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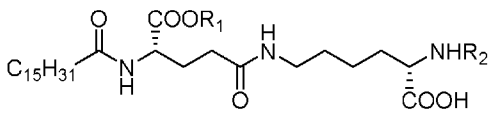
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(54) Title: IMPROVED PROCESSES FOR THE PREPARATION OF PEPTIDE INTERMEDIATES/MODIFIERS



Formula I

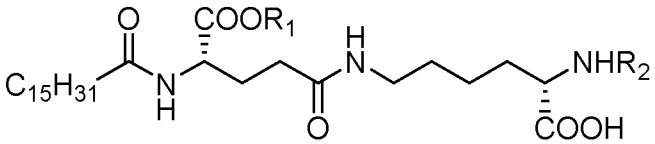
(57) Abstract: The present application relates to improved processes for the preparation of peptide intermediates/modifier compounds of Formula (I) and their use in the synthesis of peptide derivatives. The present application further provides the compound of Formula I with high chemical purity of >98% and chiral purity of >99% and its use to make pharmaceutical peptide like Liraglutide.

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IMPROVED PROCESSES FOR THE PREPARATION OF PEPTIDE INTERMEDIATES/MODIFIERS

INTRODUCTION

The present application relates to improved processes for the preparation of peptide intermediates/modifier compounds of Formula (I) and their use in the synthesis of peptide derivatives.



Formula I

The compounds of present invention have been found to be versatile intermediates for the solid phase peptide synthesis of peptide drugs which comprise a Glu-fatty alkyl side chain building block attached to a Lys-part of the peptide chain. For example, Liraglutide which is a GLP-1 analog derivative and carries a Glu-hexadecanoyl side chain building block at the Lys^{26} position.

Native peptides or analogues thereof generally have a high clearance, which is problematic if a prolonged period of biological activity is desired. Therefore in many pharmaceutical peptides like Liraglutide, Semaglutide, Insulin degludec, release properties have been modified by modifying the peptide chain or the amino acid side chains of the peptides. Modifications are often introduced on the side chains of lysine, glutamic acid, aspartic acid, amino terminal or the carboxyl terminal functions. Representative examples are Liraglutide, Semaglutide where glutamic acid bound on the side chain of a lysine with its γ-carboxyl function is used as a linker of the peptide with lipophilic groups. Such modifications are advantageous because the remaining free alpha-carboxyl function increases the water solubility of the modified peptide. Usually the peptide modification is performed post synthesis of precursor peptide.

However, modification can also be introduced during the synthesis of peptide on resin after the assembly of the desired peptide chain on a suitable resin, the selective removal side chain amino protecting group contained in the peptide sequence followed by on-resin introduction of the modifying agent.

Processes for the preparation of the compound of Formula I are described in Patent Application Publications viz., WO2013171135A1, WO2015100876A1, WO2015028966A2.

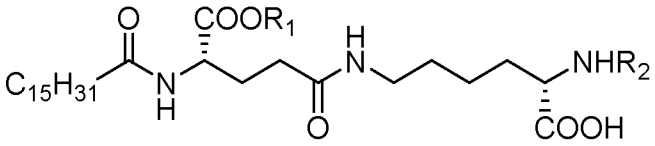
The processes reported therein for the preparation of the compound of Formula I, involve use of multi-step synthesis with protection/deprotection approach, tedious and cumbersome work-up procedures, use of not so environment friendly solvents, isolation of intermediates at various stages, multiple purifications, thus impacting the overall yield. The reported methods results in low yields, involve use of expensive Palladium complexes for deprotection that leads to contamination with residual triphenylphosphineoxide to a level of 0.8%, and also do not result in chemical purity higher than 98%. The purity of intermediate compounds play a major role in deciding the efficiency of the process for final compound, especially in case of peptides the monomers, or the peptide modifiers employed should preferably be highly pure to reduce the impurity levels at the final compound stage. At the same time, the process employed for making such monomers, peptide intermediates/modifiers should be simple, environment friendly and cost-effective. Therefore there remains a need to prepare compounds of Formula I of high purity and in good yield, while overcoming the drawbacks presented by the processes described in the art.

It is thus evident that the development of high purity drug substance preparations and pharmaceutical compositions comprising Liraglutide for use in pharmaceutical products, with both a reduced number of impurities and a decreased amount of those impurities that cannot be completely removed, is an important goal which can be substantially achieved by using highly pure intermediates. The present invention is directed to this and other important goals.

The present application relates to improved processes for the preparation of peptide intermediate/modifier compounds of Formula (I). The present application further provides the compound of Formula I with high chemical purity of >98%.

SUMMARY

In first embodiment, the present application provides improved processes for the preparation of peptide intermediates/modifier compounds of Formula (I),



Formula I

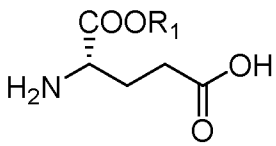
and salts thereof,

R_1 is hydrogen or an ester protecting group;

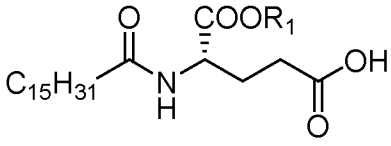
R_2 is hydrogen or an amino protecting group

the process comprising;

a) coupling palmitic acid or activated palmitic acid with a compound of Formula II in presence of inorganic base to afford compound of Formula III,



Formula II



Formula III

b) coupling compound of Formula III or its activated form with N-protected Lysine or its salts in presence of an inorganic base to afford compound of Formula I,

c) purifying the compound obtained in step b) under suitable conditions to afford highly pure compound of Formula I.

In second embodiment, the present application provides the compound of Formula I of chemical purity >98%, preferably >99%.

In third embodiment, the present application provides the compound of Formula I of chiral purity >99%.

In fourth embodiment, the present application provides compound of Formula I of high chemical and chiral purity prepared according to the process of the present invention.

Brief Description of the Drawings

FIG. 1 is a Chiral HPLC chromatogram of compound of Formula I prepared according to Example 4.

FIG. 2 is a HPLC chromatogram of compound of Formula I prepared according to Example 4.

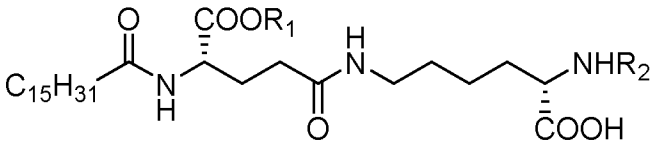
Fig. 3 is HPLC chromatogram of Liraglutide prepared by using compound of Formula I of present invention.

Fig. 4 is PXRD of compound of Formula I prepared as per Example 5.

Fig. 5 is an HPLC chromatogram of compound of Formula I prepared according to Example 6.

DETAILED DESCRIPTION

In first embodiment, the present application provides improved processes for the preparation of peptide intermediates/modifier compounds of Formula (I),



Formula I

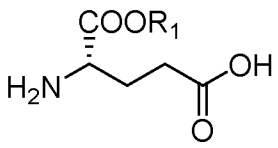
and salts thereof,

R_1 is hydrogen or an ester protecting group;

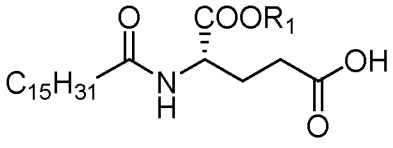
R_2 is hydrogen or an amino protecting group

the process comprising;

a) coupling palmitic acid or activated palmitic acid with a compound of Formula II in presence of an inorganic base to afford a compound of Formula III,



Formula II



Formula III

b) coupling the compound of Formula III or its activated form with N-protected Lysine or its salts in presence of an inorganic base to afford a compound of Formula I,

c) purifying the compound obtained in step b) under suitable conditions to afford highly pure compound of Formula I.

Step a) involves a coupling of palmitic acid or its activated form with glutamic acid derivative of Formula II under suitable reaction conditions.

Suitable activating agents will be familiar to the person skilled in the art. In a preferred embodiment, Palmitic acid is converted to Palmitoyl chloride before reacting with glutamic acid derivative of Formula RI.

The solvents employed in step a) Suitable solvents inert to the reaction conditions can be chosen from the list provided in the application. In a preferred embodiment, THF and water is employed.

Suitable temperatures in step b) may be less than about 40°C, or less than about 20°C, or less than about 5°C, or any other suitable temperatures.

Suitable inorganic bases that can be employed in step a) include, but are not limited to: inorganic bases such as sodium bicarbonate, sodium carbonate, sodium hydroxide, potassium carbonate, potassium hydroxide and the like. In a preferred embodiment, sodium carbonate has been employed.

The protecting groups viz., R_1 and R_2 in compound of Formula I can be the ones disclosed in T. W. Greene et al., Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, Inc., 1999, and other groups are described in the literature. In a preferred embodiment, R_1 is t-butyl and R_2 is Fmoc.

The compound of Formula III can optionally be used as such for next step, without isolation.

Step b) involves coupling compound of Formula III or its activated form with N-protected Lysine or its salts in presence of an inorganic base to afford compound of Formula I.

As mentioned above the activated form of Formula III will be familiar to the person skilled in the art. In a preferred embodiment, compound of Formula III is converted to its N-hydroxysuccinimide ester before coupling with N-protected Lysine or its salts.

Suitable Protecting groups are described by T. W. Greene et al., Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, Inc., 1999, and other groups are described in the literature.

Suitable solvent and suitable bases can be as mentioned for step a). In a preferred embodiment, THF and water is employed as solvents and sodium carbonate is employed as base.

Step c) involves purification of compound obtained in step b).

The said purification can be chromatographic separation or purification of peptides, polypeptides, analogs or derivatives thereof may be performed by any method available to those skilled in the art. Examples of these techniques include, but are not limited to reverse-phase high performance liquid chromatography (RP-HPLC), reversed-phase liquid chromatography (RP-LC), precipitation, crystallization, transforming them into a salt followed by optionally washing with an organic solvent or with an aqueous solution, and eventually adjusting pH, by slurrying in suitable solvents, or by commonly known recrystallization techniques. The suitable recrystallization techniques include, but are not limited to, steps of concentrating, cooling, stirring, or shaking a solution containing the compound, combination of a solution containing a compound with an anti-solvent, seeding, partial removal of the solvent, or combinations thereof, evaporation, flash evaporation, or the like. An anti-solvent as used herein refers to a liquid in which a compound is poorly soluble. Compounds can be subjected to any of the purification techniques more than one time, until the desired purity is attained.

The sample solution of crude compound of Formula I was loaded onto hydrophobic (BPG) column. The column was successively eluted through a gradient program using Mobile Phase A (Tris-buffer pH 7.5-8.5) and Mobile Phase B (70% acetonitrile and 30% methanol). Early and late fractions passing the minimum fraction quality criteria (NMT 0.5%) were pooled with the fractions bracketed in between them. The hydrophobic media can be selected from but not limited to C18, C8, HP20SS, SP20SS.

Suitable solvents that can be employed for recrystallization or slurrying include, but are not limited to: alcohols, such as, for example, methanol, ethanol, and 2-propanol; ethers, such as, for example, diisopropyl ether, methyl tert-butyl ether, diethyl ether, 1,4-dioxane, tetrahydrofuran (THF), and methyl THF; esters, such as, for example, ethyl acetate, isopropyl acetate, and t-butyl acetate; ketones, such as acetone and methyl isobutyl ketone; halogenated hydrocarbons, such as dichloromethane, dichloroethane, chloroform, and the like; hydrocarbons, such as toluene, xylene, and cyclohexane; nitriles, such as acetonitrile and the like; water; and any mixtures of two or more thereof.

In second embodiment, the present application provides the compound of Formula I of chemical purity >98%.

For example, compound of Formula I having impurity content of no greater than 2%, or no greater than 1%. In third embodiment, the present application provides compound of Formula I of chemical purity >99%.

The chromatographic impurity content is determined as total area percentages of impurities by HPLC by methods familiar to the skilled person.

In fourth embodiment, the present application provides a compound of Formula I of high chemical purity prepared according to the process of the present invention.

In the fifth embodiment, present invention provides a method of preparing a Liraglutide comprising use of compound of Formula I of high chemical and chiral purity. In a preferred embodiment, the present invention provides a process for preparing Liraglutide having a low level of the impurity by checking for the impurity in the compound of Formula I and then using it for the preparation of Liraglutide.

In the sixth embodiment, present invention provides a method of assaying chemical and chiral purity of a sample of compound of Formula I, wherein method comprise the steps of:

a) loading a sample of compound of Formula I onto a column,

b) eluting compound of Formula I from the column with an eluent comprising polar solvent and optionally an ion pair agent, and

c) determining the purity of the compound of Formula I.

In certain aspects of the above embodiments, the compound of Formula I has not more than 1% of total impurities.

In more specific aspects of above embodiments, the compound of Formula I has not more than 0.5% by peak area of Impurity 1 (RRT ~0.675), Impurity 2 (0.943) and Impurity 3 (Palm Di GluFmocLysine).

In more specific aspects of above embodiments, the compound of Formula I has not more than 0.5% by peak area of total chiral impurities and individual chiral impurity not more than 0.15% by peak area. In a more preferred embodiment, the compound of Formula I has not more than 0.3%by peak area of total chiral impurities.

The purity level of the compound of Formula I is measured by HPLC, wherein the HPLC method includes end capped C18 reverse-phase stationary phase and a gradient of mobile phase A containing 0.1% perchloric acid in water and mobile phase B containing mixture of Acetonitrile and water in the ratio 950: 50 (v/v).

In seventh embodiment, the invention is directed to a method for preparing a pharmaceutical composition comprising Liraglutide drug substance and one or more pharmaceutically acceptable excipients, wherein the Liraglutide drug substance is prepared by using peptide intermediate of compound of Formula I having a maximum individual chiral impurity not more than 0.15% and chemical impurity level of not more than 0.5%, as evident from Figures 1 & 2, respectively. The Liraglutide synthesized by using such highly pure compound of Formula I as prepared by process of present invention has chemical purity of >98% as shown by Fig. 3.

The chemical transformations described throughout the specification may be carried out using substantially stoichiometric amounts of reactants, though certain reactions may benefit from using an excess of one or more of the reactants.

Additionally, many of the reactions disclosed throughout the specification, may be carried out at ambient temperatures, but particular reactions may require the use of higher or lower temperatures, depending on reaction kinetics, yields, and the like. Furthermore, any of the chemical transformations may employ one or more compatible solvents, which may influence the reaction rates and yields. Depending on the nature of the reactants, the one or more solvents may be polar protic solvents, polar aprotic solvents, non-polar solvents, water or any of their combinations.

Suitable solvents inert to the reaction conditions include but are not limited to: alcohols, such as methanol, ethanol, 2-propanol, n-butanol, isoamyl alcohol and ethylene glycol; ethers, such as diisopropyl ether, dimethoxyethane, methyl tert-butyl ether, diethyl ether, 1,4-dioxane, tetrahydrofuran (THF), methyl THF, and diglyme; esters, such as ethyl acetate, isopropyl acetate, and t-butyl acetate and like; ketones, such as acetone and methyl isobutyl ketone and like; aliphatic hydrocarbons like n-hexane, cyclohexane, iso-octane and like; aromatic hydrocarbons like toluene, xylene and like; halogenated hydrocarbons, such as dichloromethane, dichloroethane, chloroform, and like; nitriles, such as acetonitrile; polar aprotic solvents, such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, dimethylsulfoxide, and the like; water; and any mixtures of two or more thereof.

The compounds obtained by the chemical transformations of the present application can be used for subsequent steps without further isolation for example in in step a) Palmitic acid can be activated and without its isolation can be coupled with Formula II to afford a compound of Formula III. Similarly, in step b) the compound of Formula III can be activated to its ester and without its isolation can be treated with lysine derivative to form compound of Formula I.

The compounds at various stages of the process may be recovered using conventional techniques known in the art. For example, useful techniques include, but are not limited to, decantation, centrifugation, gravity filtration, suction filtration, evaporation, flash evaporation, simple evaporation, rotational drying, spray drying, thin-film drying, freeze-drying, and the like. The isolation may be optionally carried out at atmospheric pressure or under a reduced pressure. The solid that is obtained may carry a small proportion of occluded mother liquor containing a higher than

desired percentage of impurities and, if desired, the solid may be washed with a solvent to wash out the mother liquor. Evaporation as used herein refers to distilling a solvent completely, or almost completely, at atmospheric pressure or under a reduced pressure. Flash evaporation as used herein refers to distilling of solvent using techniques including, but not limited to, tray drying, spray drying, fluidized bed drying, or thin-film drying, under atmospheric or a reduced pressure.

A recovered solid may optionally be dried. Drying may be suitably carried out using equipment such as a tray dryer, vacuum oven, air oven, fluidized bed dryer, spin flash dryer, flash dryer, and the like, at atmospheric pressure or under reduced pressure. Drying may be carried out at temperatures less than about 150°C, less than about 100°C, less than about 60°C, or any other suitable temperatures, in the presence or absence of an inert atmosphere such as nitrogen, argon, neon, or helium. The drying may be carried out for any desired time periods to achieve a desired purity of the product, such as, for example, from about 1 hour to about 15 hours, or longer.

The compound of this application is best characterized by the X-ray powder diffraction pattern determined in accordance with procedures that are known in the art. PXRD data reported herein was obtained using CuKα radiation, having the wavelength 1.5406 Å and were obtained using a Bruker AXS D8 Advance Powder X-ray Diffractometer and PANalytical X'Pert PRO instruments. For a discussion of these techniques see J. Haleblain, J. Pharm. Sci. 1975 64:1269-1288, and J. Haleblain and W. McCrone, J. Pharm. Sci. 1969 58:911-929.

Generally, a diffraction angle (2θ) in powder X-ray diffractometry may have an error in the range of ± 0.2°. Therefore, the aforementioned diffraction angle values should be understood as including values in the range of about ± 0.2°. Accordingly, the present application includes not only crystals whose peak diffraction angles in powder X-ray diffractometry completely coincide with each other, but also crystals whose peak diffraction angles coincide with each other with an error of about ± 0.2°. Therefore, in the present specification, the phrase "having a diffraction peak at a diffraction angle (2θ ± 0.2°) of 7.9°" means "having a diffraction peak at a diffraction angle (2 θ) of 7.7° to 8.1°". Although the intensities of peaks in the x-ray powder diffraction patterns of different batches of a compound may vary slightly, the peaks

and the peak locations are characteristic for a specific polymorphic form. Alternatively, the term "about" means within an acceptable standard error of the mean, when considered by one of ordinary skill in the art. The relative intensities of the PXRD peaks can vary depending on the sample preparation technique, crystal size distribution, various filters used, the sample mounting procedure, and the particular instrument employed. Moreover, instrument variation and other factors can affect the 2-theta values. Therefore, the term "substantially" in the context of PXRD is meant to encompass that peak assignments can vary by plus or minus about 0.2 degree. Moreover, new peaks may be observed or existing peaks may disappear, depending on the type of the machine or the settings (for example, whether a Ni filter is used or not).

DEFINITIONS

The following definitions are used in connection with the present application unless the context indicates otherwise.

The term “about” when used in the present application preceding a number and referring to it, is meant to designate any value which lies within the range of ±10%, preferably within a range of ±5%, more preferably within a range of ±2%, still more preferably within a range of ±1% of its value. For example “about 10” should be construed as meaning within the range of 9 to 11, preferably within the range of 9.5 to 10.5, more preferably within the range of 9.8 to 10.2, and still more preferably within the range of 9.9 to 10.1.

An “alcohol” is an organic compound containing a carbon bound to a hydroxyl group. “C1-C6 alcohols” include, but are not limited to, methanol, ethanol, 2-nitroethanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, hexafluoroisopropyl alcohol, ethylene glycol, 1-propanol, 2-propanol (isopropyl alcohol), 2-methoxyethanol, 1-butanol, 2-butanol, i-butyl alcohol, t-butyl alcohol, 2-ethoxyethanol, diethylene glycol, 1-, 2-, or 3-pentanol, neo-pentyl alcohol, t-pentyl alcohol, isoamyl alcohol, diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, cyclohexanol, phenol, glycerol, or the like.

A “hydrocarbon” is a liquid hydrocarbon compound, which may be linear, branched, or cyclic and may be saturated or have as many as two double bonds. A

liquid hydrocarbon compound that contains a six-carbon group having three double bonds in a ring is called “aromatic.” Examples of “C5-C8 aliphatic or aromatic hydrocarbons” include, but are not limited to, isopentane, neopentane, isohexane, 3-methylpentane, 2,3-dimethylbutane, neohexane, isoheptane, 3-methylhexane, neoheptane, 2,3-dimethylpentane, 2,4-dimethylpentane, 3,3-dimethylpentane, 3-ethylpentane, 2,2,3-trimethylbutane, n-octane, isooctane, 3-methylheptane, neooctane, cyclohexane, methylcyclohexane, cycloheptane, petroleum ethers, benzene toluene, ethylbenzene, m-xylene, o-xylene, p-xylene, trimethylbenzene, chlorobenzene, fluorobenzene, trifluorotoluene, anisole, or any mixtures thereof.

A “halogenated hydrocarbon” is an organic compound containing a carbon bound to a halogen. Halogenated hydrocarbons include, but are not limited to, dichloromethane, 1,2-dichloroethane, trichloroethylene, perchloroethylene, 1,1,1-trichloroethane, 1,1,2-trichloroethane, chloroform, carbon tetrachloride, or the like.

An “ester” is an organic compound containing a carboxyl group -(C=O)-O- bonded to two other carbon atoms. “C3-C6 esters” include, but are not limited to, ethyl acetate, n-propyl acetate, n-butyl acetate, isobutyl acetate, t-butyl acetate, ethyl formate, methyl acetate, methyl propanoate, ethyl propanoate, methyl butanoate, ethyl butanoate, or the like.

An “ether” is an organic compound containing an oxygen atom –O- bonded to two other carbon atoms. “C2-C6 ethers” include, but are not limited to, diethyl ether, diisopropyl ether, dimethoxy ethane, methyl t-butyl ether, glyme, diglyme, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, dibutyl ether, dimethylfuran, 2-methoxyethanol, 2-ethoxyethanol, anisole, or the like.

A “ketone” is an organic compound containing a carbonyl group -(C=O)- bonded to two other carbon atoms. “C3-C6 ketones” include, but are not limited to, acetone, ethyl methyl ketone, diethyl ketone, methyl isobutyl ketone, ketones, or the like.

A “polar aprotic solvent” has a dielectric constant greater than 15 and includes: amide-based organic solvents, such as hexamethyl phosphoramide (HMPA), hexamethyl phosphorus triamide (HMPT), and N-methylpyrrolidone, nitro-based organic solvents, such as nitromethane, nitroethane, nitropropane, and nitrobenzene;

ester-based organic solvents, such as γ-butyrolactone, ethylene carbonate, propylene carbonate, butylene carbonate, dimethyl carbonate, and propiolactone; pyridine-based organic solvents, such as pyridine and picoline; and sulfone-based solvents, such as dimethylsulfone, diethylsulfone, diisopropylsulfone, 2-methylsulfolane, 3-methylsulfolane, 2,4-dimethylsulfolane, 3,4-dimethylsulfolane, 3-sulfolane, and sulfolane.

A "nitrile" is an organic compound containing a cyano -(C≡N) bonded to another carbon atom. "C2-C6 Nitriles" include, but are not limited to, acetonitrile, propionitrile, butanenitrile, or the like.

Any organic solvents may be used alone, or any two or more may be used in combination, or one or more may be used in combination with water in desired ratios.

Acid addition salts are typically pharmaceutically acceptable, non-toxic addition salts with "suitable acids," including, but not limited to: inorganic acids such as hydrohalic acids (for example, hydrofluoric, hydrochloric, hydrobromic, and hydroiodic acids) or other inorganic acids (for example, nitric, perchloric, sulfuric, and phosphoric acids); organic acids, such as organic carboxylic acids (for example, xinafoic, oxalic, propionic, butyric, glycolic, lactic, mandelic, citric, acetic, benzoic, 2- or 4-methoxybenzoic, 2- or 4-hydroxybenzoic, 2- or 4-chlorobenzoic, salicylic, succinic, malic, hydroxysuccinic, tartaric, fumaric, maleic, hydroxymaleic, oleic, and glutaric acids), organic sulfonic acids (for example, methanesulfonic, trifluoromethanesulfonic, ethanesulfonic, 2-hydroxyethanesulphonic, benzenesulfonic, toluene-p-sulfonic, naphthalene-2-sulphonic, and camphorsulfonic acids), and amino acids (for example, ornithinic, glutamic, and aspartic acids).

All percentages and ratios used herein are by weight of the total composition and all measurements made are at about 25°C and about atmospheric pressure, unless otherwise designated. All temperatures are in degrees Celsius unless specified otherwise. As used herein, "comprising" means the elements recited, or their equivalents in structure or function, plus any other element or elements which are not recited. The terms "having" and "including" are also to be construed as open ended. All ranges recited herein include the endpoints, including those that recite a range "between" two values. Whether so indicated or not, all values recited herein

are approximate as defined by the circumstances, including the degree of expected experimental error, technique error, and instrument error for a given technique used to measure a value.

Terms such as "about," "generally," "substantially," and the like are to be construed as modifying a term or value such that it is not an absolute. Such terms will be defined by the circumstances and the terms that they modify as those terms are understood by those of skill in the art. This includes, at the very least, the degree of expected experimental error, technique error and instrument error for a given technique used to measure a value.

When a molecule or other material is identified herein as "pure", it generally means, unless specified otherwise, that the material has 98% purity or higher, as determined using methods conventional in the art such as high performance liquid chromatography (HPLC), gas chromatography (GC), or spectroscopic methods. In general, this refers to purity with regard to unwanted residual solvents, reaction by-products, impurities, and unreacted starting materials. In the case of stereoisomers, "pure" also means 98% of one enantiomer or diastereomer, as appropriate. "Substantially pure" refers to the same as "pure," except that the lower limit is about 98% purity or higher and, likewise, "essentially pure" means the same as "pure" except that the lower limit is about 97% purity.

The area of impurity peaks on HPLC chromatograms can be determined by standard chromatogram integration software such as, but not restricted to ChemStation from Agilent, Empower from Waters, LabSolutions from Shimadzu.

The term "RRT" as used herein is intended to indicate the relative retention time of the particular impurity against standard i.e. pure compound of Formula I (assigned an RRT value of 1) during an HPLC analysis.

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 application in any manner. Reasonable variations of the described procedures are intended to be within the scope of the present invention. While particular aspects of the present invention have been illustrated and described, it

would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

EXAMPLES

Example 1: Preparation of 5-(tert-butoxy)-5-oxo-4-palmitamidopentanoic acid (Formula III)

A flask was charged with palmitic acid (41.6 g) and toluene (285 mL) followed by addition of dimethyl formamide (0.517 g), toluene (25 mL). Then solution of thionyl chloride (prepared by adding thionyl chloride (22.84 mL) in toluene (60 mL)) was added to above reaction mixture at room temperature over a period of 1 hour. The solvents were subjected to complete distillation under vacuum. The residue was diluted with THF (60 mL). In another flask, THF (240 mL), sodium carbonate (31.3g), water (90mL) were charged and solution was cooled to 3 °C followed by addition of 4-amino-5-tert-butoxy)-5-oxopentanoic acid (30 g). To this solution, above prepared palmitoyl chloride solution was added at about 3 °C over a period of 3 hours. Then the mixture was brought to room temperature followed by addition of water (150 mL). The pH of the mixture was adjusted to 3.4 and then ethyl acetate (300 mL) was added, the ethyl acetate layer was separated and washed with water once followed by distillation under vacuum. Then heptane (90 mL) was added, heated for dissolution followed by addition of heptane (90 mL). The mixture was seeded with slurry of title compound in heptane (30 mL) followed by maintenance for 30 minutes and lot wise addition of heptane (60 mL, 90 mL, 150 mL, 240 mL). The mixture was then cooled to about 25 °C and filtered, washed with heptane (180 mL). The wet solid was taken in ethyl acetate (45 mL) and n-heptane (330 mL), heated for dissolution at about 45 °C followed by cooling, addition of seed and n-heptane (30 mL). The mixture was maintained at about 35 °C followed by lot-wise addition of n-heptane (45 mL, 90 mL,

165 mL and 240 mL). The mixture was filtered and washed with n-heptane (180 mL) to afford the title compound having chemical purity of 99.5% in 72.66% yield.

Example 2: **Preparation of 1-(tert-butyl) 5-(2,5-dioxopyrrolidin-1-yl) palmitoyl-L-glutamate**

A flask was charged with 5-(tert-butoxy)-5-oxo-4-palmitamidopentanoic acid (20 g), THF (400 mL), 1-hydroxypyrrolidine-2,5-dione (6.25 g), and the mixture was stirred at 40-50°C. To this mixture, a solution of N, N-methanediylidenebis(propan-2-amine) (6 g) in THF (20 mL) was added followed by maintenance of the mixture at the same temperature for 10-12 hours. The mixture was filtered and washed with THF (40 mL), the organic layer as such was used for next step.

Example 3: **Preparation of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid (Formula I)**

In a flask aqueous sodium carbonate (1.8 g) was prepared followed by addition of THF (800 mL) and then this mixture was cooled to 0-10 °C. To this mixture, (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanoic acid (4.59 g) was added. Then, a solution of 1-(tert-butyl) 5-(2,5-dioxopyrrolidin-1-yl) palmitoyl-L-glutamate (corresponding to 5 g of 5-(tert-butoxy)-5-oxo-4-palmitamidopentanoic acid) prepared according to example 2, was slowly added to above mixture at about 5 °C. The reaction mixture was stirred for about 2 hours at 25 °C followed by addition of water (600 mL). The solvents were subjected to distillation and then the pH of the obtained compound is adjusted to 3.0 using 5N hydrochloric acid. The mixture was stirred for 1-2 hours and resulting solid was isolated filtration, washing with water (30 mL). The obtained solid was dried under vacuum for 8 hours at about 45°C to afford title compound in ~85% yield.

Example 4: Purification of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid (Formula I)

A solution of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid is prepared by dissolving crude (9 g)

in methanol (165 mL) and filtered to get a clear solution. A preparative aliquot of the above solution was loaded onto the column (HP20SS, 50mm ID). The column was equilibrated at 50% Mobile Phase B. The desired product was eluted from the gradient of around 60-75% Mobile phase B for 11 CV until the peaks ends to absorbance UV @ 280 nm. The pooled fractions having >98% purity were adjusted to pH: 3.7 with 1N hydrochloric acid followed by addition of water and maintenance for about 30 minutes to complete the precipitation. The obtained solid was isolated by filtration and dried under vacuum to afford the title compound having HPLC purity of ~99%.

Example 5: Purification of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid (Formula I)

A solution of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid is prepared by dissolving crude in methanol (1700 mL) and filtered to get a clear solution.

A preparative aliquot of the above solution was loaded onto the C18 120A resin column. The column was subjected to gradient run by using Mobile Phase A (20 mM Tris buffer, pH 8.0) and Mobile Phase B (80% acetonitrile and 20% methanol). The desired product was eluted from the gradient of around 70-75% Mobile phase B. The pooled fractions were adjusted to pH: 3.5 with 1N hydrochloric acid followed by addition of water and maintenance for about 30 minutes to complete the precipitation. The obtained solid was isolated by filtration and dried under vacuum at about 30-35°C for overnight to afford the title compound having HPLC purity of ~99%.

Example 6: Purification of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid (Formula I)

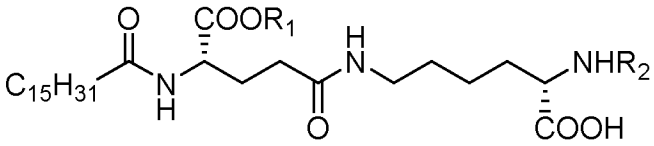
A solution of (2S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(5-(tertbutoxy)-5-oxo-4-palmitamidopentanamido)hexanoic acid is prepared by dissolving crude (170 g) in methanol (5000 mL) and filtered to get a clear solution.

A preparative aliquot of the above solution was loaded onto the C18 column. The column was subjected to gradient run by using Mobile Phase A (20 mM Tris buffer, pH 8.0) and Mobile Phase B (100% acetonitrile). The pooled fractions of the desired

product were adjusted to pH: 4.5 with 1N hydrochloric acid followed by addition of water and maintenance for about 30 minutes to complete the precipitation. The obtained solid was isolated by filtration and dried under vacuum to afford the title compound having HPLC purity of ~99%.

Claims:

Claim 1: An improved process for the preparation of peptide intermediates/modifier compounds of Formula (I),



Formula I

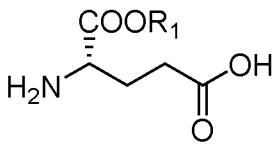
and salts thereof,

R_1 is hydrogen or an ester protecting group;

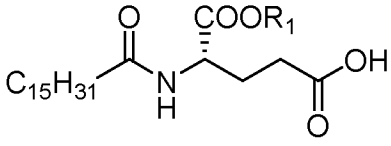
R_2 is hydrogen or an amino protecting group;

the process comprising;

a) coupling palmitic acid or activated palmitic acid with a compound of Formula II in presence of inorganic base to afford compound of Formula III,



Formula II



Formula III

b) coupling compound of Formula III or its activated form optionally without isolation, with N-protected Lysine or its salts in presence of an inorganic base to afford compound of Formula I,

c) purifying the compound obtained in step b) under suitable conditions to afford highly pure compound of Formula I.

Claim 2: The process of Claim 1, wherein palmitic acid in step a) is activated to esters, acid chloride.

Claim 3: The process of Claim 2, wherein Palmitoyl chloride is employed.

Claim 4: The process of Claim 1, wherein suitable inorganic base in step a) and step b) is selected from sodium bicarbonate, sodium carbonate, sodium hydroxide, potassium carbonate, potassium hydroxide and like.

Claim 5: The process of Claim 4, wherein sodium carbonate is employed.

Claim 6: The process of Claim 1, wherein solvents employed in step a) and step b) are selected from ethers, hydrocarbons, water and like.

Claim 7: The process of Claim 6, wherein tetrahydrofuran, water are employed.

Claim 8: The process of Claim 1, wherein the compound of Formula I have chemical purity of at least 98% and chiral purity at least 99%.

Claim 9: The compound of Formula I have chemical purity of at least 98% and chiral purity at least 99%.

Claim 10: A pharmaceutical composition comprising the compound of Formula I have chemical purity of at least 98% and chiral purity at least 99%.

Claim 11: A method for synthesis of a Liraglutide comprising using a compound of Formula I and its enantiomers, diastereomers, salts thereof, prepared according to the preceding claims.

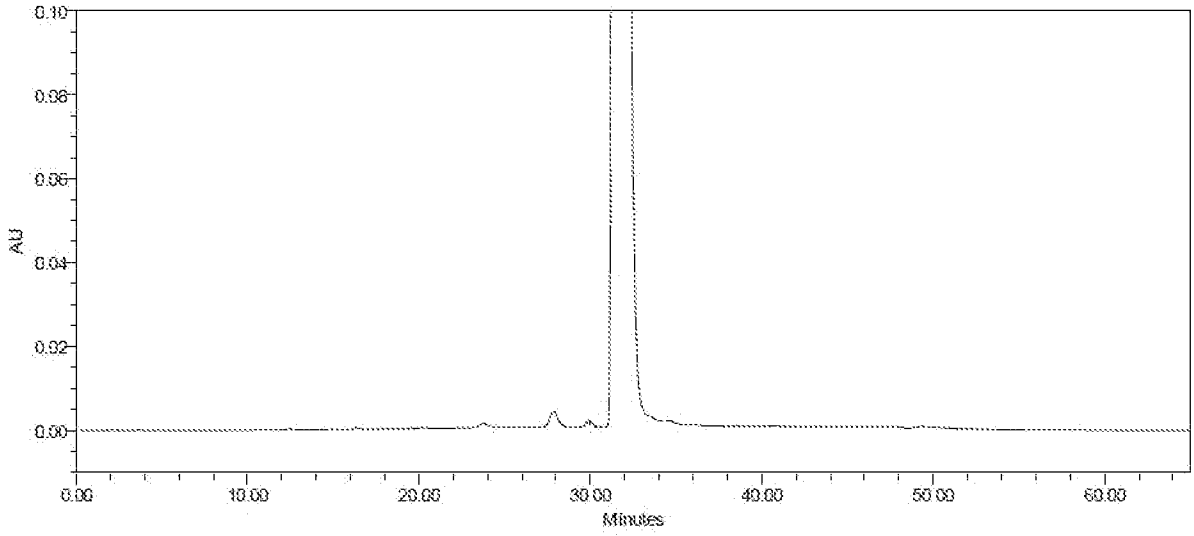


Figure 1 of 5

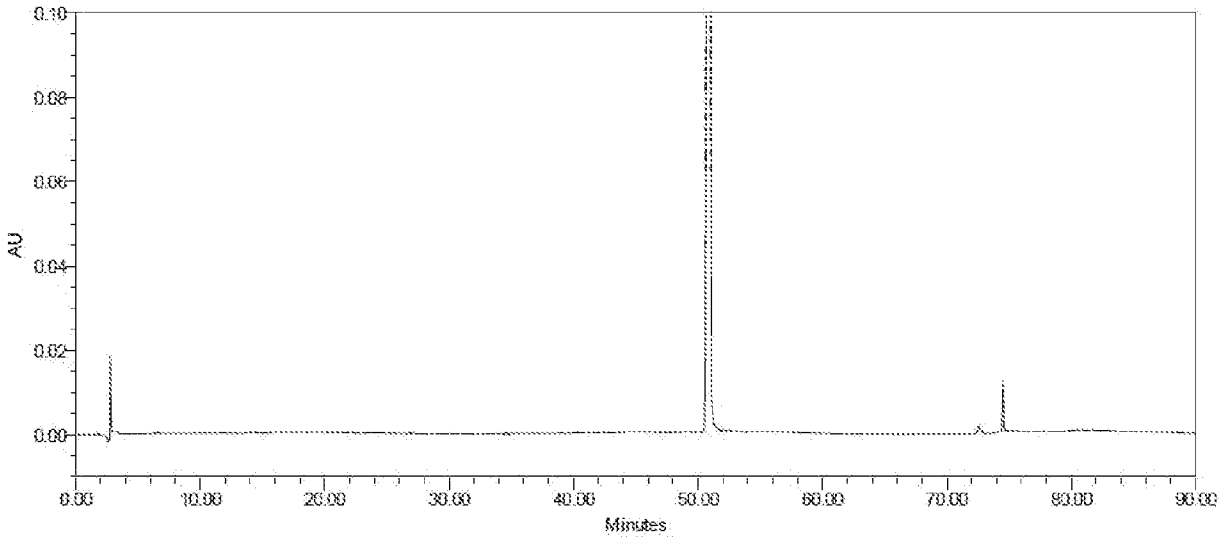


Figure 2 of 5

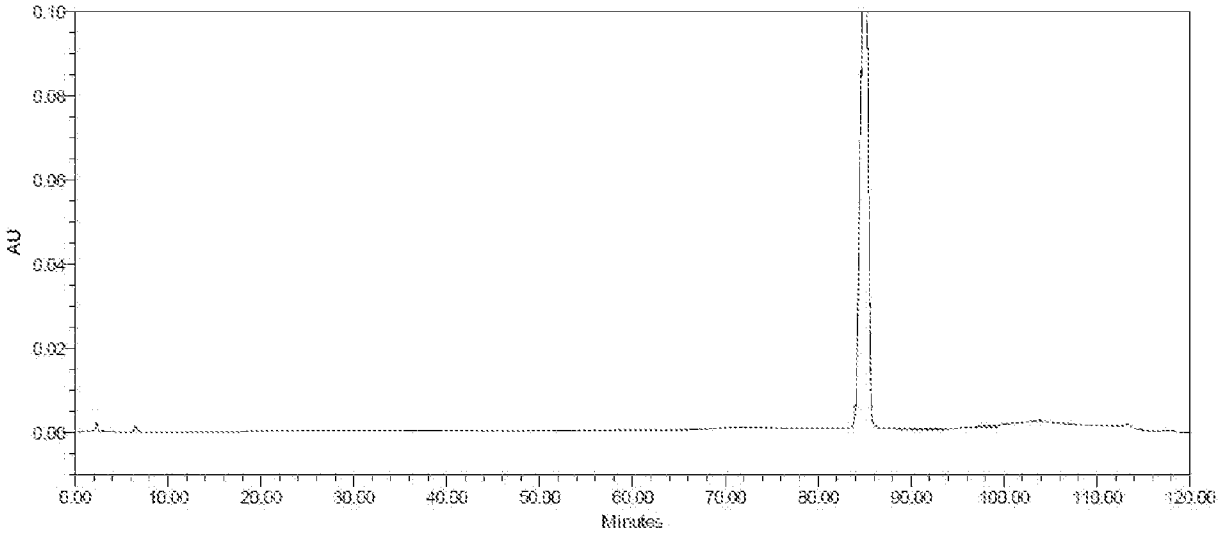


Figure 3 of 5

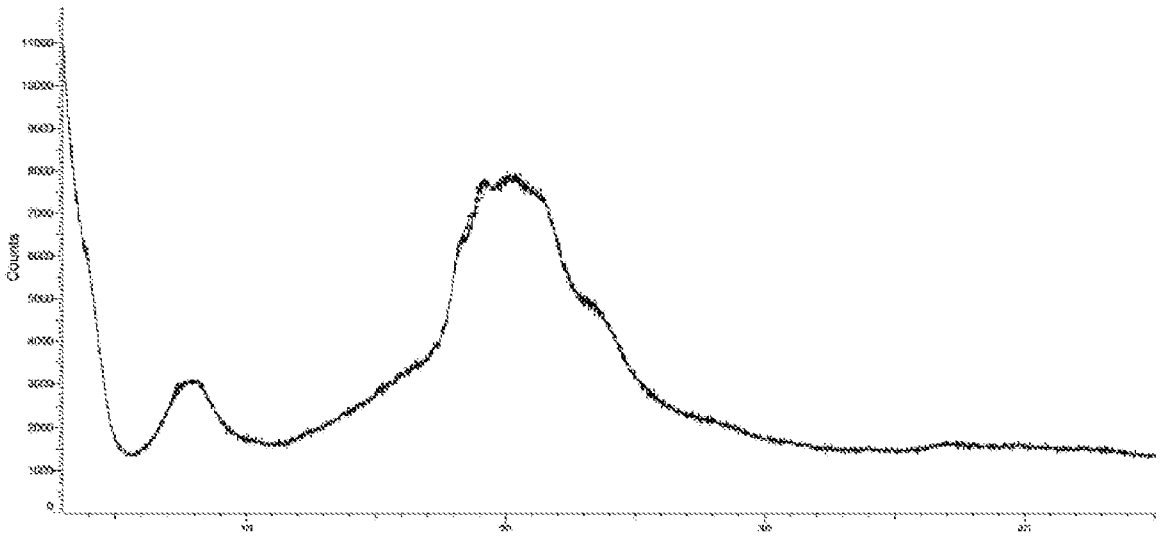


Figure 4 of 5

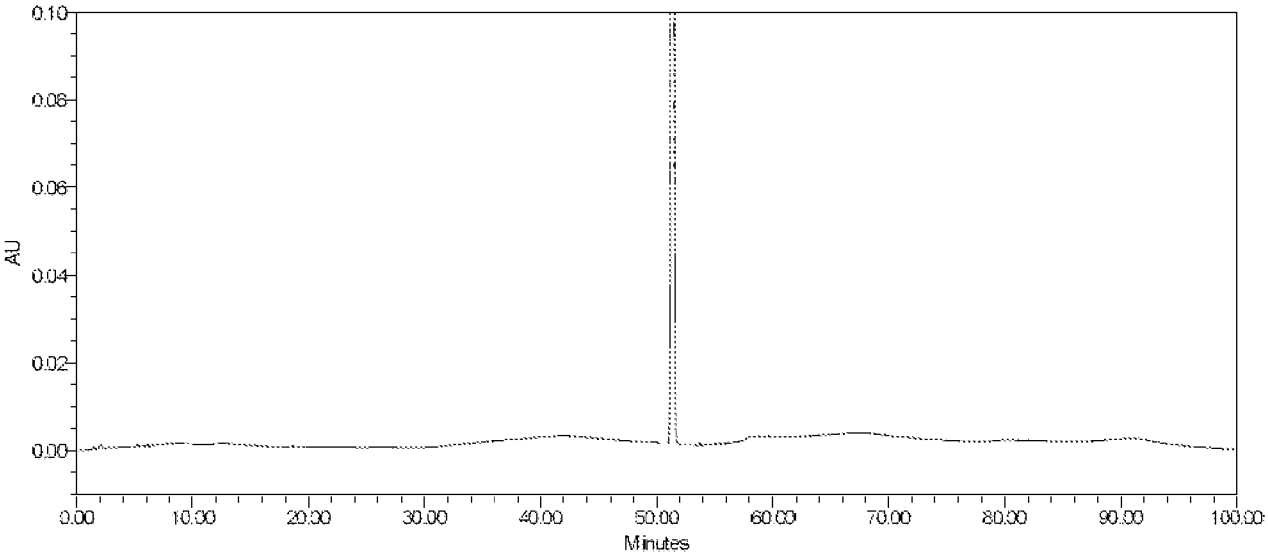

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Figure 5 of 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/054211

A. CLASSIFICATION OF SUBJECT MATTER
C07K1/06,C07K1/10 Version=2019.01

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)

C07K1, C07C

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	WO 2015028966 A2 (CHEMICAL & BIOPHARMACEUTICAL LABORATORIES OF PATRAS S.A), 05 March 2015 (05/03/2015) page 24, examples 1-6, particularly example 101, page 90. claim 26, paragraph 10, page 2	1-9, 10-11
X	EP 3127604 B1 (HOFFMANN LA ROCHE), 22 August 2018 (22/08/2018) page 2, paragraph 4	11

☐ 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
16-10-2019	16-10-2019
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Killari Appalanaid Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2019/054211

Citation	Pub.Date	Family	Pub.Date
WO 2015028966 A2	05-03-2015	EP 3038658 A2	06-07-2016
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