x DCHA [15] (1153.41 g) from Step 7 is dissolved in DMF (2.1 kg). Then, HOBt (153.2 g) is added together with AcOEt (6.9 kg) and H-Lys(iPr,Boc)-Pro-D-Ala-NH₂[21] (569.5 g) from step 9. When all solids are dissolved MSA (96.1 g) is added. The solution is cooled below 5°C and DCC (309.5 g) dissolved in AcOEt (0.810 kg) is added. The temperature is raised to 20°C and the reaction continues for 24 hours. Conversion of Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH x DCHA [15] is >96% (HPLC). AcOEt (4.95 kg) and water (5.5 L) are added, and the mixture is stirred, and filtered. While stirring, 7.5% NaHC0₃ (aq) (35 L) is added to the filtrate. The phases are separated and the organic layer is further washed with:
- 7.5% NaHC0₃
- Water at pH 3 (pH is adjusted while stirring with aqueous HCl)
- Water

The final organic phase is concentrated under reduced pressure to an oil. The oil is re-concentrated with EtOH (0.405 kg) and subsequently with AcOEt (0.45 kg). The remaining oil is dissolved in EtOH (0.405 kg), and AcOEt (0.45 kg) and AcOBu (4.6 L) are added. The solution is added to heptane (27.6 L) at 20°C for 1 hour. Then, the precipitate is filtered, washed with heptane. The solid is dried under reduced pressure at maximum to yield Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂[22]. Purity of Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂[22] is >70% (HPLC).

Final Intermediate Ac(l-10)NH₂: Ac-D-2Nai-D-4Cpa-D-3Pal-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂[24]
Step 11 (Reaction step)

Z-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂[22] (1409.67 g) from Step 10 is added to a mixture of EtOH (10.98 kg) and water (3.2 L) and stirred until the solution is homogenous. 5% Pd/C (211 g) is added. The mixture is hydrogenated at 20°C with pH-controli at pH 2.5 with aqueous HCl.

The catalyst is removed by filtration and the pH is adjusted to pH 3.8 with aqueous NaOH. The filtrate is concentrated under reduced pressure to an oil. EtOH (4.7 kg) is added to the oil and re-concentrated. Then, AcOEt (5.4 kg) is added to the oil and re-concentrated and this process is repeated again to yield H-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-leu-
Lys(iPr,Boc)-Pro-D-Ala-NH₂ [23].

H-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂ [23] is dispersed in AcOEt (1.125 g), then HOBT (153.16 g) is added and the mixture is cooled to 0°C. Ac-D-2Nal-D-4Cpa-D-3Pal-ONa [7] (609.05 g) from Step 4 is dissolved in DMSO (2.5 L), this solution is mixed with the slurry containing H-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂ [23] and DCC (309.5 g) dissolved in AcOEt (0.45 kg) is added. The mixture is stirred at 5°C for 24 hours. Conversion of [23] is >96% (HPLC).

Water (150 mL) and DMSO (0.5 L) are added and the stirring is continued at 20°C for more than 3 hours. The precipitate is filtered, and washed with a mixture of AcOEt and DMSO. The filtrates are combined, and n-butanol (17 L) is added. The organic solution is washed with:

- Water at pH 2.5 (pH is adjusted while stirring with aqueous HCl)
- 7% NaHCO₃ (aq)
- 10% aqueous NaCl (pH in the mixture is neutralised to pH 7.0, if necessary, while stirring with aqueous HCl)

DMF (4.75 kg) is added and the organic phase is concentrated under reduced pressure to an oil. The oil is slowly added to water (50 L) at 20°C for 1 hour under vigorous stirring. The precipitate is isolated on a filter, and washed twice with water. The solid is subsequently dried under reduced pressure to yield Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂ [24] [Final intermediate]. Purity of [24] is >70% (HPLC).

Example 5: Deprotection of Final Intermediate Ac(l-10)NH₂ to Crude Degarelix[25]

Step 12 (Reaction step)

Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(tBu)-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH₂ [24] (Compound of formula lib)(1844.59 g) from step 11 is dissolved in TFA (28.3 kg) at 20°C. The solution is stirred at 20°C (removal of 3 protection groups) for 24 hours. Conversion of [24] is >99% (HPLC).

The reaction mixture is then mixed with a cold solution (below 10°C) of water (74 L), AcONH₄ (19.1 kg), AcOH (18.4 L) and EtOH (14.52 kg). During mixing of the two solutions the temperature is kept below 25°C. The pH of the final solution is adjusted to pH 3 with TFA or AcONH₄, if necessary, to yield the solution of Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(L-Hor)-D-4Aph(Cbm)-Leu-Lys(iPr).Pro-D-Ala-NH₂[25][Crude Degarelix].

Step 13 (Purification and lyophilisation)

The solution of crude degarelix is pumped through a reversed phase column. Degarelix is eluted from the column with a gradient of EtOH/ 0.1% TFA in water. Fractions with a purity ≥95% are repurified on a reversed phase column using a gradient of EtOH/ 1% AcOH in water. Fractions of high purity are lyophilised.
1. A liquid-phase process for preparing Degareix having the formula Ac-AA1-AA10-NH2 or a pharmaceutically acceptable salt or solvate thereof, wherein AA1 is D-2Nal, AA2 is D-4Cpa, AA3 is D-3Pal, AA4 is Ser, AA5 is 4Aph(L-Hor), AA6 is D-Aph(Cbm), AA7 is Leu, AA8 is Lys(iPr), AA9 is Pro and AA10 is D-Ala, comprising the step of subjecting a Degareix precursor according to formula Ii, or a salt or solvate thereof to a treatment with a cleaving agent:

Wherein

P1 is an amino protecting groups or preferably acetyl;

P4 is hydrogen or a hydroxyl protecting group, preferably a hydroxy! protecting group;

P6 is hydrogen or an amino protecting groups; preferably an amino protecting groups; and

P8 is an amino protecting group.

2. The process of claim 1, wherein P1 is acetyl; P4 is a hydroxy! protecting group, P6 is hydrogen or an amino protecting groups; and P8 is an amino protecting group.

3. The process of claim 2, wherein P1 is acetyl; P4 is a tBu, P6 is hydrogen or tBu, and P8 is Boc.

4. A process for the liquid-phase manufacture of a decapeptide represented by formula (II)
wherein \( \text{AA}_1 \) is D-2Nal, \( \text{AA}_2 \) is D-4Cpa, \( \text{AA}_3 \) is D-3Pal, \( \text{AA}_4 \) is Ser, \( \text{AA}_5 \) is 4Aph(L-Hor), \( \text{AA}_6 \) is D-Aph(Cbm), \( \text{AA}_7 \) is Leu, \( \text{AA}_8 \) is Lys(iPr), \( \text{AA}_9 \) is Pro, \( \text{AA}_{10} \) is D-Ala;

\( P_1 \) is an amino protecting group or acetyl;

\( P_4 \) is hydrogen or a hydroxy! protecting group, preferably a hydroxy! protecting group;

\( P_6 \) is hydrogen or an amino protecting groups; preferably an amino protecting groups; and

\( P_8 \) is an amino protecting group,

or a pharmaceutically acceptable salt or solvate thereof, comprising the step of coupling a first polypeptide represented by formula (III)

\[
(P_i)\text{AA}_i - \text{AA}_3 (P_4) \quad (\text{III})
\]

or a salt thereof, with a second polypeptide represented by formula (IV)

\[
\text{AA}_4(P_4) - \text{AA}_5(P_6) - \text{AA}_7\text{AA}_8(P_8) - \text{AA}_{10} - \text{NH}_2 (\text{IV})
\]

or a salt thereof, in a liquid reagent medium in the presence of a peptide coupling reagent.

5. The process of claim 4, wherein \( P_1 \) is acetyl; \( P_4 \) is a hydroxy! protecting group, \( P_6 \) is hydrogen or an amino protecting groups; and \( P_8 \) is an amino protecting group.

6. The process according to claim 4 or 5, followed by a process in accordance with any one of claims 1 to 3.

7. A process for preparing a polypeptide represented by formula (IV)

\[
(P_4)\text{AA}_4 - \text{AA}_5(P_6) - \text{AA}_7\text{AA}_8(P_8) - \text{AA}_{10} - \text{NH}_2 (\text{IV})
\]

wherein

\( P_4 \) is hydrogen or a hydroxy! protecting group, preferably a hydroxy! protecting group;
P₆ is hydrogen or an amino protecting group; preferably an amino protecting group; and
P₈ is an amino protecting group,
by coupling a polypeptide represented by form ulae (V), or a salt or solvate thereof, with a
polypeptide represented by form ula (VI), or a salt or solvate thereof,

\[(P₈) \text{AA}_4(P₄)\text{-AA }₅\text{-AA }₆(P₆)\text{-AA }₇(V)\],
\[\text{AA}_8(P₈)\text{-AA}_9\text{-AA}_{10}\text{-NH}_2 \quad (VI)\]

and then removing the deprotecting group Pₙ, wherein \(n\text{AA}_4\) to \(\text{AA}_1\), \(P₄\), \(P₆\), and \(P₇\) are the
same as in claim 1, and \(Pₙ\) is a protecting group that can be removed by hydrogenation.

8. The process according to claim 7 wherein \(Pₖ\) is benzylxycoxy group which is removed by hydrogenating the compound \{(Pₙ)\text{AA}_4(P₄)\text{-AA }₅\text{-AA }₆(P₆)\text{-AA }₇\}, wherein \(n\text{AA}_4\) to \(\text{AA}_7\), \(P₄\), and \(P₆\), are the same as in claim 1, and \(Pₙ\) is a protecting group that can be
removed by hydrogenation, comprising the following steps:

(a) providing \((\text{PC})\text{-AA }₅\text{-AA }₆(P₆)\text{-AA }₇\), wherein \(P₆\) has the same meaning as above, \((Pₙ\text{AA}_2)\)
is an IM-terminal amino protecting group or hydrogen, and \((P₈)\) is a C-terminal carboxyl protecting group that can be cleaved by hydrogenation;
(b) removing the amino protecting group \((Pₙ\text{AA}_2)\), if present;
(c) hydrogenating \(\text{H}-\text{AA }₅\text{-AA }₆(P₆)\text{-AA }₇(P₈)\) to obtain \(\text{H}-\text{AA }₅\text{-AA }₆(P₆)\text{-AA }₇\); and
(d) reacting \(\text{H}-\text{AA }₅\text{-AA }₆(P₆)\text{-AA }₇\) with an activated ester of \((Pₙ)\text{AA}_4(P₄)\) to provide
\((Pₙ)\text{AA}_4(P₄)\text{-AA }₅\text{-AA }₆(P₆)\text{-AA }₇\), wherein \(AA₄\) has the same meaning as above, \((P₄)\) is a
hydroxyl protecting group or hydrogen, and \(Pₙ\) is a protecting group that can preferably be eliminated by hydrogenation.
11. The process of claim 10, wherein the degarelix solution is obtained by the process of any one of claims 1 to 3.

12. The process of one or more of the preceding claims, wherein the pH is kept below 9, preferably below 8.

13. Polypeptide compounds represented by the following formulae:

\[(P_1)AA_1\cdot AA_2\cdot AA_3,\]
\[AA_4(P_4)\cdot AA_5\cdot AA_6(P_6)\cdot AA_7\cdot AA_8(P_8)\cdot AA_9\cdot AA_{10}\cdot NH_2,\]
\[(P_N)AA_4(P_4)\cdot AA_5\cdot AA_6(P_6)\cdot AA_7\cdot AA_{10}\cdot NH_2\]
\[(P_1)AA_1\cdot AA_2\cdot AA_3\cdot AA_{10}\cdot NH_2,\]
\[AA_5(P_5)\cdot AA_9\cdot AA_{10}\cdot NH_2,\]
\[or salts or solvates,\]

wherein AA_1 is D-2Nal, AA_2 is D-4Cpa, AA_3 is D-3Pal, AA_4 is Ser, AA_5 is 4Aph(L-Hor), AA_6 is D-Aph(Cbm), AA_7 is Leu, AA_8 is Lys(tPr), AA_9 is Pro, AA_{10} is D-Ala;
P_1 is an amino protecting groups or acetyl;
P_4 is hydrogen or a hydroxyl protecting group, preferably a hydroxyl protecting group;
P_6 is hydrogen or an amino protecting groups; preferably an amino protecting groups; and
P_8 is an amino protecting group, and P_N is a protecting group.
Figure 1

Step 1 (Reaction step)

Boc-D-4Cpa-OH

Activation
1) iPrOH
2) HOSu
   DIC

Precipitation

Filtration/Drying
1) iPrOH

Boc-D-4Cpa-OSu [1]

Step 2 (Reaction step)

Boc-D-2Nal-OH

Activation
1) iPrOH
2) IBC
   NMM
3) H₂O
   iPrOH
   HOSu
4) NMM

Precipitation
1) H₂O

Filtration/drying
1) iPrOH

Boc-D-2Nal-OSu [2]
Figure 2
Step 3 (Reaction step)

H-D-3Pal-OH x 2 HCl

\[ \text{Boc-D-4Cpa-OSu [1]} \]

Reaction 1) DMSO  
2) NMM
Precipitation 1) H₂O  
2) HCl
Filtration/drying 1) H₂O

\[ \text{Boc-D-4Cpa-D-3Pal-OH [3]} \]
Figure 3

Step 4 (Reaction step)

\[ \text{Boc-D-4Cpa-D-3Pal-OH} \text{[3]} \]

**Deprotection**
1) AcOEt
   AcOH
   MSA
2) TEA

\[ \text{H-D-4Cpa-D-3Pal-OH} \text{[4]} \]

\[ \text{Boc-D-2Nal-OSu} \text{[2]} \]

**Reaction**
1) After complete coupling,
   add NH₃ and
   n-BuOH

**Extraction**
1) H₂O
2) H₂O / NaOH
3) H₂O

**Concentration**
1) AcOH

\[ \text{Boc-D-2Nal-D-4Cpa-D-3Pal-OH} \text{[5]} \]

**Dissolution**
1) H₂O
   AcOH

**Deprotection**
1) MSA

**Neutralisation**
1) TEA

**Concentration**
1) Tol

\[ \text{H-D-2Nal-D-4Cpa-D-3Pal-OH} \text{[6]} \]

**Dissolution**
1) Tol

**Reaction**
1) NAI
   Tol
2) H₂O

**Extraction**
1) n-BuOH
2) Aq. NaCl
3) MeOH/H₂O acidic
4) MeOH/H₂O alkaline
5) MeOH/H₂O/NaCl

**Precipitation**
1) Heptane

**Filtration/Drying**
1) Heptane

\[ \text{Ac-D-2Nal-D-4Cpa-D-3Pal-ONa} \text{[7]} \]
Figure 4

Step 5 (Reaction step)

Boc-D-4Aph(tBuCbm)-OH

Dissolution

1) NMP
   AcOEt

Activation

1) IBC
   NMM

Boc-D-4Aph(tBuCbm)-OAct [8]

H-Leu-OBzl x TOS

Dissolution

1) NMP
   AcOEt

Reaction

2) NMM

Extraction

1) H₂O alkaline
2) H₂O acidic
3) H₂O

Concentration

1) AcOEt

Precipitation

1) AcOEt/Heptane

Filtration/Drying

1) Heptane

Boc-D-4Aph(tBuCbm)-Leu-OBzl [9]
Figure 5

Step 6 (Reaction step)

<table>
<thead>
<tr>
<th>Boc-4Aph(L-Hor)-OH</th>
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<tbody>
<tr>
<td><strong>Dissolution</strong></td>
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</tr>
<tr>
<td><strong>Activation</strong></td>
</tr>
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**Boc-4Aph(L-Hor)-OAct [11]**

<table>
<thead>
<tr>
<th>Boc-d-4Aph(tBuCbm)-Leu-OBzl [9]</th>
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<tbody>
<tr>
<td><strong>Dissolution</strong></td>
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<td><strong>Deprotection</strong></td>
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</tr>
</tbody>
</table>

**H-d-4Aph(tBuCbm)-Leu-OBzl [10]**

| **Reaction**                    | 1) AcOEt |
|                                 | 2) n-BuOH |
| **Extraction**                  | 1) H₂O/NaHCO₃ alkaline |
|                                 | 2) H₂O/NaCl acidic |
| **Concentration**               | 1) DMF |
| **Precipitation**               | 1) H₂O |
| **Filtration/Drying**           | 1) H₂O |

| Boc-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OBzl [12] |
**Figure 6**

**Step 7 (Reaction step)**

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<thead>
<tr>
<th>Process</th>
<th>Steps</th>
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<tr>
<td>Deprotection</td>
<td>1) MSA</td>
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<td>2) 2-BuOH</td>
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<tr>
<td>Neutralisation</td>
<td>1) TEA</td>
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<td>Hydrogenation</td>
<td>1) Pd/C</td>
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<td>2-BuOH</td>
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<td>Filtration</td>
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<td><strong>H-4Aph(L-Hor)-D-4Aph(tBuCbm)-Leu-OH</strong> [13]</td>
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<td><strong>Z-Ser(tBu)-OH</strong></td>
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Figure 7

Step 8 (Reaction step)

Boc-Pro-OH

Dissolution
Activation

1) iPrOH
1) IBC
NMM

Boc-Pro-OAct [16]

Suspension

H-d-Ala-NH2 x HCl

1) iPrOH
NMM

Reaction

1) After completion of coupling, add DMEDA

Filtration
Concentration
Extraction

1) AcOEt
1) AcOEt/n-BuOH
2) H2O/NaCl acidic
3) H2O/NaCl alkaline

Concentration
Precipitation
Filtration/Drying

1) AcOEt
1) AcOEt/heptane
1) AcOEt/heptane

Boc-Pro-d-Ala-NH2 [17]
Figure 8

Step 9 (Reaction step)

Boc-Pro-d-Ala-NH$_2$ [17]

- Deprotection
  - 1) MSA
  - iPrOH

- Neutralization
  - 1) TEA

H-Pro-d-Ala-NH$_2$ [18]

Z-Lys(iPr,Boc)-OH x DCHA

- Suspension
  - 1) AcOEt
  - 1) H$_2$O/
    - NaHSO$_4$
  - 2) H$_2$O
  - 3) H$_2$O/
    - NaCl

Z-Lys(iPr,Boc)-OH [19]

- Reaction
  - 1) HOBT
  - DCC
  - AcOEt
  - 2) After complete coupling, add H$_2$O

- Filtration
  - 1) AcOEt

- Concentration

- Filtration/Extraction
  - 1) AcOEt
  - 2) H$_2$O acidic
  - 3) H$_2$O alkaline
  - 4) H$_2$O/NaCl neutral

Z-Lys(iPr,Boc)-Pro-d-Ala-NH$_2$ [20]
Z-Lys(iPr,Boc)-Pro-δ-Ala-NH₂ [20]

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H-Lys(iPr,Boc)-Pro-δ-Ala-NH₂ [21]
Figure 10

Step 10 (Reaction step)

Z(4-7)OH x DCHA [15]

Dissolution

1) DMF
2) HOBT
   AcOEt

   H-Lys(iPr,Boc)-Pro-d-Ala-NH₂ [21]

Reaction

1) MSA
2) DCC/AcOEt

Filtration

1) AcOEt/H₂O

pH-adjustment

1) H₂O/ NaHCO₃

Extraction

1) H₂O alkaline
2) H₂O acidic
3) H₂O

Concentration

1) EtOH
2) AcOEt

Precipitation

1) EtOH
   AcOEt
2) AcOBu
3) Heptane

Filtration/Drying

1) Heptane

Z-Ser(tBu)-4Aph(t-Hor)-d-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-d-Ala-NH₂ [22]
**Figure 11**

**Step 11 (Reaction step)**

Z(4-10)NH₂ [22]

| Suspension | 1) EtOH/H₂O  
| Pd/C |
| Hydrogenation | 1) H₂/HCl  
| Filtration/pH adjustment | 1) H₂O/NaOH  
| Concentration | 1) EtOH  
| AcOEt |

H-Sert(Bu)-4Aph(L-Hor)-d-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-d-Ala-NH₂ [23]

| Suspension | 1) AcOEt  
| HOBt |
| Dissolution | 1) DMSO |

Ac-d-2Nal-d-4Cpa-d-3Pal-ONa [7]

| Reaction | 1) DCC/AcOEt  
| H₂O/DMSO |
| Filtration | 1) AcOEt/DMSO  
| n-BuOH |
| Extraction | 1) H₂O acidic  
| H₂O alkaline  
| H₂O/NaCl |
| Concentration | 1) DMF |
| Precipitation | 1) H₂O |
| Filtration/Drying | 1) H₂O |

Ac-d-2Nal-d-4Cpa-d-3Pal-Sert(Bu)-4Aph(L-Hor)-d-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-d-Ala-NH₂ [24]
### Step 12 (Reaction step)

\[ \text{Ac-D-2Nal-D-4Cpa-D-3Pal-Ser(tBu)-4Aph(l-Hor)-D-4Aph(tBuCbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH}_2 \ [24] \]

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### Step 13 (Purification and Lyophilisation)

\[ \text{Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH}_2 \ [24] \]

1) Concentration on reverse phase column and purification
2) Repurification
3) Lyophilisation

\[ \text{Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(l-Hor)-D-4Aph(Cbm)-Leu-Lys(iPr,Boc)-Pro-D-Ala-NH}_2 \ [25] \]
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K1/02 C07K5/08 C07K7/06

ADD.

According to International Patent Classification (IPC) and 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 database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS, COMPENDEX, EMBASE, INSPEC, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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  * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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Date of the actual completion of the international search: 20 December 2011

Date of mailing of the international search report: 30/12/2011

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Fax. (+31-70) 340-3016

Authorized officer:
Thumb, Werner

Form PCT/ISA/210 (second sheet) (April 2005)
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