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(54) Title: SUBSTANTIALLY PURE LANREOTIDE OR ITS SALT & PROCESS THEREOF

(57) Abstract: The present invention provides substantially pure Lanreotide or its salt and preparation thereof. In another aspect present invention provides a method of preparing Lanreotide dmg product which involves measuring D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt. The present invention also provides a method of assaying purity of a sample of Lanreotide or its salt or a pharmaceutical dosage form comprising Lanreotide or its salt.

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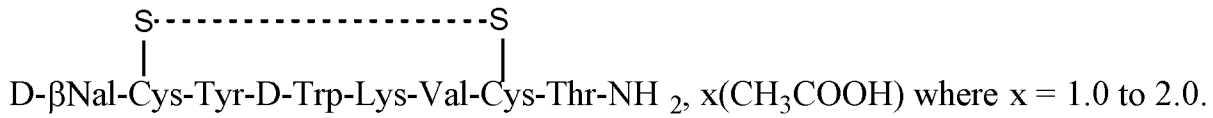
# SUBSTANTIALLY PURE LANREOTIDE OR ITS SALT & PROCESS THEREOF

## FIELD OF THE INVENTION

The present application relates to substantially pure Lanreotide or its salt and preparation thereof.

## BACKGROUND OF THE INVENTION AND DISCLOSURE OF PRIOR ART

Lanreotide acetate is a synthetic cyclic octapeptide analog of the natural hormone, somatostatin. Lanreotide acetate is chemically known as [cyclo S-S]-3-(2-naphthyl)-D-alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinyl-L-threoninamide, acetate salt. Its molecular weight is 1096.34 (base) and its amino acid sequence is:




Lanreotide acetate, developed by Ipsen with proprietary name SOMATULINE® DEPOT (Lanreotide) was first approved by USFDA on 30 August 2007. SOMATULINE DEPOT (Lanreotide) Injection 60 mg/0.2 mL, 90 mg/0.3 mL, and 120 mg/0.5 mL is a prolonged-release formulation for deep subcutaneous injection. The SOMATULINE DEPOT in the prefilled syringe is a white to pale yellow, semi-solid formulation. The mechanism of action of lanreotide is believed to be similar to that of natural somatostatin. SOMATULINE DEPOT is indicated for: a) the long-term treatment of acromegalic patients who have had an inadequate response to or cannot be treated with surgery and/or radiotherapy; b) the treatment of adult patients with unresectable, well-or moderately-differentiated, locally advanced or metastatic gastroenteropancreatic neuroendocrine tumors (GEP-NETs) to improve progression-free survival; & c) the treatment of adults with carcinoid syndrome.

US4853371, first reported Lanreotide and its salts, wherein Lanreotide was prepared by solid phase synthesis using BOC protocol on Benzhydrylamine polystyrene resin and iodine mediated cyclization.

EP0389180B1 & US5073541, disclose preparation of Lanreotide by solid phase peptide synthesis using Boc protocol and iodine mediated cyclization.

US8383770B1, also discloses preparation of Lanreotide acetate by solid phase peptide synthesis using chloromethylated polystyrene resin, aqueous solution of cesium carbonate and iodine mediated cyclization.

CN104497130A, discloses preparation of Lanreotide and its salts by fragmentation approach which involve coupling of dipeptide (Boc-D-2-Nal-Cys(Trt)) with hexapeptide (H-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Resin) using combination of solid phase & liquid phase and hydrogen peroxide mediated cyclization.

WO2013098802A, discloses preparation of Lanreotide by solid phase synthesis using resin bound Thr-amide viz. Fmoc-Thr(Resin)-NH2 which is subjected to seven sequential cycles of deprotection and coupling steps to give Boc-D-2-Nal-Cys(Trt)-Tyr(Clt)-D-Trp-Lys(Mtt)-Val-Cys(Trt)-Thr(Resin)-NH2, deprotected, cleaved from the resin, subjected to iodine mediated cylization, purified by high performance liquid chromatography and lyophilized.

CN105842362B, discloses a process for separation and detection of Lanreotide using high performance liquid chromatography, wherein the stationary phase is a mixed-mode column C18SCX.

WO2017178950A2, WO2017212390A1 and WO2019077507A1 disclose solution phase synthesis of Lanreotide acetate and iodine mediated cyclization.

WO2019184089A1 discloses use of siber resin for the preparation of Lanreotide and hydrogen peroxide mediated cyclization.

US20060276626A1, US20060148699A1, CN101298472A & CN108059667A, disclose preparation of Lanreotide and its salts by solid phase peptide synthesis using Fmoc protocol and iodine mediated cyclization.

US6503534B2, discloses solid or semi-solid pharmaceutical composition of Lanreotide acetate having a high specific surface area of at least about 4 $m^2/g$. US9352012B2 discloses preparation of sustained release pharmaceutical composition of Lanreotide acetate, comprising lyophilization of a mixture of Lanreotide salt and an aqueous acid solution in a stepwise temperature pattern.

There is a continuing need in the art to provide a substantially pure Lanreotide or its salt having reduced amounts of impurity as compared to known in the art. The present application relates to substantially pure Lanreotide or its salt and preparation thereof.

All references cited herein are incorporated by reference in their entireties for all purposes.

## SUMMARY OF THE INVENTION

In the first embodiment, present invention provides substantially pure Lanreotide or its salt.

In the one aspect of the first embodiment, present invention provides a process for the preparation of substantially pure Lanreotide or its salt.

In another aspect of the first embodiment, present invention provides pharmaceutical composition comprising substantially pure Lanreotide or its salt.

In the second embodiment, present invention provides a process for the preparation of substantially pure Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;

b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;

c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product;

d) deblocking the P1 group from the next blocked amino acid resin coupled product;

e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;

wherein:

(i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;

(ii) coupling temperature of Cys is in the range from about 40°C to about 50°C;

(iii) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;

(iv) P2 group is side-chain protecting group;

(v) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

f) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;

g) purifying the crude Lanreotide or its salt to give substantially pure Lanreotide or its salt.

In the third embodiment, present invention provides a process for the preparation substantially pure Lanreotide or its salt, wherein the process comprises use of DMSO as a cyclizing agent.

In the fourth embodiment, present invention provides a process for the preparation of Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;
b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;
c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product;
d) deblocking the P1 group from the next blocked amino acid resin coupled product;
e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;

   wherein:

   (i) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;
   (ii) P2 group is side-chain protecting group;
   (iii) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

f) cyclizing the resulting linear Lanreotide or its salt peptide using DMSO to give a crude Lanreotide or its salt;
g) purifying the crude Lanreotide or its salt to give Lanreotide or its salt.

In the fifth embodiment, present invention provides a process for the preparation Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid Fmoc-Thr(P2)-OH to a amide resin solid phase support to give first blocked amino acid amide resin coupled product;

b) deblocking the Fmoc group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;

c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by Fmoc to the deblocked amino acid resin coupled product;

d) deblocking the Fmoc group from the next blocked amino acid resin coupled product,

e) sequentially performing steps (c) and (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal Fmoc protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr and Cys;

f) coupling of Boc-D-2Nal-OH to give linear Lanreotide peptide;

wherein:

(i) P2 group is side-chain protecting group;

(ii) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

g) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;

h) purifying the crude Lanreotide or its salt to give Lanreotide or its salt.

In the sixth embodiment, present invention provides an improved process for purifying the crude Lanreotide or its salt to give substantially pure Lanreotide or its salt, the process comprising the steps of:

a) loading a crude Lanreotide or its salt sample onto a chromatographic column;

b) eluting the Lanreotide or its salt from the column using eluent comprising of acetic acid, buffer and suitable solvent;

c) collecting the fraction of desired purity of Lanreotide or its salt and pooling;

d) isolating substantially pure Lanreotide or its salt.

In the seventh embodiment, present invention provides a method for the preparation of substantially pure Lanreotide or its salt by using enantiomerically pure amino acids.

In one aspect of seventh embodiment, said enantiomerically pure amino acid can be selected from Fmoc-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Cys(Trt)-OH and Boc-D-2Nal-OH. In particular, present invention involves the purification of commercially available amino acid for attaining high purity of raw materials for preparation of Lanreotide.

In the eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;
c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.1%.

In the ninth embodiment, present invention provides a method of assaying purity of a sample of Lanreotide or its salt or a pharmaceutical dosage form comprising Lanreotide or its salt, wherein method comprise the steps of:

a) loading Lanreotide or its salt sample onto a chromatographic column;
b) eluting Lanreotide or its salt from the column with an eluent comprising polar solvent and an ion pair agent;
c) determining the purity of the Lanreotide or its salt.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a HPLC chromatogram of crude Lanreotide having content of D-Allo-Threonine Lanreotide impurity.

Figure 2 depicts a HPLC chromatogram of substantially pure Lanreotide as obtained from present invention.

Figure 3 depicts a HPLC chromatogram of substantially pure Lanreotide as obtained from present invention.

## DETAILED DESCRIPTION

Quality control of peptide active pharmaceutical ingredients prior to drug product manufacturing is an essential requirement. Generally, it is very difficult to remove impurities which are having RRT very closer to main peak. The present inventor successfully removed D-Allo-Threonine Lanreotide impurity which has RRT very close to Lanreotide or its salt.

In the first embodiment, present invention provides substantially pure Lanreotide or its salt.

In an aspect of first embodiment, present invention provides substantially pure Lanreotide acetate, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.10%.

In another aspect of first embodiment, present invention provides substantially pure Lanreotide acetate, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.080%. In another aspect of first embodiment, present invention provides substantially pure Lanreotide acetate, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.050%.

In another aspect of first embodiment, present invention provides substantially pure Lanreotide acetate, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.020%. In another aspect of first embodiment, present invention provides substantially pure Lanreotide acetate, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.010%.

In another aspect of present invention provide pharmaceutical composition comprising substantially pure Lanreotide or its salt.

In another aspect of present invention provide pharmaceutical composition comprising substantially pure Lanreotide or its salt, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.080%.

In another aspect of present invention provide pharmaceutical composition comprising substantially pure Lanreotide or its salt, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.050%.

In another aspect of present invention provide pharmaceutical composition comprising substantially pure Lanreotide or its salt, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.020%.

In another aspect of present invention provide pharmaceutical composition comprising substantially pure Lanreotide or its salt, wherein, D-Allo-Threonine Lanreotide impurity less than impurity 0.10%.

In the one aspect of the first embodiment, present invention provides a process for the preparation of substantially pure Lanreotide or its salt.

In the second embodiment, present invention provides a process for the preparation of substantially pure Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;

b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;

c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product amino;

d) deblocking the P1 group from the next blocked amino acid resin coupled product;

e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;

wherein:

(i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;

(ii) coupling temperature of Cys is in the range from about 40°C to about 50°C;

(iii) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;

(iv) P2 group is side-chain protecting group;

(v) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

f) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;

g) purifying the crude Lanreotide or its salt to give substantially pure Lanreotide or its salt.

The step a) of the second embodiment involves coupling of first amino acid P1-Thr(P2)-OH to a resin solid phase support in presence of coupling reagent and activator additive in a suitable solvent.

The step b) of the second embodiment comprises deblocking of P1 group from first blocked amino acid coupled product viz. P1-Thr(P2)-OH using suitable deblocking reagent.

The step c) of the second embodiment involves coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product in presence of coupling reagent and activator additive in a suitable solvent for the formation of amide bond.

The step d) of the second embodiment involves deblocking the P1 group from the next blocked amino acid resin coupled product using suitable deblocking reagent.

In an aspect of invention, unique coupling temperature for the preparation of Lanreotide or its salt is important for the preparation of substantially pure Lanreotide or its salt.

The step e) of the second embodiment involves sequentially performing steps (c) and (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide.

In an aspect of present invention, microwave instrument is used for the synthesis of peptide, wherein:

(i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;

(ii) coupling temperature of Cys is in the range from about 40°C to about 50°C.

The step f) of the second embodiment involves cyclizing the resulting linear Lanreotide or its salt peptide using suitable cyclizing agent to give crude Lanreotide or its salt. The suitable cyclizing agent can be selected from air, hydrogen peroxide, DMSO or iodine, preferably DMSO. In an aspect, DMSO can be used with aqueous buffer selected from ammonium acetate, acetic acetate and the like or suitable mixture thereof.

In an aspect of the third embodiment, the step g) is performed according to the sixth embodiment.

In an aspect of the present invention, substantially pure Lanreotide or its salt is prepared according to the second embodiment.

In the third embodiment, present invention provides a process for the preparation substantially pure Lanreotide or its salt, wherein the process comprises use of DMSO as a cyclizing agent.

In the fourth embodiment, present invention provides a process for the preparation of Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;

b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;

c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product;

d) deblocking the P1 group from the next blocked amino acid resin coupled product;

e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;

wherein:

(i) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;

(ii) P2 group is side-chain protecting group;

(iii) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

f) cyclizing the resulting linear Lanreotide or its salt peptide using DMSO to give a crude Lanreotide or its salt;

g) purifying the crude Lanreotide or its salt to give pure Lanreotide or its salt.

The step a) of the fourth embodiment involves coupling of first amino acid P1-Thr(P2)-OH to a resin solid phase support in presence of coupling reagent and activator additive in a suitable solvent.

The step b) of the fourth embodiment comprises deblocking of P1 group from first blocked amino acid coupled product viz. P1-Thr(P2)-OH using suitable deblocking reagent.

The step c) of the fourth embodiment involves coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product in presence of coupling reagent and activator additive in a suitable solvent for the formation of amide bond.

The step d) of the fourth embodiment involves deblocking the P1 group from the next blocked amino acid resin coupled product using suitable deblocking reagent.

The step e) of the fourth embodiment involves sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-β-Nal to give linear Lanreotide peptide;

In an aspect of present invention, microwave instrument is used for the synthesis of peptide, wherein:

(i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;

(ii) coupling temperature of Cys is in the range from about 40°C to about 50°C.

In an aspect of invention, unique coupling temperature for the preparation of Lanreotide or its salt is important for the preparation of substantially pure Lanreotide or its salt.

The step f) of fourth embodiment involves cyclizing the resulting linear Lanreotide or its salt peptide using DMSO to give crude Lanreotide or its salt. The present inventor surprisingly found that the use of DMSO as an oxidizing agent gives better purity than hydrogen peroxide of Lanreotide. Further, it is also noted that the use of iodine as an oxidizing agent for the preparation of Lanreotide or its salt leads to formation certain iodinated impurity which were difficult to control in final drug substance of Lanreotide.

In an aspect of fourth embodiment, the step g) is performed according to the sixth embodiment.

In an aspect of fourth embodiment, substantially pure Lanreotide or its salt is prepared.

In the fifth embodiment, present invention provides a process for the preparation substantially pure Lanreotide or its salt, the process comprising the steps of:

a) coupling a first amino acid Fmoc-Thr(P2)-OH to an amide resin solid phase support to give first blocked amino acid amide resin coupled product;

b) deblocking the Fmoc group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;

c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by Fmoc to the deblocked amino acid resin coupled product;

d) deblocking the Fmoc group from the next blocked amino acid resin coupled product;

e) sequentially performing steps (c) and (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal Fmoc protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr and Cys wherein Cys, Lys, D-Trp and Tyr may be optionally side-chain protected;

f) coupling of Boc-D-2Nal-OH to give linear Lanreotide peptide;

g) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;

h) purifying the crude Lanreotide or its salt to give pure Lanreotide or its salt.

In another aspect of the fifth embodiment, an amide resin can be selected from, Rink amide Rink amide AM Resin, Sieber Amide resin, MBHA Resin and the like.

The step a) of the fifth embodiment involves coupling of first amino acid Fmoc-Thr(P2)-OH to an amide resin solid phase support in presence of coupling reagent and activator additive in a suitable solvent.

The step b) of the fifth embodiment comprises deblocking of Fmoc group from first blocked amino acid coupled product viz. Fmoc-Thr(P2)-OH using suitable deblocking reagent.

The step c) of the fifth embodiment involves coupling a next amino acid having $N^{\alpha}$-terminal blocked by Fmoc to the deblocked amino acid resin coupled product in presence of coupling reagent and activator additive in a suitable solvent.

The step d) of the fifth embodiment involves deblocking the Fmoc group from the next blocked amino acid resin coupled product using suitable deblocking reagent.

The step e) of the fifth embodiment involves sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr and Cys wherein Cys, Lys, D-Trp and Tyr may be optionally side-chain protected;

The step f) of the fifth embodiment involves coupling of Boc-D-2Nal-OH in presence of coupling reagent and activator additive in a suitable solvent to give linear Lanreotide peptide.

In an aspect of present invention, microwave instrument is used for the synthesis of peptide, wherein:

(i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;

(ii) coupling temperature of Cys is in the range from about 40°C to about 50°C.

In an aspect of invention, unique coupling temperature for the preparation of Lanreotide or its salt is important for the preparation of substantially pure Lanreotide or its salt.

The step g) of the fifth embodiment involves cyclizing the resulting linear Lanreotide or its salt peptide using suitable cyclizing agent to give crude Lanreotide or its salt. The suitable cyclizing agent can be selected from air, hydrogen peroxide, DMSO or iodine. Preferably,

DMSO can be used with aqueous buffer selected from ammonium acetate, acetic acetate and the like or suitable mixture thereof.

In an aspect of the fifth embodiment, the step h) is performed according to the sixth embodiment.

In an aspect of fifth embodiment, substantially pure Lanreotide or its salt is prepared.

In another aspect of the present invention, final deprotection step is performed after preparation of Linear Lanreotide peptide. In an aspect of present invention, final deprotection step can be performed prior or later to the cyclization step which means present invention includes both 'on-resin oxidation' and 'without-resin oxidation' of linear Lanreotide peptide. The final deprotection step can be performed by using suitable cocktail mixtures.

In an aspect of present invention said 'without-resin oxidation' of linear Lanreotide peptide may further comprises steps of:

a) deprotecting all side chain protecting groups, $N^{\alpha}$-terminal protecting groups and resin from linear Lanreotide peptide;
b) cyclizing the resulting peptide by using cyclizing agent to give a crude Lanreotide or its salt.

In an aspect of present invention said 'on-resin oxidation' of linear Lanreotide peptide may further comprises steps of:

a) deprotecting all side chain protecting groups and $N^{\alpha}$-terminal protecting groups of linear Lanreotide peptide, wherein Threonine amino acid is attached to the resin, or
b) selectively deprotecting the side chain protecting group of cysteine amino acids from linear Lanreotide peptide, wherein Threonine amino acid is attached to resin, and
c) cyclizing the resulting peptide from step a) or b) using cyclizing agent to give a crude Lanreotide or its salt.

In an aspect of fifth embodiment, substantially pure Lanreotide or its salt is prepared.

In an aspect of present invention, particularly second, fourth and fifth embodiment, P1 group is protecting group attached to the $N^{\alpha}$-terminal group. In a preferred embodiment, the P1 group can be selected from Fmoc, Boc or Cbz or known to person skilled in the art.

In an aspect of present invention, particularly second, fourth and fifth embodiment, P2 group is side-chain protecting group.

In an aspect of present invention, particularly second, fourth and fifth embodiment, Thr side-chain, hydroxy group is protected by a group selected from Clt, Trt or tBu and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, Cys side-chain, thiol group is protected by a group selected from Trt, Mmt, Acm or tBu and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, Lys side-chain, N-γ amino group is protected by a group selected from Trt, Mmt, Acm or tBu and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, D-Trp side-chain, amino group is protected by a group selected from Boc, Formyl (For), Cyclohexylcabonyl (Hoc) and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, Tyr side-chain hydroxy group is protected by a group selected from Clt, Trt or tBu and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, resin can be selected from, Wang resin, Rink Amide Resin, Rink amide AM Resin, Sieber resin, HMBA-ChemMatrix® resin, MBHA Resin, HMPB-ChemMatrix® resin, Hydroxy Functionalized HypoGel® Resins, TentaGel® resin, 2-Chlorotrityl Resin, 4,4'-dimethoxy-trityl resin, 4-methyltrityl chloride resins, Rink Acid Resin and the like.

In an aspect of present invention, particularly second, fourth and fifth embodiment, the suitable coupling reagent can be selected from DCC, DIC, EDC, BOP, PyBOP, PyAOP, PyBrOP, BOP-Cl, HATU, HCTU or mixtures thereof or the like know to skilled person in the art. The suitable activator additive can be selected from Oxyma, HOBt, HOAt, 6-Cl-HOBt, NHS or suitable mixtures thereof. The amount of the coupling agents used may range from about 0.5 to about 6 molar equivalents, per molar equivalent of resin with respect to resin loading capacity.

In an aspect of present invention, particularly second, fourth and fifth embodiment, the suitable deblocking reagent used in solid phase peptide synthesis, wherein the P1 group is Fmoc can be selected from base comprising piperidine in suitable solvent selected from N-methyl pyrrolidone (NMP), dichloromethane (DCM) or dimethylformamide (DMF) or suitable mixtures thereof.

In an aspect of present invention, particularly second, fourth and fifth embodiment, the suitable deblocking reagent used in solid phase peptide synthesis, wherein the P1 group is Boc

can be selected from 25-50% TFA-DCM, 4 M HCl in dioxane, 2M $MeSO_3H$ in dioxane, 1M TMS-Cl, 1M phenol-DC or suitable mixtures thereof.

In an aspect of present invention, particularly second, fourth and fifth embodiment, the suitable deblocking reagent used in solid phase peptide synthesis, wherein the P1 group is Cbz can be selected from HF scavengers, TFMSA-TFA, H2 catalyst or suitable mixture thereof.

In an aspect of present invention, particularly second, fourth and fifth embodiment, the suitable solvent for above steps of solid-phase synthesis can be selected from organic polar aprotic solvents, such as NMP or DMF.

In the sixth embodiment, present invention provides an improved process for purifying the crude Lanreotide or its salt to give substantially pure Lanreotide or its salt, the process comprising the steps of:

a) loading a crude Lanreotide or its salt sample onto a chromatography column;
b) eluting the Lanreotide or its salt from the column using eluent comprising of acetic acid, buffer and suitable solvent;
c) collecting the fraction of desired purity of Lanreotide or its salt and pooling;
d) isolating substantially pure Lanreotide or its salt.

The step a) of sixth embodiment involves loading of crude Lanreotide or its salt sample onto a chromatography column. The suitable chromatographic column types that can be used in above step (a) can be selected from, but are not limited to the following silica gel sorbents: Daisogel™, Kromasil™ C18 100-16, Kromasil™ C18 100-10, Kromasil™ C8 100-16, Kromasil™ C4 100-16, Kromasil™ Phenyl 100-10, Kromasil™ C18 Eternity 100-5, Kromasil™ C4 Eternity 100-5, Chromatorex™ C18 SMB 100-15 HE, Chromatorex™ C8 SMB 100-15 HE, Chromatorex™ C4 SMB 100-15 HE, Daisopak™ SP 120-15 ODS-AP, Daisopak™ SP 120-10-C4-Bio, Daisopak™ SP 200-10-C4-Bio, Zeosphere™ C18 100-15, Zeosphere™ C8 100-15, Zeosphere™ C4 100-15, SepTech ST 150-10 C18, Luna C18 100-10, Gemini C18 110-10, YMC Triart C18 120-5 and YMC Triart C8 200-10. The chromatographic column can be used in a reverse phase High Performance Liquid Chromatography (HPLC).

In another aspect of above step (b), suitable solvent can be selected from acetonitrile, tetrhydrofuran, acetone, methanol, ethanol, propanol, isopropanol or suitable mixture thereof and the like.

In another aspect, the suitable buffer can be selected from ammonium acetate, sodium acetate, phosphate buffer, sodium chloride, potassium chloride, lithium chloride and the like.

In another aspect of step c), involves collecting the fractions of desired Lanreotide purity and pooling. During elution, desired fractions are collected at regular intervals and analyzed for purity. The suitable collected fractions containing the product of similar purities may be pooled together and optionally subjected to removal of acetonitrile solvent. Optionally, after completing the desired number of cycles of purification by repeating the steps a) and b), all the fractions of similar purity from each of the cycle are pooled and taken forward to the next step of the purification process. In an embodiment, pooled fractions having a purity of more than about 98% may be taken forward to the next step of the purification process.

In another aspect of Step d), the isolation of substantially pure Lanreotide or its salt can be done by well-known techniques known in the prior art. In an aspect of present invention, isolation of Lanreotide or its salt from pure pooled fraction may involve lyophilization of pure pooled fraction of Lanreotide or its salt.

In an aspect of second, fourth and fifth embodiment, the purification process of sixth embodiment is used.

In the seventh embodiment, present invention provides a method for the preparation of substantially pure Lanreotide or its salt by using enantiomerically pure amino acids.

In one aspect of seventh embodiment, said enantiomerically pure amino acid can be selected from Fmoc-Thr(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Cys(Trt)-OH and Boc-D-2Nal-OH. In particular present invention involves the purification of commercially available amino acid for attaining high purity of raw materials for preparation of Lanreotide.

In another aspect of present invention, enantiomerically pure Fmoc-Thr(tBu)-OH is used wherein, total content of D-Threonine, L-Allo-Threonine and D-Allo-Threonine is less than about 0.10%.

In the eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring the D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;

c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.10%.

In an aspect of eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring the D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;
c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.080%.

In another aspect of the eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring the D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;
c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.050%.

In another aspect of the eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring the D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;
c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.020%.

In another aspect of the eighth embodiment, present invention provides a method of preparing Lanreotide drug product, the method comprising:

a) providing a batch of Lanreotide or its salt;
b) measuring the D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;
c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.010%.

In an aspect of present invention, a batch of Lanreotide or its salt is active pharmaceutical ingredient of Lanreotide or its salt which can be further processed for making pharmaceutical composition or drug product. The drug product or pharmaceutical composition can be prepared by any process known in the art or as disclosed in US6503534B1.

In the ninth embodiment, present invention provides a method of assaying purity of a sample of Lanreotide or its salt or a pharmaceutical dosage form comprising Lanreotide or its salt, wherein method comprise the steps of:

a) loading Lanreotide or its salt sample onto a chromatographic column;
b) eluting Lanreotide or its salt from the column with an eluent comprising polar solvent and an ion pair agent;
c) determining the purity of the Lanreotide or its salt.

In an aspect of above step (a), the chromatographic column can be selected from, but are not limited to the following silica gel sorbents: Daisogel™, Kromasil™ C18 100-16, Kromasil™ C18 100-10, Kromasil™ C8 100-16, Kromasil™ C4 100-16, Kromasil™ Phenyl 100-10, Kromasil™ C18 Eternity 100-5, Kromasil™ C4 Eternity 100-5, Chromatorex™ C18 SMB 100-15 HE, Chromatorex™ C8 SMB 100-15 HE, Chromatorex™ C4 SMB 100-15 HE, Daisopak™ SP 120-15 ODS-AP, Daisopak™ SP 120-10-C4-Bio, Daisopak™ SP 200-10-C4-Bio, Zeosphere™ C18 100-15, Zeosphere™ C8 100-15, Zeosphere™ C4 100-15, SepTech ST 150-10 C18, Luna C18 100-10, Gemini C18 110-10, YMC Triart C18 120-5 and YMC Triart C8 200-10. The chromatographic column can be used in a reverse phase High Performance Liquid Chromatography (HPLC) or Ultra performance Liquid chromatography.

In an aspect of above step (b), the ion pair agent can be selected from tetra butyl ammonium hydrogen sulfate, dodecyltrimethylammonium bromide, dodecyltrimethylammonium hydrogen sulfate, heptafluorobutyric acid, hexadecyltrimethylammonium bisulfate, hexadecyltrimethylammonium bromide, myristyltrimethylammonium bromide, tetrabutylammonium bisulfate, tetrabutylammonium bromide, tetrabutylammonium chloride, tetrabutylammonium iodide,tetrabutylammonium phosphate, tetraethylammonium bromide, tetraheptylammonium bromide, tetraethylammonium hydrogen sulfate, tetrahexylammonium bromide, tetrahexylammonium hydrogensulfate, tetrakis(decyl)ammonium bromide, tetramethylammonium bisulfate, tetramethylammonium bromide, tetramethylammonium sulfate tetraoctylammonium bromide, tetrapentylammonium bromide, tetrapropylammonium bisulfate,

tetrapropylammonium bromide, trimethyltetradecylammonium hydrogen sulfate or suitable mixture thereof and the like.

**Definitions:**

The following definitions can be used in connection with the words or phrases used in the present application unless the context indicates otherwise.

The term "amino acid" as used herein refers to an organic compound comprising at least one amino group and at least one acidic group. The amino acid may be a naturally occurring amino acid or be of synthetic origin, or an amino acid derivative or amino acid analog.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Likewise nucleotides may be referred to by their commonly accepted single-letter codes. Amino acids mentioned herein in the specification if does not represent any chirality means that amino acid is in its natural or L form.

The term “D” as used herein before amino acids refers to chirality of the amino acid that follows as a D-amino acid;

The term "sequentially" refers to the method of peptide synthesis where any of the amino acids contained in the peptide chain is introduced individually. The method may or may not involve an intermediate purification step.

The term "peptide" as used herein refers to any peptide comprising two or more amino acid residues connected by peptide linkage.

The term "RRT" as used herein is intended to indicate the relative retention time of the particular impurity against a pure Lanreotide or its salt standard (assigned an RRT value of 1) during an HPLC analysis.

The term “substantially pure Lanreotide or its salt” as used herein refers to Lanreotide or its salt having purity greater than about 99% and free from D-Allo-Threonine Lanreotide impurity. In an embodiment, the term “free from D-Allo-Threonine Lanreotide impurity” means that D-Allo-Threonine Lanreotide impurity is less than about 0.10% (w/w) or less than 0.080% (w/w) or less than 0.050% (w/w) or less than 0.02% (w/w) or less than 0.010% (w/w) in pure Lanreotide or its salt.

In an embodiment RRT 1.1 impurity is D-Allo-Threonine Lanreotide which is confirmed from spiking studies after synthesizing D-Allo-Threonine Lanreotide using D-Allo Theronine as stating material.

The term "Lanreotide or its salt" as used herein means Lanreotide free base or Lanreotide salts. Lanreotide salts according to present invention are preferably pharmaceutically acceptable salts of organic acids, such as those of acetic, lactic, malic, ascorbic, succinic, benzoic, methanesulphonic acids, or pharmaceutically acceptable salts of inorganic acids, such as those of hydrochloric, hydrobromic, hydriodic, sulphuric or phosphoric acids. In particular, Lanreotide salt is Lanreotide acetate. In an aspect of present invention, Lanreotide salts also include trifluoroacetic acid salts which can be prepared at intermediate stage.

The term "enantiomerically pure" as used herein means, solid or solution that consists of a single enantiomer and not its mirror image is called enantiomerically pure.

The term “Drug Product or pharmaceutical composition” as used herein refers to a finished dosage form, for example, a tablet, capsule or solution that contains an active pharmaceutical ingredient, generally, but not necessarily, in association with inactive ingredients.

The term “Active Pharmaceutical Ingredient or API” is any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body.

The carboxyl and amino groups that participate in the formation of the peptide amide bond are called “non-side chain” carboxyl group or amino group, respectively. The term “$N^{\alpha}$-terminal group of amino acid” is non-side chain amino group that participate in the formation of the peptide amide bond. On the other hand, any functional groups of an amino acid which are not involved in formation of a peptide amide bond are called “side chain or side-chain” functionalities.

The term “Acm” as used herein refers to acetamidomethyl.

The term “Boc” as used herein refers to tetra-butyloxycarbonyl.

The term “BOP” as used herein refers to Benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate.

The term “BOP-Cl” as used herein refers to Bis(2-oxo-3-oxazolidinyl)phosphinic Chloride

The term “Cbz” as used herein refers to carboxy benzyl.

The term “Clt” as used herein refers to chlorotrityl.

The term “DCC” as used herein refers to Dicyclohexylcarbodiimide.

The term “DIC” as used herein refers to Diisopropylcarbodiimide.

The term “DCM” as used herein refers to dichloromethane

The term “DMF” as used herein refers to dimethylformamide

The term “EDC” as used herein refers to 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide.

The term “Fmoc” as used herein refers to 9 Fluorenylmethoxy carbony

The term “HOBt” as used herein refers to 1-Hydroxybenzotriazole.

The term “HOSu” as used herein refers to N-Hydroxysuccinimide.

The term “HOAt” as used herein refers to 1-Hydroxy-7-aza-1H-benzotriazole.

The term “HATU” as used herein refers to 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate.

The term “tBu” as used herein refers to tert-butyl

The term “Trt” as used herein refers to trityl

The term “Mmt” as used herein refers to monomethoxytrityl

The term “NHS” as used herein refers to N-hydroxy succinamide

The term “NMP” as used herein refers to N-methylpyrrolidone

The term “Oxyma” as used herein refers to ethyl 2-cyano-2-(hydroxyimino)acetate

The term “PyBrOP” as used herein refers to Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate

The term “PyAOP” as used herein refers to 7-Aza-benzotriazol-1-yloxy-tripyrrolidinophosphoniumhexafluorophosphate

The term “PyBOP” as used herein refers to (Benzotriazol-1-yloxy-tripyrrolidino-phosphonium hexafluorophosphate

The term “DODT” as used herein refers to 2,2’-(Ethylenedioxy)-diethanethiol

The term “DMSO” as used herein refers to dimethyl sulfoxide

The term “TIPS” as used herein refers to Triisopropylsilane

The term "TFA" as used herein refers to trifluoroacetic acid

The term "MTBE" as used herein refers to methyl tert-butyl ether

The term "NH4OAc" as used herein refers to ammonium acetate

The term "AcOH" as used herein refers to acetic acid.

The term "IPA" as used herein refers to isopropyl alcohol.

Although the exemplified procedures herein illustrate the practice of the present invention in some of its embodiments, the procedures should not be construed as limiting the scope of the invention. Modifications from consideration of the specification and examples within the ambit of current scientific knowledge will be apparent to one skilled in the art.

**EXAMPLE 1: Preparation of Lanreotide acetate:**

**Step a) Preparation of Boc-D-2-Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)--Lys(Boc)-Val-Cys(Trt)-Thr(tBu)- NH-Rink Amide AM Resin:**

In microwave instrument, Fmoc protected NH-Rink amide AM resin (375 mmol) was swelled in DMF and Fmoc group was deprotected by 20% piperidine in DMF at 90°C. Fmoc-Thr(tBu)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF. The coupling was performed at 90°C for two minutes to give Fmoc-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-Cys(Trt)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 50°C to give Fmoc-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-Val-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 90°C for two minutes to give Fmoc-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-Lys(Boc)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in

DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 90°C for two minutes to give Fmoc-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-D-Trp(Boc)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 90°C for two minutes to give Fmoc-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-Tyr(tBu)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 90°C for two minutes to give Fmoc-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Fmoc-Cys(Trt)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 50°C to give Fmoc-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 90°C. The next amino acid Boc-D-2Nal-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 90°C for two minutes to give Boc-D-2Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. The resultant linear lanreotide peptide was washed with IPA to give titled product of 1.2 Kg.

**Step b) Deprotection of Boc-D-2Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin to give D-2-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$bis TFA salt**

450 gm of step a) product was treated with cleavage cocktail mixture (DODT, TIPS, Water, and TFA) and stirred in glass reactor. Resultant reaction mixture was filtered, washed with TFA and air dried. Further, charged combined TFA filtrate to vessel and MTBE was added and stirred for 1 hour. The precipitate was filtered, washed with MTBE and dried in a vacuum tray drier to afford 145 gm of titled product.

**Step c) Oxidation of D-2-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$bis TFA salt to give Lanreotide**

To a reactor vessel 142 gm of step b) product and DMSO (2.1L) was added and stirred the reaction mixture. Further, 20 mmol NH4OAc/acetic acid was added to the reaction mixture and stirred to afford a solution of 2.5 mg/mL of Lanreotide product.

**Step d) Purification of crude Lanreotide:**

The Reverse phase C18 media was equilibrated with 0.2% Acetic acid + 1M NaCl buffer. The crude Lanreotide solution was loaded onto the column and the gradient elution was performed using acetonitrile as mobile phase B. The desired fractions was collected whose purity was greater than 99.5% and pooled together. The acetonitrile was removed by distillation from eluted product and subjected to lyophilization to obtain substantially pure Lanreotide acetate.

**Step e): Assaying the purity of Lanreotide acetate sample**

The lanreotide acetate sample was prepared using 5 mg of Lanreotide acetate and water and loaded on column of Ultra performance liquid chromatography. Further, the loaded column was eluted with gradient programme using mobile phase A and mobile phase B and impurities were determined by relative area normalization method.

Preparation of Mobile phase A: 10mm Tetra-n-butyl ammonium hydrogen sulfate

Preparation of Mobile phase B: The Mobile phase B was prepared by mixing mobile phase A, acetonitrile and methanol.

The purity of Lanreotide acetate sample according to above method is 99.69% and D-Allo-Threonine Lanreotide impurity less than 0.010%. The HPLC chromatograph is depicted as Figure 3.

**EXAMPLE 2: Preparation of Lanreotide acetate:**

**Step a) Preparation of Boc-D-2-Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)--Lys(Boc)-Val-Cys(Trt)-Thr(tBu)- NH-Rink Amide AM Resin**

In microwave instrument, Fmoc protected NH-Rink amide AM resin (258 mmol) was swelled in DMF and Fmoc group was deprotected by 20% piperidine in DMF at 65°C. Fmoc-Thr(tBu)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF. The coupling was performed at 85°C for two minutes to give Fmoc-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-Cys(Trt)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 40°C to give Fmoc-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-Val-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 85°C for two minutes to give Fmoc-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-Lys(Boc)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 85°C for two minutes to give Fmoc-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-D-Trp(Boc)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 85°C for two minutes to give Fmoc-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-Tyr(tBu)-OH was dissolved in

DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 85°C for two minutes to give Fmoc-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Fmoc-Cys(Trt)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 40°C to give Fmoc-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF at 65°C. The next amino acid Boc-D-2Nal-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5M DIC solution in DMF & 1M oxyma pure solution in DMF were added to the reaction vessel. The coupling was performed at 85°C for two minutes to give Boc-D-2Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys-(Trt)-Thr(tBu)-NH-Rink amide AM resin. The resultant linear Lanreotide peptide was washed with IPA to give titled product of 0.88Kg.

Step b), step c), step d) and step e) was performed according to example 1 to give substantially pure Lanreotide.

**Example 3: Preparation of Lanreotide acetate**

**Step a) Preparation of Boc-D-2-Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)--Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink Amide AM Resin**

Fmoc protected NH-Rink amide AM resin (0.74 mmol/g) was swelled in DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF. The resin was washed repeatedly with DMF. Fmoc-Thr(tBu)-OH was dissolved in DMF (0.4 M solution) and added to the deprotected resin along with 5 M DIC solution in DMF & 1 M oxyma pure solution in DMF. The resultant reaction mixture was stirred to give Fmoc-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF. The resin was washed repeatedly with DMF. The next amino acid Fmoc-Cys(Trt)-OH was dissolved in DMF (0.4 M solution) and then 5 M DIC solution in DMF & 1 M oxyma pure solution in DMF were added to the resin. The

resultant mixture was stirred to give Fmoc-Cys(Trt)-Thr(tBu)-NH-Rink amide AM resin. After coupling, the resultant resin was washed with DMF. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF. These steps of coupling and deprotection were repeated sequentially using Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Cys(Trt)-OH and Boc-D-2Nal-OH. The resultant coupled amino acid resin was washed with DMF and IPA to give 1.22 Kg of Boc-D-2-Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)-NH-Rink Amide AM Resin.

**Step b) Cleavage of Boc-D-2-Nal-Cys(Trt)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(tBu)- NH-Rink Amide AM Resin to give D-2-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$ bis TFA salt:**

200 gm of step a) product was treated with cleavage cocktail mixture (DODT, TIPS, Water, and TFA) and stirred for 2 hours in glass reactor. Then the reaction mixture was filtered, washed with TFA and air dried. Further, charged combined TFA filtrate to vessel and MTBE (8000 mL) was added and stirred for 1 hour. The precipitate was filtered, washed with MTBE and dried in a vacuum tray drier to afford 79.8 gm of titled product.

**Step c) Oxidation of D-2-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$ bis TFA salt to give Lanreotide:**

To a reactor vessel, 3.75 gm of step b) product and DMSO (5.7 mL) were added. The resultant mixture was stirred. Afterward, NH4OAc/AcOH (69.3 ml) was added to the reaction mixture and stirred for 15 hours to afford a solution of 3 mg/mL of Lanreotide product.

**Step d) Purification of crude Lanreotide:**

The Reverse phase C18 media was equilibrated with 0.2% Acetic acid + 1M NaCl buffer. The crude Lanreotide solution of step c) was loaded onto the column and the gradient elution was performed using acetonitrile as mobile phase B. The desired fractions was collected whose purity was greater than 99.5% and pooled together. The acetonitrile was removed by distillation from eluted product and subjected to lyophilization to obtain substantially pure Lanreotide.

**Step e) Assaying the purity of Lanreotide sample:**

The lanreotide acetate sample was prepared using 5 mg of Lanreotide acetate and water and loaded on column of Ultra performance liquid chromatograph. Further, the loaded Lanreotide column was eluted with gradient programme using mobile phase A and mobile phase B and impurities were determined by relative area normalization method.

Preparation of Mobile phase A: 10mm Tetra-n-butyl ammonium hydrogen sulfate

Preparation of Mobile phase B: The Mobile phase B was prepared by mixing mobile phase A, acetonitrile and methanol.

The purity of Lanreotide sample according to above method is 99.412% and D-Allo-Threonine impurity and less than 0.010%. The HPLC chromatograph is depicted as Figure 2.

We Claim:

1. A process for preparation of substantially pure Lanreotide or its salt, the process comprising the steps of:
   a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;
   b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;
   c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product amino;
   d) deblocking the P1 group from the next blocked amino acid resin coupled product;
   e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;
      wherein:
      (i) coupling temperature of Thr, Val, Lys, D-Trp, Tyr and D-2Nal is in the range from about 85°C to about 90°C;
      (ii) coupling temperature of Cys is in the range from about 40°C to about 50°C;
      (iii) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;
      (iv) P2 group is side-chain protecting group;
      (v) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;
   f) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;
   g) purifying the crude Lanreotide or its salt to give substantially pure Lanreotide or its salt.
2. The process as claimed in claim 1, wherein in step (f), DMSO is used for cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt.
3. The substantially pure Lanreotide or its salt prepared according to the process of claim 1.
4. Substantially pure Lanreotide or its salt.
5. A pharmaceutical composition comprising, substantially pure Lanreotide or its salt.

6. A process for the preparation of Lanreotide or its salt, the process comprising the steps of:
   a) coupling a first amino acid P1-Thr(P2)-OH to a resin solid phase support to give first blocked amino acid resin coupled product;
   b) deblocking the P1 group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;
   c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by P1 to the deblocked amino acid resin coupled product;
   d) deblocking the P1 group from the next blocked amino acid resin coupled product;
   e) sequentially performing step (c) and step (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal P1-protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr, Cys, D-2-Nal to give linear Lanreotide peptide;
      wherein:
      (i) P1 group is protecting group attached to the $N^{\alpha}$-terminal group;
      (ii) P2 group is side-chain protecting group;
      (iii) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;
   f) cyclizing the resulting linear Lanreotide or its salt peptide using DMSO to give a crude Lanreotide or its salt;
   g) purifying the crude Lanreotide or its salt to give pure Lanreotide or its salt.
7. A process for the preparation of Lanreotide or its salt, the process comprising the steps of:
   a) coupling a first amino acid Fmoc-Thr(P2)-OH to a amide resin solid phase support to give first blocked amino acid amide resin coupled product;
   b) deblocking the Fmoc group from the first blocked amino acid resin coupled product to give a deblocked amino acid resin coupled product;
   c) coupling a next amino acid having $N^{\alpha}$-terminal blocked by Fmoc to the deblocked amino acid resin coupled product;
   d) deblocking the Fmoc group from the next blocked amino acid resin coupled product;

e) sequentially performing steps (c) and (d) after the formation of the first deblocked coupled resin product of H-Thr(P2)-resin obtained in step b) using $N^{\alpha}$-terminal Fmoc protected amino acids in the order of Cys, Val, Lys, D-Trp, Tyr and Cys;

f) coupling of Boc-D-2Nal-OH to give linear Lanreotide peptide;
wherein:

(i) P2 group is side-chain protecting group;

(ii) optionally, Cys, Lys, D-Trp and Tyr is side-chain protected;

g) cyclizing the resulting linear Lanreotide or its salt peptide to give a crude Lanreotide or its salt;

h) purifying the crude Lanreotide or its salt to give pure Lanreotide or its salt.

8. A process for the preparing substantially pure Lanreotide or its salt, comprising use of enantiomerically pure Fmoc-Thr(tBu)-OH, wherein, total content of D-Threonine, L-Allo-Threonine and D-Allo-Threonine is less than about 0.10%.

9. A method of preparing Lanreotide drug product, the method comprising;

a) providing a batch of Lanreotide or its salt;

b) measuring D-Allo-Threonine Lanreotide impurity content in the Lanreotide or its salt;

c) processing the batch of Lanreotide or its salt as a drug product, if D-Allo-Threonine Lanreotide impurity is less than 0.10%.

10. A method of assaying purity of a sample of Lanreotide or its salt or a pharmaceutical dosage form comprising Lanreotide or its salt, wherein the method comprises the steps of;

a) loading Lanreotide or its salt sample onto a column;

b) eluting Lanreotide or its salt from the column with an eluent comprising polar solvent and an ion pair agent;

c) determining the purity of the Lanreotide or its salt.

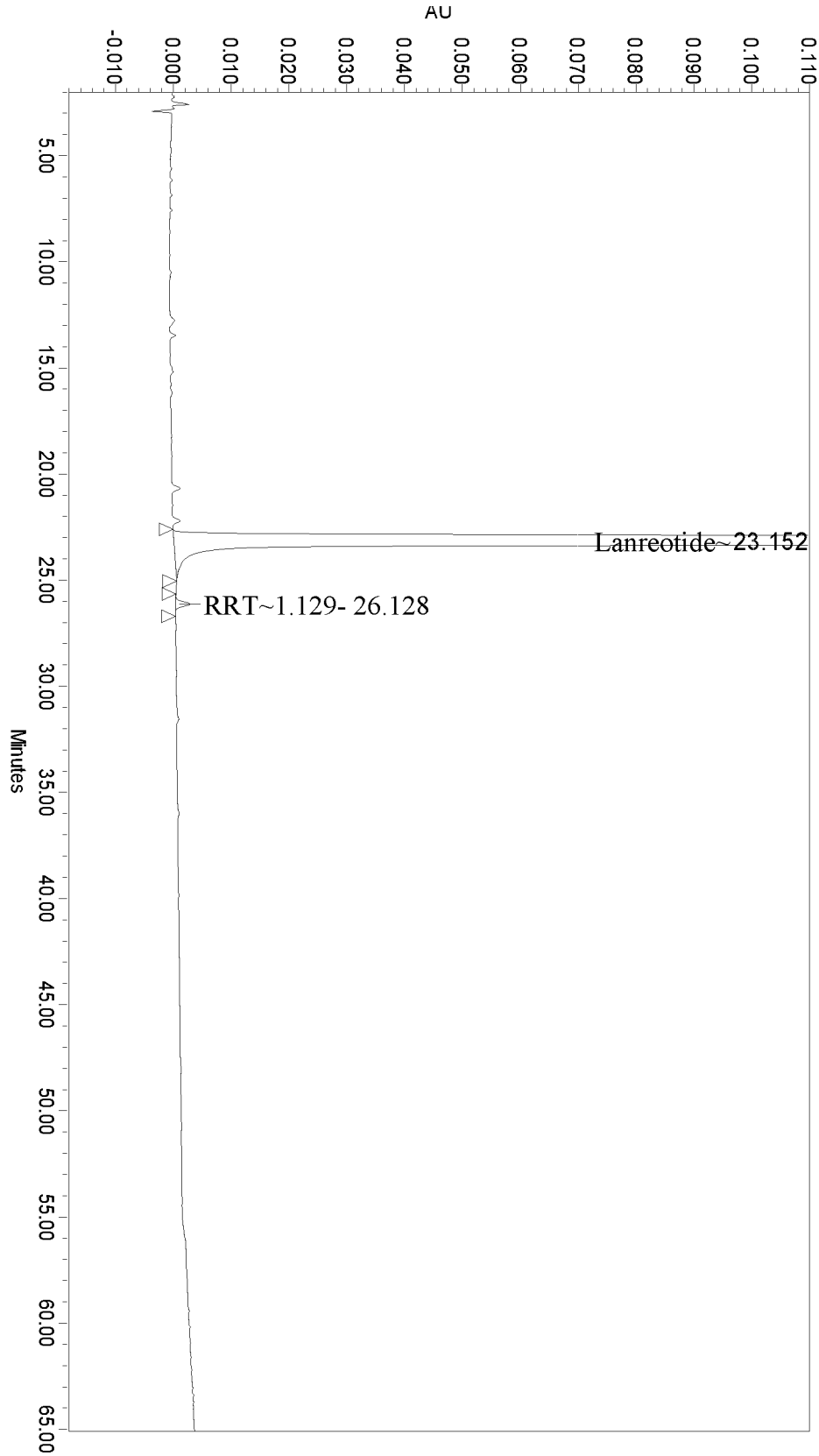

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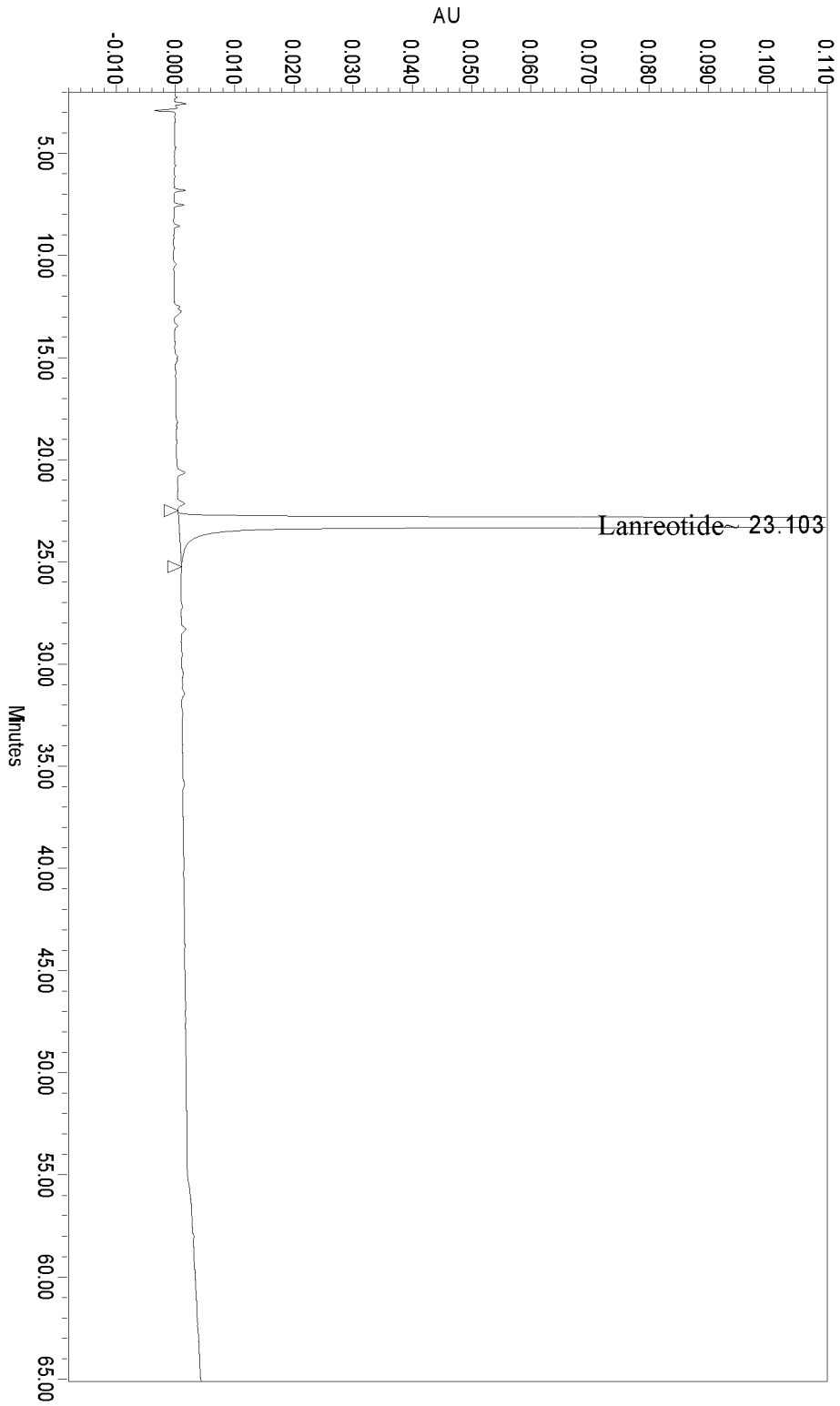
**Figure 1**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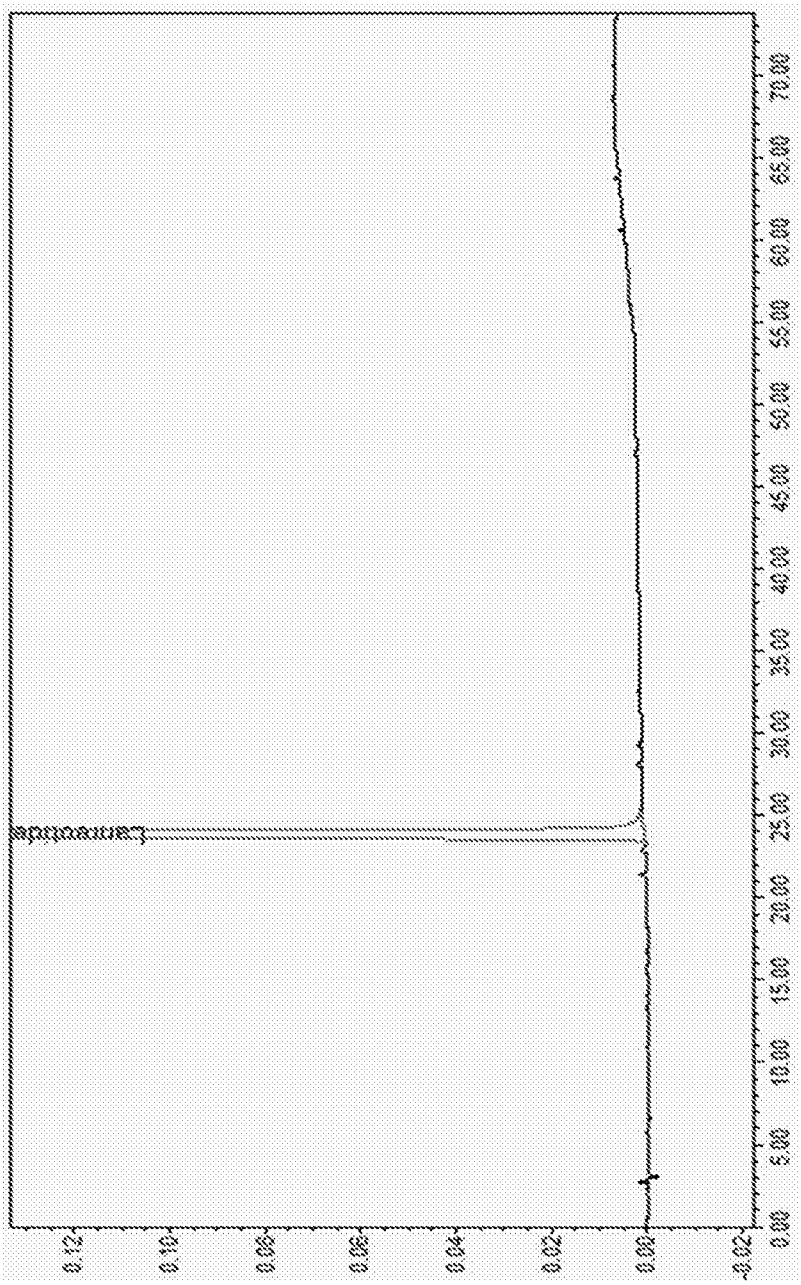
Lanreotide~ 23.103

**Figure 2**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70.00

Figure 3

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2020/051420

A. CLASSIFICATION OF SUBJECT MATTER
A61K38/31,C07K5/10,C07K1/06,C07K1/14 Version=2020.01

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)

A61K, 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 practicable, search terms used)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US6503534 B1 (IPSEN PHARMA S.A.S. [FR]) 07 JANUARY 2003 (07.01.2003) *col. 5, lines 2-3; examples 1-4* | 4-5 |
| Y | *examples 1-4* | 9 |
| X | US 2006/0148699 A1 (TEVA PHARMACEUTICALS INC. [US]) 06 JULY 2006 (06.07.2006) *page 6, col. 2, line 9; examples 1-2* | 10 |
| Y | WO 2013/098802 A2 (CHEMICAL & BIOPHARMACEUTICAL LABORATORIES OF PATRAS S.A. [GR]) 04 JULY 2013 (04.07.2013) *para [0026] [0063-0079]; examples; claims 1-9* FAMILY: [NONE] | 1-3, 6-7 |
| Y | WO 2006/119388 A2 (TEVA PHARMACEUTICALS INC. [US]) 9 NOVEMBER 2006 (09.11.2006) *SEQ. ID. no. 17; pages 21-27; examples 44-45* | 1-3, 6-7, 9 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20-07-2020 | 20-07-2020 |
| Name and mailing address of the ISA/<br>Indian Patent Office<br>Plot No.32, Sector 14,Dwarka,New Delhi-110075<br>Facsimile No. | Authorized officer<br>Dr. Komal Choudhary<br>Telephone No. +91-1125300200 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2020/051420

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The application consists of the following group of inventions:

Group I: Claims 1-3 and 6-7

These claims relate to a process for the preparation of substantially pure Lanreotide or its salt thereof.

Group II: Claims 4 and 5

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Observations where unity of invention is lacking(Box III)

These claims relate to substantially pure Lanreotide or its salt and a pharmaceutical composition comprising, substantially pure Lanreotide or its salt.

Group III: Claim 8

The claim 8 relates to a process for the preparing substantially pure Lanreotide or its salt, comprising use of enantiomerically pure Fmoc-Thr(tBu)-OH.

Group IV: Claim 9

The claim 9 relates to a method of preparing Lanreotide drug product.

Group V: Claim 10

Claim 10 relates to a method of assaying purity of a sample of Lanreotide or its salt or a pharmaceutical dosage form comprising Lanreotide or its salt.

These groups of inventions are not so linked as to form a single general inventive concept as required under Rule 13.1 of PCT for the following reasons.

The special technical feature is an essential feature common to all embodiments of the claimed invention (and responsible for the inventive effect) and which defines a contribution with each of the claimed inventions over prior art (Rule 13.2 of PCT). Upon prior art search, it was found that the Lanreotide or its salt, is already known from the prior arts.

The only linking feature in the group of inventions is the Lanreotide or its salt, which is already known.

Hence, here it is considered that the common technical link in the above-mentioned groups is not novel. Therefore, the above-mentioned groups lack a common feature which could be regarded as the special technical feature providing unity to the claims. Consequently, the application may be objected for lacking unity 'a posteriori'.

**INTERNATIONAL SEARCH REPORT**
Information on patent family members

International application No.
PCT/IB2020/051420

| Citation | Pub.Date | Family | Pub.Date |
|---|---|---|---|
| US US6503534 B1 | 07-01-2003 | WO 1999048517 A1 | 30-09-1999 |
| US 20060148699 A1 | 06-07-2006 | WO 2006041945 A2 | 20-04-2006 |
| WO 2006/119388 A2 | 09-11-2006 | US 20060276626 A1 | 07-12-2006 |