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(54) **Title:** PROCESS FOR THE PREPARATION OF LIRAGLUTIDE

(57) **Abstract:** The present invention relates to an improved process for the synthesis of Iiraglutide or pharmaceutically acceptable salts by hybrid approach. The fragments are synthesized by solid phase synthesis approach and the Iiraglutide is synthesized by making use of the fragments in solution phase.



“PROCESS FOR THE PREPARATION OF LIRAGLUTIDE”

FIELD OF THE INVENTION:

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

BACK GROUND OF THE INVENTION:

- 10 Liraglutide, a glucagon-like peptide -1 (G LP-1) receptor agonist, as a subcutaneous formulation, can play a good role in lowering blood glucose. Liraglutide is marketed under the brand name VICTOZA® in the U.S, India, Canada, Europe and Japan. The peptide sequence of liraglutide can be represented in terms of chemical formula (I) as follows:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys(Glu-palmitoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH.

15 **Formula (I)**

Liraglutide was prepared mainly through genetic engineering and other biological methods of preparation, which involves technical difficulties, high production costs and is not conducive to large-scale production.

- 20 Liraglutide is first disclosed in U.S. Patent No. 6,268,343, in which Liraglutide is prepared by solid-liquid synthetic method. The intermediate GLP-I (7-37)-OH is purified by reverse phase HPLC purification and then reacted with N^α-hexadecanoyl-Glu (ONSu)-OtBu under liquid phase condition to get liraglutide. The deprotection of side chain causes a lot of impurities and its purification is difficult due to the long chain peptide.

- 25 PCT Publication No. WO2013037266A1 discloses solid phase synthetic method by 2-CTC resin or Wang resin. According to this method, the liraglutide main chain peptide sequence is prepared by coupling N terminal Fmoc protected and side chain protected amino acids, including lysine Fmoc-Lys (Alloc)-OH; removal of Alloc protecting group of lysine side chain and coupling with Palmitoyl-Glu-OtBu; followed by deprotection and cleavage of resin to obtain crude liraglutide and further purification to get pure liraglutide.

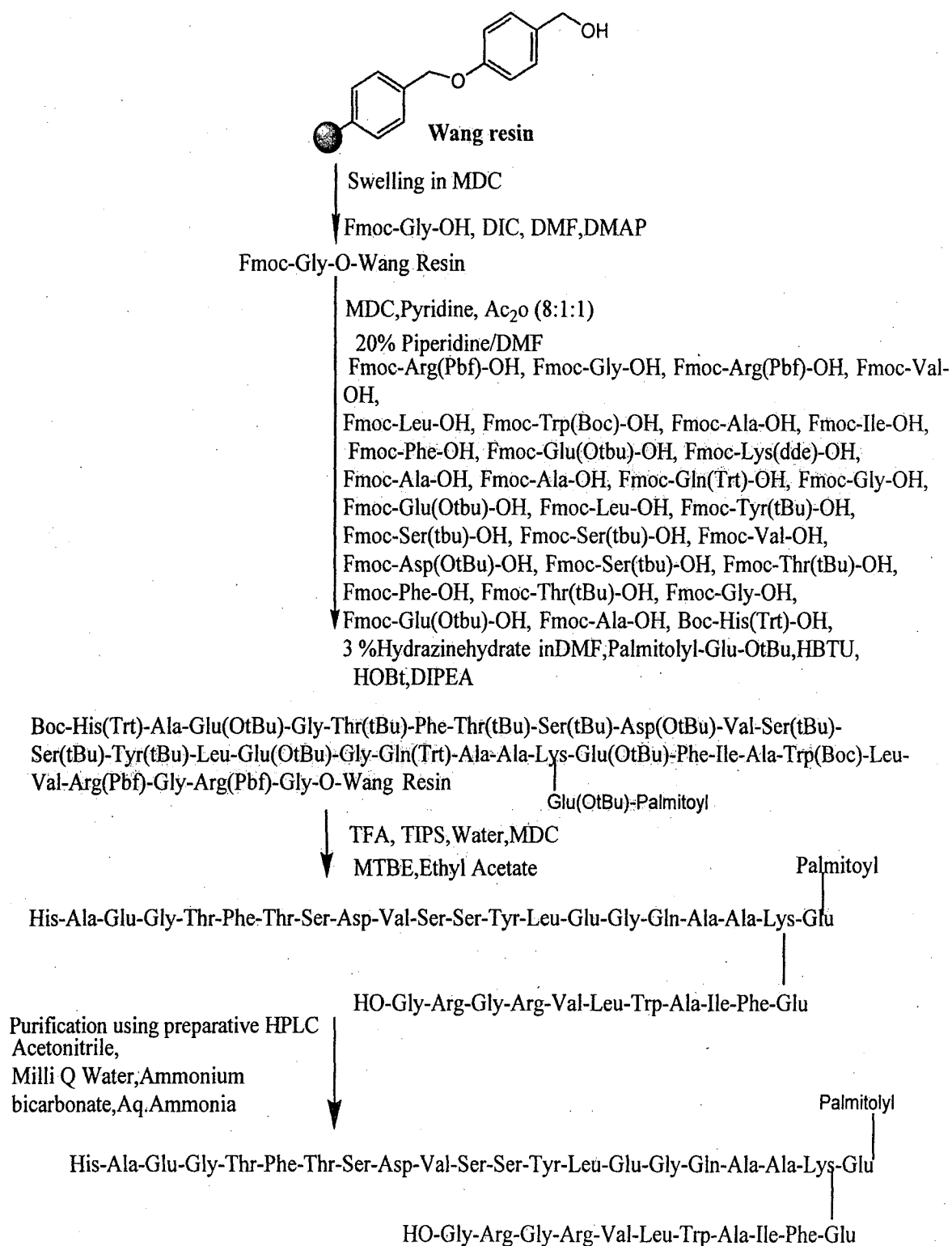
SUMMARY OF THE INVENTION

5 The present invention relates to an improved process for the preparation of liraglutide by a hybrid approach.

The main aspect of the present invention relates to an improved process for obtaining liraglutide by means of solid phase synthesis using Wang resin. The process will involve the coupling of appropriate protected amino acids in a required sequence, cleavage and
10 deprotection, followed by purification to get liraglutide. The schematic description of the process is as shown in scheme-1.

Yet another aspect of the present invention is to provide an improved process for the preparation of liraglutide by coupling appropriate fragments in a required sequence,
15 deprotection and condensing them in solution phase, followed by purification to get liraglutide. The schematic description of the process is as shown in scheme-2.

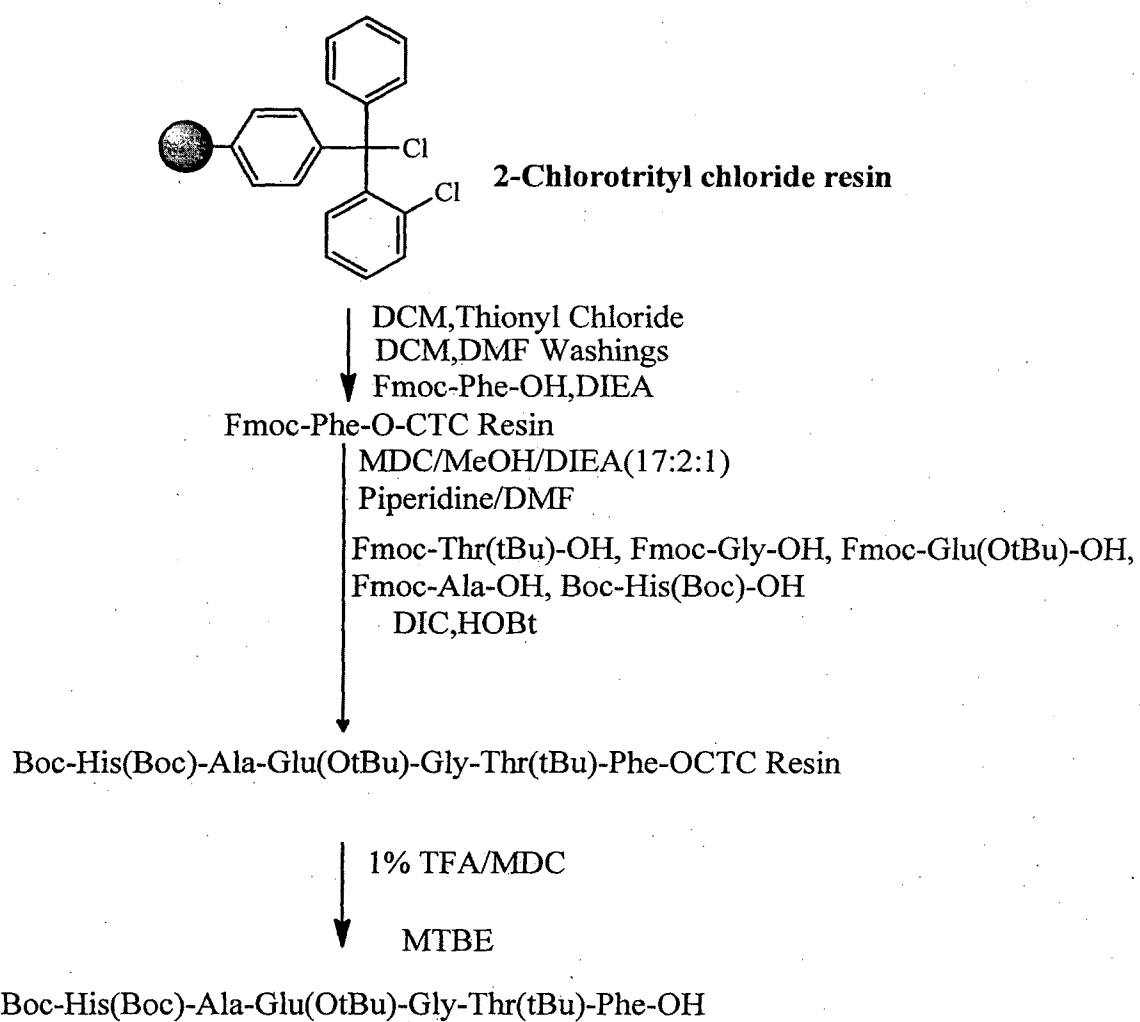
Scheme-1



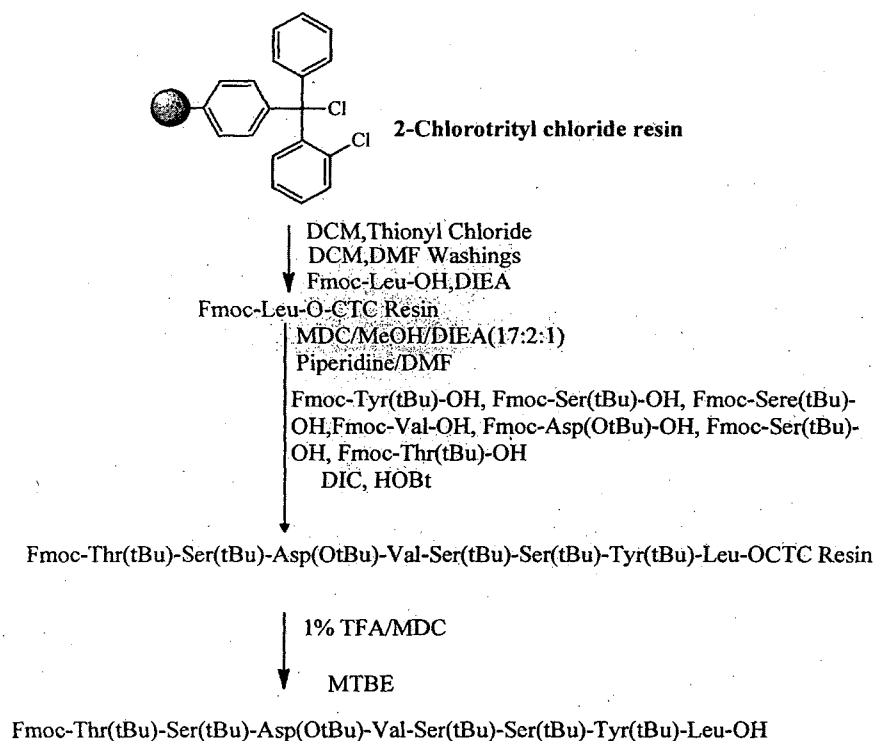
Scheme-2

5

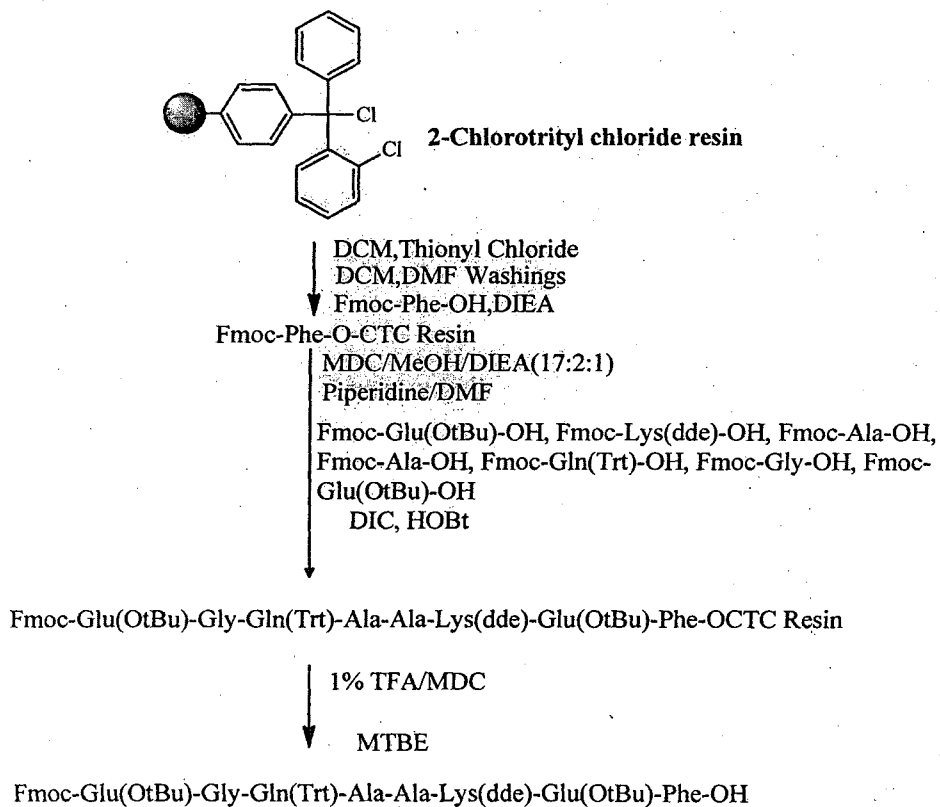
Synthesis of Fragment-1



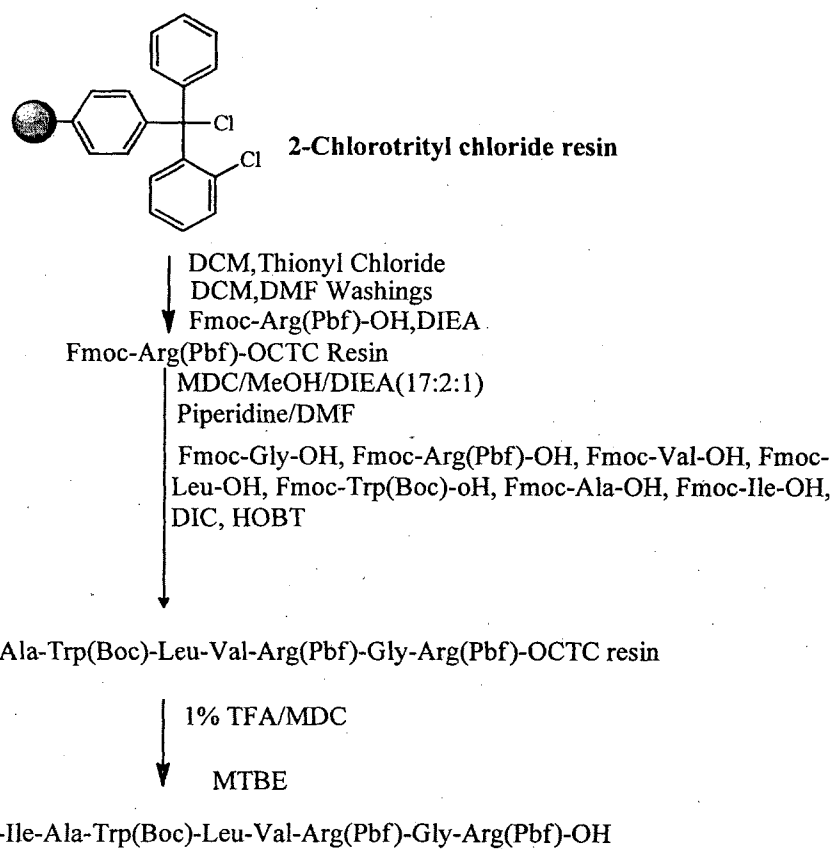
10 Synthesis of Fragment-2



Synthesis of Fragment-3

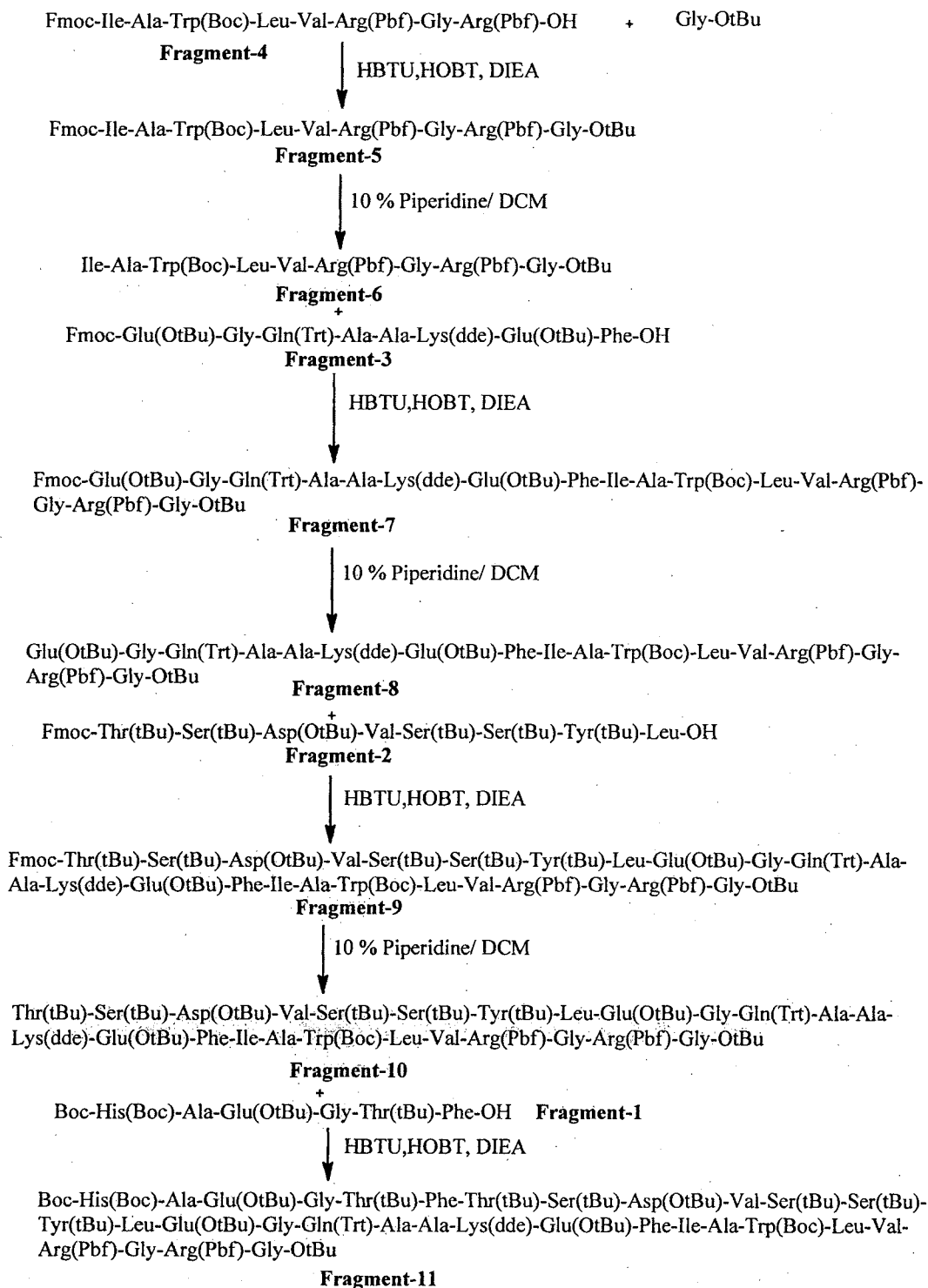


Synthesis of Fragment-4



Synthesis of Liraglutide

10



Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-
Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(dde)-Glu(OtBu)-Phe-Ile-Ala-
Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-OtBu

Fragment-11

3% Hydrazinehydrate in DMF
Palmitoyl-Glu-OtBu, HBTU, HOBT

Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-
Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(Palmitoyl-Glu(OtBu))-Glu(OtBu)-Phe-
Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-OtBu Protected Peptide

TFA, TIPS, Water 90:5:5%

Purification using HPLC

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys(Glu-
Palmitoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

Liraglutide

DETAILED DESCRIPTION OF THE INVENTION

- 5 The present invention relates to an improved process for the preparation of liraglutide by making appropriate fragments using a solid and solution phase (hybrid) approach. The present invention also provides a process for the preparation of liraglutide by the solid phase method.

10 **Abbreviations:**

	Boc	Di-tert-butyl-dicarbonate
	tBu	tert-butyl
	DCM	dichloromethane
15	DIC	N,N'-diisopropylcarbodiimide
	DMF	N,N'-Dimethylformamide
	DMAP	Dimethyl amino pyrimidine
	DIEA	Diisopropylethylamine
	2-CTC resin	2-Chlorotriyl chloride resin
20	Fmoc	9-fluorenylmethoxycarbonyl
	HOBT	N-hydroxybenzotriazole
	HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-

		hexafluoro-phosphate
	MTBE	Methyl tert-butyl ether
	SPPS	solid phase peptide synthesis
	Phe	Phenylalanine
5	Trp	Tryptophan
	Lys	Lysine
	Thr	Threonine
	TFA	trifluoroacetic acid
	TIPS	triisopropylsilane
10	HIS	Histidine
	Ala	Alanine
	Glu	Glutamic acid
	Gly	Glycine
	Ser	Serine
15	Asp	Aspartic acid
	Val	Valine
	Tyr	Tyrosine
	Leu	Leucine
	Gln	Glutamine
20	Ile	Isoleucine
	Arg	Arginine
	Pbf	pentmethyldihydrobenzofuransulfonyl
	Trt	Trityl
25	Dde	4,4-dimethyl-2,6-dioxocyclohex-1-ylidene

One embodiment of the present invention is to provide a process for the preparation of liraglutide comprising the steps of:

- a. anchoring thirty first protected terminal amino acids to a resin,
- b. capping the resin obtained in step a),
- 30 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 peptide sequence,
- f. removal of the lysine side chain protecting group dde, followed by coupling with
- 35 Palmitoyl-Glu-OtBu,

- g. cleaving the peptide with a cocktail mixture from the resin to isolate the straight chain of crude liraglutide, and
- h. purifying by preparative HPLC to isolate pure liraglutide.

5 According to the present invention, the resin used for synthesis of peptide undergoes swelling in presence of a solvent selected from dichloromethane, *N, N*-dimethylformamide, and *N*-methyl-2-pyrrolidone or mixtures. The resin used is selected from Wang resin or 2-chlorotriyl chloride resin.

10 The swelled resin is treated with *N*-terminus protected amino acids in the presence of DIC, HOBt and DMAP for a desired period of time for the esterification of the Wang resin. The solvent used is selected from dichloromethane, tetrahydrofuran, *N, N*-dimethylformamide, *N, N*-dimethylacetamide, *N*-methyl-2-pyrrolidone or mixtures thereof.

15 Before proceeding to the next step, the unreacted linkers on the resin (polymer) are protected (capped) to avoid the undesired peptide chain formation. The capping is carried out in the presence of 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 the
20 presence of a solvent. The solvent used is selected from *N, N*-dimethylformamide, methylene chloride, tetrahydrofuran, *N*-methyl pyrrolidine or a mixture thereof.

Removal of the dde protecting group from the lysine side chain is then carried out selectively in the presence of 3% hydrazine hydrate and DMF.

25 The liraglutide main chain peptide sequential coupling having the N terminal Fmoc protected and side chain protected amino acids, including lysine Fmoc-Lys (dde)-OH, then undergoes the removal of the dde protecting group from the lysine side chain, followed by the coupling with Palmitoyl-Glu-OtBu to give linear protected liraglutide. This sequential linear peptide is
30 cleaved, deprotected and purified to give liraglutide.

The selectively deprotected amino group is then coupled with next *N*-protected amino acid in a solvent in the presence of a coupling reagent. The solvent used for the coupling reaction is selected from dichloromethane, tetrahydrofuran, dimethylformamide, *N*-methylpyrrolidone or
35 a mixture thereof. The coupling agent used for the coupling of the amino acids is selected from DIC/6-Cl-HOBt, DIC/HOBt, HBTU/HOBt/DIEA or DIC/Oxyma.

According to the present invention, the 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 the 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 or mixtures thereof. In a preferred embodiment, the cocktail mixture used for the cleavage of the peptide from resin is 90% TFA, 5% water, 5% TIPS.

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 before proceeding to the next step.

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

Another embodiment of the present invention is to provide a process for obtaining liraglutide as shown in scheme-2, comprising the steps of:

- a) condensing Fragment 4 with Gly-OtBu in the presence of a coupling agent to give Fragment 5; followed by deprotection of Fmoc to get Fragment 6;
- b) condensing Fragment 6 with Fragment 3 in the presence of a coupling agent to give Fragment 7; followed by deprotection of Fmoc to get Fragment 8;
- c) condensing Fragment 8 with Fragment 2 in the presence of a coupling agent to give Fragment 9; followed by deprotection of Fmoc to get Fragment 10;
- d) condensing Fragment 10 with Fragment 1 in the presence of a coupling agent to give Fragment 11; followed by removal of the lysine side chain protecting base dde and the lysine side chain coupling with Palmitoyl-Glu-OtBu to get protected liraglutide;
- e) deprotecting the protected liraglutide with a cocktail mixture consisting of (TFA/TIPS/Water 90%/5%/5%) to afford crude liraglutide; and
- f) purifying by preparative HPLC to isolate pure liraglutide.

According to the invention, the novel fragments 1, 2, 3, and 4 are as follows.

- a) Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-OH (Fragment 1)
- b) Fmoc-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH (Fragment 2)
- c) Fmoc-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(dde)-Glu(OtBu)-Phe-OH (Fragment 3)
- 5 d) Fmoc-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-OH (Fragment 4)

In another embodiment, the process for the preparation of Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-OH (Fragment 1) comprises the steps of:

- a) anchoring the first protected terminal amino acid to a resin,
- 10 b) selectively deprotecting the amino group,
- c) coupling carboxyl terminus of the next *N*-protected amino acid to the amine group,
- d) repeating steps b) and c) to form a peptide sequence, and
- e) cleaving the fragment 1 from the resin using a 1% TFA in DCM.

15 In yet another embodiment, the process for the preparation of Fmoc-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH (Fragment 2) comprises the steps of:

- a) anchoring the first protected terminal amino acid to a resin,
- b) selectively deprotecting the amino group,
- c) coupling carboxyl terminus of the next *N*-protected amino acid to the amine group,
- 20 d) repeating steps b) and c) to form a peptide sequence, and
- e) cleaving the fragment 2 from the resin using a 1% TFA in DCM.

In yet another embodiment, the process for the preparation of Fmoc-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(dde)-Glu(OtBu)-Phe-OH (Fragment 3) comprising the steps of:

- 25 a) anchoring the first protected terminal amino acid to a resin,
- b) selectively deprotecting the amino group,
- c) coupling carboxyl terminus of the next *N*-protected amino acid to the amine group,
- d) repeating steps b) and c) to form a peptide sequence, and
- e) cleaving the fragment 3 from the resin using a 1% TFA in DCM.

30

In yet another embodiment, the process for the preparation of Fmoc-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-OH (Fragment 4) comprising the steps of:

- a) anchoring first protected terminal amino acid to a resin,
- b) selectively deprotecting the amino group,
- 35 c) coupling carboxyl terminus of the next *N*-protected amino acid to the amine group,
- d) repeating steps b) and c) to form a peptide sequence, and

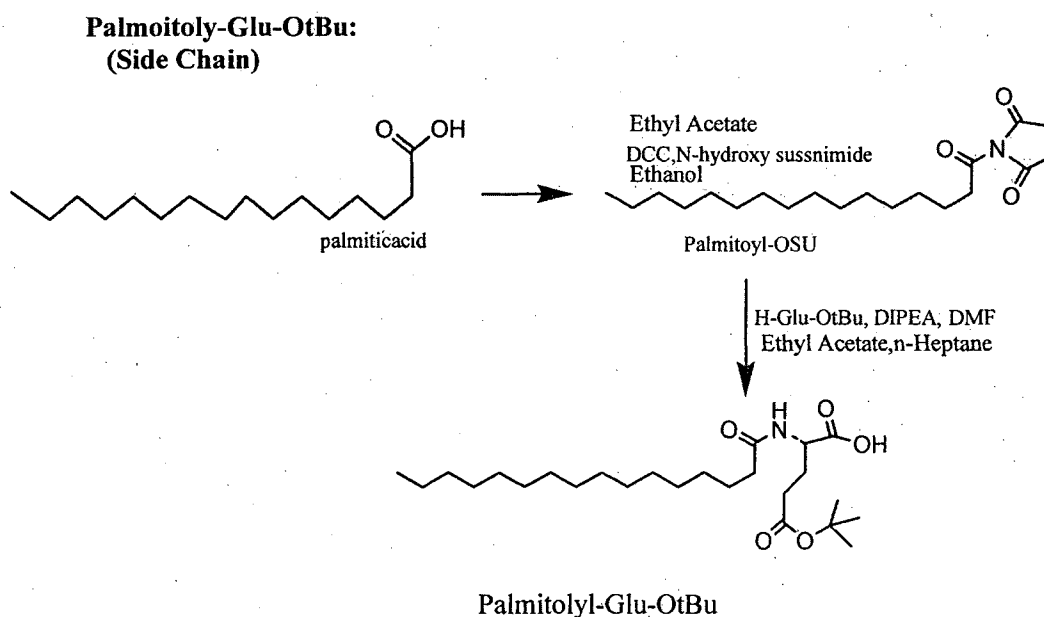
e) cleaving the fragment 4 from the resin using a 1% TFA in DCM.

According to the present invention, the resin used for the synthesis of the peptide undergoes swelling in the presence of a solvent selected from dichloromethane, *N,N*-dimethylformamide, and *N*-methyl-2-pyrrolidone or mixtures. The resin used is selected from Wang resin or 2-chlorotriyl chloride resin.

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*-methylpyrrolidine or a mixture thereof.

The removal of dde protecting group from the lysine side chain is carried out selectively in the presence of 3% hydrazine hydrate and DMF. The selectively deprotected amino group is coupled next with *N*-protected amino acid in a solvent in the presence of a coupling reagent. The solvent used for the coupling reaction is selected from dichloromethane, tetrahydrofuran, dimethylformamide, *N*-methylpyrrolidone or mixtures thereof. The coupling agent used for the coupling of the amino acids is selected from DIC/6-Cl-HOBt, DIC/HOBt, HBTU/HOBt/DIEA or DIC/Oxyma.

The side chain Palmitoyl-Glu-OtBu can be prepared by the following procedure



Yet another embodiment of the present invention is to provide a pharmaceutical composition comprising liraglutide and pharmaceutically acceptable carrier.

- 5 Certain specific aspects and embodiments of the present application will be explained in greater detail with reference to the following examples, which are provided only for purposes of illustration and should not be construed as limiting the scope of the disclosure in any manner. Reasonable variations of the described procedures are intended to be within the scope of the present application. While particular aspects of the present application have
10 been illustrated and described, it would be apparent to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the disclosure. It is therefore intended to encompass all such changes and modifications that are within the scope of this disclosure.

15

Examples:

Example-1: Preparation of liraglutide by solid phase synthesis using the Wang resin

Stage-I:

- 20 Liraglutide was synthesized manually on Wang resin by standard Fmoc solid phase synthesis strategy. The resin was soaked in the DCM for the swelling. Fmoc-Gly-OH was treated with the swelled Wang resin in DCM in the presence of DIC, HOBt and catalytic amount of DMAP; substitution level was determined by weight gain measurements and also by UV Method. This process of capping is performed after anchoring the first protected
25 amino acid to the resin. The complete synthesis was achieved by stepwise coupling of Fmoc-Amino acids to the growing peptide chain on the resin. All the couplings were carried out in HBTU, HOBt, DIEA and DMF as a solvent. The N-terminal Fmoc group was removed with 20% piperidine in DMF. The efficiency of the coupling was monitored using the Kaiser Ninhydrin test. The coupling step was repeated if Kaiser test was found positive. The
30 sequence of addition for the synthesis of liraglutide was Fmoc-Arg(pbf)-OH, Fmoc-Gly-OH, Fmoc-Arg(pbf)-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Lys(dde)-OH, Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Gln(trt)-OH, Fmoc-Gly-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH; Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ala-OH, Boc-His(trt)-OH.

Stage -II:

The stage-I resin was swelled with dichloromethane and deprotection of dde with 3% hydrazine hydrate in DMF, followed by coupling with Palmitoyl-Glu-OtBu-OH in the presence of DMF, HBTU, HOBT and DIEA for 4hrs at room temperature to give protected liraglutide anchored to Wang resin.

Stage-III:

The protected peptide resin (stage II) along with global protection was cleaved by cocktail mixture consisting of TFA/TIPS/Water (90%/5%/5%) to afford crude liraglutide, and the obtained reaction mixture was stirred for 2.5 hours at 25-30°C under nitrogen atmosphere. The reaction mixture was filtered and washed the resin with TFA. The obtained filtrate was charged into cold MTBE under stirring and allowing the temperature to rise more than 5° C. The reaction mixture was stirred for 45-75 minutes at 0-5°C. The precipitate was filtered, washed with MTBE and dried under vacuum at 40°C for 2 hrs to obtain a crude liraglutide. The resultant peptide was further purified in preparative HPLC to get a title compound.

Stage-IV:

Dissolve 3 g of Liraglutide crude in 90 ml of 1 M ammonium bicarbonate solution and filter the un- dissolved matter. The filtrate is injected into preparative HPLC through feed pump. The peptide was eluted using a gradient method comprising of 0.01M Ammonium bicarbonate as mobile phase A and 50% of Mobile phase A and 50% of Acetonitrile as B. Stabilize the preparative HPLC column at flow rate of 60ml/min with mobile phase A and B in ration of 50:50. Inject 90 ml of above filtered sample solution into preparative HPLC. Elute mobile phase at flow rate of 65ml/min and collect the fraction of Liraglutide. Pool all Liraglutide fractions and evaporate completely and lyophilize the product using freeze dryer till moisture content not more than 10%.

Example-2:**Preparation of liraglutide by fragment method:****Preparation of Fragment 1**

The 2-chloro tritylchloride resin (10g, loading 0.7mmol/g) was allowed to swell for 30 min. in DCM (50 mL). The mixture was filtered and fresh DCM (50 mL), Fmoc-Phe-OH, diisopropylethylamine (DIEA, 14mL, 78.6mmol) were added. The mixture was stirred at room

temperature for 2 hrs, filtered and washed with DMF and DCM. Subsequently, the mixture was stirred with a solution of 5% DIEA and 10% methanol in DCM for 30 min. The resin was washed with DMF and DCM, dried in vacuum to yield the loaded resin. The loading was determined using Beer-Lambert law and found to be 0.5mmol/g. After resin loading and prior to first deprotection, the resin was allowed to swell in DMF for 1 h. The Fmoc group was deprotected with 20% piperidine in DMF and subsequent amino acid couplings were done using DIC/HOBt as coupling agent. After every coupling and deprotecting, the washings was done prior to proceed for next amino acid. All the couplings and deprotections were monitored using ninhydrin test.

Preparation of Fragment 2

The 2-chloro tritylchloride resin (10g, loading 0.7mmol/g) was allowed to swell for 30 min. in DCM (50 mL). The mixture was filtered and fresh DCM (50 mL), Fmoc-Leu-OH, diisopropylethylamine (DIEA, 13mL, 73.02 mmol) were added. The mixture was stirred for 2 hrs. at room temperature, filtered and washed with DMF and DCM. Subsequently the mixture was stirred with a solution of 5% DIEA and 10% methanol in DCM for 30 min. The resin was washed with DMF and DCM and dried in vacuum to yield the loaded resin. The loading was determined using Beer-Lambert law and found to be 0.5mmol/g. After resin loading and prior to first deprotection; the resin was allowed to swell in DMF for 1 h. The Fmoc group was deprotected with 20% piperidine in DMF and subsequent amino acid couplings were done using DIC/HOBt as coupling agent. After every coupling and deprotecting, the washings was done prior to proceed for next amino acid. All the couplings and deprotections were monitored using ninhydrin test.

Preparation of Fragment 3

The 2-chloro tritylchloride resin (10g, loading 0.7mmol/g) was allowed to swell for 30 min. in DCM (50 mL). The mixture was filtered and fresh DCM (50 mL), Fmoc-Phe-OH, diisopropylethylamine (DIEA, 13mL, 73.02 mmol) were added. The mixture was stirred for 2 h. at room temperature, filtered and washed with DMF and DCM. Subsequently the mixture was stirred with a solution of 5% DIEA and 10% methanol in DCM for 30 min. The resin was washed with DMF and DCM and dried in vacuum to yield the loaded resin. The loading was determined using Beer-Lambert law and found to be 0.5mmol/g. After resin loading and prior to first deprotection; the resin was allowed to swell in DMF for 1 h. The Fmoc group was deprotected with 20% piperidine in DMF and subsequent amino acid couplings were done using DIC/HOBt as coupling agent. After every coupling and deprotecting, the washings

were done prior to proceed for next amino acid .All the couplings and deprotections were monitored using ninhydrin test.

Preparation of Fragment 4

- 5 The 2-chloro tritylchloride resin (10g, loading 0.7mmol/g) was allowed to swell for 30 min. in DCM (50 mL). The mixture was filtered and fresh DCM (50 mL), Fmoc-Arg(Pbf)-OH, diisopropylethylamine (DIEA, 13mL, 73.02 mmol) were added. The mixture was stirred for 2 h. at room temperature, filtered and washed with DMF and DCM. Subsequently the mixture was stirred with a solution of 5% DIEA and 10% methanol in DCM for 30 min. The resin was washed with DMF and DCM and dried in vacuum to yield the loaded resin. The loading was determined using Beer-Lambert law and found to be 0.5mmol/g. After resin loading and prior to first deprotection; the resin was allowed to swell in DMF for 1 h. The Fmoc group was deprotected with 20% piperidine in DMF and subsequent amino acid couplings were done using DIC/HOBt as coupling agent. After every coupling and deprotecting, the washings was done prior to proceed for next amino acid. All the couplings and deprotections were monitored using ninhydrin test.

Preparation of Fragment 5 & 6

- 20 To a stirred solution of Fragment-4 in DMF was coupled with Gly-OtBu in the presence of HBTU, HOBT and DIPEA at 10° and stirred for 15 min. The reaction mixture was stirred for overnight at room temperature; cool the reaction mixture and then add water, stirred for 30 min's. The precipitated solid was filtered, washed with water and dried under vacuum at 45°C for 2 hrs to give Fragment-5, removal of Fmoc protecting group from Fragment-5 by 10% piperidine in DCM to get Fragment 6.

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Preparation of Fragment 7 & 8

- 30 To a stirred solution of Fragment-6 in DMF was coupled with fragment-3 in the presence of HBTU, HOBT and DIPEA at 10° and stirred for 15 min. The reaction mixture was stirred for overnight at room temperature; cool the reaction mixture and then add water, stirred for 30 min's. The precipitated solid was filtered, washed with water and dried under vacuum at 45°C for 2 hrs to give Fragment-7, removal of Fmoc protecting group from Fragment-7 by 10% piperidine in DCM to get Fragment 8.

Preparation of Fragment 9 &10

- 35 To a stirred solution of Fragment-8 in DMF was coupled with Fragment-2 in the presence of HBTU, HOBT and DIPEA at 10° and stirred for 15 min. The reaction mixture was stirred for

overnight at room temperature; cooled the reaction mixture and then add water, stirred for 30 min's. The precipitated solid was filtered, washed with water and dried under vacuum at 45°C for 2 hrs to give Fragment-9, removal of Fmoc protecting group from fragment-9 by 10% piperidine in DCM to get Fragment 10.

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Preparation of Fragment 11 and protected liraglutide

To a stirred solution of Fragment-10 in DMF was coupled with Fragment-1 in the presence of HBTU, HOBT and DIPEA at 10° and stirred for 15 min. The reaction mixture was stirred for overnight at room temperature; cooled the reaction mixture and then add water, stirred for 30 min's. The precipitated solid was filtered, washed with water and dried under vacuum at 45°C for 2 hrs to give Fragment-11, followed by removal of the lysine side chain protecting base dde of Fragment-11 and the lysine side chain coupling with Palmitoyl-Glu-OtBu to get protected liraglutide.

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Preparation of crude liraglutide

The side chain functional protecting groups of the protected liraglutide were cleaved using cocktail mixture consisting of TFA/TIPS/Water (95%/5%/5%) in presence of DCM at 10°C. The resultant reaction mass was stirred for 5 hrs at room temperature, allow to cooled, the chilled MTBE was added and stirred for 30 min. The obtained solid was filtered and washed with MTBE to get crude liraglutide.

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Preparative HPLC purification of liraglutide

The crude Liraglutide was dissolved in 0.5M ammonium bicarbonate loaded on to preparative C8 column (50X250mm, 100 Å). The peptide was purified using a linear gradient of aqueous TFA (0.1%) and acetonitrile : methanol (8 :1 , 0.1% TFA) from 40% to 90% over 60 min. The pure fraction containing the liraglutide was pooled. The acetonitrile was evaporated and the aqueous layer was lyophilized to give the liraglutide as white solid. The peptide analyzed by RP-HPLC and confirmed by MALDI or LC-MS.

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Purity by HPLC - > 97%

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We claim:

1. An improved process for the preparation of liraglutide comprising the steps of:
 - a) anchoring thirty first protected terminal amino acid to a resin;
 - 5 b) capping the resin obtained in step a);
 - c) selectively deprotecting the amino group;
 - d) coupling the carboxyl terminus of the next N-protected amino acid to the amine group in the presence of a coupling reagent;
 - e) repeating steps c) and d) to form a peptide sequence;
 - 10 f) removal of the lysine side chain protecting group dde, followed by coupling with Palmitoyl-Glu-OtBu;
 - g) cleaving the peptide with a cocktail mixture from the resin to isolate the straight chain of crude liraglutide; and
 - h) purifying by preparative HPLC to isolate pure liraglutide.
- 15 2. An improved process for the preparation of liraglutide comprising the steps of:
 - a) condensing a Fragment 4 with Gly-OtBu in the presence of a coupling agent to give a Fragment 5, followed by the deprotection of Fmoc to produce a Fragment 6;
 - 20 b) condensing the Fragment 6 with a Fragment 3 in presence of a coupling agent to give a Fragment 7, followed by deprotection of Fmoc to get a Fragment 8;
 - c) condensing the Fragment 8 with a Fragment 2 in presence of a coupling agent to give a Fragment 9, followed by deprotection of Fmoc to get a Fragment 10;
 - 25 d) condensing the Fragment 10 with a Fragment 1 in presence of coupling agent to give a Fragment 11, followed by removal of a lysine side chain protecting base dde and a lysine side chain coupling with Palmitoyl-Glu-OtBu to get protected liraglutide;
 - e) deprotecting the protected liraglutide with a cocktail mixture consisting of (TFA/TIPS/Water 90%/5%/5%) to produce crude liraglutide; and
 - 30 f) purifying by preparative HPLC to isolate pure liraglutide.
3. The process according to the claim 2, wherein the process for preparation of Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-OH (Fragment 1) comprising the steps of:
 - a) anchoring first protected terminal amino acid to a resin;
 - 35 b) selectively deprotecting the amino group;

- c) coupling the carboxyl terminus of the next N-protected amino acid to the amine group;
- d) repeating steps b) and c) to form a peptide sequence; and
- e) cleaving the fragment 1 from the resin using a 1% TFA in DCM.

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4. The process according to the claim 2, wherein the process for preparation of Fmoc-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH (Fragment 2) comprising the steps of:

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- a) anchoring first protected terminal amino acid to a resin;
- b) selectively deprotecting the amino group;
- c) coupling carboxyl terminus of the next N-protected amino acid to the amine group;
- d) repeating steps b) and c) to form a peptide sequence; and
- e) cleaving the fragment 2 from the resin using a 1% TFA in DCM.

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5. The process according to the claim 2, wherein the process for preparation of Fmoc-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(dde)-Glu(OtBu)-Phe-OH(Fragment3) comprising the steps of:

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- a) anchoring first protected terminal amino acid to a resin;
- b) selectively deprotecting the amino group;
- c) coupling carboxyl terminus of the next N-protected amino acid to the amine group;
- d) repeating steps b) and c) to form a peptide sequence; and
- e) cleaving the fragment 3 from the resin using a 1% TFA in DCM.

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6. The process according to the claim 2, wherein the process for preparation of Fmoc-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-OH (Fragment 4) comprising the steps of:

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- a) anchoring first protected terminal amino acid to a resin;
- b) selectively deprotecting the amino group;
- c) coupling carboxyl terminus of the next N-protected amino acid to the amine group;
- d) repeating steps b) and c) to form a peptide sequence; and
- e) cleaving the fragment 4 from the resin using a 1% TFA in DCM.

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7. The process according to claims 1 and 2, wherein the resin used is selected from the Wang resin or 2-chlorotrityl chloride resin, and the cocktail mixture used for the cleavage of the peptide from resin is 90% TFA, 5% water, 5% TIPS.

5 8. The process according to claims 1 and 2, wherein the deprotection of the amino acid attached to the resin is done selectively in the presence of a nucleophilic base such as 20% piperidine and in the presence of a solvent like *N,N*-dimethyl formamide, methylene chloride, tetrahydrofuran or *N*-methyl pyrrolidine.

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9. The process according to claims 1 and 2, wherein the removal of the dde protecting group from lysine side chain is carried out selectively in the presence of 3% hydrazine hydrate and DMF.

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10. The novel fragments 1, 2, 3, and 4 are as follows.

a) Boc-His(Boc)-Ala-Glu(OtBu)-Gly-Thr(tBu)-Phe-OH (Fragment 1);

b) Fmoc-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-OH (Fragment 2);

20 c) Fmoc-Glu(OtBu)-Gly-Gln(Trt)-Ala-Ala-Lys(dde)-Glu(OtBu)-Phe-OH (Fragment 3);

d) Fmoc-Ile-Ala-Trp(Boc)-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-OH (Fragment 4)

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