Claims

1. A method of manufacture of degarelix, Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-4Aph(Hor)-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein degarelix comprises 0.3 % by weight or less, in particular 0.1 % by weight or less, most particularly 0.01 % by weight or less, of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine, the method comprising step-wise synthesis on a solid support comprising an amino group linked to the support, wherein a step comprises providing a solution of an amino acid or peptide of which the α-amino group is protected by Fmoc; contacting the support with the solution in the presence of reagent for forming a peptide bond between a carboxyl group of the dissolved amino acid or peptide and the amino group linked to the support for a time sufficient to form said peptide bond; removing Fmoc by contacting the support with an organic base selected from piperidine and C-alkyl substituted piperidine, in particular 2- alkylpiperidine, 3-alkylpiperidine, 2,4-dialkylpiperidine, 2,5-dialkylpiperidine, 2,6-dialkylpiperidine, wherein alkyl is branched or straight chain from 1 to 6 carbon, in particular methyl or ethyl, most particularly methyl, in an organic solvent.

2. The method of claim 1, wherein the organic base is piperidine.

3. The method of claim 1, wherein the organic base is C-alkyl substituted piperidine.

4. The method of claim 3, wherein the C-alkyl substituted piperidine is 2- alkylpiperidine, 3-alkylpiperidine, 2,4-dialkylpiperidine, 2,5-dialkylpiperidine or 2,6-dialkylpiperidine, wherein alkyl is branched or straight chain from 1 to 6 carbon.

5. The method of claim 4, wherein the alkyl is methyl or ethyl.

6. The method of claim 5, wherein the alkyl is methyl.

7. The method of any one of claims 1 to 6, wherein the organic solvent is dimethyl formamide.

8. The method of any one of claims 1 to 7, wherein the reagent for forming a peptide bond comprises N,N'-diisopropylcarbodiimide.

9. The method of any one of claims 1 to 8, wherein the amino group linked to the support is an α-amino group of a fragment of degarelix linked to the support.

10. The method of any one of claims 1 to 9, wherein the peptide protected by Fmoc is a fragment of degarelix.

11. The method of any one of claims 1 to 10, wherein the support is selected from Rink amide AM resin and Rink amide MBHA resin.

12. The method of any one of claims 1 to 11, wherein degarelix is released from the support by acid treatment.

13. The method of any one of claims 1 to 12, wherein the degarelix comprises 0.1 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

14. The method of any one of claims 1 to 12, wherein the degarelix comprises 0.01 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

15. Degarelix prepared by the method of any one of claims 1 to 12, containing 0.3 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

16. Degarelix prepared by the method of any one of claims 1 to 12, containing 0.1 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

17. Degarelix prepared by the method of any one of claims 1 to 12, containing 0.01 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

18. Use of Fmoc in solid phase synthesis for preparing degarelix containing 0.3 % by weight or less, more preferred 0.1 % by weight or less, most preferred 0.01 % by weight or less, of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

19. The use of claim 18, wherein the degarelix contains 0.1 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

20. The use of claim 18, wherein the degarelix contains 0.01 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

Claims

1. A method of manufacture of degarelix, Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-4Aph(Hor)-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein degarelix comprises 0.3 % by weight or less, in particular 0.1 % by weight or less, most particularly 0.01 % by weight or less, of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine, the method comprising step-wise synthesis on a solid support comprising an amino group linked to the support, wherein a step comprises providing a solution of an amino acid or peptide of which the α-amino group is protected by Fmoc; contacting the support with the solution in the presence of reagent for forming a peptide bond between a carboxyl group of the dissolved amino acid or peptide and the amino group linked to the support for a time sufficient to form said peptide bond; removing Fmoc by contacting the support with an organic base selected from piperidine and C-alkyl substituted piperidine, in particular 2- alkylpiperidine, 3-alkylpiperidine, 2,4-dialkylpiperidine, 2,5-dialkylpiperidine, 2,6-dialkylpiperidine, wherein alkyl is branched or straight chain from 1 to 6 carbon, in particular methyl or ethyl, most particularly methyl, in an organic solvent.

2. The method of claim 1, wherein the organic base is piperidine.

3. The method of claim 1, wherein the organic base is C-alkyl substituted piperidine.

4. The method of claim 3, wherein the C-alkyl substituted piperidine is 2- alkylpiperidine, 3-alkylpiperidine, 2,4-dialkylpiperidine, 2,5-dialkylpiperidine or 2,6-dialkylpiperidine, wherein alkyl is branched or straight chain from 1 to 6 carbon.

5. The method of claim 4, wherein the alkyl is methyl or ethyl.

6. The method of claim 5, wherein the alkyl is methyl.

7. The method of any one of claims 1 to 6, wherein the organic solvent is dimethyl formamide.

8. The method of any one of claims 1 to 7, wherein the reagent for forming a peptide bond comprises N,N'-diisopropylcarbodiimide.

9. The method of any one of claims 1 to 8, wherein the amino group linked to the support is an α-amino group of a fragment of degarelix linked to the support.

10. The method of any one of claims 1 to 9, wherein the peptide protected by Fmoc is a fragment of degarelix.

11. The method of any one of claims 1 to 10, wherein the support is selected from Rink amide AM resin and Rink amide MBHA resin.

12. The method of any one of claims 1 to 11, wherein degarelix is released from the support by acid treatment.

13. The method of any one of claims 1 to 12, wherein the degarelix comprises 0.1 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.

14. The method of any one of claims 1 to 12, wherein the degarelix comprises 0.01 % by weight or less of Ac-D-2Nal-D-Phe(4Cl)-D-3Pal-Ser-X-D-4Aph(Cbm)-Leu-ILys-Pro-D-Ala-NH_2, wherein X is 4-([2-(5-hydantoyl)]acetylamino)-phenylalanine.