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(71) Applicant: **MYLAN LABORATORIES LIMITED** [IN/IN]; Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN).

(72) Inventors: **KUPPANNA, Ananda**; Mylan Laboratories Ltd, Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN). **JAYACHANDRA, Sureshbabu**; Mylan Laboratories Ltd, Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN). **TRIPATHI, Anil Kumar**; Mylan Laboratories Ltd, Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN). **KAMANA, Bulliraju**; Mylan Laboratories Ltd, Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN). **SHANIGARAM, Chandrashekhar**; Mylan Laboratories Ltd, Plot No 564/A/22, Road No 92, Jubilee Hills, Hyderabad 500033 (IN).

(74) Agent: **HASAN, Afzal** et al.; HASAN AND SINGH, No. 04, Sree Nilayam, Plot No. 12, Camelot Layout, Kondapur, Hyderabad 500084 (IN).

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(54) Title: PROCESS FOR THE PREPARATION OF ABALOPARATIDE

(57) Abstract: The present invention relates to an improved process for the preparation of Abaloparatide or pharmaceutically acceptable salts.

PROCESS FOR THE PREPARATION OF ABALOPARATIDE

CROSS-REFERENCE TO RELATED APPLICATIONS:

This application claims the benefit of the filing date of Indian Provisional Application No. 201941012325 filed on March 29, 2019, the contents of which are incorporated herein in their entirety by reference.

FIELD OF THE INVENTION:

The present invention relates to an improved process for the preparation of Abaloparatide or pharmaceutically acceptable salts.

BACKGROUND OF THE INVENTION:

Abaloparatide is used for the treatment of postmenopausal women with osteoporosis under the brand name of TYMLOS, marketed by Radius Health Inc. TYMLOS injection for subcutaneous administration contains abaloparatide, a synthetic 34 amino acid peptide. Abaloparatide is an analog of human parathyroid hormone related peptide, PTHrP(1-34). It has 41% homology to hPTH(1-34) (human parathyroid hormone 1-34) and 76% homology to hPTHrP(1-34) (human parathyroid hormone-related peptide 1-34).

Abaloparatide has a molecular formula of $C_{174}H_{300}N_{56}O_{49}$ and a molecular weight of 3961 daltons with the amino acid sequence shown below:

Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2.

Abaloparatide is disclosed in U.S Patent No. 5,969,095 B2, which is hereby incorporated by reference.

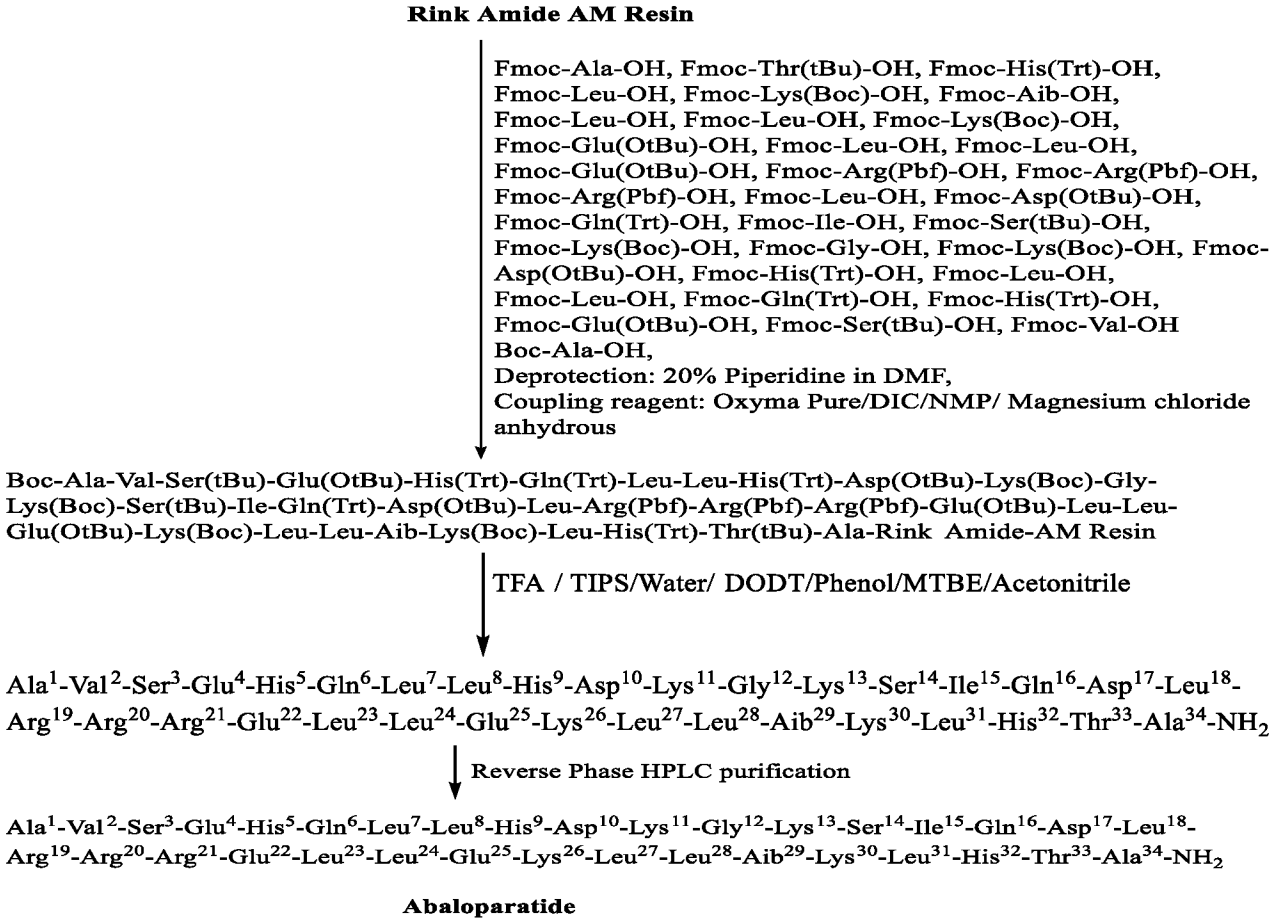
There is no sufficient data available in the literature for the preparation of Abaloparatide such as loading capacity of the resin. Therefore, there exists a need to develop an alternate and improved process for the preparation of Abaloparatide with improved yield and purity. Further the process is simple, convenient and cost-effective for large scale production.

SUMMARY OF THE INVENTION

The present invention relates to an improved process for the preparation of Abaloparatide.

The main aspect of the present invention is to provide an improved process for the preparation of Abaloparatide by solid phase peptide synthesis, followed by purification to get Abaloparatide. The flow chart description of the process is as shown in scheme-1

Scheme-1



DETAILED DESCRIPTION OF THE INVENTION

The main object of the present invention is to provide an improved process for the preparation of Abaloparatide.

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

Abbreviations:

Boc	Di-tert-butyl-dicarbonate
tBu	tert-butyl
DCM	dichloromethane
DMF	N,N'-Dimethylformamide
DIEA	Diisopropylethylamine
DIC	*N,N*-diisopropylcarbodiimide
DODT	2,2′-(ethylenedioxy) diethanethiol
Fmoc	9-fluorenylmethoxycarbonyl
HOBt	N-hydroxybenzotriazole
HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
MTBE	Methyl tert-butyl ether
NMP	N-Methyl-2-pyrrolidone
DVB	Divinylbenzene
Lys	Lysine
Thr	Threonine
TFA	trifluoroacetic acid
TIPS	triisopropylsilane
His	Histidine
Ala	Alanine
Glu	Glutamic acid
Gly	Glycine
Ser	Serine
Asp	Aspartic acid
Val	Valine
Tyr	Tyrosine
Aib	2-Aminoisobutyric acid
Leu	Leucine
Gln	Glutamine
Ile	Isoleucine
Arg	Arginine
Pbf	pentmethyldihydrobenzofuransulfonyl

Trt	Trityl
Oxyma Pure	Ethyl cyano(hydroxyimino)acetate

In yet another embodiment of the present invention is to provide a process for the preparation of Abaloparatide comprising the steps of:

a) anchoring first protected terminal amino acid to a resin;
b) capping the resin obtained in step a);
c) selectively deprotecting the amino group;
d) coupling carboxyl terminus of the next *N*-protected amino acid to the amine group in presence of a coupling reagent;
e) repeating steps c) and d) to form a thirty-four peptide sequence;
f) cleaving the peptide with cocktail mixture from the resin to isolate straight chain of crude Abaloparatide; and
g) purifying by reverse phase HPLC to isolate pure Abaloparatide.

According to the present invention, the resin used for synthesis of peptide undergoes swelling in presence of a solvent selected from dichloromethane and *N*, *N*-dimethylformamide. The resin used is selected from Rink-Amide resin.

The swelled resin is treated with 20% piperidine in DMF to get N-terminal free Rink-Amide-AM resin, subsequently protected amino acid washed in presence of anhydrous magnesium chloride ,NMP, Oxyma Pure, DIC, HOBT, HBTU and DIPEA for a desired period of time to form peptide bond. The solvent used is selected from dichloromethane, tetrahydrofuran, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, *N*-methyl-2-pyrrrolidone or mixtures thereof.

Before proceeding to the next step, the unreacted linkers on the resin (polymer) are protected (capped) to avoid the undesired peptide chain formation. The reagent used for the capping is acetic anhydride, pyridine and dichloromethane.

According to the present invention, the deprotection of the amino acid attached to the resin is done selectively in the presence of a nucleophilic base such as 20% piperidine in presence of a solvent. The solvent used is selected from *N*, *N*-dimethylformamide, methylene chloride, tetrahydrofuran, *N*-methyl pyrrolidine or mixture thereof.

According to the present invention, selectively deprotected the amino group is coupled with next *N*-protected amino acid in a solvent in presence of a coupling reagent. The solvent used for the coupling reaction is selected from dichloromethane, tetrahydrofuran, dimethylformamide, *N*-methylpyrolidone or mixture thereof. The coupling agent used for the coupling of the amino acids is selected from Oxyma pure, HBTU, HOBt and DIEA.

According to the present invention, the resin cleavage and global deprotection (a process for deprotecting the protected amino acid in the peptide, which has additional functional groups) of the peptide is carried out with a cocktail mixture. The cleavage of the peptide from the resin involves treating the protected peptide anchored to the resin with an acid having at least one scavenger. The acid utilized in the cleavage reagent is TFA. The amount of TFA used for the purpose of cleavage of peptide from the resin and global deprotection in the cocktail mixture may range from 80-90%. The scavengers used are selected from TIPS, phenol, thioanisole, water, DODT or mixtures thereof. The particular cocktail mixture used for the cleavage of the peptide from resin is 90% TFA, 5% water, 5% TIPS and DODT.

The two cocktail mixture for the cleavage of the peptide from resin are disclosed in the prior art and they are.

a) TFA/EDT/Thioanisole/DCM/TIPS in the ratio of about 80%/5%/5%/5%/5% or 80%/5%/3.5%/3.5%/8% respectively and

b) TFA/phenol/Thioanisole/DCM/TIPS/H20 in the preferred volumes of 80%/5%/3.33%/3.33%/5%/3.33% respectively or 80%/5%/3.5%/3.5%/8% respectively.

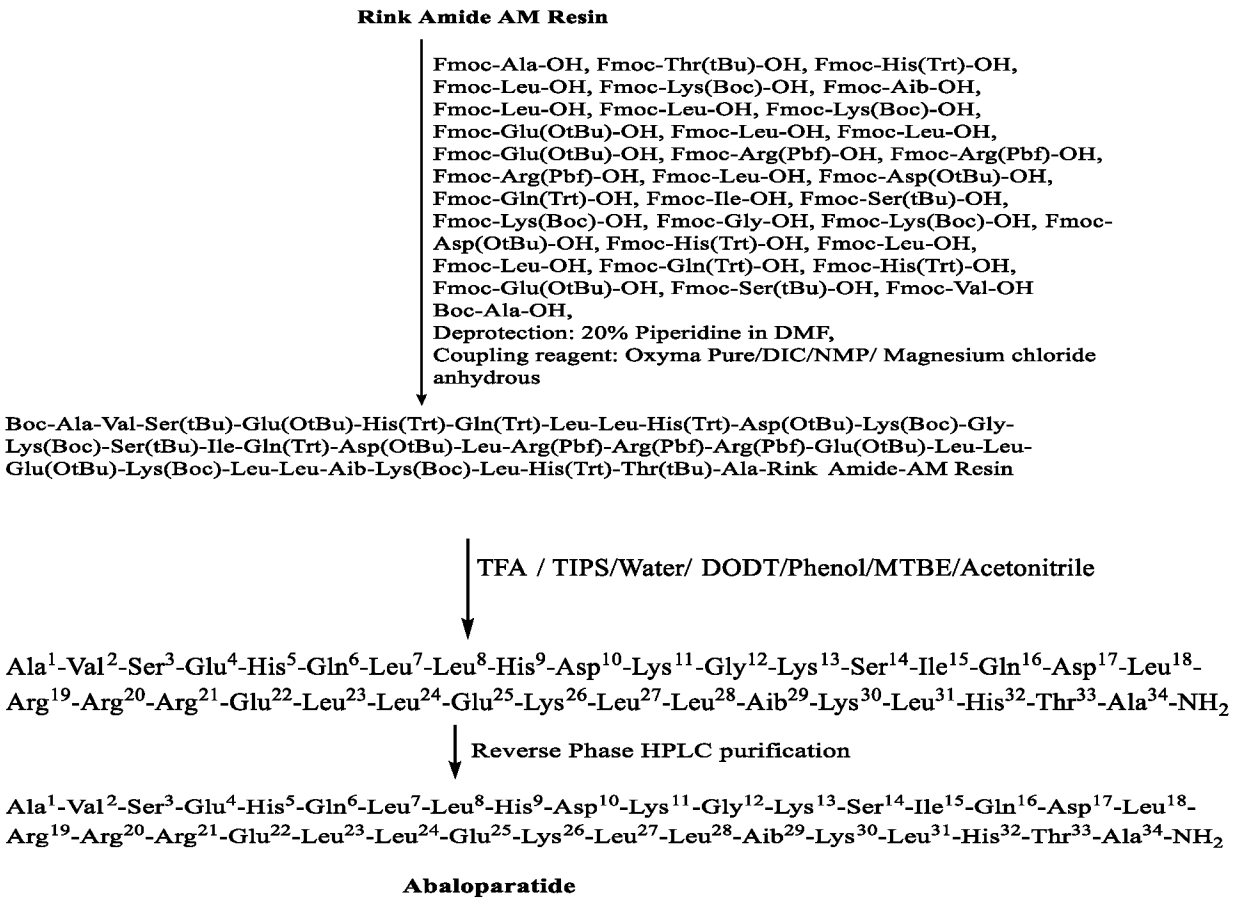
However, it does not disclose the use of the above conditions for the preparation of abaloparatide.

According to the present invention, the resin after the completion of the reaction is optionally washed with solvents such as DMF and DCM to remove residual reagents and byproducts. The process is repeated if desired and before proceeding to next step.

According to present invention, the isolation of Abaloparatide is carried out by precipitating with ether solvent to get Abaloparatide as a solid. Ether solvents that are used for precipitation is selected from methyl tert-butyl ether, diethyl ether, t-butyl methyl ether, isopropyl ether or mixtures thereof.

The process for the preparation of abaloparatide is summarized in synthetic scheme-1 depicted below.

Scheme-1



According to the present invention, solid phase of peptide synthesis for preparation of protected Abaloparatide-Rink Amide AM resin, the loading of first protected amino acid (Fmoc-Ala-OH) to Rink amide resin plays a significant role. It has been observed that when the solid phase peptide synthesis reaction has carried out loading of first protected amino acid with matrix about 0.50 to 0.6 mmol/g, then the crude purity of Abaloparatide was about 50% only. However, when loading of first protected amino acid with matrix about 0.35 to 0.45 mmol/g was used, the crude purity was obtained about 70-73%. The results are summarized in the following table.

The Rink amide AM resin used for the peptide synthesis and their loading capacity are listed below.

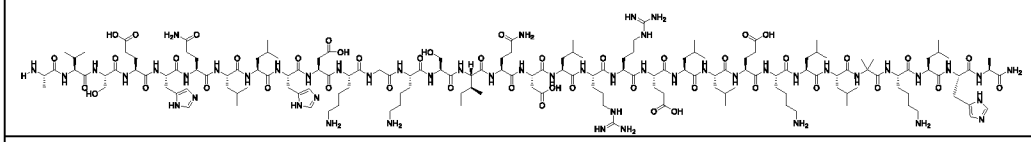
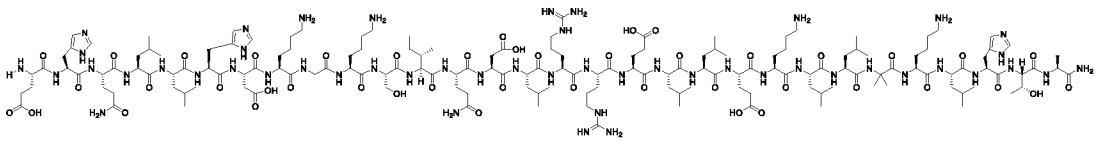
Resin	Matrix	Loading	Purity
Rink amide AM resin	Amino methyl polystyrene crosslinked with 1% DVB	0.52mmol/g	53.0

Rink amide AM resin	Amino methyl polystyrene crosslinked with 1% DVB	0.40mmol/g	71.0
Rink amide AM resin	Amino methyl polystyrene crosslinked with 1% DVB	0.40mmol/g	73.0

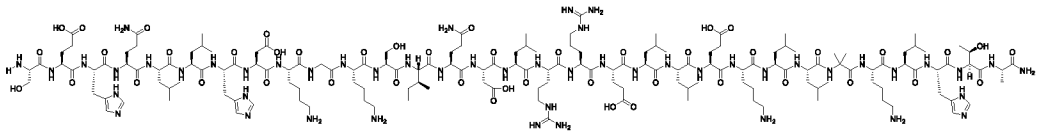
The resin used for the preparation of Abaloparatide as support material is Rink amide AM resin (0.40mmol/g). The selection of polymeric support and attached linker is very critical for overall outcome of the solid phase peptide synthesis. Rink amide AM resin is found to be very effective for the preparation of Abaloparatide and are comprising of grafted copolymers consisting of a low cross-linked polystyrene, but the loading capacity is 0.40 mmol/g.

The Rink amide AM resin is used in the process of Abaloparatide in low load in 0.40 mmol/g with amino methyl polystyrene type linker has advantages over with high loading in 0.52 mmol/g in achieving the better purity. The advantage of using low load resin is that significantly more peptide for unit measure of beads could be produced. This is a consequence of the fact that higher concentrations of reagents and reactants can be achieved with low load resins. Smaller vessel sizes could be employed to generate a given amount of peptide and at least 50% less wash solvents needed while using low loaded resins. For the scale up of the solid phase attractive it is important to reduce the large amounts of reagents typically employed in solid phase peptide synthesis.

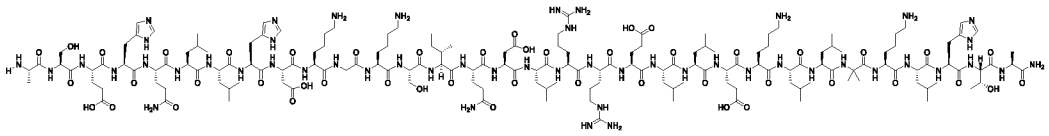
Yet another embodiment of the present invention is to provide a process for the preparation of Abaloparatide with reduced level of below listed impurities.

Impurity: Name and Structure
Des-Thr^{33}-Abaloparatide: Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-ArgArg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Ala-NH_2 
(4-34)-Abaloparatide: Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp -Leu-Arg-ArgArg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2 

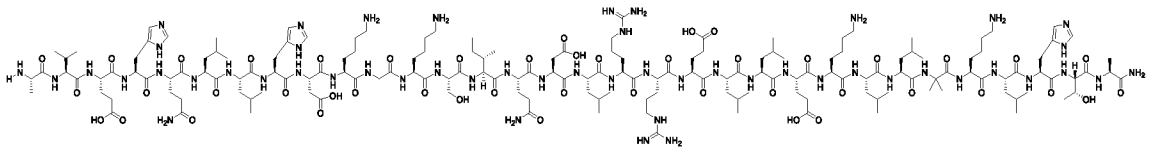
(3-34)-Abaloparatide: Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-ArgArg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2



Des-Val^2-Abaloparatide: Ala-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-ArgArg-Glu-Leu-Leu-Glu-Lys -Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2



Des-Ser^3-Abaloparatide: Ala-Val-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-ArgArg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2



Yet another embodiment of the present invention is to provide a process for the preparation of Abaloparatide with HPLC purity of at least 99.0%.

Yet another embodiment of the present invention is to provide, Abaloparatide containing low levels of impurities selected from des-Thr^{33}-Abaloparatide, (4-34)-Abaloparatide, (3-34)-Abaloparatide, des-Val^2-Abaloparatide and Des-Ser^3-Abaloparatide.

Certain embodiment of the present invention is to provide Abaloparatide contains less than 0.10% Des-Thr^{33}-Abaloparatide impurity.

Certain embodiment of the present invention is to provide Abaloparatide contains less than 0.20% (4-34)-Abaloparatide impurity.

Certain embodiment of the present invention is to provide Abaloparatide contains less than 0.10% (3-34)-Abaloparatide impurity.

Certain embodiment of the present invention is to provide Abaloparatide contains less than 0.10% Des-Val^2-Abaloparatide impurity.

Certain embodiment of the present invention is to provide Abaloparatide contains less than 0.10% Des-Ser^3-Abaloparatide impurity.

Certain embodiment of the present invention is to provide a pharmaceutical composition comprising Abaloparatide and pharmaceutically acceptable carrier.

The following example is provided to illustrate the process of the present invention. However, they are not intended to limit the scope of an invention.

Examples:

Example-1: Preparation of Protected Abaloparatide-Rink Amide Resin

In a clean dry peptide synthesizer vessel charged 100 g of Rink-Amide AM Resin and 1000 ml of DCM. Allowed the resin to swell for 1 hr. Drain the solvent. Deprotect the Fmoc group by using 20% piperidine (2×1000 ml) followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. Dissolve the 1.5 eq. of Fmoc-Ala-OH, 1.5 eq. of 1-Hydroxybenzotriazole (HOBT) anhydrous in 1000 ml of *N*-Methyl-2-pyrrolidone (NMP) at temp. 20-25°C and added 2.0 eq. of *N,N*-diisopropyl carbodiimide (DIC). Charged the above solution into peptide synthesizer and stirred the reaction mass for 2 to 3 hrs. at temp. 20-25°C. Drain the solvent and resin was washed with 3×1000 ml of DMF followed by 1×1000 ml of DCM. Checked the loading of first amino acid. (Limit: 0.3 to 0.7mmol/g). To end cap the resin, prepared a mixture of 880 ml of (DCM), 60 ml of acetic anhydride and 60 ml of pyridine and charged the above solution into peptide synthesizer vessel. Stirred the mass for 1 hr. Drain the solvent and washed the resin with 3×1000 ml of DMF followed by 1×1000 ml of DCM. Deprotect the Fmoc group by using 20% piperidine in DMF (2×1000 ml) followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. The Fmoc deprotection was monitored by using Kaiser Test. Continue the coupling by dissolving the 2.0 eq. of Fmoc-Thr(tBu)-OH, 2.0 eq. of 1-Hydroxybenzotriazole (HOBT) anhydrous, 2.0 eq. *N, N, N', N'*-Tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate (HBTU) in 1000 ml of *N*-Methyl-2-pyrrolidone (NMP) at temp. 20-25°C to get a clear solution followed by addition of 2.0 eq. of *N,N*-diisopropyl ethyl amine (DIPEA). Charged the above solution into peptide synthesizer vessel and stirred the reaction mass for 2 to 3 hrs. at temp. 20-25°C. The progress of reaction was monitored by using Kaiser test. After completion of reaction, drain the solvent and resin was washed with 1000 ml of DMF. The Fmoc was deprotected by using 20% piperidine in DMF (2×1000 ml) followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. After deprotection of Fmoc group, the coupling sequence of subsequent amino acids, i.e. Fmoc-Thr(tBu)-OH (2.0 eq.), Fmoc-His(Trt)-OH (2.0 eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Lys(Boc)-OH (2.0 eq.), Fmoc-

Aib-OH (4eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Lys(Boc)-OH (2.0 eq.), Fmoc-Glu(OtBu)-OH(2.0 eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Glu(OtBu)-OH (2.0 eq.), Fmoc-Arg(Pbf)-OH (2.0 eq.), Fmoc- Arg(Pbf)-OH (2.0 eq.), Fmoc-Arg(Pbf)-OH (2.0 eq.), Fmoc-Leu-OH (2.0 eq.), Fmoc-Asp(OtBu)-OH (2.0 eq.), Fmoc-Gln(Trt)-OH (2.0 eq.), Fmoc-Ile-OH (2.0 eq.), Fmoc-Ser(tBu)-OH (3.0 eq.), Fmoc-Lys(Boc)-OH (3.0 eq.), Fmoc-Gly-OH (3.0 eq.), Fmoc-Lys(Boc)-OH (3.0 eq.), Fmoc-Asp(OtBu)-OH (3.0 eq.), Fmoc-His(Trt)-OH (3.0 eq.), Fmoc-Leu-OH (3.0 eq.), Fmoc-Leu-OH (3.0 eq.), Fmoc-Gln(Trt)-OH (3.0 eq.), Fmoc-His(Trt)-OH (3.0 eq.), Fmoc-Glu(OtBu)-OH (3.0 eq.), Fmoc-Ser(tBu)-OH (3.0 eq.), Fmoc-Val-OH (3.0 eq.) and Boc-Ala-OH(3.0 eq.) were performed. The coupling reagent was used as 1-Hydroxybenzotriazole (HOBT) anhydrous, *N, N, N', N'*-Tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate (HBTU), *N,N*-diisopropyl ethyl amine (DIPEA) as base and *N*-Methyl-2-pyrrolidone (NMP) as a solvent. The deprotection of Fmoc was carried out using 20% piperidine in DMF as a solvent. After completion of synthesis washed the resin with methanol (2×1000 ml) followed by MTBE (2 ×1000 ml). Finally, the resin containing the peptide was dried under vacuum tray dryer for 2 hrs., temp.40-45°C. Wt. of Protected Abaloparatide Resin: 300 g

Molar yield: 82.6%

Example 2: Preparation of Crude Abaloparatide

In a round bottom flask charged 800 ml of trifluoroacetic acid (TFA), 80 ml of triisopropylsilane (TIPS), 80 ml of 2,2'-(ethylenedioxy) diethanethiol (DODT) and 50 ml of water. Cooled the mixture to 0 to 10ºC. Slowly added the Protected Abaloparatide 100 g over a period for 5 min. Stir the reaction mass for 30 min at temp. 0 to 10ºC. Raised the temperature of reaction mass to 20-25ºC and stirred the reaction mass for 3 hrs. Filter the reaction mass and keep the mother liquor aside. In another round bottom flask charged 3000 ml of MTBE and cooled the mass to 0 to -10ºC. Slowly added the TFA solution (mother liquor) into cooled MTBE solution over a period of 30 min. at temp. 0 to -10 ºC. The product was precipitate out. Maintain the temperature of reaction mass 0 to -10ºC for 30 min. Filtered the reaction mass and washed the cake with 3×1000 ml MTBE. Charged the wet cake into flask and added 600 ml of ethyl acetate and 30 ml of acetic acid. Stirred the mass for 12 hrs. The product was isolated by filtration and washed the cake with 100 ml of ethyl acetate. Dry the product under reduced pressure at temperature 40-45ºC. Dry weight of Crude Abaloparatide: 36 g

Threotical yield: 39 g

Molar yield: 78.0%

Purity: 70%

Impurity content:

Des-Thr^{33}-Abaloparatide:0.60%.

(4-34)-Abaloparatide:1.2 %.

(3-34)-Abaloparatide:1.1%.

Des-Val^{2}-Abaloparatide:1.1%.

Des-Ser^{3}-Abaloparatide:1.2%.

Example-3: Preparation of Protected Abaloparatide-Rink Amide Resin

In a clean dry peptide synthesizer vessel charged 250 g of Rink-Amide AM Resin (vendor loading 0.3 to 0.7 mmol/g) and 1000 ml of DCM. Allowed the resin to swell for 1 hr. Filter the solvent. Deprotect the Fmoc group by using mixture of 20% piperidine (2×1000 ml) at temp. 25±5ºC followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. Mix 31.1 g (0.4 eq.) of Fmoc-Ala-OH, 21.3 g (1.5 eq.) of oxyma pure, 78 ml (5.0 eq.) of DIC (isopropyl carbodiimide) in 1000 ml of *N*-Methyl-2-pyrrolidone (NMP) at temp. 20-25°C under nitrogen atmosphere. Charged the above solution into peptide synthesizer and stirred the reaction mass for 4 to 5 hrs. at temp. 20-25°C. Filter the mass and washed the resin with 3×1000 ml of DMF followed by 1×1000 ml of DCM. The loading of first amino acid was 0.4 mmol/g (Limit: 0.3 to 0.5 mmol/g). Caped the active site of Rink Amide AM resin using a mixture of 1000 ml of (DCM), 60 ml of acetic anhydride and 60 ml of pyridine at temp. 25±5ºC. Charged the above solution into peptide synthesizer vessel. Stirred the mass for 1 hr. at temp. 20-25°C. Filter the solvent and repeat the capping operation twice. After filtration of solvent, washed the resin with 3×1000 ml of DMF followed by 1×1000 ml of DCM. Check the Kaiser test (Colorless of beads). Deprotect the Fmoc group by using mixture of 20% piperidine (2×1000 ml) and 1% (w/v) of HOBT at temp. 25±5ºC followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. The Fmoc deprotection was monitored by Kaiser Test. Continue the coupling with subsequent protected amino acids (typically 2 to 6 mol. eq.), coupling reagent Oxyma Pure /DIC typically 2 to 6 eq., additive 250 mg of anhydrous magnesium chloride and NMP was used as a solvent. When the coupling of protected amino acid is not complete, repeated the coupling reaction. The reaction was monitored by Kaiser test. Still if reaction is not complete, capped the unreacted site of peptide using acetic anhydride/ pyridine and DCM was used as a solvent. The coupling reaction of subsequent amino acids, i.e. Fmoc-Thr(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys (Boc)-OH, Fmoc-Aib-OH, Fmoc-Leu-OH, Fmoc-Leu-

OH (OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln (Trt)-OH, Fmoc-Ile-OH, Fmoc-Ser(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH and Boc-Ala-OH were performed. Typically coupling reaction has been performed at temperature 36-40°C. For Fmoc-His (Trt)-OH the coupling reaction has been performed at ambient temperature. The Fmoc was deprotected by stirring the resin with mixture of 20% piperidine in DMF (2×1000 ml) and 1% (w/v) of HOBT for 15 min. at temp. 25±5ºC followed by washing the resin with 3×1000 ml of DMF and 1×1000 ml of DCM. After completion of synthesis washed the resin with DMF (2×1000 ml) and 1000 ml of DCM followed by MTBE (2×1000 ml). Finally, the resin containing the peptide was dried under vacuum tray dryer for 3 to 4 hrs., temp. not more than 40-45°C.

Wt. of Protected Abaloparatide Resin: 780 g

Molar yield: 86.6%

Example-4: Preparation of Crude Abaloparatide

In a round bottom flask charged 800 ml of trifluoroacetic acid (TFA), 80 ml of triisopropylsilane (TIPS), 50 ml of phenol, 80 ml of 2,2′-(ethylenedioxy) diethanethiol (DODT) and 50 ml of water. Cooled the mixture to 0 to 10ºC. Slowly added the Protected Abaloparatide 100 g over a period for 5 min. at 0 to 10ºC. Stir the reaction mass for 30 min at temp. 0 to 10ºC. Raised the temperature of reaction mass to 20-25ºC and stirred the reaction mass for 3 to 4 hrs. Filter the reaction mass and keep the mother liquor aside. In another round bottom flask charged 3000 ml of MTBE and cooled the mass -10 to -20ºC. Slowly added the TFA solution (mother liquor) into cooled MTBE solution over a period of 30 min. at temp. 0 to -10ºC. The product was precipitate out. Maintain the temperature of reaction mass -0 to -10ºC for 30 min. Filtered the reaction mass and washed the cake with 3×1000 ml of MTBE. Charged the wet cake into flask and charge 600 ml of acetonitrile. Stirred the mass for about 2 hrs. The product was isolated by filtration and washed the cake with 500 ml of MTBE. Dry the product under reduced pressure at temperature 25-35ºC.

Weight of crude Abaloparatide: 39 g

Molar yield: 85.2%

Purity: 70%

Impurity content:

Des-Thr^{33}-Abaloparatide:0.50%.

(4-34)-Abaloparatide:1.0 %.

(3-34)-Abaloparatide:1.0%.

Des-Val^{2}-Abaloparatide:1.0%.

Des-Ser^{3}-Abaloparatide:1.0%.

Example-5: Purification of Abaloparatide

The purification process is accomplished by preparative HPLC using reverse phase C18 material as the support. Preparation of the column (equilibration) is accomplished by washing with 0.1%

aqueous TFA to avoid elution of the product in the loading phase. The peptide is loaded onto the column and eluted with mobile phase gradient buffers and the organic modifier acetonitrile. The fractions are monitored by analytical UPLC as they elute from the column. The purification process for Abaloparatide is divided into three stages. The first stage is purification of the crude peptide using gradient elution buffers consisting of 0.1% TFA and acetonitrile. The second stage is purification of the first stage fractions with peptide purity ≥ 97% (main pool) using 0.10% acetic acid and acetonitrile. Third stage is salt exchange using 0.1M ammonium acetate (NH_4OAc) buffer and gradient elution buffers consisting of 0.05% acetic acid and acetonitrile while simultaneously converting the peptide into the acetate salt form. Upon completion of the final purification stage, fractions that meet the purity criteria of ≥ 98.0% are lyophilized and the yield of the purified peptide is determined and recorded. By using 100 g of crude Abaloparatide 10g of purified Abaloparatide was obtained.

Yield: 10 g

Molar yield: 10.0%

Purity: 99.5%

Impurity content:

Des-Thr^{33}-Abaloparatide:0.10%

(4-34)-Abaloparatide:0.20%

(3-34)-Abaloparatide:0.10%

Des-Val^{2}-Abaloparatide:0.10%.

Des-Ser^{3}-Abaloparatide:0.10%.

We claim:

1. A process for the preparation of Abaloparatide comprising the steps of:
    a) anchoring first protected terminal amino acid to a resin,
    b) capping the resin obtained in step a),
    c) selectively deprotecting the amino group,
    d) coupling carboxyl terminus of the next N-protected amino acid to the amine group in presence of a coupling reagent,
    e) repeating steps c) and d) to form a thirty-four peptide sequence,
    f) cleaving the peptide with cocktail mixture from the resin to isolate straight chain of crude Abaloparatide, and
    g) purifying by reverse phase HPLC to isolate pure Abaloparatide.

2. The process according to claim 1, wherein resin is selected from Rink-Amide AM resin.

3. The process according to claim 1, wherein the coupling agent used for the coupling of the amino acids is selected from O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 1-Hydroxybenzotriazole (HOBt), ethyl cyano(hydroxyimino)acetate (Oxyma Pure) and Diisopropylethylamine (DIEA).

4. The process according to claim 1, isolation of Abaloparatide is carried out by precipitating with ether solvent selected from methyl tert-butyl ether, diethyl ether, t-butyl methyl ether, isopropyl ether or mixtures thereof.

5. The process according to claims 1-4, wherein the obtained Abaloparatide having a purity of at least 99.0%.

6. The process according to claims 1-5, wherein the obtained Abaloparatide containing low levels of impurities selected from des-Thr^{33}-Abaloparatide, (4-34)-Abaloparatide, (3-34)-Abaloparatide, des-Val^{2}-Abaloparatide and Des-Ser^{3}-Abaloparatide.

7. The process according to claims 1-5, wherein Abaloparatide contains less than 0.10% Des-Thr^{33}-Abaloparatide.

8. The process according to claims 1-5, wherein Abaloparatide contains less than 0.20% (4-34)-Abaloparatide.

9. The process according to claims 1-5, wherein Abaloparatide contains less than 0.10% (3-34)-Abaloparatide.

10. The process according to claims 1-5, wherein Abaloparatide contains less than 0.10% Des-Val^{2}-Abaloparatide.

11. The process according to claims 1-5, wherein Abaloparatide contains less than 0.10% Des-Ser^{3}-Abaloparatide.

12. Abaloparatide contains the impurities less than 0.10% Des-Thr^{33}-Abaloparatide, less than 0.20% (4-34)-Abaloparatide, less than 0.10% (3-34)-Abaloparatide, less than 0.10% Des-Val^{2}-Abaloparatide and less than 0.10% Des-Ser^{3}-Abaloparatide.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2020/050277

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/635
ADD.

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

B. FIELDS SEARCHED

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

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 969 095 A (DONG ZHENG XIN [US]) 19 October 1999 (1999-10-19) cited in the application column 9; claim 1	1-12
Y	CN 106 146 648 A (SHENZHEN HYBIO PHARMACEUTICAL) 23 November 2016 (2016-11-23) embodiments 1,7; paragraph [0058] - paragraph [0061]; claims 1-8; table 2	1-12

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

[X] 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

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Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2020	21/09/2020

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Unknown: "Assessment report Eladynos International non-proprietary name: abaloparatide", , 26 July 2018 (2018-07-26), XP055729703, Retrieved from the Internet: URL:https://www.ema.europa.eu/en/documents/assessment-report/eladynos-epar-refusal-public-assessment-report_en.pdf [retrieved on 2020-09-10] pages 12-16,22	1-12
X,P	WO 2019/175173 A1 (FRESENIUS KABI IPSUM S R L [IT]) 19 September 2019 (2019-09-19) claims 1-12	1-12
X,P	CN 109 734 794 A (UNIV SUZHOU SCI & TECHNOLOGY) 10 May 2019 (2019-05-10) claims 1-10; example 5	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2020/050277

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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CN 106146648 A	23-11-2016	NONE	
WO 2019175173 A1	19-09-2019	NONE	
CN 109734794 A	10-05-2019	NONE	