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भौगोलिक उपदर्शन

**जिस किसी से संबन्धित हो
TO WHOMSOEVER IT MAY CONCERN**

मैं, अधोहस्ताक्षरी जो पेटेंट अधिनियम, 1970 की धारा 73(3) के तहत महानियंत्रक एकस्व, अभिकल्प एवं व्यापार चिह्न की ओर से प्रमाणपत्र हस्ताक्षर व जारी करने के लिए प्राधिकृत अधिकारी हूँ, एतद्वारा यह प्रमाणित करता(ती) हूँ कि निम्नलिखित पेटेंट आवेदन के संबंध में फाइल दस्तावेज़(जों) की सही प्रतिलिपि इसके साथ संलग्न है:

I, the undersigned, being an officer duly authorized to sign and issue the certificate on behalf of the Controller General of Patents, Designs and Trademarks in accordance with the provisions of Section 73(3) of the Patents Act, 1970, hereby certify that annexed hereto is a True Copy of the document(s) as filed in connection with the following Patent Application:

- क) आवेदन संख्या / a) Application Number: **201821013109**
ख) फाइल करने की तारीख / b) Date of Filing: **05/04/2018**
ग) अनुरोधित दस्तावेज़(जों) का नाम / C) Name of the document(s) requested:

- 1) **Application for Grant of Patent (Form-1)**
2) **Provisional Specification filed on 05/04/2018**

यह प्रमाणपत्र पेटेंट अधिनियम, 1970 की धारा 147(1) के अधीन मुझमें निहित शक्तियों के तहत जारी किया गया है।

This certificate is issued under the powers vested in me U/S 147(1) of The Patents Act, 1970.

दिनांक / Dated this th 16 day of April, 2019

(VIJAY T. DOYE)

सहायक नियंत्रक पेटेंट व डिजाइन / Assistant Controller of Patents and Designs

(प्राधिकृत हस्ताक्षरी / Authorized Signatory)

"FORM 1 THE PATENTS ACT 1970 (39 OF 1970) and THE PATENT RULES, 2003 APPLICATION FOR GRANT OF PATENT (See Section 7, 54 & 135 and sub-rule (1) of rule 20)				(FOR OFFICE USE ONLY)	
				Application No.	
				Filing date :	
				Amount of Fee paid:	
				CBR No.	
				Signature:	
1. APPLICANT'S REFERENCE/ IDENTIFICATION NO. (AS ALLOTTED BY OFFICE)					
2. TYPE OF APPLICATION [Please tick (✓) at the appropriate category]					
Ordinary (☒)		Convention ()		PCT-NP ()	
Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()
3A. APPLICANT(S)					
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				Pin Code	400063
3B. CATEGORY OF APPLICANT [Please tick (✓) at the appropriate category]					
Natural Person ()		Other than Natural Person			
		Small Entity ()	Startup ()	Others (☒)	
4. INVENTORS (S) [Please tick (✓) at the appropriate category]					
Are all the inventor(s) same as the applicant (s) named above?		Yes ()			No (☒)
If "No", furnish the details of the inventor(s)					
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			State	GUJARAT
			Country	India
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5. TITLE OF THE INVENTION- NOVEL GLP-1 ANALOGUE				
6. AUTHORISED REGISTERED PATENT AGENT(S)	IN/PA No.			
	Name			
	Mobile No.			
7. ADDRESS FOR SERVICE OF	Name		Dr. Bishram Singh Chouhan	

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8. IN CASE OF APPLICANT CLAIMING PRIORITY OF APPLICATION FILED IN CONVENTION COUNTRY, PARTICULARS OF CONVENTION APPLICATION

Country	Application Number	Filing date	Name of the applicant	Title of the invention	IPC (as classified in the convention country)
-NA-	-NA-	-NA-	-NA-	-NA-	-NA-

9. IN CASE OF PCT NATIONAL PHASE APPLICATION, PARTICULARS OF INTERNATIONAL APPLICATION FILED UNDER PATENT CO-OPERATION TREATY (PCT)

International application number	International filing date
-NA-	-NA-

10. IN CASE OF DIVISIONAL APPLICATION FILED UNDER SECTION 16, PARTICULARS OF ORIGINAL (FIRST) APPLICATION

Original (first) application No.	Date of filing of original (first) application
-NA-	-NA-

11. IN CASE OF PATENT OF ADDITION FILED UNDER SECTION 54, PARTICULARS OF MAIN APPLICATION OR PATENT

Main application/patent No.	Date of filing of main application
-NA-	-NA-

12. DECLARATIONS

(i) Declaration by the inventor(s)

(In case the applicant is an assignee : the inventors(s) may sign herein below or the applicant may upload the assignment or enclose the assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period).

I/We, the above named inventor(s) is/are the true & first inventor(s) for this Invention and

declare that the applicant(s) herein is/are my/our assignee or legal representative.

(a) Date: April 04, 2018

(b) Signature(s)

1) Rajamannar

2) N. Chaturvedi

3) Burade

4) [Signature]

5) Kunal Pandya

6) Patel BR

7) Zalawadia

8) [Signature]

(c) Name(s)

1) THENNATI RAJAMANNAR

2) CHATURVEDI NISHITH

3) BURADE VINOD

4) PATEL VIPULKUMAR S

5) PANDYA KUNAL L

6) PATEL BRIJESHKUMAR

7) ZALAWADIA RISHIT

8) JOSHI DHIREN

(ii) Declaration by the applicant(s) in the convention country:

(In case the applicant in India is different than the applicant in the convention country: the applicant in the convention country may sign herein below or applicant in India may upload the assignment form the applicant in the convention country or enclose the said assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period)

I/We the applicant(s) in the convention country declare that the applicant(s) herein is/are my/our assignee or legal representative.

(a) Date

(b) Signature(s)

-NA-

(c) Name(s) of the signatory

iii) Declaration by the applicant(s):

I/we the applicant(s) hereby declare(s) that: -

- ☒ I am/We are in possession of the above mentioned invention
- ☒ The provisional /complete specification relating to the invention is filed with this application
 - ☐ The invention as disclosed in the specification uses the biological material from India and the necessary permission from the competent authority shall be submitted by me/us before the grant of patent to me/us
- ☒ There is no lawful ground of objection(s) to the grant of the patent to me/us.
 - ☐ I am/we are the true & first inventor(s).
- ☒ I am/we are the assignee or legal representative of true and first inventor(s).
 - ☐ The application or each of the applications, particular of which are given in para-8 was the first application in convention country/countries in respect of my/our invention(s).
 - ☐ I/we claim the priority from the above mentioned application (s) filed in convention country/countries and state that no application for protection in respect of the invention had been made in a convention country before that date by me/us or by any person from which I/we derive the title
 - ☐ My/our application in India is based on international application under patent cooperation treaty (PCT) as mentioned in Para-9.
 - ☐ The application is divided out of my/our application particulars of which is given in para-10 and prays that this application may be treated as deemed to have been filed on DD/MM/YY under sec 16 of the Act.
 - ☐ The said invention is an improvement in or modification of the invention particulars of which are given in Para- 11.

13. FOLLOWING ARE THE ATTACHMENTS WITH THE APPLICATION

(a) Form 2

Item	Details	Fee	Remarks
Complete/provisional	No. of pages. - 20+1	Rs. 8000/-	

specification)#			
No. of claim(s)	No. of claims ¹ and No. of pages: <u>1</u>	-	
Abstract	No. of pages- <u>1</u>	-	No. of pages included in the total no. of pages of provisional specification.
No. of Drawing(s)	No. of drawings and No. of pages- <u>6</u>	-	-

In case of a complete specification, if the applicant desires to adopt the drawings filed with his provisional specification as the drawings or part of the drawings for the complete specification under rule 13(4), the number of such pages with the provisional specification are required to be mentioned here.

~~(b) Complete specification (in conformation with the international application/as amended before the international Preliminary Examination Authority (IPEA), as applicable (2 copies).~~

~~(c) Sequence listing in electronic form~~

~~(d) Drawings (in confirmation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2 copies).~~

~~(e) Priority document(s) or a request to retrieve the priority document(s) from DAS (Digital Access Service) if the applicant had already requested the office of first filing to make the priority document(s) available to DAS.~~

~~(f) Translation of priority document/Specification/International Search Report/International Preliminary Report on Patentability.~~

~~(g) Statement and Undertaking on Form 3~~

~~(h) Declaration of Inventorship on Form 5~~

~~(i) Power of Authority~~

~~(j)~~

Total fee ₹ 8000 via online transfer Cash/ Banker's Cheque /Bank Draft bearing No.

I/We hereby declare that to the best of my/our knowledge, information and belief the fact and matters stated herein are correct and I/we request that a patent may be granted to me/us for the said invention.

Dated this 4th day of Apr, 2018

Signature: 

Name: Dr. RATNESH SHRIVASTAVA
VICE PRESIDENT,
SUN PHARMACEUTICAL INDUSTRIES LTD.

To,
The Controllers of Patents
The Patent Office, at MUMBAI

FORM 2

THE PATENTS ACT, 1970

[39 of 1970]

&

PATENTS RULES, 2003

PROVISIONAL SPECIFICATION

(Sec section 10 and rule 13)

NOVEL GLP-1 ANALOGUE

SUN PHARMACEUTICAL INDUSTRIES LTD.

A company incorporated under the laws of India having their office at
SUN HOUSE, 201 B/1, WESTERN EXPRESS HIGHWAY, GOREGOAN (E), MUMBAI-
400063 MAHARASHTRA, INDIA.

The following specification describes invention.

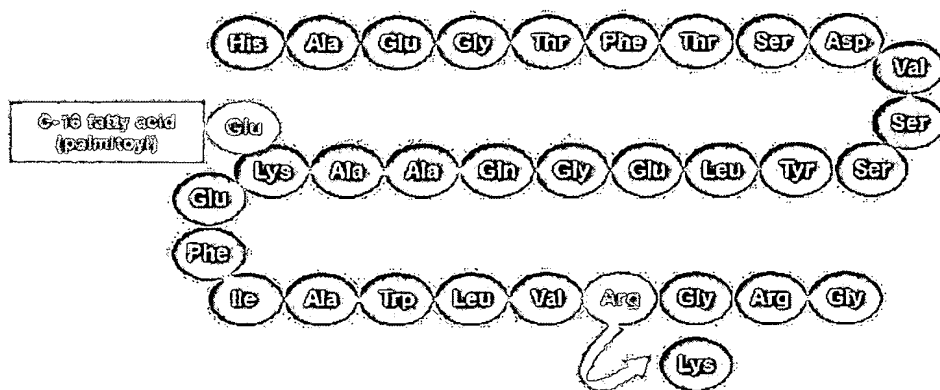
FIELD OF THE INVENTION

The present invention pertains to a novel Glucagon like Peptide-1 (GLP-1) (7-38) analogue for use in treatment of diabetes and obesity.

BACKGROUND OF THE INVENTION

Glucagon-like peptide-1 (GLP-1) is a hormone that is mainly produced in enteroendocrine L cells of the gut and is secreted into the blood stream when food containing fat, protein hydrolysate and/or glucose enters the duodenum. GLP-1 is derived from the cell-specific post-translational processing of the preproglucagon gene. Initially, the peptide GLP-1(1-37) was identified from this processing, but it was the two *N*-terminally truncated products, GLP-1(7-37) and GLP-1(7-36)amide, that were found to recognize the pancreatic receptor and which were determined to be the active species *in vivo*. GLP-1 has been found to stimulate insulin secretion, thereby causing glucose uptake by cells and decreased serum glucose levels.

Various GLP-1 analogues have been known. The first GLP-1 analogue to be developed was liraglutide which was disclosed in United States Patent No. US6268343. Liraglutide was approved by USFDA in Jan 2010 and marketed under the brand Victoza®. Victoza® is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus and to reduce the risk of major adverse cardiovascular events in adults with type 2 diabetes mellitus and established cardiovascular disease. Liraglutide is GLP-1(7-37) analogue with one amino acid substitution i.e. arginine for lysine at position 34 and with addition of C-16 fatty acid chain (palmitic acid) with a glutamic acid spacer on the lysine residue at position 26. The structure of liraglutide is as shown below (Source: Victoza® US FDA Label):

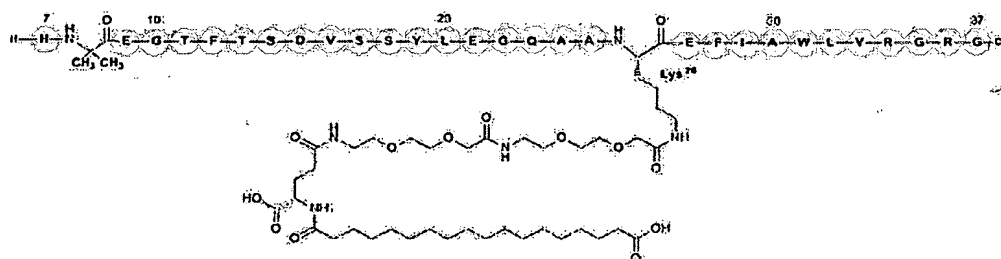


Structure of Liraglutide

Liraglutide is administered by subcutaneous injection once-daily. There are other GLP-1 receptor agonists that have been approved by USFDA, including exenatide (Byetta®), lixisenatide (Adlyxin®), dulaglutide (Trulicity®), albiglutide (Tanzeum®). Exenatide and lixisenatide are analogues of exendin-4, which is a peptide that was isolated from the Gila monster venom. Dulaglutide is a GLP-1 agonist consisting of GLP-1(7-37) covalently linked to an Fc fragment of human IgG4. It is used for the treatment of type 2 diabetes that can be used once weekly. Albiglutide is a peptide consisting of 645 proteinogenic amino acids with 17 disulfide bridges, amino acids 1–30 and 31–60 constitute two copies of modified human GLP-1, (the alanine at position 2 having been exchanged for a glycine) for better DPP-4 resistance. The remaining sequence is human albumin. It is administered as once-weekly injection for treatment of type 2 diabetes.

Recently, Semaglutide, a GLP-1 analogue was approved by USFDA. Semaglutide is marketed under the brand name Ozempic®. It is administered as a once-weekly subcutaneous injection. The peptide was first disclosed in WIPO patent publication WO2006097537A2, as a GLP-1 (7-37) analogue i.e. an Aib⁸, Arg³⁴-GLP-1(7-37) analogue substituted on the ε-amino group of the lysine residue in position 26 with an (S)-22,40-dicarboxy-10,19,24-trioxo-3,6,12,15-tetraoxa-9,18,23-triazatetracontan-1-oyl side chain. The side chain consists of two 8-amino-3,6-dioxaoctanoic acid (ADO) spacers, one γ-glutamic acid (Glu) spacer,

and a fatty diacid (1,18-octadecanedioic acid). The structure of semaglutide is as shown below (Source: Ozempic® US FDA Label). An oral formulation of semaglutide has also been made and is in Phase 3 clinical trials.



Structure of semaglutide

Various other GLP-1 derivatives are disclosed in patent applications such as WIPO publication number WO2005027978A2, WO1998008871A1, WO1999043705A1, WO1999043706A1, WO1999043707A1, WO1999043708A1, WO2000034331A2, WO2009030771A1, WO2011080103A1, WO2012140117A1, WO2012062803A1, WO2012062804A1, WO2013037690A1, WO2014202727A1, WO2015000942A1, WO2015022400A1, WO2016083499A1, WO2016097108A1 and WO2017149070A1. There is a need for GLP-1 analogue with improved activity and duration of action.

SUMMARY OF THE INVENTION

In one aspect the present invention provides a peptide compound of Formula I

[illegible]

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Schematic representation of preparation of Intermediate 3.

Figure 2: Result of Oral glucose tolerance test (OGTT) in rats; single injection;

1mg/kg glucose AUC 0-120min

Figure 3: Reduction in blood glucose levels in db/db type 2 diabetic mice after chronic treatment

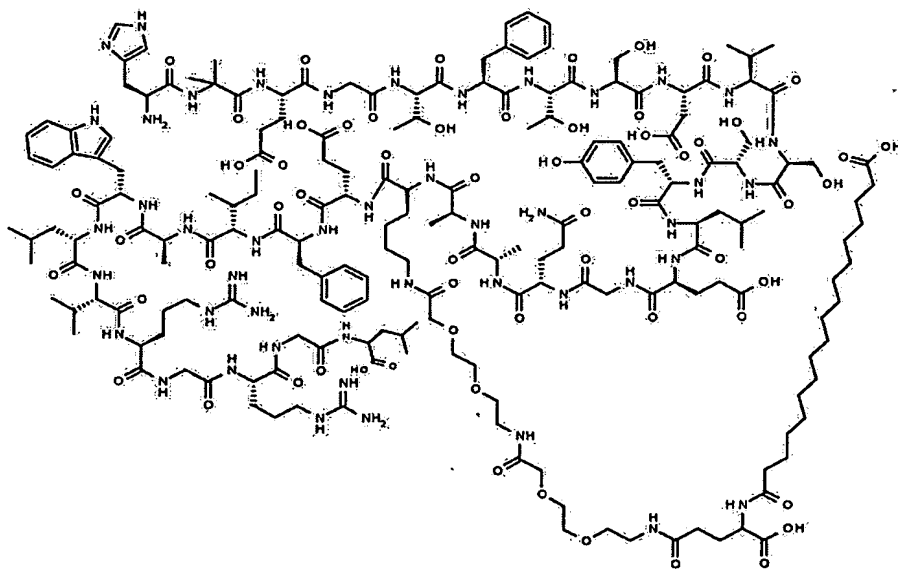
Figure 4: Reduction in food Intake in db/db mice

Figure 5: Efficacy in db/db mice in reduction of body weight

Figure 6: Reduction in Hb1Ac in db/db mice

DESCRIPTION OF THE INVENTION

The present invention provides a GLP-1 (7-38) analogue of Formula I



Formula I

The GLP-1 (7-38) analogue of Formula I has a long duration of action than the parent peptide, GLP-1 (7-37). It has a potential utility in treating diabetes and

obesity. The compound of formula I has shown significant glucose lowering effect and reduction of HbA1c values in animal model of diabetes.

In the following description, the abbreviations used for amino acids, protecting groups and peptides follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature as described in J. Biol. Chem. 250:3215 (1975); all optically active amino acids are of the L variety unless otherwise specified. The term analogue has been used interchangeably with the term derivative.

The GLP-1(7-38) analogue of the formula I can be prepared by methods described herein below. The process involves two steps, involving preparation of the parent peptide i.e Aib⁸, Arg³⁴, Leu³⁸ GLP-1(7-38) and subsequent attachment of fatty acid chain to the parent peptide.

There are many well-known methods for peptide synthesis. The peptide described herein may be produced by chemical synthesis using various solid-phase techniques such as those described in G. Barany and R. B. Merrifield, "The Peptides: Analysis, Synthesis, Biology"; Volume 2—"Special Methods in Peptide Synthesis, Part A", pp. 3-284, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980; and in J. M. Stewart and J. D. Young, "Solid-Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Co., Rockford, Ill., 1984. The desired strategy is based on the Fmoc (9-Fluorenylmethyl methyl-oxycarbonyl) group for temporary protection of the α -amino group, in combination with the tert-butyl group for temporary protection of the amino acid side chains (see for example E. Atherton and R. C. Sheppard, "The Fluorenylmethoxycarbonyl Amino Protecting Group", in "The Peptides: Analysis, Synthesis, Biology"; Volume 9—"Special Methods in Peptide Synthesis, Part C", pp. 1-38, S. Udenfriend and J. Meienhofer, Eds., Academic Press, San Diego, 1987).

The peptides can be synthesized in a stepwise manner on an insoluble polymer support (also referred to as "resin") starting from the C-terminus of the peptide. A synthesis is begun by appending the C-terminal amino acid of the peptide to the

resin through formation of an amide or ester linkage. This allows the eventual release of the resulting peptide as a C-terminal amide or carboxylic acid, respectively

The C-terminal amino acid and all other amino acids used in the synthesis are required to have their α -amino groups and side chain functionalities (if present) differentially protected such that the α -amino protecting group may be selectively removed during the synthesis. The coupling of an amino acid is performed by activation of its carboxyl group as an active ester and reaction thereof with the unblocked α -amino group of the N-terminal amino acid appended to the resin. The sequence of α -amino group deprotection and coupling is repeated until the entire peptide sequence is assembled. The peptide is then released from the resin with concomitant deprotection of the side chain functionalities, usually in the presence of appropriate scavengers to limit side reactions. The resulting peptide is finally purified by reverse phase HPLC.

The non-natural amino acid present at position 8 was incorporated into the peptide chain in one of two methods. In the first approach a Boc- or Fmoc-protected non-natural amino acid was prepared in solution using appropriate organic synthetic procedures. The resulting derivative was then used in the step-wise synthesis of the peptide. Alternatively the required non-natural amino acid was built on the resin directly using synthetic organic chemistry procedures.

Alternatively, a part of the parent peptide i.e Arg³⁴, Leu³⁸ GLP-1(9-38) can be produced by using recombinant technology, which comprises culturing a host cell containing a DNA sequence encoding the polypeptide and capable of expressing the polypeptide in a suitable nutrient medium under conditions permitting the expression of the peptide, after which the resulting peptide is recovered from the culture. The parent peptide can then be prepared by following the solid phase synthesis or using principles of organic chemistry as mentioned above.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells. Suitable media are available from commercial suppliers or may be prepared of published recipes (e.g. in catalogues of the American Type Culture Collection). The peptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like. The DNA sequence encoding the parent peptide may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the peptide by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (see, for example, Sambrook, J, Fritsch, EF and Maniatis, T, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). The DNA sequence encoding the peptide may also be prepared synthetically by established standard methods, e.g. the phosphoramidite method described by Beaucage and Caruthers, *Tetrahedron Letters* 22 (1981), 1859-1869, or the method described by Matthes et al., *EMBO Journal* 3 (1984), 801-805. The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in U.S. Pat. No. 4,683,202 or Saiki et al., *Science* 239 (1988), 487-491. The DNA sequence may be inserted into any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. The vector is preferably an expression vector in which the DNA sequence encoding the peptide is operably linked to additional segments required for transcription of the DNA, such as a promoter, a terminator, polyadenylation signals, transcriptional enhancer sequences, and translational enhancer sequences and a secretory signal sequence to direct the parent peptide of the present invention into the secretory pathway of the host cells.

The parent peptide can then be coupled to the fatty acid chain by coupling the protected fatty acid chain with the parent peptide. The fatty acid chain may be made by methods well known in organic chemistry. For eg the fatty acid chain can be made using solid phase synthetic methods which enables preparation of linear fatty acid chains.

The GLP-1(7-38) analogue of formula I is useful in treatment or prevention of hyperglycemia, type-2 diabetes, impaired glucose tolerance, obesity, hypertension, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, stroke, coronary heart disease and other cardiovascular disorders, inflammatory bowel syndrome, dyspepsia, gastric ulcers and combinations thereof comprising the step of: administering to the mammal a compound of Formula I and one or more pharmaceutically acceptable excipients. The GLP-1 analogue of Formula I is may be used in a method for treating a mammal having Type-2 diabetes associated with obesity, the method comprising administering to the mammal a compound of Formula I and one or more pharmaceutically acceptable excipients.

The treatment with a compound according to the present invention may also be combined with a one or more pharmacologically active substances, e.g. selected from antidiabetic agents, antiobesity agents, appetite regulating agents, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity. Examples of these pharmacologically active substances are: Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake,

RXR agonists and agents acting on the ATP-dependent potassium channel of the β -cells; Cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; β -blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and β -blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonists, mixed PYY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β 3 agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyrotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR β agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

Pharmaceutical compositions containing the compound according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen. The pharmaceutical

composition of the compound according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment. Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension or a powder for the administration of the compound of the present invention in the form of a nasal or pulmonal liquid or powder spray. As a still further option, the pharmaceutical compositions containing the compound of the invention can also be adapted to transdermal administration, e.g. by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, e.g. buccal, administration. The compounds of the present invention can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebulizers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien Y W. Pulmonary drug delivery: Physiologic and mechanistic aspects. Crit. Rev Ther. Drug Carr. Sys 14(4) (1997) 395-453).

Pharmaceutical compositions of the peptide compound of formula I may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, micro-emulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in

situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants. The compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound of the present invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions thereof, well-known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

The compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of compounds of the present invention, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all devices being well known to those skilled in the art.

The compositions of the current invention are specifically useful in the controlled, sustained, protracting, retarded, and slow release of compound of formula I. More specifically, the compositions of compound of Formula I are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to

those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneously. Without limiting the scope of the invention, examples of useful controlled release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles. Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high pressure homogenisation, encapsulation, spray drying, microencapsulating, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made to Handbook of Pharmaceutical Controlled Release (Wise, D. L., ed. Marcel Dekker, New York, 2000) and Drug and the Pharmaceutical Sciences vol. 99: Protein Formulation and Delivery (MacNally, E. J., ed. Marcel Dekker, New York, 2000).

The parenteral injection may be of a composition of GLP-1(7-38) analogue which may be a powder or a liquid. Pharmaceutical compositions containing GLP-1 analogue of the present invention may be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, 1985 or in Remington: The Science and Practice of Pharmacy, 19th edition, 1995. Thus, the injectable compositions of the GLP-1 analogue of the present invention can be prepared using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product. According to one procedure, the GLP-1 analogue of the present invention is dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonic agent, a preservative and a suitable buffer is added as required and the pH value of the solution is adjusted, if necessary, using an acid, e.g. hydrochloric acid, or a base, e.g. aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concentration of the ingredients. It is possible that other ingredients may be present in the pharmaceutical formulation of the GLP-1

analogue of the present invention. Such additional ingredients may include: surfactants, wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

The present invention is further illustrated in detail with reference to the following examples. It is desired that the example be considered in all respect as illustrative and are not intended to limit the scope of the claimed invention.

EXAMPLES

Example 1: Preparation of GLP-1 (7-38) analogue of Formula I

A: Synthesis of the parent peptide Aib⁸, Arg³⁴, Leu³⁸ GLP-1(7-38)

The parent peptide was synthesized by solid-phase method. The starting resin used for synthesis of GLP-1 peptide analogue, the title compound, was Wang resin. Fmoc protected Leucine was used for coupling with the Wang resin. The couplings were performed by using diisopropylcarbodiimide, N-hydroxybenzotriazole (DIC-HOBT) as coupling reagent in presence of 4-dimethyl aminopyridine (DMAP) which yields Fmoc-Leu-Wang Resin. Selective De-blocking of amino group of Fmoc-Leu-Wang Resin using piperidine followed by coupling of Fmoc-Gly-OH using of 1-Hydroxybenzotriazole(HOBT) and N,N'-Diisopropylcarbodiimide (DIPC) which yields Fmoc-Gly-Leu-Wang Resin. This completes one cycle. Acetic anhydride and Diisopropylethyl amine/Pyridine was used to terminate the uncoupled amino groups at every amino acid coupling.

The above 2 steps i.e selective deblocking of Fmoc- protection of amino acid attached to the resin and coupling of next amino acid residue in sequence with Fmoc-protected amino group is repeated for remaining 30 amino acid residues.

The side chain of the Fmoc-protected amino acids were protected orthogonally, for eg. hydroxyl group in of Serine, Tyrosine or Threonine are protected with -tBu group, amino and guanido group of Lysine and Arginine are protected using -Boc and -Pbf group respectively, the imidazole of Histidine with -Trt and carboxylic acid groups of Aspartic acid or glutamic acid are protected with -tBu group. The above mentioned two steps of selective deblocking and coupline are performed using 1-Hydroxybenztriazole(HOBT) and N,N'-Diisopropylcarbodiimide (DIPC)) to get Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-resin.

De-blocking of Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-Resin using piperidine followed by cleavage and de-protection using Trifluoroacetic Acid with Ethane-1,2-dithiol resulted in crude H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH (Aib⁸, Arg³⁴, Leu³⁸ GLP-1 (7-38) peptide) which was purified by HPLC.

B : Synthesis of the fatty acid side chain Intermediate-3 ((N-17-carboxy-1-oxoheptadecyl)-L-gamma-glutamyl-2-(2-(2-aminoethoxy)ethoxy)acetyl-2-(2-(2-aminoethoxy)ethoxy)acetyl-succinimide ester)

The fatty acid side chain was prepared using solid phase synthesis using 2-Chlorotrityl Chloride Resin (2-Cl-Trt-Resin). as schematically represented in Fig 1. {(2-(2-Fmoc-amino-ethoxy)-ethoxy}-acetic acid (intermediate 1) was attached to 2-Chlorotrityl Chloride Resin in presence of N,N'-Di-isopropylethylamine (DIPEA) which yields {(2-(2-Fmoc-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin. Intermediate 1 can be prepared by coupling of [2-(2-amino ethoxy)-ethoxy]

acetic acid with Fmoc N-hydroxysuccinimide ester. Alternatively, the intermediate is available commercially and can be procured as such. The Fmoc protecting group was removed by selective de-blocking of amino group of {(2-(2-Fmoc-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin} using piperidine. The free amino group of {(2-(2-amino-ethoxy)-ethoxy)-Acetic Acid-2-Cl-Trt-Resin} was then coupled to {(2-(2-Fmoc-amino-ethoxy)-ethoxy)-Acetic Acid} using 1-Hydroxybenzotriazole(HOBT) and N,N'-Di-isopropylcarbodiimide (DIPC) which yields {(Fmoc-amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking of amino group of {(Fmoc-amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin using piperidine. The free amino group of {(2-amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-resin was then coupled to Fmoc-Glu-OtBu using 1-Hydroxybenzotriazole(HOBT) and N,N'-Di-isopropylcarbodiimide (DIPC) which yielded Fmoc-Glu({(amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin)-OtBu. The resultant Fmoc-Glu({(amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin)-OtBu was selectively deblocked using piperidine to yield NH₂-Glu({(amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin)-OtBu. The free amino group of NH₂-Glu({(amino-ethoxy)-ethoxy}-acetyl-((-amino-ethoxy)-ethoxy)-acetic acid-2-Cl-Trt-Resin)-OtBu was coupled with Octadecanedioic acid mono t-butyl ester to give intermediate 2. Intermediate-2 was then cleaved from 2-Cl-Trt-Resin using Trifluoroethanol:DCM (1:1). The resultant compound was then reacted with N-Hydroxysuccinimide (HOSu) in presence of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMP) to yield succinimide protected Intermediate 3, which was de-protected with trifluoroacetic acid to yield Intermediate-3.

C. Synthesis of GLP-1(7-38) analogue of formula I

Grafting of Intermediate-3 over purified (Linear Peptide) H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-

Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH obtained in Step A, in acetonitrile at pH about 10 results in crude title peptide of formula I which on final purification with preparative HPLC yielded pure title peptide of formula I with a MW of 4226.8. The pure title peptide of formula I was obtained after lyophilization

Example 2: Oral Glucose tolerance test (OGTT) in rats; Single Injection; 1mg/kg

Animals were divided into three groups – a normal control group, a test group and a third semaglutide group, with 4 animals in each group. The animals were fasted for 12 hours before initiation of OGTT. To the test group animals, the GLP-1 analogue of Formula I was injected subcutaneously at 1 mg/kg dose. To the semaglutide group, a dose of 1 mg/kg was injected subcutaneously. After 22 hrs and after 46 hours of subcutaneous injection of test drug or semaglutide, blood glucose was measured with blood glucose meter (time 0 measurements). All the animals were then given 2 g/kg of glucose solution orally. Blood glucose was measured at 20, 40, 60, 90 and 120 minutes following glucose challenge. Body weight and food intake was recorded. Blood glucose data was analyzed using Two way ANOVA followed by Bonferroni posttests using PRISM (Graph Pad version 5.03). Data of Blood glucose AUC0-120 min, was analyzed using t test.

The GLP-1 analogue of Formula I has shown significant glucose lowering effect compared to the control group when studied in Oral Glucose Tolerance Test (OGTT) in rats. Fig. 2 provides change in blood glucose level from time 0 to 120 min after 22 h and 46 h in the test group and the semaglutide treatment group. At 22 hours after single dosing, the test group showed statistically significant reduction in blood glucose level with $p < 0.001$ vs normal control in ANOVA followed by Bonferroni's posttests. The glucose lowering effect of the test compound was superior than the glucose lowering effect observed with semaglutide (See Fig 2A). The superiority of glucose lowering effect of the GLP-1 analogue of the present invention was observed even after 46 hours of

subcutaneous administration (Fig.2 B). Further, both the test compound and semaglutide showed statistically significant reduction in food intake as compared to control when observed on day 2 as well as on day 4 (See Table 1&2). The reduced food intake shown by test compound on day 4 was greater than semaglutide.(See Table 2). In terms of body weight reduction, only the test compound showed significant reduction on body weight on day 4.

Table 1: Effect of treatment on food intake and body weight on Day 2

	Food Consumption (g) (day 0 to day 2)		Body Weight (g) (Day 2)		
	Mean	SD	Mean	SD	% Change vs Baseline
Normal Control	33.0	4.7	480.6	15.6	8.89
GLP-1 Analogue	2.63***	2.5	365.6	21.0	-9.33
Semaglutide	5.03***	3.4	428.1	42.3	-7.65

*p<0.05, **p<0.01, ***p<0.001 vs Normal Control; one way ANOVA followed by Bonferroni's posttests

Table 2 : Effect of treatment on food intake and body weight on Day 4

	Food Consumption (g) (day 0 to day 4)		Body Weight (g) (Day 4)		
	Mean	SD	Mean	SD	% Change vs Baseline
Normal Control	90.5	5.6	491.3	16.9	2.1
GLP-1 Analogue	43.25***,##	2.8	436.2*	21.1	-7.5
Semaglutide	55.73***	4.1	427.2	42.3	-5.3

*p<0.05, **p<0.01, ***p<0.001 vs Normal Control; one way ANOVA followed by Bonferroni's posttests

#p<0.05, ##p<0.01, ###p<0.001 vs Semaglutide; one way ANOVA followed by Bonferroni's posttests

Example 3: Reduction of HbA1c in db/db type 2 diabetic mice after chronic treatment

This study was done in diabetic mouse model. The animals were divided into three treatment groups- a diabetic control group, a test group and a semaglutide treatment group. The GLP-1 analogue of Formula I was injected subcutaneously at 0.3 mg/kg dose once a day for 3 days (qd*3) followed by 0.1 mg/kg dose every alternate day for 7 doses (q2d*7) followed by 0.1 mg/kg dose once every four days for two dose cycles (q4d*2). Same dosage regimen was administered in

semaglutide treatment group. Measurements of blood glucose levels and body weight were done daily. %HbA1c was measured on Day 0, day 7, day 14 and day 27 by column chromatography. Cumulative food intake was calculated on day 27. %HbA1C data was analyzed by two way ANOVA followed by Bonferroni's posttests using PRISM (Graph Pad version 5.03).

The test group animals administered with the GLP-1 analogue of Formula I showed statistically significant reduction in blood glucose levels as compared to the diabetic control group (See Fig 3), and the effect was superior to semaglutide treatment group in latter phase of the study. The test group animals administered with the GLP-1 analogue of Formula I showed significant reduction in food intake as can be seen in the results provided in Figure 4. Figure 4 provides cumulative food intake from 0 to 27th day by control and db/db mice treated with the test compound. Both the test compound and semaglutide showed a statistically significant reduction in food intake as compared to the diabetic control group. Further, the test compound showed a significantly lower food intake, as compared to semaglutide. In the same study, the GLP-1 analogue of formula I has also shown significant reduction in body weight when compared to diabetic control group. Figure 4 provides the result of % change in body weight for control and test group from day 0 to day 27th. The test compound showed a significant reduction of -16% as compared to -8% as observed in semaglutide treatment group (see Fig.5).

In diabetes mellitus, higher amounts of HbA1c, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy. In a 27 day study, the compound of Formula I has shown statistically significant reduction in HbA1c level in db/db type 2 diabetic mice after chronic treatment. Table 3 below and Figure 6 provide the level of HbA1c at 0 and 27 days in Diabetic control group and group after chronic treatment with the GLP-1 analogue of Formula I. The effect was statistically significant even when compared to semaglutide.

Table 3: Effect of treatment on % HbA1c levels in db/db mice

Time Point (Days)	% HbA1c										
	Diabetic Control (DC)			GLP-1 analogue of Formula I				Semaglutide			
	Mean	SD	n	Mean	SD	n	% Change vs DC	Mean	SD	n	% Change vs DC
0	4.70	0.52	6	4.87	0.59	6	-	5.05	0.35	5	-
27	7.30	1.17	6	3.73***##	0.86	6	-3.57	5.03***	0.69	5	-2.26

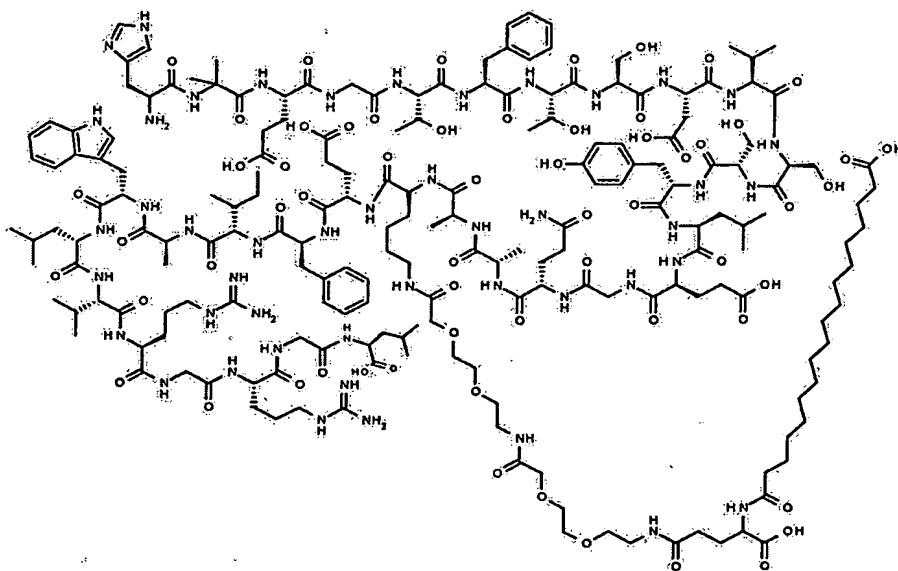
*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control; two way ANOVA followed by Bonferroni's posttests

#p<0.05, ##p<0.01, ###p<0.001 vs Semaglutide; one way ANOVA followed by Bonferroni's posttests

These results demonstrate that the compound of present invention can find potential use for the treatment of diabetes and obesity.

Provisional Claims:

1. A compound of Formula I



Formula I

Dated this 4th day of Apr, 2018

(Signature) Ratnesh Shrivastava

Dr. RATNESH SHRIVASTAVA
VICE PRESIDENT,
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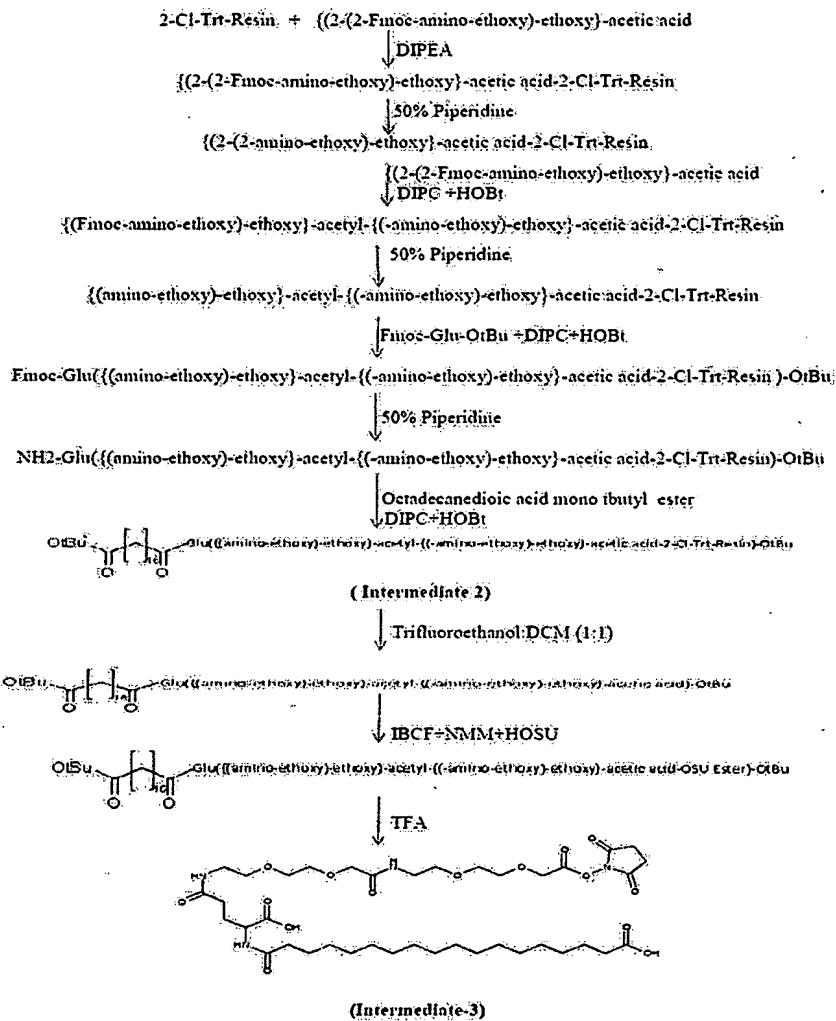


Fig 1

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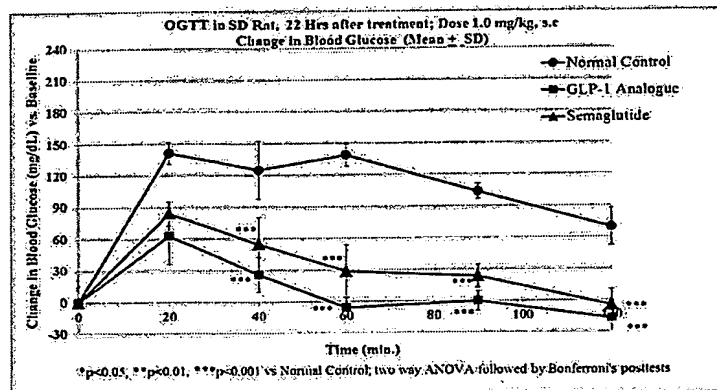


Figure 2 (A)

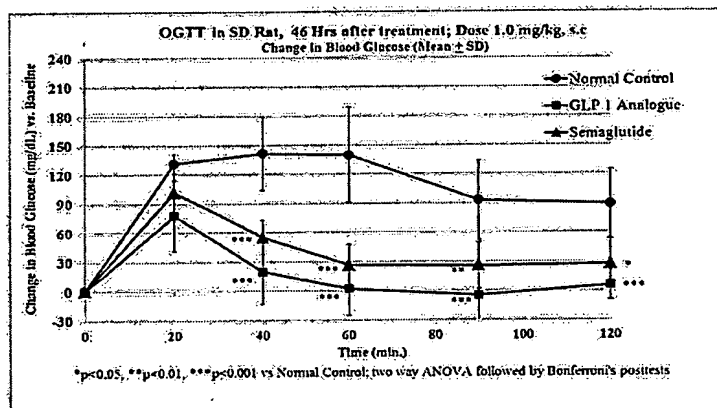


Fig 2 (B)

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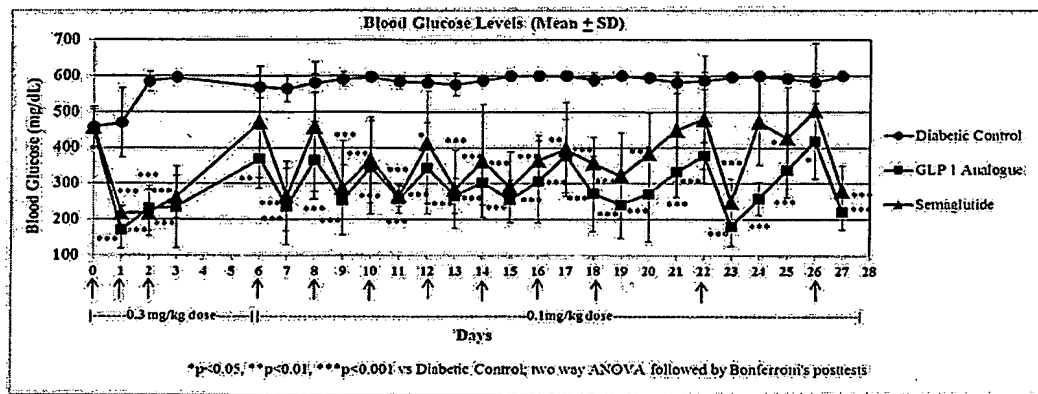


Fig 3

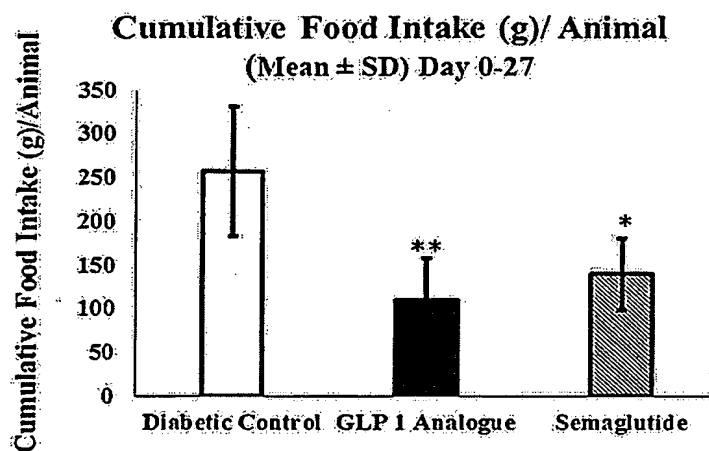
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*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control; one way ANOVA followed by Dunnett's posttests

Fig 4

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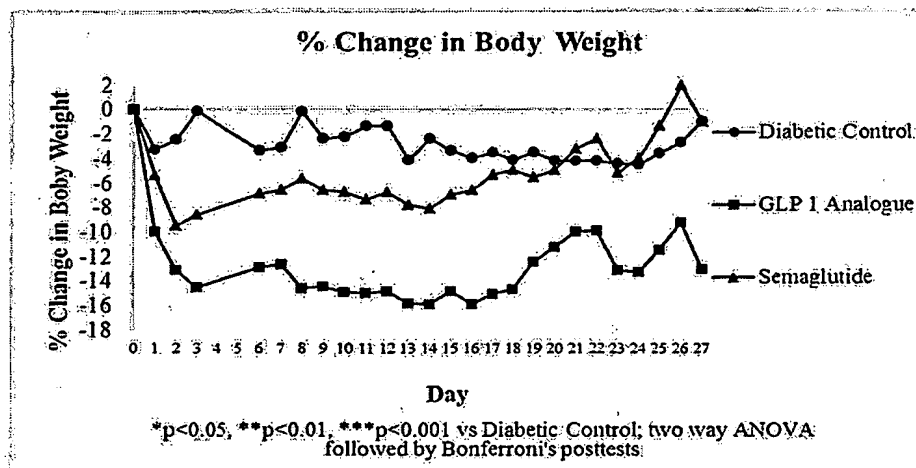


Fig 5

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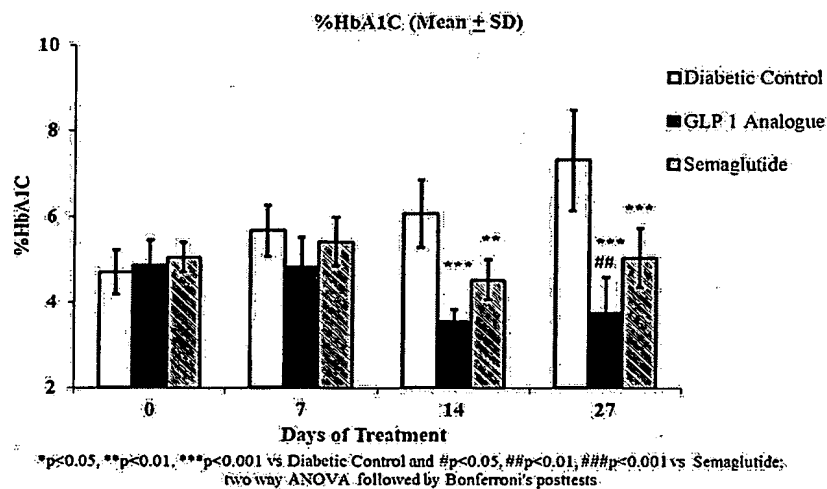


Fig 6

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ABSTRACT

NOVEL GLP-1 ANALOGUE

5 The present invention pertains to a novel GLP-1 (7-38) analogue for use in treatment of diabetes and obesity.

10

Dated this ^{4th} day of Apr, 2018

(Signature).....*Ratnesh Shrivastava*.....

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**जिस किसी से संबन्धित हो
TO WHOMSOEVER IT MAY CONCERN**

मैं, अधोहस्ताक्षरी जो पेटेंट अधिनियम, 1970 की धारा 73(3) के तहत महानियंत्रक एकस्व, अभिकल्प एवं व्यापार चिह्न की ओर से प्रमाणपत्र हस्ताक्षर व जारी करने के लिए प्राधिकृत अधिकारी हूँ, एतद्वारा यह प्रमाणित करता(ती) हूँ कि निम्नलिखित पेटेंट आवेदन के संबंध में फाइल दस्तावेज़(जों) की सही प्रतिलिपि इसके साथ संलग्न है:

I, the undersigned, being an officer duly authorized to sign and issue the certificate on behalf of the Controller General of Patents, Designs and Trademarks in accordance with the provisions of Section 73(3) of the Patents Act, 1970, hereby certify that annexed hereto is a True Copy of the document(s) as filed in connection with the following Patent Application:

- क) आवेदन संख्या / a) Application Number: **201821040474**
ख) फाइल करने की तारीख / b) Date of Filing: **26/10/2018**
ग) अनुरोधित दस्तावेज़(जों) का नाम / C) Name of the document(s) requested:

- 1) Application for Grant of Patent (Form-1)**
2) Provisional Specification filed on 26/10/2018

यह प्रमाणपत्र पेटेंट अधिनियम, 1970 की धारा 147(1) के अधीन मुझमें निहित शक्तियों के तहत जारी किया गया है।

This certificate is issued under the powers vested in me U/S 147(1) of The Patents Act, 1970.

दिनांक / Dated this 22nd day of April, 2019


(VIJAY T. DOYE)

सहायक नियंत्रक पेटेंट व डिजाइन / Assistant Controller of Patents and Designs
(प्राधिकृत हस्ताक्षरी / Authorized Signatory)

"FORM 1 THE PATENTS ACT 1970 (39 OF 1970) and THE PATENT RULES, 2003 APPLICATION FOR GRANT OF PATENT (See Section 7, 54 & 135 and sub-rule (1) of rule 20)		(FOR OFFICE USE ONLY)	
		Application No.	
		Filing date :	
		Amount of Fee paid:	
		CBR No.	
		Signature:	
1. APPLICANT'S REFERENCE/ IDENTIFICATION NO. (AS ALLOTTED BY OFFICE)			
2. TYPE OF APPLICATION [Please tick (✓) at the appropriate category]			
Ordinary (☒)		Convention ()	
PCT-NP ()			
Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()
3A. APPLICANT(S)			
Name in Full	Nationality	Country of Residence	Address of the Applicant
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			City MUMBAI
			State MAHARASHTRA
			Country INDIA
			Pin Code 400063
3B. CATEGORY OF APPLICANT [Please tick (✓) at the appropriate category]			
Natural Person ()		Other than Natural Person	
Small Entity ()		Startup ()	Others (☒)
4. INVENTORS (S) [Please tick (✓) at the appropriate category]			
Are all the inventor(s) same as the applicant (s) named above?		Yes ()	No (☒)
If "No", furnish the details of the inventor(s)			
Name in Full	Nationality	Country of Residence	Address of the Inventor

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			State	GUJARAT
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				ENCLAVE, TANDALJA ROAD,
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			Country	India
			Pin Code	390 012
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			City	BARODA
			State	GUJARAT
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			City	BARODA
			State	GUJARAT
			Country	India

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			Country	India
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			City	BARODA
			State	GUJARAT
			Country	India
TIWARI ABHISHEK	Indian	India	Pin Code	390 012
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			Street	NIMA COMPOUND, NEAR PRATHAM ENCLAVE, TANDALJA ROAD,
			City	BARODA
			State	GUJARAT
			Country	India
5. TITLE OF THE INVENTION- ACYLATED ANALOGUES OF GLP-1				
6. AUTHORISED REGISTERED PATENT AGENT(S)	IN/PA No.			
	Name			
	Mobile No.			
7. ADDRESS FOR SERVICE OF APPLICANT IN INDIA	Name	Dr. Bishram Singh Chouhan		
	Postal Address	General Manager, Sun Pharmaceutical Industries Ltd., Intellectual Property Cell, 17/B, Mahal Industrial Estate, Mahakali Caves Road, Andheri (East), Mumbai – 400093, India.		
	Telephone No.	91-22-6645- 5645		
	Mobile No.	+91-9727786543		
	Fax No.	91-22-6645-5685		

		E-mail ID		Bishram.Chouhan@sunpharma.com	
8. IN CASE OF APPLICANT CLAIMING PRIORITY OF APPLICATION FILED IN CONVENTION COUNTRY, PARTICULARS OF CONVENTION APPLICATION					
Country	Application Number	Filing date	Name of the applicant	Title of the invention	IPC (as classified in the convention country)
-NA-	-NA-	-NA-	-NA-	-NA-	-NA-
9. IN CASE OF PCT NATIONAL PHASE APPLICATION, PARTICULARS OF INTERNATIONAL APPLICATION FILED UNDER PATENT CO-OPERATION TREATY (PCT)					
International application number			International filing date		
-NA-			-NA-		
10. IN CASE OF DIVISIONAL APPLICATION FILED UNDER SECTION 16, PARTICULARS OF ORIGINAL (FIRST) APPLICATION					
Original (first) application No.			Date of filing of original (first) application		
-NA-			-NA-		
11. IN CASE OF PATENT OF ADDITION FILED UNDER SECTION 54, PARTICULARS OF MAIN APPLICATION OR PATENT					
Main application/patent No.			Date of filing of main application		
-NA-			-NA-		
12. DECLARATIONS					
(i) Declaration by the inventor(s) (In case the applicant is an assignee : the inventors(s) may sign herein below or the applicant may upload the assignment or enclose the assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period). I/We, the above named inventor(s) is/are the true & first inventor(s) for this Invention and declare that the applicant(s) herein is/are my/our assignee or legal representative. (a) Date: Oct 26, 2018 (b) Signature(s) 1) <u>Najam</u> 2) <u>N. Chaturvedi</u>					

3) Wismada

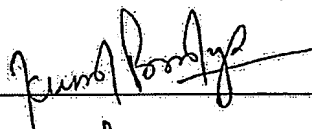
4) Iskaka

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6) amb

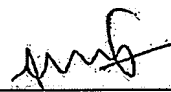
7) Zabinsadia

8) 

9) 

10) 

11) 

12) 

(c) Name(s)

- 1) THENNATI RAJAMANNAR
- 2) CHATURVEDI NISHITH
- 3) BURADE VINOD SAMPATRAO
- 4) SHAHI PRADEEP DINESH
- 5) NATARAJAN MUTHUKUMARAN
- 6) NAGARAJA RAVISHANKARA MADAVATI
- 7) ZALAWADIA RISHIT MANSUKHLAL
- 8) PATEL VIPULKUMAR SHANKARBHAI
- 9) PANDYA KUNAL
- 10) JOSHI DHIREN RAMESHCHANDRA
- 11) SONI KRUNAL HARISHBHAI
- 12) TIWARILABHISHEK

(ii) Declaration by the applicant(s) in the convention country:

(In case the applicant in India is different than the applicant in the convention country: the applicant in the convention country may sign herein below or applicant in India may upload the assignment form the applicant in the convention country or enclose the said assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period)

I/We the applicant(s) in the convention country declare that the applicant(s) herein is/are my/our assignee or legal representative.

(a) Date

(b) Signature(s)

-NA-

(c) Name(s) of the signatory

iii) Declaration by the applicant(s):

I/we the applicant(s) hereby declare(s) that: -

- ☒ I am/We are in possession of the above mentioned invention
- ☒ The provisional/~~complete~~ specification relating to the invention is filed with this application
- ☒ The invention as disclosed in the specification uses the biological material from India and the necessary permission from the competent authority shall be submitted by me/us before the grant of patent to me/us
- ☒ There is no lawful ground of objection(s) to the grant of the patent to me/us.
- ☒ I am/we are the true & first inventor(s).
- ☒ I am/we are the assignee or legal representative of true and first inventor(s).
- ☒ The application or each of the applications, particular of which are given in para-8 was the

first application in convention country/countries in respect of my/our invention(s).

☒ I/we claim the priority from the above mentioned application (s) filed in convention country/countries and state that no application for protection in respect of the invention had been made in a convention country before that date by me/us or by any person from which I/we derive the title

☒ My/our application in India is based on international application under patent cooperation treaty (PCT) as mentioned in Para-9.

☒ The application is divided out of my/our application particulars of which is given in para-10 and prays that this application may be treated as deemed to have been filed on DD/MM/YY under sec 16 of the Act.

☒ The said invention is an improvement in or modification of the invention particulars of which are given in Para- 11.

13. FOLLOWING ARE THE ATTACHMENTS WITH THE APPLICATION

(a) Form 2

Item	Details	Fee	Remarks
Complete/provisional specification)#	No. of pages -37	Rs. 16800 /-	Fees including 4 pages of Sequence listing.
No. of claim(s)	No. of claims and No. of pages: None	-	
Abstract	No. of pages- 01	-	No. of pages included in the total no. of pages of provisional specification.
No. of Drawing(s)	No. of drawings and No. of pages- None	-	No. of pages included in the total no. of pages of provisional specification.

In case of a complete specification, if the applicant desires to adopt the drawings filed with his provisional specification as the drawings or part of the drawings for the complete specification under rule 13(4), the number of such pages with the provisional specification are required to be mentioned here.

(b) Complete specification (in conformation with the international application/as amended before the international Preliminary Examination Authority (IPEA), as applicable (2 copies).

(c) Sequence listing in electronic form

(d) Drawings (in confirmation with the international application/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2 copies).

(e) Priority document(s) or a request to retrieve the priority document(s) from DAS (Digital Access Service) if the applicant had already requested the office of first filing to make the priority document(s) available to DAS.

(f) Translation of priority document/Specification/International Search Report/International Preliminary Report on Patentability.

(g) Statement and Undertaking on Form 3

(h) Declaration of Inventorship on Form 5

(i) Power of Authority

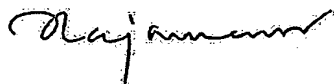
(j)

Total fee Rs. 16800/- via online transfer Cash/ Banker's Cheque /Bank Draft bearing No.

I/We hereby declare that to the best of my/our knowledge, information and belief the fact and matters stated herein are correct and I/we request that a patent may be granted to me/us for the said invention.

Dated this 26th day of Oct, 2018:

Signature:



Name: Dr. RAJAMANNAR THENNATI
EXECUTIVE VICE PRESIDENT,
SUN PHARMACEUTICAL INDUSTRIES LTD.

To,
The Controllers of Patents
The Patent Office, at MUMBAI

Note:-

- Repeat boxes in case of more than one entry.
- To be signed by the applicant(s) or by authorized registered patent agent otherwise where mentioned.
- Tick (✓)/cross (×) whichever is applicable/not applicable in declaration in paragraph-12.
- Name of the inventor and applicant should be given in full, family name in the beginning.
- Strike out the portion which is/are not applicable.

For fee: See First Schedule".

FORM 2

THE PATENTS ACT, 1970

[39 of 1970]

&

PATENTS RULES, 2003

PROVISIONAL SPECIFICATION

(Sec section 10 and rule 13)

ACYLATED ANALOGUES OF GLP-1

SUN PHARMACEUTICAL INDUSTRIES LTD.

A company incorporated under the laws of India having their office at
SUN HOUSE, 201 B/1, WESTERN EXPRESS HIGHWAY, GOREGOAN (E), MUMBAI-
400063 MAHARASHTRA, INDIA.

The following specification describes invention.

ACYLATED ANALOGUES OF GLP-1

FIELD OF THE INVENTION

The present invention pertains to a novel Glucagon like Peptide-1 (GLP-1) (7-38) analogs
5 which are acylated with a protracting moiety having fatty acid chain and a linker. The
moieties of the present invention afford compounds which have long duration of activity. The
new analogs are potent GLP-1 agonist with reduced adverse effect and improved duration of
action. The analogs of present invention may be useful in treatment of diabetes and obesity.

10 BACKGROUND OF THE INVENTION

Glucagon-like peptide-1 (GLP-1) is a hormone that is mainly produced in enteroendocrine L
cells of the gut and is secreted into the blood stream when food containing fat, protein
hydrolysate and/or glucose enters the duodenum. GLP-1 is derived from the cell-specific
post-translational processing of the preproglucagon gene. Initially, the peptide GLP-1(1-37)
15 was identified from this processing, but it was the two *N*-terminally truncated products, GLP-
1(7-37) and GLP-1(7-36)amide, that were found to recognize the pancreatic receptor and
which were determined to be the active species *in vivo*. GLP-1 has been found to stimulate
insulin secretion, thereby causing glucose uptake by cells and decreased serum glucose
levels. GLP-1 agonists are available for the treatments for Type 2 Diabetes Mellitus (T2DM)
20 as favored drugs as they are devoid of hypoglycemia and with a positive benefit of weight
loss. The endogenous substance, GLP-1(7-37) and GLP-1(7-36)amide, are cleaved by
peptidases and thus have very short half-life. Efforts were made to improve the performance
by developing GLP-1 analogues with improved half-life. The first drug approved in 2005 is
Exenatide with twice a day dosing at dose level 10 mcg and was found to show a significant
25 improvement in HbA1c, a marker of glucose control. Further, Novo Nordisk developed
Liraglutide (United States Patent No. US6268343) with once a day dosing of 1.8 mg, s.c./day
and approved in 2010. Further research and development produced once a week products
like, Albiglutide developed by GSK and Dulaglutide developed by Eli Lilly. Recently,
Semaglutide (WO2006097537A2), a GLP-1 analogue was approved by USFDA.
30 Semaglutide is marketed under the brand name Ozempic®. It is administered as a once-
weekly subcutaneous injection.

Many attempts to make GLP-1 analogs having improved potency and duration of action are
reported in literature. United States patent No. US7291594B2 (the US '594 patent) discloses

GLP-1 (7-35) derivatives having added several residues of arginine and/or lysine to the C-terminus thereof to provide high bioavailability via mucous membranes. The US '594 patent further discloses that these derivative can be conferred with resistance to dipeptidyl peptidase IV (DPP-IV) by substituting amino acid 8 in its GLP-1 amino acid sequence with Ser, or with
5 resistance to trypsin by substituting amino acids 26 and 34 with Gln and Asn, respectively.

United States patent No. US 7893017B2 (the US '017 patent) discloses acylated GLP-1 analog wherein the said GLP-1 analog is stabilized against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1 (7-37) and wherein said acylation is a diacid attached directly to the C-terminal amino acid residue of
10 said GLP-1 analog.

United States patent No. US 8951959B2 (the US '959 patent) discloses DPP-IV resistant GLP-1 (7-37) analogue having a non-proteogenic amino acid residue containing trifluoromethyl group in position 8 relative to the sequence GLP-1, and is acylated with a
15 moiety comprising two acidic groups to the lysine residue in position 26.

United States patent No. US7084243B2 (the US '243 patent) discloses GLP-1 (7-37) analogues having Val or Gly at position 8 relative to the sequence GLP-1 (7-37) as DPP-IV resistant peptides.
20

WO2017149070A1(the WO '070) discloses GLP-1 analogues having a Trp at a position corresponding to position 8 of GLP-1 (7-37) and these Trp8 compounds were shown to be very stable against degradation by DPP-IV.

25 WO2004103390A2 (the WO '390) discloses that the modification at the P₁ position (corresponding to 9 position in case of GLP-1 (7-37)) can produce GLP-1 analogues with greatly reduce susceptibility to enzyme-mediated (such as DPP-IV) cleavage relative to the native substrate, yet retain the biological activity of the native substrate. The WO '390 further discloses GLP-1 (7-37) analogues having an amino acid with tetrasubstituted C β carbon
30 (such as *tert*-leucine) at position 9 provides GLP-analogues with resistant to degradation by DPP-IV.

WO2015086686A2 (the WO'686 publication) discloses that incorporation of alpha-methyl-functionalized amino acids directly into the main chain of GLP-1 analogues has been determined to produce protease-resistant (includes DPP-IV resistant) peptides.

Various other DPP-IV resistant GLP-1 agonists are disclosed in patent publications such as
5 WO2007030519A2, WO2004078777A2, WO2007039140A1, WO2014209886A1,
WO2012016419A1, WO2017211922A2, WO2016198544A1 and WO2013051938A2.

Various patent applications disclose C-terminally extended GLP-1 analogues with increased stability and longer duration of action. For e.g. United States patents US7482321B2, US9498534B2 and US7897566B2.

10 Various patent applications disclose acylated GLP-1 analogs wherein the GLP-1 analogues are attached to lipophilic substituent optionally via a linker to provide longer duration of action. United States patent No. US8603972B2 discloses monoacylated derivatives of GLP-1 analogues wherein Lys residue at position 37 or 38 of GLP-1 analogue is acylated. United
15 States patent No. US8648041B2, US9758560B2, US9006178B2, US9266940B2, US9708383B2 and the United States patent application publications US20150152157A1, US20150133374A1 discloses di-acylated derivatives of GLP-1 analogues. United States patent application publication US20160200791A1 discloses triacylated derivatives of GLP-1 analogues.

20 WIPO patent publication WO2016083499A1, WO2016097108A1 and WO2014202727A1 discloses acylated GLP-1 analogues wherein the Lys residue of GLP-1 analogues is attached to two protracting moieties via a branched linker. WIPO patent publication WO2009030771A1 and WO2018083335A1 discloses various acylating agents (side chain)
25 which can be attached to Lys residue of GLP-1 analogues to provide longer duration of action.

WIPO patent publication WO2013186240A2 discloses exendin-4 peptide analogues having Gly, Ser or functionalized Ser, e.g. Ser (OCH₃), D-Ser or functionalized D-Ser, e.g. D-Ser(OCH₃), Aib, Ala, or D-Ala at position 2 of exendin-4 amino acid sequence.
30

Various other GLP-1 analogues are disclosed in patent applications such as WIPO publication number WO2005027978A2, WO1998008871A1, WO1999043705A1, WO1999043706A1, WO1999043707A1, WO1999043708A1, WO2000034331A2,

WO2009030771A1, WO2011080103A1, WO2012140117A1, WO2012062803A1,
WO2012062804A1, WO2013037690A1, WO2014202727A1, WO2015000942A1,
WO2015022400A1, WO2016083499A1, WO2016097108A1 and WO2017149070A1.

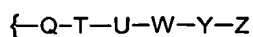
- 5 Still, there is need to develop a GLP-1 analog which have optimum desired properties in terms of stability and duration of action.

SUMMARY OF THE INVENTION

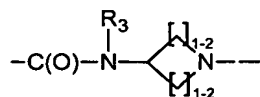
- 10 In one aspect the present invention provides a polypeptide comprising the amino acid sequence:

H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-G-R-G-X33-X34

- wherein X2 is Ser, Ser(Me), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;
15 X3 is absent or Gln;
X4 is Gln or Glu;
X16 is Glu or Asp;
X24 is Ile or Val or Leu;
20 X33 Leu, Ile, Tyr or absent;
X34 is Ser, Lys, Ala or absent and
X21 is Lys wherein the side chain amino (ε amino) group of Lys is acylated with a moiety:



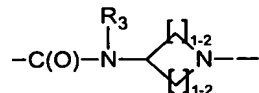
- wherein Q, T, U and W are absent or selected from a group consisting of -C(O)-M-NH-
25 wherein M is a C₅₋₁₀ alk chain interrupted with 1, 2 or 3 oxygen atoms, -C(O)-N(R₁)-C₂₋₅ alk-N(R₁)-, -C(O)-C(CH₃)₂-N(R₂)-and



wherein R₁, R₂ or R₃ is independently selected from hydrogen or C₁₋₃ alkyl;
provided that:

- 30 i. at least one of Q, T, U and W is always present and,

ii. at least one of Q, T, U and W is always selected from groups C(O)-N(R₁)-C₂₋₅ alk-N(R₁)-, -C(O)-C(CH₃)₂-N(R₂)—and



Y is either absent or -C(O)-(CH₂)_m-CH(COOH)NH-- wherein m is an integer selected from
5 1 to 2 and -- is point of attachment with the group Z;

Z is -C(O)-(CH₂)_n-COOH or -C(O)-(CH₂)_n-CH₃ wherein n is an integer from 10 to 20.

The polypeptides of the present invention are potent GLP-1 agonist with fewer adverse
10 effects. Further, the polypeptides of the present invention are stable and have long duration of action and are suitable for oral administration.

DESCRIPTION OF FIGURES

Figure 1: Preparation of Moiety C-OSu

15 Figure 2: Preparation of Moiety D-OSu

Figure 3: Preparation of Moiety E-OSu

Figure 4: Preparation of Moiety F-OSu

Figure 5: Preparation of Moiety G-OSu

20 ABBREVIATIONS

Aib: 2-Aminoisobutyric acid

ADO: 8-Amino-3,6-dioxo-octanoic acid

OGTT: Oral glucose tolerance test

DIPEA: *N,N'*-Di-isopropylethylamine

25 HOBt: 1-Hydroxybenztriazole

DIPC: *N,N'*-Di-isopropylcarbodiimide

HOSu: *N*-Hydroxysuccinimide

IBCF: Isobutyl chloroformate

NMM: *N*-Methylmorpholine

30 THF: Tetrahydrofuran

DCM: Dichloromethane

DMAP: 4-Dimethylaminopyridine

DCC: Dicyclohexyl carbodiimide

DMAc: Dimethylacetamide

DESCRIPTION OF THE INVENTION

5

The present invention provides a stable long acting GLP-1 analog which does not require frequent subcutaneous dosing and is also suitable for oral administration. The applicants have developed moieties which can be appended to peptides which are analogs of GLP-1(7-37) via acylation reaction to afford compounds with significantly improved potency and longer

10 duration of action. The protracting moieties of present invention have more stable bonds which are less susceptible to cleavage by biological enzymes. Thus the compounds of present invention are more stable and require less frequent administration adding to patient compliance. Accordingly, the present invention provides a polypeptide comprising the amino acid sequence:

15 H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-G-R-G-X33-X34

wherein X2 is Ser, Ser(Me), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

X3 is absent or Gln;

20 X4 is Gln or Glu;

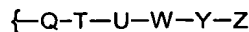
X16 is Glu or Asp;

X24 is Ile or Val or Leu;

X33 Leu, Ile, Tyr or absent;

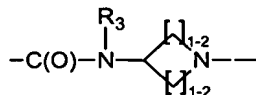
X34 is Ser, Lys, Ala or absent and

25 X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q, T, U and W are absent or selected from a group consisting of $-C(O)-M-NH-$

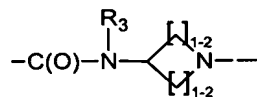
wherein M is a C₅₋₁₀ alk chain interrupted with 1, 2 or 3 oxygen atoms, $-C(O)-N(R_1)-C_{2-5} alk-N(R_1)-$, $-C(O)-C(CH_3)_2-N(R_2)-$ and



wherein R₁, R₂ or R₃ is independently selected from hydrogen or C₁₋₃ alkyl;

provided that:

- i. at least one of Q, T, U and W is always present and,
- ii. at least one of Q, T, U and W is always selected from groups $C(O)-N(R_1)-C_{2-5} \text{ alk-}$
 $N(R_1)-$, $-C(O)-C(CH_3)_2-N(R_2)-$ and



5

Y is either absent or $-C(O)-(CH_2)_m-CH(COOH)NH-$ wherein m is an integer selected from 1 to 2 and -- is point of attachment with the group Z;

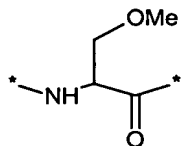
Z is $-C(O)-(CH_2)_n-COOH$ or $-C(O)-(CH_2)_n-CH_3$ wherein n is an integer from 10 to 20.

10

Unless stated otherwise, the specification intends to cover both L and D isomers of the amino acids in the sequence.

Ser(OMe) as described herein in the specification is amino acid serine with its hydroxyl group methylated and has following structure.

15



In another aspect, the present invention provides a polypeptide comprising the amino acid sequence:

H-X2-X3-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-X21-E-F-I-A-W-L-V-R-G-R-G-X33-

20 X34

wherein X2 is Ser, Ser(OMe), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C_{3-8} cycloalkyl) carboxylic acid, Trp or Thr;

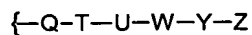
X3 is absent or Gln;

X4 is Glu;

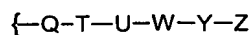
25 X33 is Leu or Ile;

X34 is absent or Ala and

X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety with following structure:



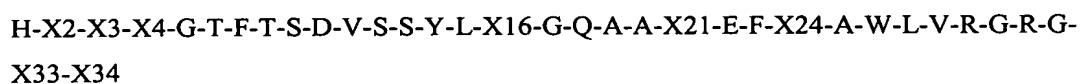
The moiety



is also referred as the fatty acid side chain and protracting moiety in the specification.

5

In another aspect the present invention provides polypeptide comprising the amino acid sequence:



10 wherein X2 is Ser, Ser(OMe) , Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

X3 is absent or Gln;

X4 is Gln or Glu;

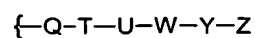
X16 is Glu or Asp;

15 X24 is Ile or Val or Leu;

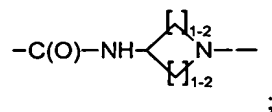
X33 is Leu, Ile or Tyr;

X34 is absent or Ala and

X21 is Lys wherein the side chain amino (ε amino) group of Lys is acylated with a moiety:

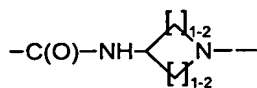


20 wherein Q, T, U and W are absent or selected from a group consisting of $-C(O)-M-NH-$ wherein M is a C₅₋₁₀ alk chain interrupted with 1, 2 or 3 oxygen atoms, $-C(O)-NH-C_{2-5}$ alk-NH-, $-C(O)-C(CH_3)_2-NH-$ and



provided that:

- 25
- i. at least one of Q, T, U and W is always present and,
 - ii. at least one of Q, T, U and W is always selected from a group consisting of $-C(O)-NH-C_{2-5}$ alk-NH-, $-C(O)-C(CH_3)_2-NH-$ and



The bond depicted as "--" is point of attachment with the group on the right hand side of the structure. For example when Q is $-C(O)-M-NH-$, the $NH-$ group is attached to T.

- 5 In the group $-C(O)-M-NH-$, M is a C_{5-10} alk chain interrupted with 1, 2 or 3 oxygen atoms. The term "interrupted" means that between two carbons of the alkyl chain an oxygen atom is inserted. The oxygen atom cannot be at the end of the chain (terminal position) and two oxygen atoms cannot be present adjacent (peroxide linkage) to each other. The chain length is excluding the number of oxygen atoms i.e. if a C_5 alkyl chain is interrupted with two oxygen atoms then the total chain length will be of total 7 atoms.
- 10

In another preferred embodiment, the group $-C(O)-NH-C_{2-5}$ alk- $NH-$ is $-C(O)-NH-C_3H_7-NH-$. In another preferred embodiment, the group $-C(O)-NH-C_{2-5}$ alk- $NH-$ is $-C(O)-NH-C_4H_9-NH-$.

15

In an embodiment, the amino acid at X2 is selected from Ser, Ser(OMe), Ala or Aib.

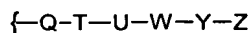
In another embodiment, X3 is absent.

In another embodiment, X3 is absent and X4 is Gln.

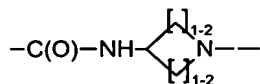
In a preferred embodiment, X33 is Leu or Ile and X34 is Ala.

- 20 In another preferred embodiment, X33 is Leu or Ile and X34 is absent.

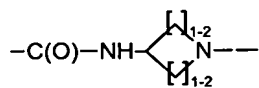
In another embodiment X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



- In another embodiment, W is absent and T and W are $-C(O)-M-NH-$ and U is selected from a group consisting of $-C(O)-M-NH-$, $-C(O)-NH-C_{2-5}$ alk- $NH-$, $-C(O)-C(CH_3)_2-NH-$ and
- 25

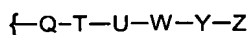


In another embodiment, T is $-C(O)-M-NH-$; U is selected from a group consisting of $-C(O)-M-NH-$, $-C(O)-NH-C_{2-5}$ alk- $NH-$, $-C(O)-C(CH_3)_2-NH-$ and

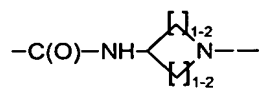


and Q and W are absent.

- In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino)
 5 group of Lys is acylated with a moiety:



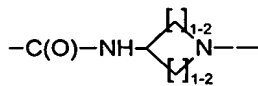
wherein Q is absent; T is selected from a group consisting of $-C(O)-NH-C_{2-5} \text{ alk-NH--}$, $-C(O)-C(CH_3)_2-NH--$ and



and

- 10 U and W are $-C(O)-M-NH--$.

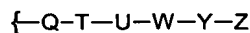
The protracting moieties of the present invention have bonds which are less susceptible to cleavage by the biological enzymes thus affording compounds with long duration of action. For example, when Q, T, U or W is selected from $-C(O)-NH-C_{2-5} \text{ alk-NH--}$ or



15

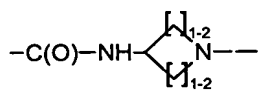
it forms a $-NH-C(O)-NH--$ (urea) linkage which is relatively difficult to get cleaved by the enzymes when compared to simple amide bonds. Similarly, when Q, T, U or W is $-C(O)-C(CH_3)_2-NH--$ (Aib) it forms an amide linkage which is relatively more stable.

- 20 Accordingly, in an embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino) group of Lys is acylated with a moiety:

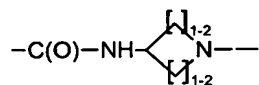


wherein

- i. at least one of Q, T, U and W is always present and,
 25 ii. at least one of Q, T, U and W is always selected from groups $-C(O)-NH-C_{2-5} \text{ alk-NH--}$, $-C(O)-C(CH_3)_2-NH--$ and



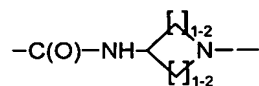
In another embodiment, Q and T are $-\text{C}(\text{O})-\text{M}-\text{NH}-$ and U is selected from a group consisting of $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5} \text{ alk}-\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and



and W is absent.

5

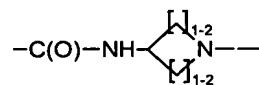
In another embodiment, Q is $-\text{C}(\text{O})-\text{M}-\text{NH}-$; T is selected from a group consisting of $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5} \text{ alk}-\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and



and

U and W are absent.

- 10 In another embodiment, Q is selected from a group consisting of $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5} \text{ alk}-\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and



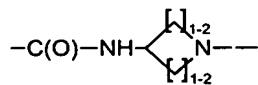
;

T and U are $-\text{C}(\text{O})-\text{M}-\text{NH}-$ and W is absent.

- 15 In a preferred embodiment, $-\text{C}(\text{O})-\text{M}-\text{NH}-$ group is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$.

In another embodiment, wherein Q is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is $-\text{C}(\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}-$ and U and W are absent.

- 20 In another embodiment, Q is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is



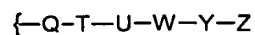
; and U and W are absent.

In another embodiment, Q is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and U and W are absent.

In another embodiment, Q and U are $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and W is absent.

5

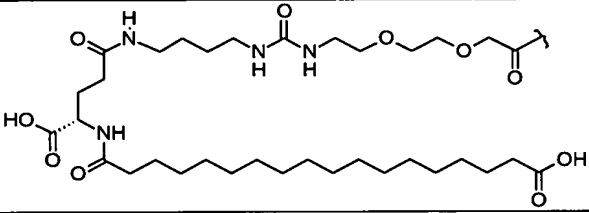
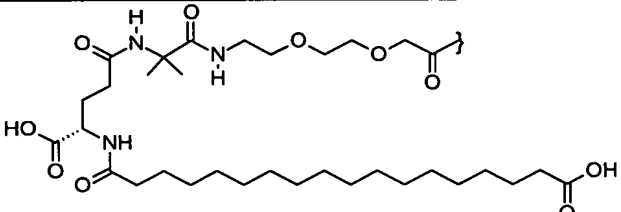
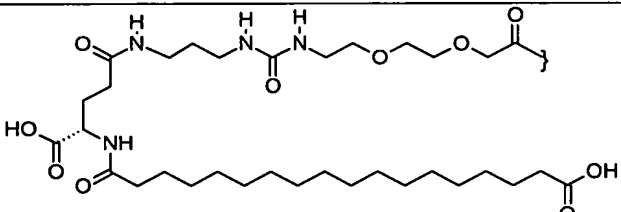
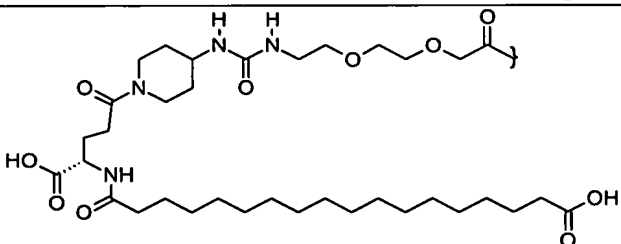
In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety

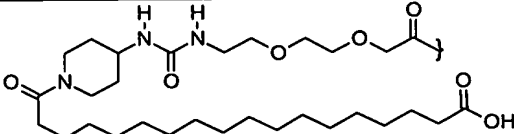
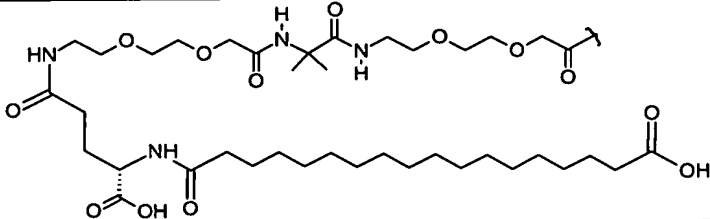
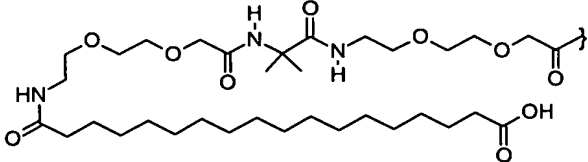
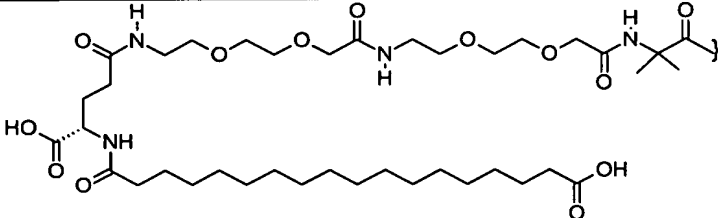
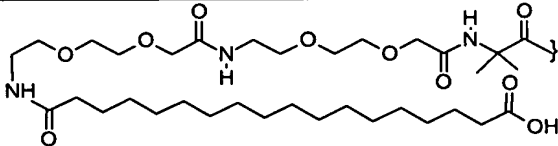
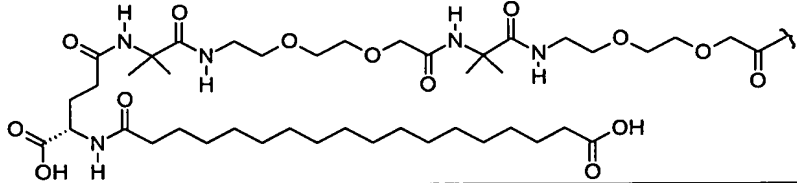
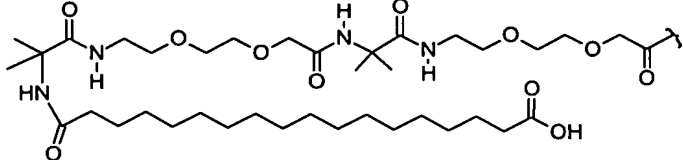


which is represented by the moieties provided in Table 1

10

Table 1: Representative moieties for group $\{-\text{Q}-\text{T}-\text{U}-\text{W}-\text{Y}-\text{Z}\}$

Designation	Moiety
Moiety C	
Moiety D	
Moiety E	
Moiety F	

Moiety G	
Moiety H	
Moiety I	
Moiety J	
Moiety K	
Moiety L	
Moiety M	

In another aspect the present invention provides a polypeptide comprising the amino acid sequence:

H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-G-R-G-X33-X34

wherein X2 is Ser, Ser(OMe), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

5 X3 is absent or Gln;

X4 is Gln or Glu;

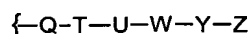
X16 is Glu or Asp;

X24 is Ile or Val or Leu;

X33 is Leu, Ile, Tyr, Ser, Lys, amide or absent;

10 X34 is Ser, Lys, Ala, amide or absent and

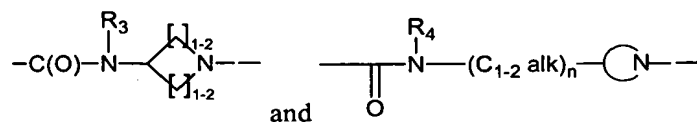
X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q, T, U and W are absent or selected from a group consisting of $-C(O)-M-NH-$

wherein M is a C₅₋₁₀ alk chain interrupted with 1, 2 or 3 oxygen atoms, $-C(O)-N(R_1)-C_{2-5}$ alk-

15 $N(R_1)-$, $-C(O)-C(CH_3)_2-N(R_2)-$,

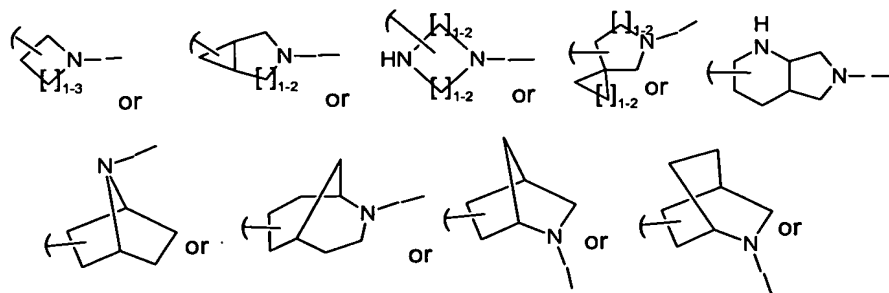


wherein R₁, R₂, R₃ or R₄ is independently selected from hydrogen or C₁₋₃ alkyl;

n is an integer selected from 0 or 1 and

ring

20 N is selected from following moieties:

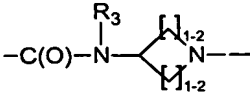


wherein '(' denotes point of attachment to the ϵ amino group of Lys;

provided that:

i. at least one of Q, T, U and W is always present and,

ii. at least one of Q, T, U and W is always selected from groups C(O)-N(R₁)-C₂₋₅ alk-N(R₁)-, -C(O)-C(CH₃)₂-N(R₂)-and



Y is either absent or -C(O)-(CH₂)_m-CH(COOH)NH-- wherein m is an integer selected from
5 1 to 2 and -- is point of attachment with the group Z;

Z is -C(O)-(CH₂)_n-COOH or -C(O)-(CH₂)_n-CH₃ wherein n is an integer from 10 to 20.

In another embodiment, the present invention provides a polypeptide according to anyone of
10 the preceding embodiments, which is selected from the peptides provided in Table 2:

Table 2: Representative polypeptide compounds of present invention

Comp. #	Structure*	Seq ID
1	Structure 1: A polypeptide chain with a branched N-terminus (isopropylglycine) and a side chain containing a long alkyl amine group attached to the side chain of the 10th residue (Lysine). The sequence is E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH₂. The side chain is -CH₂-CH₂-CH₂-CH₂-CH₂-NH₂.	Seq ID:05
2	Structure 2: A polypeptide chain with a linear N-terminus (Glycine) and a side chain containing a long alkyl amine group attached to the side chain of the 10th residue (Lysine). The sequence is H-S-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH₂. The side chain is -CH₂-CH₂-CH₂-CH₂-CH₂-NH₂.	Seq ID:06
3	Structure 3: A polypeptide chain with a branched N-terminus (isopropylglycine) and a side chain containing a long alkyl amine group attached to the side chain of the 10th residue (Lysine). The sequence is E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH₂. The side chain is -CH₂-CH₂-CH₂-CH₂-CH₂-NH₂.	Seq ID:07
4	Structure 4: A polypeptide chain with a linear N-terminus (Glycine) and a side chain containing a long alkyl amine group attached to the side chain of the 10th residue (Lysine). The sequence is H-S-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH₂. The side chain is -CH₂-CH₂-CH₂-CH₂-CH₂-NH₂.	Seq ID:05

5		Seq ID:05
6		Seq ID:05
7		Seq ID:05
8		Seq ID:05
9		Seq ID:05
10		Seq ID:05
11		Seq ID:05
12		Seq ID:08

* Unless stated otherwise, all the amino acids in the structures have L configuration at α position.

The polypeptides of the present invention surprisingly showed significant reduction in the blood glucose when subjected to oral glucose tolerance test (OGTT) in SD rats. It was observed that the compounds of present invention maintained the glucose lowering effect even after 166 hrs of administration. This long duration of activity makes the compounds suitable for development of compositions which will require less frequent administration. The result of the OGTT experiment is further discussed under biological study section.

The present invention is further illustrated in detail with reference to the following examples. It is desired that the example be considered in all respect as illustrative and are not intended to limit the scope of the claimed invention.

General method of preparation:

The polypeptide compound of the present invention can be prepared by methods described herein below. The process involves two steps, involving preparation of the parent linear peptide and subsequent attachment of fatty acid chain to the parent peptide.

The peptides described herein may be prepared by chemical synthesis using solid-phase techniques such as those described in G. Barany and R. B. Merrifield, "The Peptides: Analysis, Synthesis, Biology"; Volume 2—"Special Methods in Peptide Synthesis, Part A", pp. 3-284, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980; and in J. M. Stewart and J. D. Young, "Solid-Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Co., Rockford, Ill., 1984. The desired strategy is based on the Fmoc (9-Fluorenylmethyl methoxycarbonyl) group for temporary protection of the α -amino group, in combination with protecting groups such as *tert*-butyl (-tBu), *tert*-butoxycarbonyl (-Boc), trityl (-Trt) groups for temporary protection of the amino acid side chains (see for example E. Atherton and R. C. Sheppard, "The Fluorenylmethoxycarbonyl Amino Protecting Group", in "The Peptides: Analysis, Synthesis, Biology"; Volume 9—"Special Methods in Peptide Synthesis, Part C", pp. 1-38, S. Udenfriend and J. Meienhofer, Eds., Academic Press, San Diego, 1987).

The peptides can be synthesized in a stepwise manner on an insoluble polymer support (also referred to as "resin") starting from the C-terminus of the peptide. A synthesis is begun by appending the C-terminal amino acid of the peptide to the resin through formation of an

amide or ester linkage. This allows the eventual release of the resulting peptide as a C-terminal amide or carboxylic acid, respectively.

5 The C-terminal amino acid and all other amino acids used in the synthesis are required to have their α -amino groups and side chain functionalities (if present) differentially protected such that the α -amino protecting group may be selectively removed during the synthesis. The coupling of an amino acid is performed by activation of its carboxyl group as an active ester and reaction thereof with the unblocked α -amino group of the N-terminal amino acid appended to the resin. The sequence of α -amino group deprotection and coupling is repeated
10 until the entire peptide sequence is assembled. The peptide is then released from the resin with concomitant deprotection of the side chain functionalities, usually in the presence of appropriate scavengers to limit side reactions. The resulting peptide is finally purified by reverse phase HPLC.

15 The parent peptide can then be coupled to the fatty acid chain by coupling the activated fatty acid chain with the parent peptide. The fatty acid chain may be made by methods well known in organic chemistry. For e.g. the fatty acid chain can be made using solid phase synthetic methods which enables preparation of linear fatty acid chains.

20 The linear peptides synthesized were purified by preparative HPLC procedure as outlined below:

Preparative HPLC: WATERS 2555 Quaternary gradient module (Max Total Flow: 300 mL/min, Max Pressure: 3000 psi) or

Shimadzu LC-8A (Max Total Flow: 150 mL: Max Pressure: 20 Mpa)

25 Column: C18, 10 μ

Flow: 75 mL/min

Mobile Phase: Mobile Phase A: pH 7.5 Phosphate buffer

Mobile Phase B: Acetonitrile

Gradient: 10 to 40% Mobile Phase-B in 300 min.

30

The final compounds of the present invention were purified by preparative HPLC procedure as outlined below:

Preparative HPLC: WATERS 2555 Quaternary gradient module (Max Total Flow: 300 mL/min, Max Pressure: 3000 psi) or

Shimadzu LC-8A (Max Total Flow: 150 mL, Max Pressure: 20 Mpa)

Column: C18, 10 μ

Flow: 75mL/min

Mobile Phase: Mobile Phase A: 1% Acetic acid in water

5 Mobile Phase B: 1% Acetic acid in Acetonitrile:n-Propanol (50:50)

Gradient: 15 to 45% Mobile Phase-B in 300 min.

The purity of the compounds of the present invention was analysed by RP-HPLC method as outlined below:

10 **HPLC Method:**

Column: YMC-Pack Pro C18 (4 mm x 250 mm, 3 μ)

Eluent: Mobile Phase A: Buffer: Acetonitrile (900:100)

Mobile phase B: Buffer: Acetonitrile (300:700)

15 Buffer: Potassium dihydrogen orthophosphate in water, pH adjusted to 3.0 $\pm$ 0.1 with orthophosphoric acid

Flow rate: 1.0 mL/min

Detection: UV detection at 210 nm

Column Temperature: 50 °C

Run Time: 38 min.

20 **Gradient:**

Time	Mobile Phase A %	Mobile Phase B %
0	100	0
5	100	0
30	0	100
32	0	100
32.1	100	0
38	100	0

The compounds of the present invention were analyzed by the LCMS as outlined below:

25 Mass spectra were recorded on LCMS using Waters Acquity® QDa®, Waters Micromass Quattro Micro API or Thermo scientific LCQ Fleet™. The test solution was prepared by dissolving suitable quantity of analyte in diluent with final concentration from 1 μ g/ml to 50 μ g/ml depending on the ionization of analyte. The test solution was infused at a rate of about 10 μ l to 50 μ l per minute into LCMS for 1 min and mass spectra were recorded in Electro Spray Ionization (ESI) positive or negative mode and in an appropriate mass range.

Examples:

Preparation of activated fatty acid side chains:

1. Preparation of 18-[[[(1S)-1-carboxy-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]butylamino]-4-oxo-butyl]amino]-18-oxo-

5 octadecanoic acid (Moiety C-OSu)

The activated fatty acid side chain, Moiety C-OSu was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 1. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of *N,N'*-di-isopropylethylamine (DIPEA) to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then activated using *p*-nitrophenylchloroformate in THF and DIPEA followed by reaction with Fmoc-amino butylamine hydrochloride salt in THF: DMAc in presence of DIPEA which yielded 2-[2-[2-(4-Fmoc-aminobutylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the free amino group was then coupled to Fmoc-Glu-OtBu using 1-hydroxybenzotriazole (HOBt) and *N,N'*-diisopropylcarbodiimide (DIPC) which yielded 2-[2-[2-[4-[[[(4S)-4-Fmoc-amino-5-tert-butoxy-5-oxo-pentanoyl]amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the free amino group was then coupled to Fmoc-Glu-OtBu using 1-hydroxybenzotriazole (HOBt) and *N,N'*-diisopropylcarbodiimide (DIPC) which yielded 2-[2-[2-[4-[[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl]amino]-5-oxo-pentanoyl]amino] butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[4-[[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl]amino]-5-oxo-pentanoyl]amino] butylcarbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 814.56 ($M+H^+$)). The resultant compound was then reacted with *N*-hydroxysuccinimide (HOSu) in presence of dicyclohexyl carbodiimide (DCC) to yield succinimide protected intermediate, which was de-protected with trifluoroacetic acid (TFA) to yield the title compound (Moiety C-OSu).

30 2. Preparation of 18-[[[(1S)-1-carboxy-4-[[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]amino]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety D-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 2. 2-[2-(2-Fmoc-aminoethoxy)

ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine followed by coupling with Fmoc-Aib-OH in THF: DMAc using DIPC and HOBt which yielded 2-[2-[(2-Fmoc-amino-2-methyl-propanoyl)amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the free amino group was coupled with Fmoc-Glu-OtBu using HOBt and DIPC to yield 2-[2-[2-[[2-[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]-2-methyl-propanoyl] amino] ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group of the resultant compound was selectively de-blocked using piperidine and the free amino group was then coupled with octadecanedioic acid mono *tert* butyl ester to give 2-[2-[2-[[2-[(4*S*)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]- amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[[2-[(4*S*)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid (LCMS= *m/z*: 786.39 (*M*+*H*⁺)). The resultant compound was then reacted with HOSu in presence of DCC to yield succinimide protected intermediate, which was de-protected with trifluoroacetic acid to yield the title compound (Moiety D-OSu).

3. Preparation of 18-[[[(1*S*)-1-carboxy-4-[3-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]propylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety E-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 3. 2-[2-(2-Fmoc-aminoethoxy) ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then activated using *p*-nitrophenylchloroformate in THF and DIPEA followed by reaction with 1,3-diaminopropane in THF: DMAc in presence of DIPEA using HOBt to form NH₂-(CH₂)₃-NH-C(O)-{(2-(2-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin. The free amino group was then coupled to Fmoc-Glu-OtBu using HOBt and DIPC which yielded 2-[2-[2-[3-[[2-[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino] propylcarbamoylamino] ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant 2-[2-[2-[3-[[2-[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]- ethoxy]acetic acid-2-

Cl-Trt-Resin was selectively deblocked using piperidine and then coupled with octadecanedioic acid mono tert butyl ester to give 2-[2-[2-[3-[[*(4S)*-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-propylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[3-[[*(4S)*-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-propylcarbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 801.41 ($M+H^+$)). The resultant compound was then reacted with HOSu in presence of dicyclohexyl carbodiimide (DCC) to yield succinimide protected intermediate, which was de-protected with trifluoroacetic acid to yield the title compound (Moiety E-OSu).

4. Preparation of 18-[[*(1S)*-1-carboxy-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]-1-piperidyl]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety F-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 4. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then activated using *p*-nitrophenylchloroformate in THF and DIPEA followed by reaction with 4-amino-Boc-piperidine in THF: DMAc in presence of DIPEA using HOBt which yielded (2-[2-[2-(4-Boc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid -2-Cl-Trt-Resin. The resultant compound on cleavage using trifluoro acetic acid yielded 2-[2-[2-(4-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid which on further reaction with Fmoc-OSu in presence of triethylamine (TEA) yielded 2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid. The obtained compound was then further attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the free amino group was then coupled with Fmoc-Glu-OtBu using HOBt and DIPC which yielded 2-[2-[2-[[1-[(*4S*)-4-amino-5-*tert*-butoxy-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant compound was selectively de-blocked using piperidine and was then coupled with octadecanedioic acid mono tert-butyl ester to give 2-[2-[2-[[1-[(*4S*)-5-*tert*-butoxy-4-[(18-*tert*-

butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. Intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[1-[(4*S*)-5-tert-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 827.40 ($M+H^+$)). The resultant compound was then reacted with HOSu in presence of DCC to yield succinimide protected intermediate, which was de-protected using trifluoroacetic acid to yield the title compound, Moiety F-OSu.

5. Preparation of 18-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]-1-piperidyl]-18-oxo-octadecanoic acid (Moiety G-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 5. 2-[2-[2-(4-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid (intermediate prepared as disclosed in the preparation of Moiety F-OSu) was coupled with octadecanedioic acid mono *tert*-butyl ester to give 2-[2-[2-[[1-(18-*tert*-butoxy-18-oxo-octadecanoyl)-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 642.26 ($M+H^+$)). This intermediate was then reacted with HOSu in presence of DCC to yield succinimide protected intermediate which was de-protected using trifluoroacetic acid to yield the title compound, Moiety G-OSu.

Example 1: Preparation of Compound 1

Part A: Synthesis of the parent linear peptide Aib⁸, Arg³⁴, Leu³⁸ GLP-1(7-38)

The parent peptide was synthesized by solid-phase method. The starting resin used for synthesis was Wang resin. Fmoc protected Leucine was used for coupling with the Wang resin. The coupling was performed by using diisopropylcarbodiimide, *N*-hydroxybenzotriazole (DIC-HOBt) as coupling reagent in presence of 4-dimethylaminopyridine (DMAP) which yielded Fmoc-Leu-Wang Resin. Selective de-blocking of amino group of Fmoc-Leu-Wang Resin using piperidine followed by coupling with Fmoc-Gly-OH using HOBt/DIPC yielded Fmoc-Gly-Leu-Wang Resin. This completes one cycle. Acetic anhydride and diisopropylethyl amine/pyridine was used to terminate the uncoupled amino groups at every amino acid coupling.

The above 2 steps i.e selective deblocking of Fmoc- protection of amino acid attached to the resin and coupling of next amino acid residue in sequence with Fmoc-protected amino group were repeated for remaining 30 amino acid residues. The selective deblocking i.e.

deprotection of Fmoc group was done using piperidine and coupling with next Fmoc protected amino acid was done using HOBt/DIPC. The side chain of the Fmoc-protected amino acids were protected orthogonally, for eg. hydroxyl group of Serine, Tyrosine or Threonine were protected with *tert*-butyl(-tBu) group, amino and guanido group of Lysine and Arginine were protected with *tert*-butyloxycarbonyl (-Boc) and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (-Pbf) group respectively, the imidazole of histidine was protected with trityl (-Trt) and carboxylic acid groups of aspartic acid or glutamic acid were protected with -tBu group. The above mentioned two steps i.e. selective deblocking and then coupling with next Fmoc protected amino acid were performed to get Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-resin.

De-blocking of Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-Resin using piperidine followed by cleavage and de-protection using trifluoroacetic acid with ethane-1,2-dithiol resulted in crude H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH (Aib⁸, Arg³⁴, Leu³⁸ GLP-1 (7-38) peptide) which was purified by HPLC.

Part B:

Grafting of activated fatty acid chain, Moiety C-OSu over purified (Linear Peptide) H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH obtained in Part A, in acetonitrile at pH about 10 resulted in crude title peptide which was purified by preparative HPLC. The characterization of the compounds is provided in Table 3.

Example 2: Preparation of Compound 8

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A except here Fmoc protected Glycine was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide afforded the Compound 8 by following the analogous process of Example 1, Part B.

Example 3: Preparation Compound 2

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A. The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide afforded the Compound 2 by following the analogous process of Example 1, Part B.

Example 4: Preparation Compound 3

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A except here Fmoc protected Isoleucine was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide afforded the Compound 3 by following analogous process of Example 1, Part B.

Example 5: Preparation Compound 4

The grafting of activated fatty acid chain, Moiety D-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 4.

Example 6: Preparation Compound 5

The grafting of activated fatty acid chain, Moiety E-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 5.

Example 7: Preparation Compound 6

The grafting of activated fatty acid chain, Moiety F-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 6.

Example 8: Preparation Compound 7

The grafting of activated fatty acid chain, Moiety G-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 7.

The characterization data of the synthesized compounds of the present invention are provided below in following table.

Table 3. Characterization data of synthesized compounds of the present invention

Comp. #	LCMS Data	HPLC Purity
1	m/z = 1049.59 (MH ₄ ⁴⁺), Calculated Mass= 4194.33	96.01 % (Method B2), RT=25.16 min.
2	m/z = 1050.13 (MH ₄ ⁴⁺), Calculated Mass= 4196.49	92.06 % (Method B2), RT=24.55 min.
3	m/z = 1049.61 (MH ₄ ⁴⁺), Calculated Mass= 4194.41	94.41 % (Method B2), RT=24.82 min.
4	m/z = 1042.34 (MH ₄ ⁴⁺), Calculated Mass= 4165.32	94.56 % (Method B2), RT=25.26 min.
5	m/z = 1046.18 (MH ₄ ⁴⁺), Calculated Mass: 4180.72	94.33 % (Method B2), RT=25.17 min.
6	m/z = 1052.77 (MH ₄ ⁴⁺), Calculated Mass: 4207.08	93.12 % (Method B2), RT=24.92 min.
7	m/z = 1018.66 (M-4H) ⁴⁺ , Calculated Mass: 4078.67	92.00 % (Method B2), RT=25.84 min.
8	m/z = 1361.88 (MH ₄ ⁴⁺), Calculated Mass= 4082.62	92.38 % (Method B2), RT=24.55 min.

5

Biological Study:

Oral Glucose tolerance test (OGTT) in rats; Single Injection; 1mg/kg

Animals were divided into three groups – a normal control group, a test group and a third semaglutide group, with 4 animals in each group. The animals were fasted for 12 hours before initiation of OGTT. To the test group animals, the test compound was injected subcutaneously at 1 mg/kg dose. To the semaglutide group, a dose of 1 mg/kg was injected subcutaneously. To the normal control group, equivalent volume of vehicle was injected subcutaneously. After 166 hrs of subcutaneous injection of test drug or semaglutide or vehicle, blood glucose was measured with blood glucose meter (time 0 measurements). All the animals were then given 2 g/kg of glucose solution orally. Blood glucose was measured at 20min, 40 min, 60 min, 90 min and 120 min following glucose challenge.

The compounds with the protracting moieties of the present invention showed significantly greater reduction in blood glucose when compared to semaglutide which was used as standard. All the compounds of present invention showed at least 50 % more reduction in blood glucose AUC_{0-120min} as compared to semaglutide.

20

Provisional claims:

1. A polypeptide comprising the amino acid sequence:

H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-
G-R-G-X33-X34

wherein X2 is Ser, Ser(OMe), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

X3 is absent or Gln;

X4 is Gln or Glu;

X16 is Glu or Asp;

X24 is Ile or Val or Leu;

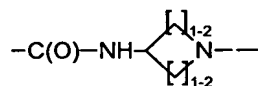
X33 is Leu, Ile, Tyr, Ser, Lys, amide or absent;

X34 is Ser, Lys, Ala, amide or absent and

X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:

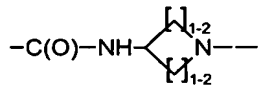
{-Q-T-U-W-Y-Z

wherein Q, T, U and W are absent or selected from a group consisting of -C(O)-M-NH-- wherein M is a C₅₋₁₀ alk chain interrupted with 1, 2 or 3 oxygen atoms, -C(O)-NH-C₂₋₅ alk-NH--, -C(O)-C(CH₃)₂-NH--and



provided that:

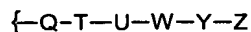
- at least one of Q, T, U and W is always present and,
- at least one of Q, T, U and W is always selected from a group consisting of -C(O)-NH-C₂₋₅ alk-NH--, -C(O)-C(CH₃)₂-NH--and



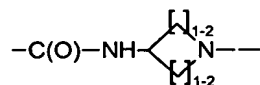
Y is either absent or -C(O)-(CH₂)_m-CH(COOH)NH-- wherein m is an integer selected from 1 to 2 and -- is point of attachment with the group Z;

Z is $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ or $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{CH}_3$ wherein n is an integer from 10 to 20.

2. The polypeptide as in provisional claim 1, wherein
 5 X2 is Ser, Ser(OMe), Aib or Ala;
 X16 is Glu and
 X24 is Ile.
3. The polypeptide as in provisional claim 2, wherein
 10 X34 is Ala or absent.
4. The polypeptide as in provisional claims 1, 2 or 3, wherein
 X3 and X34 both are absent.
5. The polypeptide as in any one of the claims 1-4, wherein
 15 X3, X33 and X34 are absent.
6. The polypeptide as in any one of the provisional claims 1-4, wherein
 X3 is absent;
 20 X33 is Leu or Ile and
 X34 is Ala or absent.
7. The polypeptide as in any one of the provisional claims 1-6, wherein X21 is lipid
 modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a
 25 moiety:

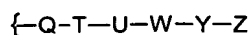


wherein Q and T are $-\text{C}(\text{O})-\text{M}-\text{NH}-$ and U is selected from a group consisting of $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5}\text{ alk}-\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and

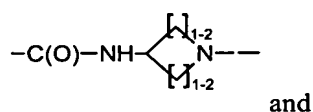


and W is absent.

8. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:

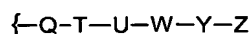


- 5 wherein Q is $-C(O)-M-NH-$; T is selected from a group consisting of $-C(O)-NH-C_{2-5} alk-NH-$, $-C(O)-C(CH_3)_2-NH-$ and

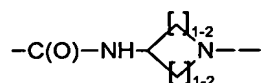


U and W are absent.

- 10 9. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is selected from a group consisting of $-C(O)-NH-C_{2-5} alk-NH-$, $-C(O)-C(CH_3)_2-NH-$ and

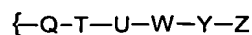


- 15 ;
T and U are $-C(O)-M-NH-$ and W is absent.

10. The polypeptide as in any one of the provisional claims 7 to 9, wherein Y is absent.

11. The polypeptide as in any one of the provisional claims 7 to 10, wherein $-C(O)-M-NH-$ group is $-C(O)-CH_2O-C_2H_5OC_2H_5NH-$.

- 20 12. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:

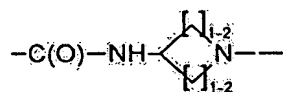


- 25 wherein Q is $-C(O)-CH_2O-C_2H_5OC_2H_5NH-$; T is $-C(O)-NH-(CH_2)_{3-4}NH-$ and U and W are absent.

13. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is



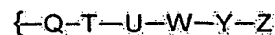
; and U and W are absent.

14. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and U and W are absent.

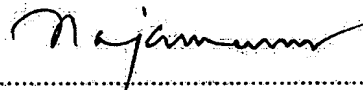
15. The polypeptide in any one of the provisional claims 1-6, wherein X21 is lipid modified Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



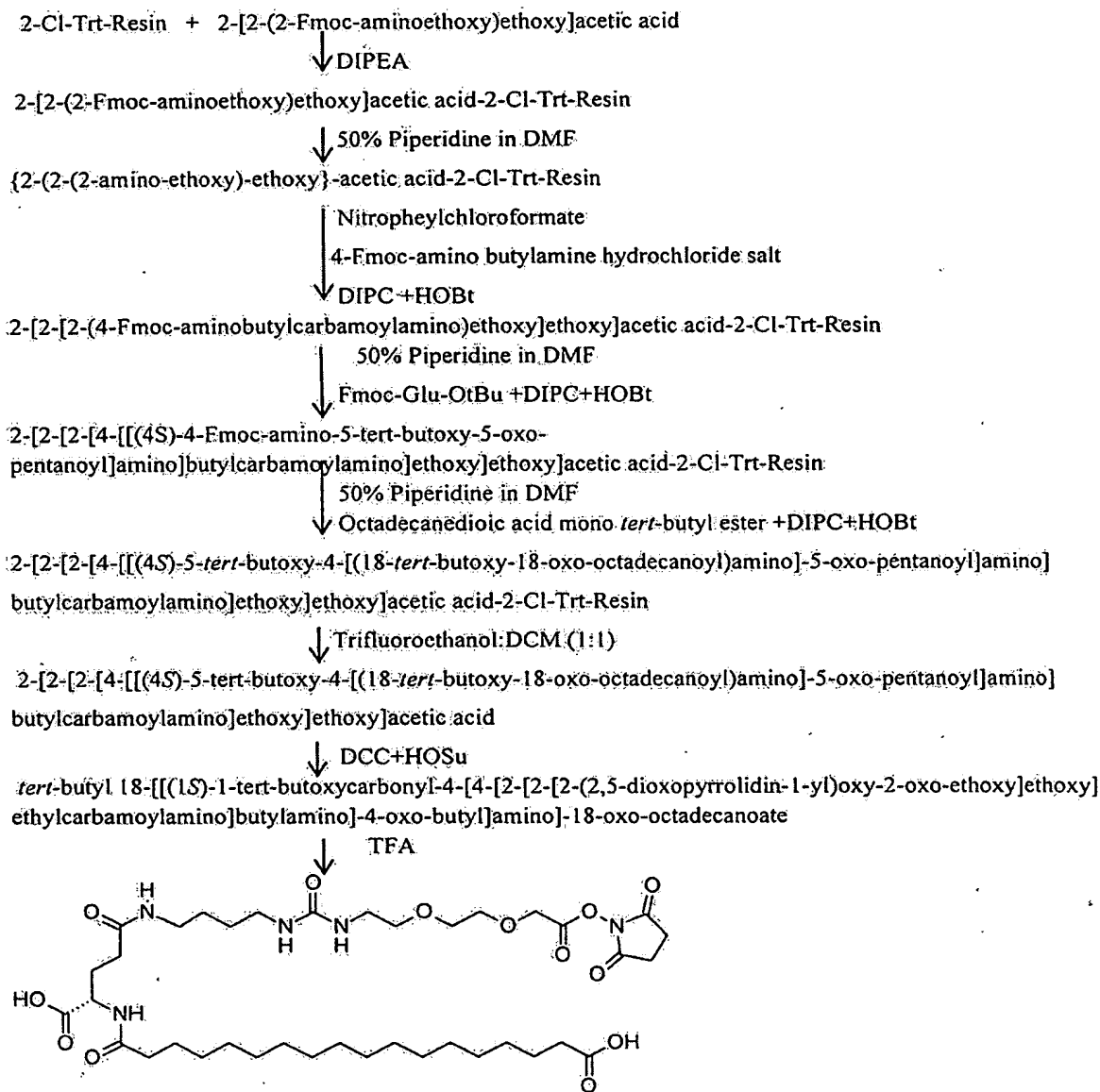
wherein Q and U are $-\text{C}(\text{O})-\text{CH}_2\text{O}-\text{C}_2\text{H}_5\text{OC}_2\text{H}_5\text{NH}-$; T is $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and W is absent.

Dated 26th Day of October 2018.

(Signature).....



Dr. RAJAMANNAR THENNATI
EXECUTIVE VICE PRESIDENT,
SUN PHARMACEUTICAL INDUSTRIES LTD.



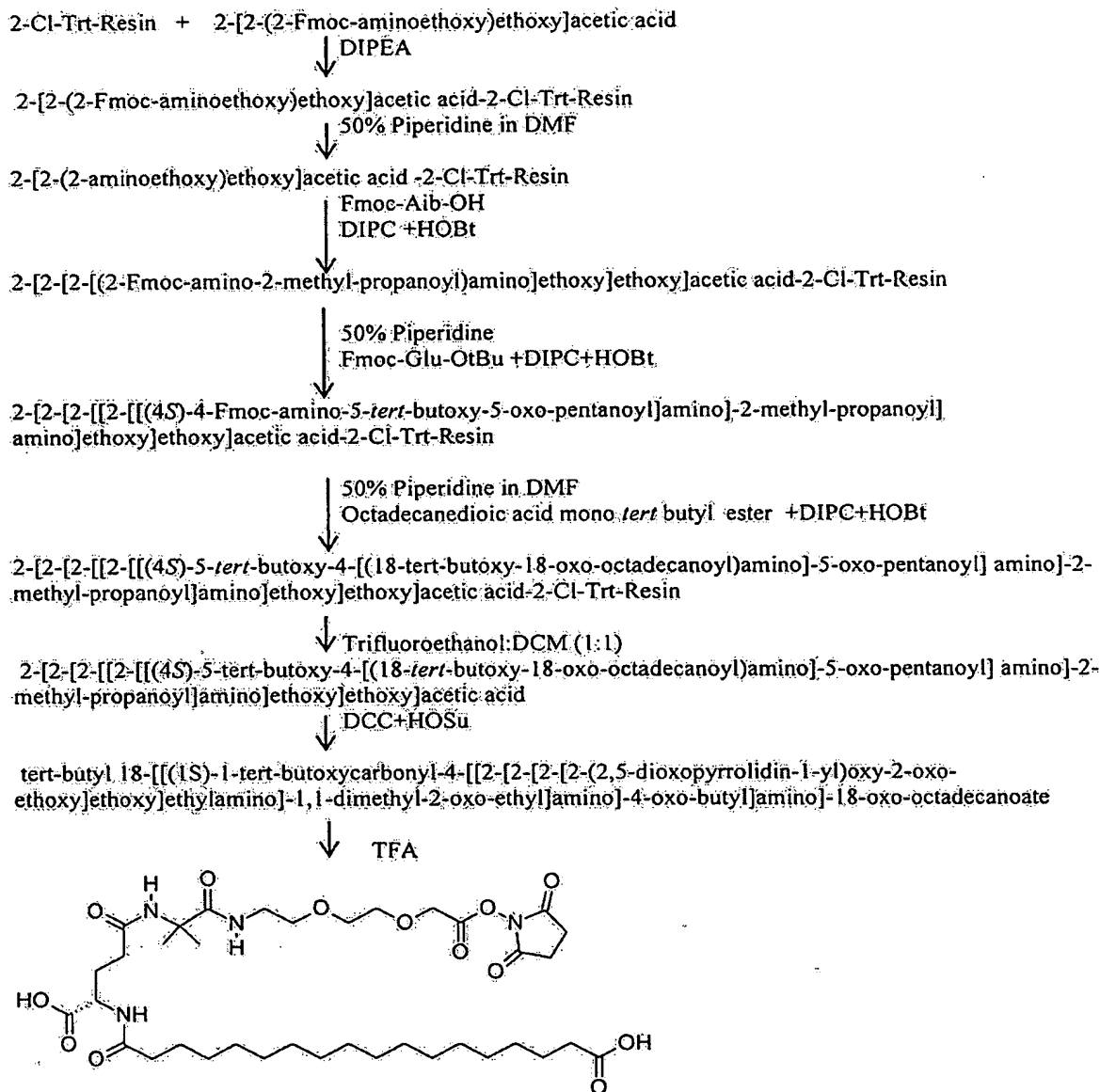
Moiety C-OSu

Figure 1: Preparation of Moiety C-OSu

Dated 26th Day of October 2018

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 SUN PHARMACEUTICAL INDUSTRIES LTD.



Moiety D-OSu

Figure 2: Preparation of Moiety D-OSu

Dated 26th Day of October 2018

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 SUN PHARMACEUTICAL INDUSTRIES LTD.

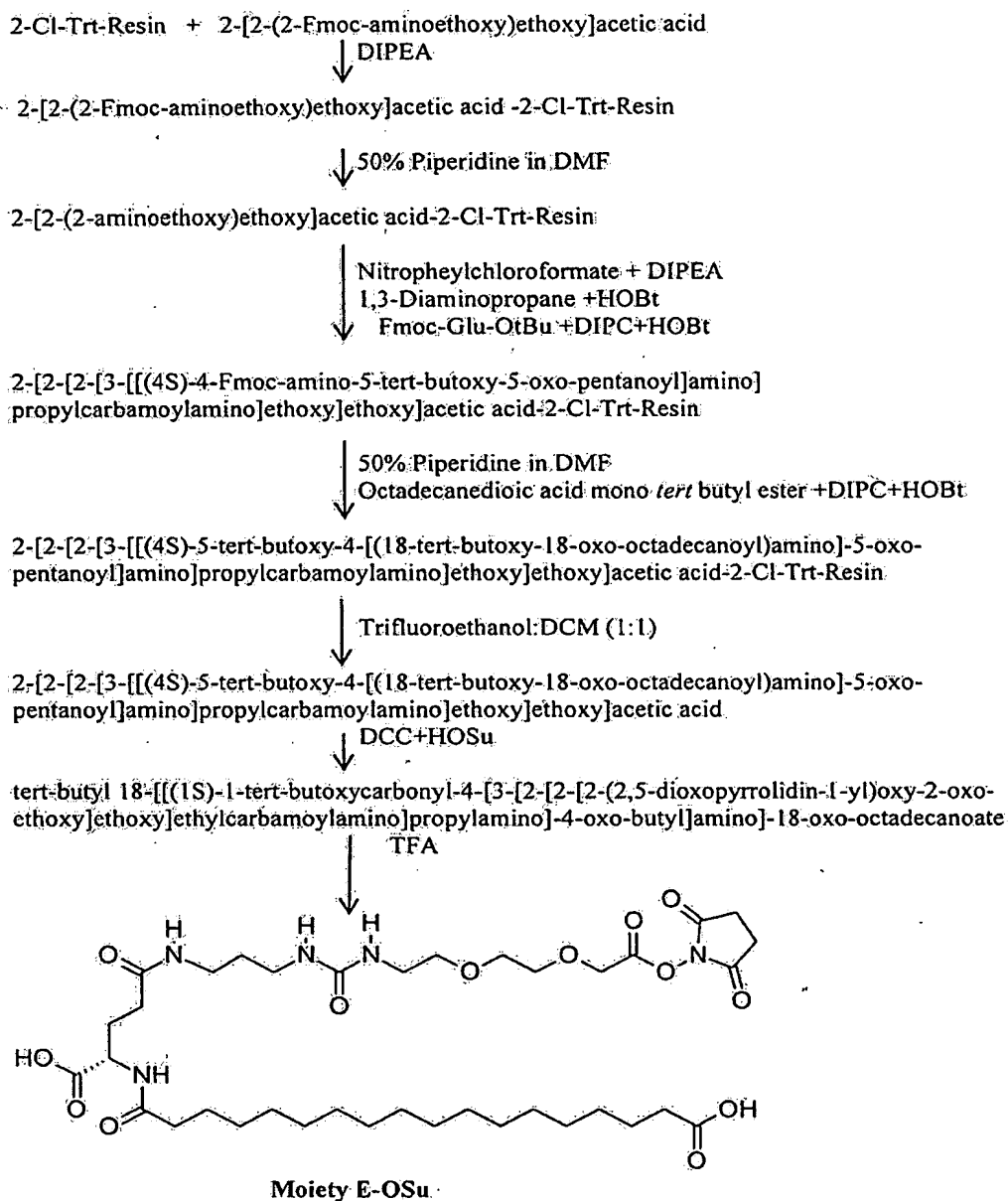
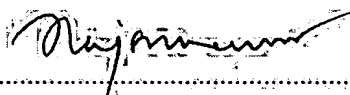


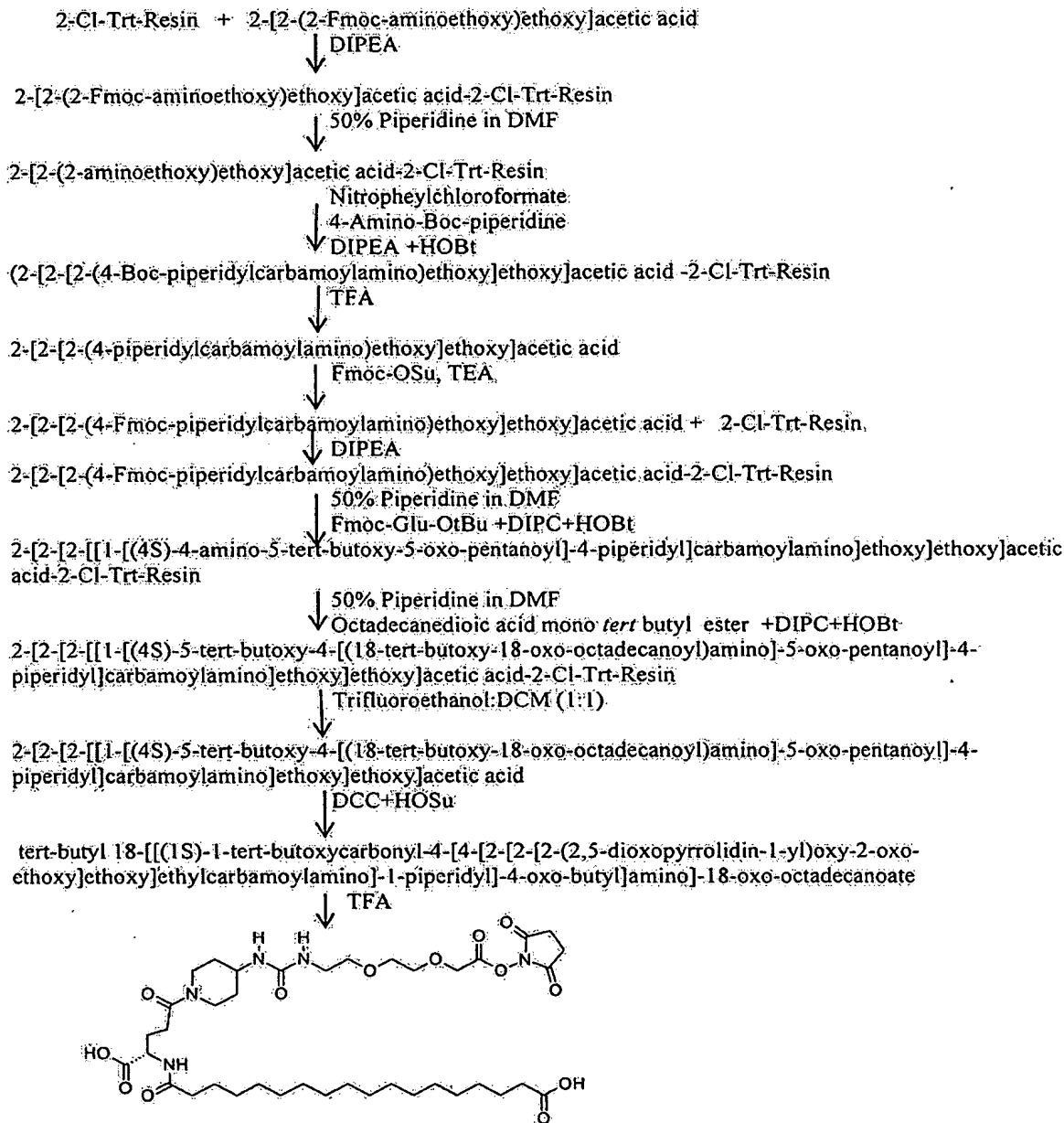
Figure 3: Preparation of Moiety E-OSu

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Moiety F-OSu

Figure 4: Preparation of Moiety F-OSu

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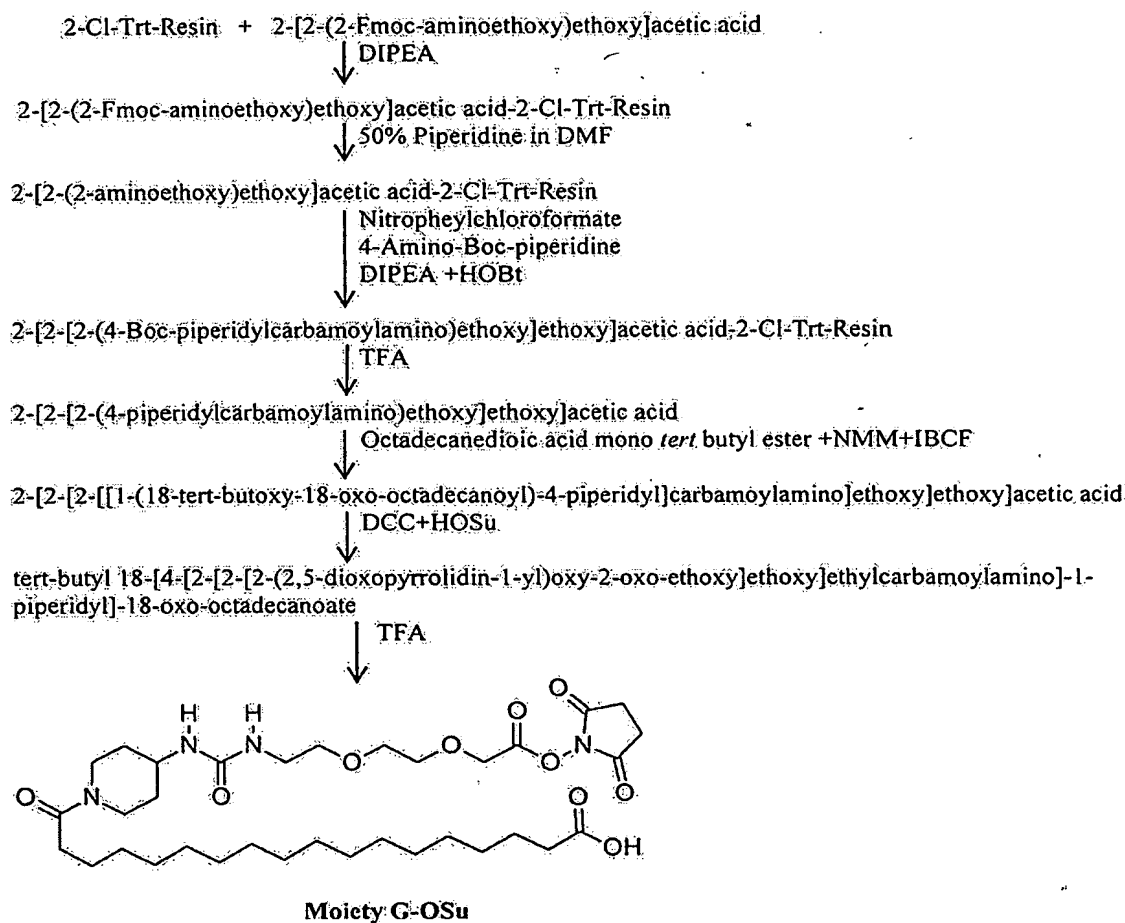
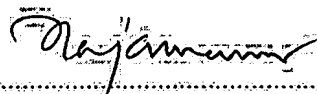


Figure 5: Preparation of Moiety G-OSu

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ABSTRACT

ACYLATED ANALOGUES OF GLP-1

The present invention pertains to a novel Glucagon like Peptide-1 (GLP-1) (7-38) analogs which are acylated with a protracting moiety having fatty acid chain and a linker. The moieties of the present invention afford compounds which have long duration of activity. The new analogs are potent GLP-1 agonist with reduced adverse effect and improved duration of action. The analogs of present invention may be useful in treatment of diabetes and obesity.

SEQUENCE LISTING

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<170> PatentIn version 3.5

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Gln	Ala	Ala	Lys	Glu	Phe	Ile	Ala	Trp	Leu	Val	Lys	Gly	Arg	Gly
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भारत सरकार

GOVERNMENT OF INDIA

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पेटेंट कार्यालय

THE PATENT OFFICE

बौद्धिक संपदा भारत

एकस्व अभिकल्प व्यापार चिह्न

भौगोलिक उपदर्शन

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TO WHOMSOEVER IT MAY CONCERN

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I, the undersigned, being an officer duly authorized to sign and issue the certificate on behalf of the Controller General of Patents, Designs and Trademarks in accordance with the provisions of Section 73(3) of the Patents Act, 1970, hereby certify that annexed hereto is a True Copy of the document(s) as filed in connection with the following Patent Application:

- क) आवेदन संख्या / a) Application Number: **201821040468**
ख) फाइल करने की तारीख / b) Date of Filing: **26/10/2018**
ग) अनुरोधित दस्तावेज़(जों) का नाम / C) Name of the document(s) requested:

- 1) Application for Grant of Patent (Form-1)
- 2) Provisional Specification filed on 26/10/2018

यह प्रमाणपत्र पेटेंट अधिनियम, 1970 की धारा 147(1) के अधीन मुझमें निहित शक्तियों के तहत जारी किया गया है।

This certificate is issued under the powers vested in me U/S 147(1) of The Patents Act, 1970.

दिनांक / Dated this 22nd day of April, 2019

(VIJAY T. DOYE)

सहायक नियंत्रक पेटेंट व डिजाइन / Assistant Controller of Patents and Designs

(प्राधिकृत हस्ताक्षरी / Authorized Signatory)

"FORM 1 THE PATENTS ACT 1970 (39 OF 1970) and THE PATENT RULES, 2003 APPLICATION FOR GRANT OF PATENT (See Section 7, 54 & 135 and sub-rule (1) of rule 20)		(FOR OFFICE USE ONLY)	
		Application No.	
		Filing date :	
		Amount of Fee paid:	
		CBR No.	
		Signature:	
1. APPLICANT'S REFERENCE/ IDENTIFICATION NO. (AS ALLOTTED BY OFFICE)			
2. TYPE OF APPLICATION [Please tick (✓) at the appropriate category]			
Ordinary (☒)		Convention ()	
PCT-NP ()			
Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()
3A. APPLICANT(S)			
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			City MUMBAI
			State MAHARASHTRA
			Country INDIA
Pin Code 400063			
3B. CATEGORY OF APPLICANT [Please tick (✓) at the appropriate category]			
Natural Person ()		Other than Natural Person	
Small Entity ()		Startup ()	Others (☒)
4. INVENTORS (S) [Please tick (✓) at the appropriate category]			
Are all the inventor(s) same as the applicant (s) named above?		Yes ()	No (☒)
If "No", furnish the details of the inventor(s)			
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			Pin Code	390 012
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			City	BARODA
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			Pin Code	390 012
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			State	GUJARAT
			Country	India
			Pin Code	390 012
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				ENCLAVE, TANDALJA ROAD,
			City	BARODA
			State	GUJARAT
			Country	India
			Pin Code	390 012
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			City	BARODA
			State	GUJARAT
			Country	India
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			City	BARODA
			State	GUJARAT
			Country	India
			Pin Code	390 012
			House No.	SUN PHARMACEUTICAL INDUSTRIES LIMITED
			Street	NIMA COMPOUND, NEAR PRATHAM ENCLAVE, TANDALJA ROAD,
			City	BARODA
			State	GUJARAT
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			City	BARODA
PANDYA KUNAL	Indian	India	State	GUJARAT
			Country	India
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			City	BARODA
			State	GUJARAT
			Country	India

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			City	BARODA
			State	GUJARAT
			Country	India
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			Street	NIMA COMPOUND, NEAR PRATHAM ENCLAVE, TANDALJA ROAD,
			City	BARODA
			State	GUJARAT
			Country	India
TIWARI ABHISHEK	Indian	India	Pin Code	390.012
			House No.	SUN PHARMACEUTICAL INDUSTRIES LIMITED
			Street	NIMA COMPOUND, NEAR PRATHAM ENCLAVE, TANDALJA ROAD,
			City	BARODA
			State	GUJARAT
			Country	India
5. TITLE OF THE INVENTION- EXTENDED GLP-1 DERIVATIVES				
6. AUTHORISED REGISTERED PATENT AGENT(S)	IN/PA No.			
	Name			
	Mobile No.			
7. ADDRESS FOR SERVICE OF APPLICANT IN INDIA	Name	Dr. Bishram Singh Chouhan		
	Postal Address	General Manager, Sun Pharmaceutical Industries Ltd., Intellectual Property Cell, 17/B, Mahal Industrial Estate, Mahakali Caves Road, Andheri (East), Mumbai – 400093, India.		
	Telephone No.	91-22-6645- 5645		
	Mobile No.	+91-9727786543		
	Fax No.	91-22-6645-5685		

E-mail ID		Bishram.Chouhan@sunpharma.com			
8. IN CASE OF APPLICANT CLAIMING PRIORITY OF APPLICATION FILED IN CONVENTION COUNTRY, PARTICULARS OF CONVENTION APPLICATION					
Country	Application Number	Filing date	Name of the applicant	Title of the invention	IPC (as classified in the convention country)
-NA-	-NA-	-NA-	-NA-	-NA-	-NA-
9. IN CASE OF PCT NATIONAL PHASE APPLICATION, PARTICULARS OF INTERNATIONAL APPLICATION FILED UNDER PATENT CO-OPERATION TREATY (PCT)					
International application number			International filing date		
-NA-			-NA-		
10. IN CASE OF DIVISIONAL APPLICATION FILED UNDER SECTION 16, PARTICULARS OF ORIGINAL (FIRST) APPLICATION					
Original (first) application No.			Date of filing of original (first) application		
-NA-			-NA-		
11. IN CASE OF PATENT OF ADDITION FILED UNDER SECTION 54, PARTICULARS OF MAIN APPLICATION OR PATENT					
Main application/patent No.			Date of filing of main application		
-NA-			-NA-		
12. DECLARATIONS					
(i) Declaration by the inventor(s) (In case the applicant is an assignee : the inventors(s) may sign herein below or the applicant may upload the assignment or enclose the assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period). I/We, the above named inventor(s) is/are the true & first inventor(s) for this Invention and declare that the applicant(s) herein is/are my/our assignee or legal representative. (a) Date: Oct 26, 2018 (b) Signature(s) 1) <u>Najam</u> 2) <u>N. Chaturvedi</u>					

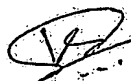
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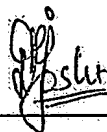
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6) amb

7) Zalcsadia

8) 

9) Funf Boonye

10) 

11) KnQui

12) pus

(c) Name(s)	1) THENNATI RAJAMANNAR 2) CHATURVEDI NISHITH 3) BURADE VINOD SAMPATRAO 4) SHAHI PRADEEP DINESH 5) NATARAJAN MUTHUKUMARAN 6) NAGARAJA RAVISHANKARA MADAVATI 7) ZALAWADIA RISHIT MANSUKHLAL 8) PATEL VIPULKUMAR SHANKARBHAI 9) PANDYA KUNAL 10) JOSHI DHIREN RAMESHCHANDRA
	11) SONI KRUNAL HARISHBHAI 12) TIWARI ABHISHEK
	(ii) Declaration by the applicant(s) in the convention country: (In case the applicant in India is different than the applicant in the convention country: the applicant in the convention country may sign herein below or applicant in India may upload the assignment form the applicant in the convention country or enclose the said assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period) I/We the applicant(s) in the convention country declare that the applicant(s) herein is/are my/our assignee or legal representative. (a) Date _____ (b) Signature(s) _____ -NA- (c) Name(s) of the signatory _____
	iii) Declaration by the applicant(s): I/we the applicant(s) hereby declare(s) that: - ☒ I am/We are in possession of the above mentioned invention ☒ The provisional /complete specification relating to the invention is filed with this application ☒ The invention as disclosed in the specification uses the biological material from India and the necessary permission from the competent authority shall be submitted by me/us before the grant of patent to me/us ☒ There is no lawful ground of objection(s) to the grant of the patent to me/us. ☒ I am/we are the true & first inventor(s). ☒ I am/we are the assignee or legal representative of true and first inventor(s). ☒ The application or each of the applications, particular of which are given in para-8 was the

first application in convention country/countries in respect of my/our invention(s).

- ☒ I/we claim the priority from the above mentioned application (s) filed in convention country/countries and state that no application for protection in respect of the invention had been made in a convention country before that date by me/us or by any person from which I/we derive the title
- ☒ My/our application in India is based on international application under patent cooperation treaty (PCT) as mentioned in Para-9.
- ☒ The application is divided out of my/our application particulars of which is given in para-10 and prays that this application may be treated as deemed to have been filed on DD/MM/YY under sec 16 of the Act.
- ☒ The said invention is an improvement in or modification of the invention particulars of which are given in Para- 11.

13. FOLLOWING ARE THE ATTACHMENTS WITH THE APPLICATION

(a) Form 2

Item	Details	Fee	Remarks
Complete/provisional specification)#	No. of pages -40	Rs. 22400 /-	Fees including 8 pages of Sequence listing.
No. of claim(s)	No. of claims and No. of pages: None	-	
Abstract	No. of pages- 01	-	No. of pages included in the total no. of pages of provisional specification.
No. of Drawing(s)	No. of drawings and No. of pages- 6	-	No. of pages included in the total no. of pages of provisional specification.

In case of a complete specification, if the applicant desires to adopt the drawings filed with his provisional specification as the drawings or part of the drawings for the complete specification under rule 13(4), the number of such pages with the provisional specification are required to be mentioned here:

~~(b) Complete specification (in conformation with the international application/as amended before the international Preliminary Examination Authority (IPEA), as applicable (2 copies).~~

~~(c) Sequence listing in electronic form~~

~~(d) Drawings (in confirmation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2 copies).~~

~~(e) Priority document(s) or a request to retrieve the priority document(s) from DAS (Digital Access Service) if the applicant had already requested the office of first filing to make the priority document(s) available to DAS.~~

~~(f) Translation of priority document/Specification/International Search Report/International Preliminary Report on Patentability.~~

~~(g) Statement and Undertaking on Form 3~~

~~(h) Declaration of Inventorship on Form 5~~

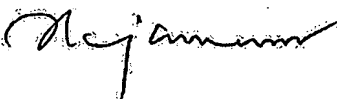
~~(i) Power of Authority~~

~~(j)~~

Total fee Rs.22400/- via online transfer Cash/ Banker's Cheque /Bank Draft bearing No.

I/We hereby declare that to the best of my/our knowledge, information and belief the fact and matters stated herein are correct and I/we request that a patent may be granted to me/us for the said invention.

Dated this 26th day of Oct, 2018

Signature: 

Name: Dr. RAJAMANNAR THENNATI
EXECUTIVE VICE PRESIDENT,
SUN PHARMACEUTICAL INDUSTRIES LTD.

To,
The Controllers of Patents
The Patent Office, at MUMBAI

Note:-

- Repeat boxes in case of more than one entry.
- To be signed by the applicant(s) or by authorized registered patent agent otherwise where mentioned.
- Tick (✓)/cross (×) whichever is applicable/not applicable in declaration in paragraph-12.
- Name of the inventor and applicant should be given in full, family name in the beginning.
- Strike out the portion which is/are not applicable.

For fee: See First Schedule".

FORM 2

THE PATENTS ACT, 1970

[39 of 1970]

&

PATENTS RULES, 2003

PROVISIONAL SPECIFICATION

(Sec section 10 and rule 13)

EXTENDED GLP-1 DERIVATIVES

SUN PHARMACEUTICAL INDUSTRIES LTD.

A company incorporated under the laws of India having their office at
SUN HOUSE, 201 B/1, WESTERN EXPRESS HIGHWAY, GOREGOAN (E), MUMBAI-
400063 MAHARASHTRA, INDIA.

The following specification describes invention.

EXTENDED GLP-1 DERIVATIVES

FIELD OF THE INVENTION

The present invention pertains to a novel Glucagon like Peptide-1 (GLP-1) (7-38) analogs having an amino acid sequence with Leu or Ile or Leu/Ile-X at the C-terminal. The new analogs are potent GLP-1 agonist with reduced adverse effect and improved duration of action. The present invention further relates to acylated derivatives of the new analogs which have further improved potency and duration of action and are suitable for oral administration. The analogs of present invention may be useful in treatment of diabetes and obesity.

BACKGROUND OF THE INVENTION

Glucagon-like peptide-1 (GLP-1) is a hormone that is mainly produced in enteroendocrine L cells of the gut and is secreted into the blood stream when food containing fat, protein hydrolysate and/or glucose enters the duodenum. GLP-1 is derived from the cell-specific post-translational processing of the preproglucagon gene. Initially, the peptide GLP-1(1-37) was identified from this processing, but it was the two *N*-terminally truncated products, GLP-1(7-37) and GLP-1(7-36)amide, that were found to recognize the pancreatic receptor and which were determined to be the active species *in vivo*. GLP-1 has been found to stimulate insulin secretion, thereby causing glucose uptake by cells and decreased serum glucose levels. GLP-1 agonists are available for the treatments for Type 2 Diabetes Mellitus (T2DM) as favored drugs as they are devoid of hypoglycemia and with a positive benefit of weight loss. The endogenous substance, GLP-1(7-37) and GLP-1(7-36)amide, are cleaved by peptidases and thus have very short half-life. Efforts were made to improve the performance by developing GLP-1 analogues with improved half-life. The first drug approved in 2005 is Exenatide with twice a day dosing at dose level 10 mcg and was found to show a significant improvement in HbA1c, a marker of glucose control. Further, Novo Nordisk developed Liraglutide (United States Patent No. US6268343) with once a day dosing of 1.8 mg, s.c./day and approved in 2010. Further research and development produced once a week products like, Albiglutide developed by GSK and Dulaglutide developed by Eli Lilly. Recently, Semaglutide (WO2006097537A2), a GLP-1 analogue was approved by USFDA. Semaglutide is marketed under the brand name Ozempic®. It is administered as a once-weekly subcutaneous injection.

Many attempts to make GLP-1 analogs having improved potency and duration of action are reported in literature. United States patent No. US7291594B2 (the US '594 patent) discloses GLP-1 (7-35) derivatives having added several residues of arginine and/or lysine to the C-terminus thereof to provide high bioavailability via mucous membranes. The US '594 patent
5 further discloses that these derivative can be conferred with resistance to dipeptidyl peptidase IV (DPP-IV) by substituting amino acid 8 in its GLP-1 amino acid sequence with Ser, or with resistance to trypsin by substituting amino acids 26 and 34 with Gln and Asn, respectively. United States patent No. US 7893017B2 (the US '017 patent) discloses acylated GLP-1 analog wherein the said GLP-1 analog is stabilized against DPP-IV by modification of at
10 least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1 (7-37) and wherein said acylation is a diacid attached directly to the C-terminal amino acid residue of said GLP-1 analog.

United States patent No. US 8951959B2 (the US '959 patent) discloses DPP-IV resistant
15 GLP-1 (7-37) analogue having a non-proteogenic amino acid residue containing trifluoromethyl group in position 8 relative to the sequence GLP-1, and is acylated with a moiety comprising two acidic groups to the lysine residue in position 26.

United States patent No. US7084243B2 (the US '243 patent) discloses GLP-1 (7-37)
20 analogues having Val or Gly at position 8 relative to the sequence GLP-1 (7-37) as DPP-IV resistant peptides.

WO2017149070A1(the WO '070) discloses GLP-1 analogues having a Trp at a position
25 corresponding to position 8 of GLP-1 (7-37) and these Trp8 compounds were shown to be very stable against degradation by DPP-IV.

WO2004103390A2 (the WO '390) discloses that the modification at the P₁ position (corresponding to 9 position in case of GLP-1 (7-37)) can produce GLP-1 analogues with greatly reduce susceptibility to enzyme-mediated (such as DPP-IV) cleavage relative to the native substrate, yet retain the biological activity of the native substrate. The WO '390 further
30 discloses GLP-1 (7-37) analogues having an amino acid with tetrasubstituted C β carbon (such as *tert*- leucine) at position 9 provides GLP-analogues with resistant to degradation by DPP-IV.

WO2015086686A2 (the WO'686 publication) discloses that incorporation of alpha-methyl-functionalized amino acids directly into the main chain of GLP-1 analogues has been determined to produce protease-resistant (includes DPP-IV resistant) peptides.

Various other DPP-IV resistant GLP-1 agonists are disclosed in patent publications such as
5 WO2007030519A2, WO2004078777A2, WO2007039140A1, WO2014209886A1, WO2012016419A1, WO2017211922A2, WO2016198544A1 and WO2013051938A2.

Various patent applications disclose C-terminally extended GLP-1 analogues with increased stability and longer duration of action. For e.g. United States patents US7482321B2, US9498534B2 and US7897566B2.

- 10 Various patent applications disclose acylated GLP-1 analogs wherein the GLP-1 analogues are attached to lipophilic substituent optionally via a linker to provide longer duration of action.

United States patent No. US8603972B2 (the US '972) discloses monoacylated derivatives of GLP-1 analogues wherein Lys residue at position 37 or 38 of GLP-1 analogue is acylated.

- 15 United States patent No. US8648041B2, US9758560B2, US9006178B2, US9266940B2, US9708383B2 and the United States patent application publications US20150152157A1, US20150133374A1 discloses di-acylated derivatives of GLP-1 analogues.

United States patent application publication US20160200791A1 discloses triacylated derivatives of GLP-1 analogues.

- 20 WIPO patent publication WO2016083499A1, WO2016097108A1 and WO2014202727A1 discloses acylated GLP-1 analogues wherein the Lys residue of GLP-1 analogues is attached to two protracting moieties via a branched linker.

- WIPO patent publication WO2009030771A1 and WO2018083335A1 discloses various acylating agents (side chain) which can be attached to Lys residue of GLP-1 analogues to
25 provide longer duration of action.

WIPO patent publication WO2013186240A2 discloses exendin-4 peptide analogues having Gly, Ser or functionalized Ser, e.g. Ser (OCH₃), D-Ser or functionalized D-Ser, e.g. D-Ser(OCH₃), Aib, Ala, or D-Ala at position 2 of exendin-4 amino acid sequence.

30

Various other GLP-1 analogues are disclosed in patent applications such as WIPO publication number WO2005027978A2, WO1998008871A1, WO1999043705A1, WO1999043706A1, WO1999043707A1, WO1999043708A1, WO2000034331A2, WO2009030771A1, WO2011080103A1, WO2012140117A1, WO2012062803A1,

Still, there is need to develop a GLP-1 analog which have optimum desired properties in
5 terms of stability and duration of action.

In one aspect the present invention provides a polypeptide comprising the amino acid
10 sequence:

wherein X2 is Ser, Ser(OMe) , Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl)
15 carboxylic acid, Trp or Thr;

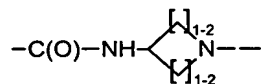
X4 is Gln or Glu;

X24 is Ile or Val or Leu;

X34 is absent or Ala and

{-T-U-W-Y-Z

wherein T, U and W are absent or selected from a group consisting of $-C(O)-M-NH-$
 25 wherein M is a C_{5-10} alkyl chain interrupted with 1, 2 or 3 oxygen atoms, $-C(O)-NH-C_{2-5}$
 alkyl-NH-, $-C(O)-C(CH_3)_2-NH-$ —and



30

4

The polypeptides of the present invention are potent GLP-1 agonist with fewer adverse effects. Further, the polypeptides of the present invention are stable and have long duration of action and are suitable for oral administration.

5

DESCRIPTION OF FIGURES

Figure 1: Preparation of Moiety A-OSu

Figure 2: Preparation of Moiety C-OSu

10 Figure 3: Preparation of Moiety D-OSu

Figure 4: Preparation of Moiety E-OSu

Figure 5: Preparation of Moiety F-OSu

Figure 6: Preparation of Moiety G-OSu

15 ABBREVIATIONS

Aib: 2-Aminoisobutyric acid

ADO: 8-Amino-3,6-dioxo-octanoic acid

OGTT: Oral glucose tolerance test

DIPEA: *N,N'*-Di-isopropylethylamine

20 HOBT: 1-Hydroxybenztriazole

DIPC: *N,N'*-Di-isopropylcarbodiimide

HOSu: *N*-Hydroxysuccinimide

IBCF: Isobutyl chloroformate

NMM: *N*-Methylmorpholine

25 THF: Tetrahydrofuran

DCM: Dichloromethane

DMAP: 4-Dimethylaminopyridine

DCC: Dicyclohexyl carbodiimide

DMAc: Dimethylacetamide

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DESCRIPTION OF THE INVENTION

The present invention provides a stable long acting GLP-1 analog which does not require frequent subcutaneous dosing and is also suitable for oral administration. In the quest to

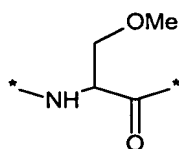
prepare potent GLP-1 agonistic compounds the applicant surprisingly found that addition of an extra Leu at the C terminal of the sequence gave peptides with significantly improved potency and duration of action when compared to parent peptide. The peptides with extra Ile also showed similar effect of improved potency and duration of action when compared to the parent peptide. Accordingly, the present invention provides a polypeptide comprising the

H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-G-R-G-X33-X34

- wherein X2 is Ser, Ser(OMe) , Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;
- X3 is absent or Gln;
- X4 is Gln or Glu;
- X16 is Glu or Asp;
- X24 is Ile or Val or Leu;
- X33 is Leu, Ile or Tyr;
- X34 is absent or Ala and
- X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated.

Unless stated otherwise, the specification intends to cover both L and D isomers of the amino acids in the sequence.

Ser(OMe) as described herein in the specification is amino acid serine with its hydroxyl group methylated and has following structure.



In another aspect, the present invention provides a polypeptide comprising the amino acid sequence:

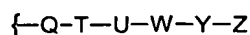
H-X2-X3-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-X21-E-F-I-A-W-L-V-R-G-R-G-X33-X34

- wherein X2 is Ser, Ser(OMe) , Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

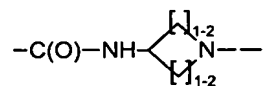
- X3 is absent or Gln;
 X4 is Glu;
 X33 is Leu or Ile;
 X34 is absent or Ala and
 5 X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated.

In another aspect, the X21 Lys is acylated at its side chain amino (ϵ amino) group with a moiety comprising a fatty acid group. The fatty acid group may be attached to X21 Lys via a linker. Accordingly, in another aspect the present invention provides polypeptide comprising
 10 the amino acid sequence:

- H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-G-R-G-
 X33-X34
 wherein X2 is Ser, Ser(OMe), Aib, Ala, Gly, Val, Leu, Ile, (1-amino C_{3-8} cycloalkyl)
 15 carboxylic acid, Trp or Thr;
 X3 is absent or Gln;
 X4 is Gln or Glu;
 X16 is Glu or Asp;
 X24 is Ile or Val or Leu;
 20 X33 is Leu, Ile or Tyr;
 X34 is absent or Ala and
 X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



- wherein Q, T, U and W are absent or selected from a group consisting of $-C(O)-M-NH-$
 25 wherein M is a C_{5-10} alkyl chain interrupted with 1, 2 or 3 oxygen atoms, $-C(O)-NH-C_{2-5}$
 alkyl-NH--, $-C(O)-C(CH_3)_2-NH-$ and



- Y is either absent or $-C(O)-(CH_2)_m-CH(COOH)NH-$ wherein m is an integer selected from
 1 to 2 and -- is point of attachment with the group Z;

- 30 Z is $-C(O)-(CH_2)_n-COOH$ or $-C(O)-(CH_2)_n-CH_3$ wherein n is an integer from 10 to 20.

The bond depicted as “--” is point of attachment with the group on the right hand side of the structure. For example when T is $-\text{C}(\text{O})-\text{M}-\text{NH}-$, the $\text{NH}-$ group is attached to U and so on.

- 5 In the group $-\text{C}(\text{O})-\text{M}-\text{NH}-$, M is a C_{5-10} alkyl chain interrupted with 1, 2 or 3 oxygen atoms. The term “interrupted” means that between two carbons of the alkyl chain oxygen is inserted. The oxygen atom cannot be at the end of the chain (terminal position) and two oxygen atoms cannot be present adjacent (peroxide linkage) to each other.

- 10 In a preferred embodiment, the $-\text{C}(\text{O})-\text{M}-\text{NH}-$ group is 8-amino-3,6-dioxo-octanoic acid (ADO).

In another preferred embodiment, the group $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5}$ alkyl- $\text{NH}-$ is $-\text{C}(\text{O})-\text{NH}-\text{C}_3\text{H}_7-\text{NH}-$. In another preferred embodiment, the group $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5}$ alkyl- $\text{NH}-$ is $-\text{C}(\text{O})-\text{NH}-$

- 15 $\text{C}_4\text{H}_8-\text{NH}-$.

In an embodiment, the amino acid at X2 is selected from Ser, Ser(OMe), Ala or Aib.

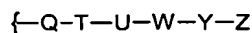
In another embodiment, X3 is absent.

In another embodiment, X3 is absent and X4 is Gln.

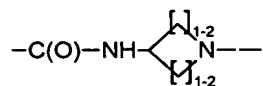
- 20 In a preferred embodiment, X33 is Leu or Ile and X34 is Ala.

In another preferred embodiment, X33 is Leu or Ile and X34 is absent.

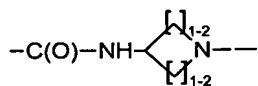
In another embodiment X21 is Lys wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



- 25 In another embodiment, W is absent and T and W are $-\text{C}(\text{O})-\text{M}-\text{NH}-$ and U is selected from a group consisting of $-\text{C}(\text{O})-\text{M}-\text{NH}-$, $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5}$ alkyl- $\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and

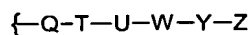


In another embodiment, T is $-\text{C}(\text{O})-\text{M}-\text{NH}-$; U is selected from a group consisting of $-\text{C}(\text{O})-\text{M}-\text{NH}-$, $-\text{C}(\text{O})-\text{NH}-\text{C}_{2-5}$ alkyl- $\text{NH}-$, $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ and

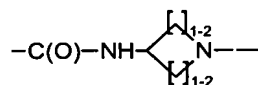


and Q and W are absent.

- In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino)
 5 group of Lys is acylated with a moiety:



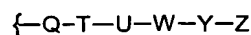
wherein Q is absent; T is selected from a group consisting of $-C(O)-NH-C_{2-5}$ alkyl-NH--, $-C(O)-C(CH_3)_2-NH--$ and



and

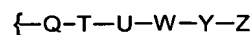
- 10 U and W are $-C(O)-M-NH--$.

In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino)
 group of Lys is acylated with a moiety:



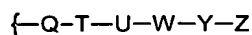
- 15 wherein Y is absent.

In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino)
 group of Lys is acylated with a moiety:



- 20 wherein Q, T, U and W are absent.

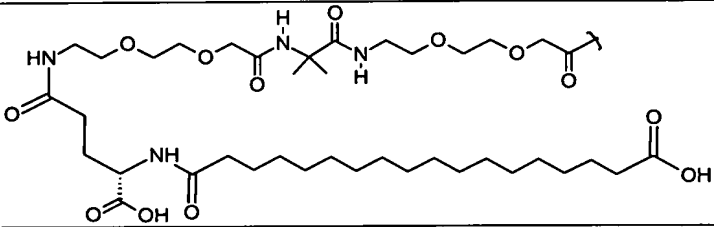
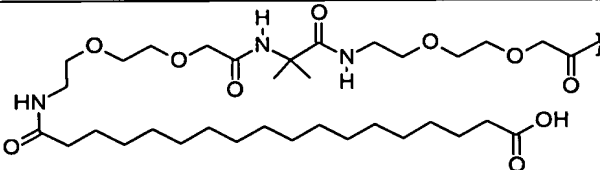
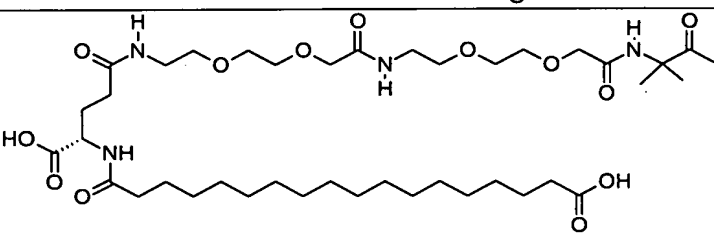
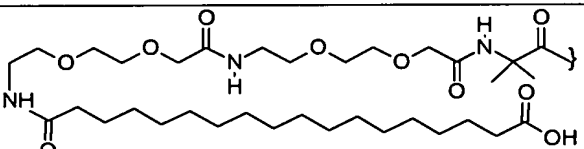
In another embodiment, X21 is lipid modified Lys wherein the side chain amino (ε amino)
 group of Lys is acylated with a moiety



- 25 which is represented by the moieties provided in Table 1

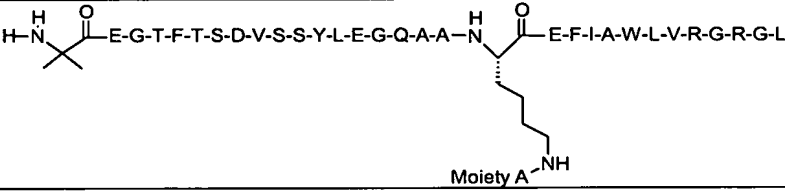
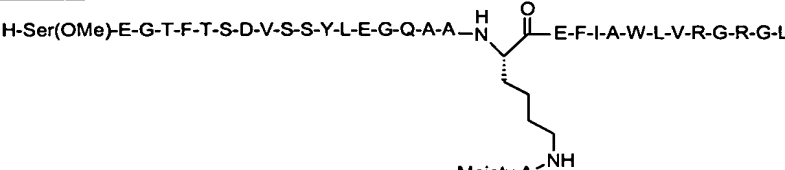
Table 1: Representative moieties for group {-Q-T-U-W-Y-Z

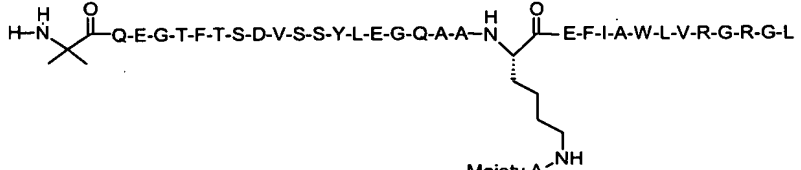
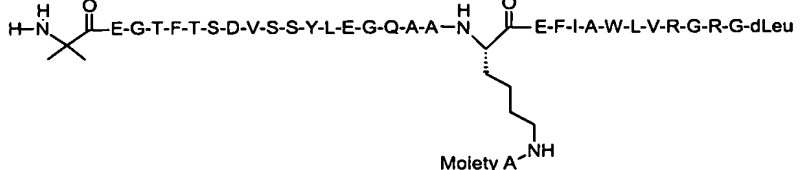
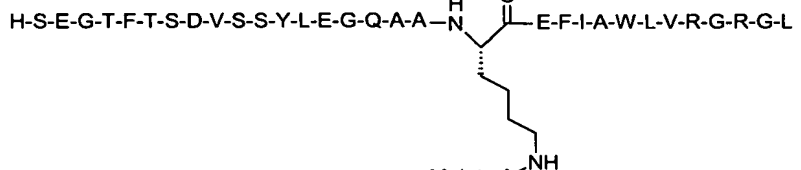
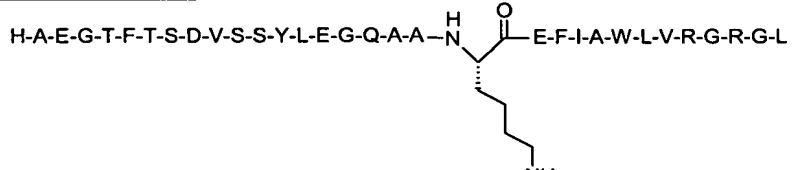
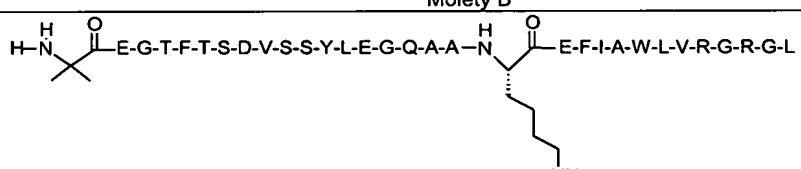
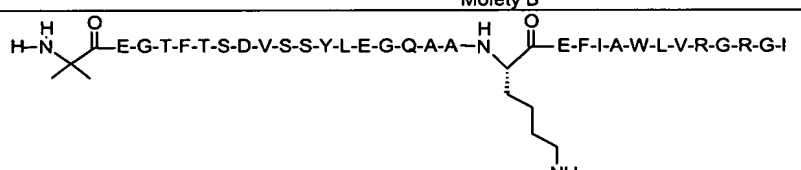
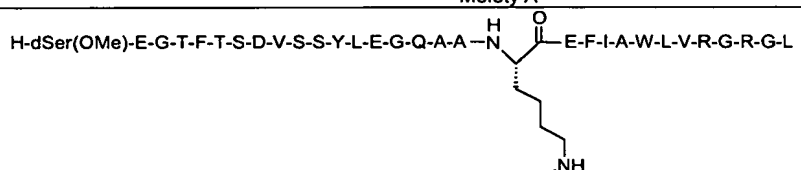
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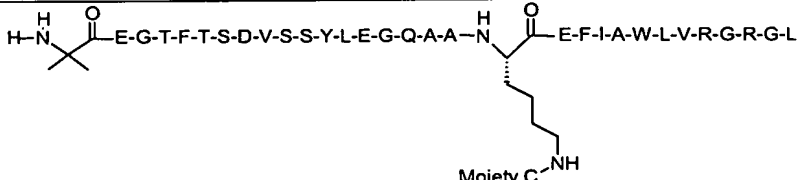
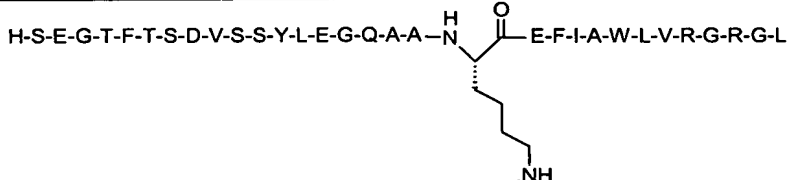
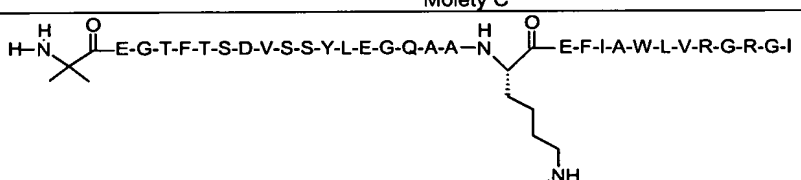
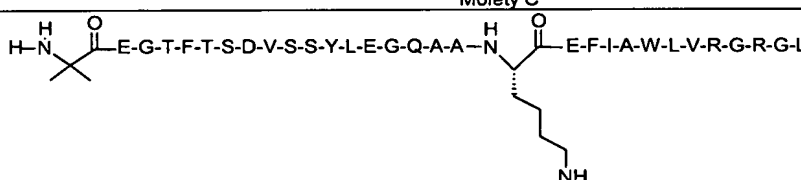
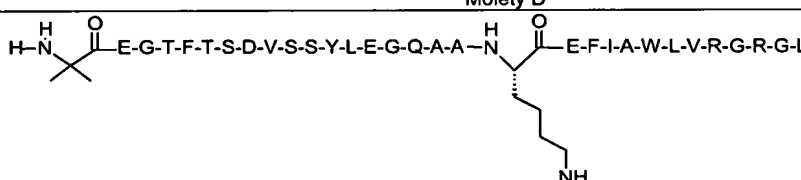
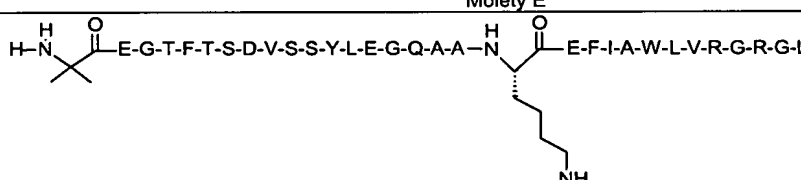
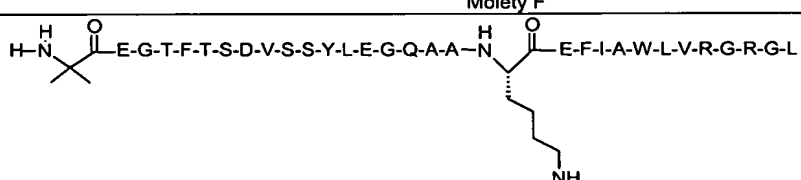
In another embodiment, the present invention provides a polypeptide according to anyone of the preceding embodiments, which is selected from the peptides provided in Table 2:

5 Table 2: Representative polypeptide compounds of present invention

Comp. #	Structure*	Seq ID
1		Seq. ID No: 05
2		Seq. ID No: 06

3		Seq. ID No: 07
4		Seq. ID No: 08
5		Seq. ID No: 09
6		Seq. ID No: 10
7		Seq. ID No: 05
8		Seq. ID No: 11
9		Seq. ID No: 12

10	$\text{H-dSer-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-L}$ Moietly A-NH	Seq. ID No: 13
11	$\text{H-N} \begin{array}{c} \text{H} \\ \parallel \\ \text{---} \end{array} \text{E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-L-A}$ Moietly A-NH	Seq. ID No: 14
12	$\text{H-N} \begin{array}{c} \text{H} \\ \parallel \\ \text{---} \end{array} \text{Q-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-dLeu}$ Moietly A-NH	Seq. ID No: 15
13	$\text{H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-L}$ Moietly A-NH	Seq. ID No: 10
14	$\text{H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-Y}$ Moietly A-NH	Seq. ID No: 16
15	$\text{H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-Y}$ Moietly B-NH	Seq. ID No: 16
16	$\text{H-N} \begin{array}{c} \text{H} \\ \parallel \\ \text{---} \end{array} \text{Q-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-NH} \begin{array}{c} \text{O} \\ \parallel \\ \text{---} \end{array} \text{E-F-I-A-W-L-V-R-G-R-G-L}$ Moietly A-NH	Seq. ID No: 17

17	 Moiety C-NH	Seq. ID No: 05
18	 Moiety C-NH	Seq. ID No: 09
19	 Moiety C-NH	Seq. ID No: 11
20	 Moiety D-NH	Seq. ID No: 05
21	 Moiety E-NH	Seq. ID No: 05
22	 Moiety F-NH	Seq. ID No: 05
23	 Moiety G-NH	Seq. ID No: 05

24		Seq. ID No: 05
25		Seq. ID No: 05
26		Seq. ID No: 05
27		Seq. ID No: 05

* Unless stated otherwise, all the amino acids in the structures have L configuration at α position.

- The polypeptides of the present invention surprisingly showed significant reduction in the blood glucose when subjected to oral glucose tolerance test (OGTT) in SD rats. The percentage reduction of blood glucose in SD rats when challenged with oral glucose was significantly lower than the corresponding polypeptides which lacked the additional Leu or Ile at X33 position. The result of the OGTT experiment is further discussed under biological study section.
- 10 The present invention is further illustrated in detail with reference to the following examples. It is desired that the example be considered in all respect as illustrative and are not intended to limit the scope of the claimed invention.

General method of preparation:

- 15 The polypeptide compound of the present invention can be prepared by methods described herein below. The process involves two steps, involving preparation of the parent linear peptide and subsequent attachment of fatty acid chain to the parent peptide.

The peptides described herein may be prepared by chemical synthesis using solid-phase techniques such as those described in G. Barany and R. B. Merrifield, "The Peptides: Analysis, Synthesis, Biology"; Volume 2—"Special Methods in Peptide Synthesis, Part A", pp. 3-284, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980; and in J. M. Stewart and J. D. Young, "Solid-Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Co., Rockford, Ill., 1984. The desired strategy is based on the Fmoc (9-Fluorenylmethyl methoxycarbonyl) group for temporary protection of the α -amino group, in combination with protecting groups such as *tert*-butyl (-tBu), *tert*-butoxycarbonyl (-Boc), trityl (-Trt) groups for temporary protection of the amino acid side chains (see for example E. Atherton and R. C. Sheppard, "The Fluorenylmethoxycarbonyl Amino Protecting Group", in "The Peptides: Analysis, Synthesis, Biology"; Volume 9—"Special Methods in Peptide Synthesis, Part C", pp. 1-38, S. Udenfriend and J. Meienhofer, Eds., Academic Press, San Diego, 1987).

The peptides can be synthesized in a stepwise manner on an insoluble polymer support (also referred to as "resin") starting from the C-terminus of the peptide. A synthesis is begun by appending the C-terminal amino acid of the peptide to the resin through formation of an amide or ester linkage. This allows the eventual release of the resulting peptide as a C-terminal amide or carboxylic acid, respectively.

The C-terminal amino acid and all other amino acids used in the synthesis are required to have their α -amino groups and side chain functionalities (if present) differentially protected such that the α -amino protecting group may be selectively removed during the synthesis. The coupling of an amino acid is performed by activation of its carboxyl group as an active ester and reaction thereof with the unblocked α -amino group of the N-terminal amino acid appended to the resin. The sequence of α -amino group deprotection and coupling is repeated until the entire peptide sequence is assembled. The peptide is then released from the resin with concomitant deprotection of the side chain functionalities, usually in the presence of appropriate scavengers to limit side reactions. The resulting peptide is finally purified by reverse phase HPLC.

The parent peptide can then be coupled to the fatty acid chain by coupling the activated fatty acid chain with the parent peptide. The fatty acid chain may be made by methods well known

in organic chemistry. For e.g. the fatty acid chain can be made using solid phase synthetic methods which enables preparation of linear fatty acid chains.

5 The linear peptides synthesized were purified by preparative HPLC procedure as outlined below:

Preparative HPLC: WATERS 2555 Quaternary gradient module (Max Total Flow: 300 mL/min, Max Pressure : 3000 psi) or

Shimadzu LC-8A (Max Total Flow: 150 mL: Max Pressure : 20 Mpa)

Column: C18, 10 μ

10 Flow: 75 mL/min

Mobile Phase: Mobile Phase A: pH 7.5 Phosphate buffer

Mobile Phase B: Acetonitrile

Gradient: 10 to 40% Mobile Phase-B in 300 min.

15 The final compounds of the present invention were purified by preparative HPLC procedure as outlined below:

Preparative HPLC: WATERS 2555 Quaternary gradient module (Max Total Flow: 300 mL/min, Max Pressure: 3000 psi) or

Shimadzu LC-8A (Max Total Flow: 150 mL, Max Pressure: 20 Mpa)

20 Column: C18, 10 μ

Flow: 75mL/min

Mobile Phase: Mobile Phase A: 1% Acetic acid in water

Mobile Phase B: 1% Acetic acid in Acetonitrile:n-Propanol (50:50)

Gradient: 15 to 45% Mobile Phase-B in 300 min.

25 The purity of the compounds of the present invention was analysed by RP-HPLC method as outlined below:

Method B1:

Column: YMC Pack-Ph (4.6 mm X 150 mm 3 μ)

30 Eluent: Mobile Phase A: 0.1 % Trifluoroacetic acid in Water

Mobile phase B: 0.1 % Trifluoroacetic acid in Acetonitrile

Flow rate: 1.5 mL/min

Detection: UV detection at 210 nm

Column Temperature: 50 °C

Run Time: 50 min.

Gradient:

Time	Mobile Phase B%
0.01	10
35.0	80
40.0	80
41.0	10
50.0	10

HPLC Method B2:

- 5 Column: YMC-Pack Pro C18 (4 mm x 250 mm, 3 μ)

Eluent: Mobile Phase A: Buffer: Acetonitrile (900:100)

Mobile phase B: Buffer: Acetonitrile (300:700)

Buffer: Potassium dihydrogen orthophosphate in water, pH adjusted to 3.0 $\pm$ 0.1 with orthophosphoric acid

- 10 Flow rate: 1.0 mL/min

Detection: UV detection at 210 nm

Column Temperature: 50 °C

Run Time: 38 min.

Time	Mobile Phase A %	Mobile Phase B %
0	100	0
5	100	0
30	0	100
32	0	100
32.1	100	0
38	100	0

- 15 **HPLC Method B3:**

Column: Waters X-Select CSH-C18 (150mm X 4.6 mm; 2.5 μ)

Eluent: Mobile Phase A: Buffer: Acetonitrile (900:100)

Mobile phase B: Buffer: Acetonitrile (300:700)

Buffer: Potassium dihydrogen orthophosphate in water, pH adjusted to 1.5 $\pm$ 0.1 with orthophosphoric acid

- 20

Flow rate: 0.9 mL/min

Detection: UV detection at 210 nm

Column Temperature: 40 °C

Run Time: 100 min.

Time	Mobile Phase A	Mobile Phase B
0	50	50
40	43	57

55	43	57
90	0	100
91	50	50
100	50	50

The compounds of the present invention were analyzed by the LCMS as outlined below:

Mass spectra were recorded on LCMS using Waters Acquity® QDa®, Waters Micromass Quattro Micro API or Thermo scientific LCQ Fleet™. The test solution was prepared by dissolving suitable quantity of analyte in diluent with final concentration from 1 µg/ml to 50µg/ml depending on the ionization of analyte. The test solution was infused at a rate of about 10 µl to 50 µl per minute into LCMS for 1 min and mass spectra were recorded in Electro Spray Ionization (ESI) positive or negative mode and in an appropriate mass range.

10 Examples:

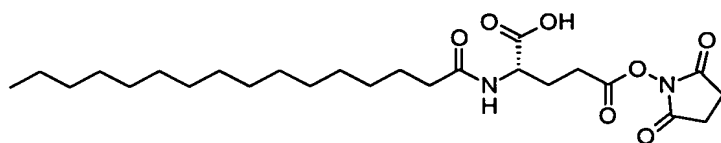
Preparation of activated fatty acid side chains:

1. Preparation of 18-[[[(1S)-1-carboxy-4-[2-[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety A-OSu)

- 15 The activated fatty acid side chain, Moiety A-OSu was prepared by solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 1. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of *N,N'*-di-isopropylethylamine (DIPEA) which yielded 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then coupled to 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid using 1-hydroxybenzotriazole(HOBt) and *N,N'*-di-isopropylcarbodiimide (DIPC) to obtain 2-[2-[2-[[2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was then removed by selective de-blocking of amino group using piperidine and the free amino group was then coupled to Fmoc-Glu-OtBu using HOBt and DIPC to obtain 2-[2-[2-[[2-[2-[2-[[[(4S)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant 2-[2-[2-[[2-[2-[2-[[[(4S)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin was selectively deblocked using piperidine and then coupled with octadecanedioic acid mono *tert*-butyl ester to give intermediate 2-[2-[2-[[2-[2-[2-[[[(4S)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-

pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy] acetic acid]- 2-Cl-Trt-Resin. The intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1). The resultant compound was then reacted with *N*-hydroxysuccinimide (HOSu) in presence of isobutyl chloroformate (IBCF) and *N*-methylmorpholine (NMM) followed by de-protection with trifluoroacetic acid to yield the title compound (Moiety A-OSu).

2. Preparation of *N*-palmitoyl-L- γ -glutamyl succinimide ester (Moiety B-OSu)



Moiety B-OSu

L-Glutamic acid alpha-*tert*-butyl ester (H-Glu-OtBu) was reacted with palmitic acid in presence of IBCF and NMM to yield $\text{CH}_3\text{-(CH}_2\text{)}_{14}\text{-C(O)-Glu-OtBu}$, which was then reacted with HOSu in presence of IBCF and NMM to yield $\text{CH}_3\text{-(CH}_2\text{)}_{14}\text{-C(O)-Glu(OSu)-OtBu}$, which was then de-protected with trifluoroacetic acid to yield Moiety B-OSu.

3. Preparation of 18-[[[(1*S*)-1-carboxy-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]butylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety C-OSu)

The activated fatty acid side chain, Moiety C-OSu was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 2. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then activated using *p*-nitrophenylchloroformate in THF and DIPEA followed by reaction with Fmoc-amino butylamine hydrochloride salt in THF: DMAc and DIPEA which yielded 2-[2-[2-(4-Fmoc-aminobutylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the free amino group was then coupled to Fmoc-Glu-OtBu using of HOBt and DIPC which yielded 2-[2-[2-[4-[[[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant 2-[2-[2-[4-[[[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]-butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin was selectively deblocked using piperidine and then

coupled with octadecanedioic acid mono *tert*-butyl ester to give intermediate 2-[2-[2-[4-
 [[(4*S*)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]
 amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate
 was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[4-
 5 [[(4*S*)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]
 amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= *m/z*: 814.56 (*M*+*H*⁺)). The
 resultant compound was then reacted with HOSu in presence of dicyclohexyl carbodiimide
 (DCC) to yield succinimide protected intermediate, which was de-protected with
 trifluoroacetic acid to yield the title compound (Moiety C-OSu).

10

4. Preparation of 18-[[[(1*S*)-1-carboxy-4-[[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo- ethoxy]ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]amino]-4-oxo-butyl]amino]-18- oxo-octadecanoic acid (Moiety D-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl
 15 chloride resin as schematically represented in Figure 3. 2-[2-(2-Fmoc-
 aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of
 DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc
 protecting group was removed by selective de-blocking of amino group using piperidine
 followed by coupling with Fmoc-Aib-OH in THF: DMAc using DIPC and HOBt which
 20 yielded 2-[2-[2-[(2-Fmoc-amino-2-methyl-propanoyl)amino]ethoxy]ethoxy]acetic acid-2-Cl-
 Trt-Resin. The Fmoc group was removed by selective de-blocking using piperidine and the
 free amino group was coupled with Fmoc-Glu-OtBu using HOBt and DIPC to yield 2-[2-[2-
 [[2-[[[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]-2-methyl-propanoyl] amino]
 ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group of the resultant compound was
 25 selectively de-blocked using piperidine and the free amino group was then coupled with
 octadecanedioic acid mono *tert* butyl ester to give 2-[2-[2-[[2-[[[(4*S*)-5-*tert*-butoxy-4-[(18-
tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]-
 amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate was then cleaved from 2-
 Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[[2-[[[(4*S*)-5-*tert*-butoxy-4-
 30 [(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-
 propanoyl]amino]ethoxy]ethoxy]acetic acid (LCMS= *m/z*: 786.39 (*M*+*H*⁺)). The resultant
 compound was then reacted with HOSu in presence of DCC to yield succinimide protected
 intermediate, which was de-protected with trifluoroacetic acid to yield the title compound
 (Moiety D-OSu).

5. Preparation of 18-[[[(1S)-1-carboxy-4-[3-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]propylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety E-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 4. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free amino group was then activated using p-nitrophenylchloroformate in THF and DIPEA followed by reaction with 1,3-diaminopropane in THF: DMAc in presence of DIPEA using HOBt to form $\text{NH}_2-(\text{CH}_2)_3-\text{NH}-\text{C}(\text{O})-\{(2-(2\text{-amino-ethoxy})\text{-ethoxy})\}$ -acetic acid-2-Cl-Trt-Resin. The free amino group was then coupled to Fmoc-Glu-OtBu using HOBt and DIPC which yielded 2-[2-[2-[3-[[[(4S)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant 2-[2-[2-[3-[[[(4S)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]-ethoxy]acetic acid-2-Cl-Trt-Resin was selectively deblocked using piperidine and then coupled with octadecanedioic acid mono *tert* butyl ester to give 2-[2-[2-[3-[[[(4S)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl]amino]-5-oxo-pentanoyl]amino]-propylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[3-[[[(4S)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl]amino]-5-oxo-pentanoyl]amino]-propylcarbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 801.41 ($\text{M}+\text{H}^+$)). The resultant compound was then reacted with HOSu in presence of dicyclohexyl carbodiimide (DCC) to yield succinimide protected intermediate, which was de-protected with trifluoroacetic acid to yield the title compound (Moiety E-OSu).

6. Preparation of 18-[[[(1S)-1-carboxy-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]-1-piperidyl]-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (Moiety F-OSu)

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 5. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine and the free

amino group was then activated using p-nitrophenylchloroformate in THF and DIPEA followed by reaction with 4-amino-Boc-piperidine in THF: DMAc in presence of DIPEA using HOBt which yielded (2-[2-[2-(4-Boc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid -2-Cl-Trt-Resin. The resultant compound on cleavage using trifluoro acetic acid yielded

5 2-[2-[2-(4-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid which on further reaction with Fmoc-OSu in presence of triethylamine (TEA) yielded 2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid. The obtained compound was then further attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc group

10 was removed by selective de-blocking using piperidine and the free amino group was then coupled with Fmoc-Glu-OtBu using HOBt and DIPC which yielded 2-[2-[2-[[1-[(4S)-4-amino-5-*tert*-butoxy-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. The resultant compound was selectively de-blocked using piperidine and was then coupled with octadecanedioic acid mono *tert*-butyl ester to give 2-[2-[2-[[1-[(4S)-5-

15 *tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. Intermediate was then cleaved from 2-Cl-Trt-Resin using trifluoroethanol:DCM (1:1) to obtain 2-[2-[2-[[1-[(4S)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid (LCMS= m/z : 827.40 ($M+H^+$)). The resultant

20 compound was then reacted with HOSu in presence of DCC to yield succinimide protected intermediate, which was de-protected using trifluoroacetic acid to yield the title compound, Moiety F-OSu.

7. Preparation of 18-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]-1-piperidyl]-18-oxo-octadecanoic acid (Moiety G-OSu)

25

The fatty acid side chain was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented in Figure 6. 2-[2-[2-(4-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid (intermediate prepared as disclosed in the preparation of Moiety F-OSu) was coupled with octadecanedioic acid mono *tert*-butyl ester to give 2-[2-[2-[[1-(18-*tert*-butoxy-18-oxo-octadecanoyl)-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic

30 acid (LCMS= m/z : 642.26 ($M+H^+$)). This intermediate was then reacted with HOSu in presence of DCC to yield succinimide protected intermediate which was de-protected using trifluoroacetic acid to yield the title compound, Moiety G-OSu.

Example 1: Synthesis of Compound 1:

N- ϵ^{26} -[2-(2-[2-(2-[2-(2-[4-(17-Carboxyheptadecanoylamino)-4(S)carboxybutyrylamino]ethoxy)ethoxy]acetylamino)ethoxy]ethoxy)acetyl)][(Aib⁸, Arg³⁴, Leu³⁸ GLP-1(7-38) peptide

5 Part A: Synthesis of the parent linear peptide Aib⁸, Arg³⁴, Leu³⁸ GLP-1(7-38)

The parent peptide was synthesized by solid-phase method. The starting resin used for synthesis was Wang resin. Fmoc protected Leucine was used for coupling with the Wang resin. The coupling was performed by using diisopropylcarbodiimide, *N*-hydroxybenzotriazole (DIC-HOBt) as coupling reagent in presence of 4-dimethylaminopyridine (DMAP) which yielded Fmoc-Leu-Wang Resin. Selective de-
10 blocking of amino group of Fmoc-Leu-Wang Resin using piperidine followed by coupling with Fmoc-Gly-OH using HOBt/DIPC yielded Fmoc-Gly-Leu-Wang Resin. This completes one cycle. Acetic anhydride and diisopropylethyl amine/pyridine was used to terminate the uncoupled amino groups at every amino acid coupling.

15

The above 2 steps i.e selective deblocking of Fmoc- protection of amino acid attached to the resin and coupling of next amino acid residue in sequence with Fmoc-protected amino group were repeated for remaining 30 amino acid residues. The selective deblocking i.e. deprotection of Fmoc group was done using piperidine and coupling with next Fmoc
20 protected amino acid was done using HOBt/DIPC. The side chain of the Fmoc-protected amino acids were protected orthogonally, for eg. hydroxyl group of Serine, Tyrosine or Threonine were protected with *tert*-butyl(-tBu) group, amino and guanido group of Lysine and Arginine were protected with *tert*-butoxycarbonyl (-Boc) and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (-Pbf) group respectively, the imidazole of
25 histidine was protected with trityl (-Trt) and carboxylic acid groups of aspartic acid or glutamic acid were protected with -tBu group. The above mentioned two steps i.e. selective deblocking and then coupling with next Fmoc protected amino acid were performed to get Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-
30 Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-resin.

De-blocking of Fmoc-His(Trt)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Val-Ser(tBu)-Ser(tBu)-Tyr(tBu)-Leu-Glu(OtBu)-Gly-Gln-Ala-Ala-Lys(Boc)-Glu(OtBu)-Phe-Ile-Ala-Trp-Leu-Val-Arg(Pbf)-Gly-Arg(Pbf)-Gly-Leu-Resin using piperidine

followed by cleavage and de-protection using trifluoroacetic acid with ethane-1,2-dithiol resulted in crude H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH (Aib⁸, Arg³⁴, Leu³⁸ GLP-1 (7-38) peptide) which was purified by HPLC.

5

Part B:

Grafting of activated fatty acid chain, Moiety A-OSu over purified (Linear Peptide) H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-Leu-OH obtained in Part A, in acetonitrile at pH
10 about 10 resulted in crude title peptide which was purified by preparative HPLC. The characterization of the compounds is provided in Table 3.

Example 2: Preparation of Compound 2, 3, 5, 9, 10, 13, 16

The linear peptides of the compounds 2, 3, 5, 9, 10, 13 and 16 were prepared by solid phase
15 method as per the analogous process given for Example 1, Part A. Grafting of activated fatty acid chain, Moiety A-OSu by following the process of Example 1, Part B over the respective linear peptides afforded compound 2, 3, 5, 9, 10, 13 and 16.

Example 3: Preparation of Compound 4 and 12:

20 The linear peptides of the Compound 4 and 12 were prepared by solid phase method as per the analogous process given for example 1, part A except here Fmoc protected D-Leucine was first coupled with Wang resin and then sequentially other amino acids were coupled. Grafting of activated fatty acid chain, Moiety A-OSu over the respective linear peptide by following the process of Example 1, Part B afforded the Compound 4 and 12.

25

Example 4: Preparation of Compound 8

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, part A except here Fmoc protected Isoleucine was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid
30 chain, Moiety A-OSu over the linear peptide by following the process of Example 1, Part B afforded the Compound 8.

Example 4: Preparation of Compound 11

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A starting with Fmoc protected Alanine was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety A-OSu over the linear peptide afforded the Compound 11 by following the process of Example 1, Part B.

Example 5: Preparation of Compound 14

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A starting with Fmoc protected Tyrosine (OH orthogonally protected with – tBu) was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety A-OSu over the linear peptide by following the process of Example 1, Part B afforded the Compound 14.

Example 6: Preparation of Compound 6

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A. The grafting of activated fatty acid chain, Moiety B-OSu over the linear peptide by following analogous process of Example 1, Part B afforded the Compound 6.

Example 7: Preparation of Compound 7

The grafting of activated fatty acid chain, Moiety B-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 7.

Example 8: Preparation of Compound 15

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A starting with Fmoc protected Tyrosine (OH orthogonally protected with – tBu) was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety B-OSu over the linear peptide by following analogous process of Example 1, Part B afforded the Compound 15.

Example 9: Preparation of Compound 17

The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 17.

Example 10: Preparation of Compound 18

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A. The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide by following analogous process of Example 1, Part B afforded the Compound 18.

Example 11: Preparation of Compound 19

The linear peptide was prepared by solid phase method as per the analogous process given for Example 1, Part A starting with Fmoc protected Isoleucine was first coupled with Wang resin and then sequentially other amino acids were coupled. The grafting of activated fatty acid chain, Moiety C-OSu over the linear peptide by following analogous process of Example 1, Part B afforded the Compound 19.

Example 12: Preparation of Compound 20

The grafting of activated fatty acid chain, Moiety D-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 20.

Example 13: Preparation of Compound 21

The grafting of activated fatty acid chain, Moiety E-OSu over the linear peptide of the Example 1, Part A by following analogous process of Example 1, Part B afforded the Compound 35.

Example 14: Preparation of Compound 22

The grafting of activated fatty acid chain, Moiety F-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 22.

Example 15: Preparation of Compound 23

The grafting of activated fatty acid chain, Moiety G-OSu over the linear peptide of the Example 1, Part A, by following analogous process of Example 1, Part B afforded the Compound 23.

The characterization data of the synthesized compounds of the present invention are provided below in following table 3.

Table 3: Characterization data of synthesized compounds of the present invention

Comp. #	LCMS Data	HPLC Purity
1	m/z = 1057.52 (MH ₄ ⁴⁺), Calculated Mass= 4226.05	98.70 % (Method B2), RT=24.73 min.
2	m/z = 1061.74 (MH ₄ ⁴⁺), Calculated Mass= 4242.93	99.02 % (Method B1), RT=18.53 min.
3	m/z = 1087.65 (M-4H) ⁴⁺ , Calculated Mass=4354.63	98.12 % (Method B1), RT=18.48 min.
4	m/z = 1055.68 (M-4H) ⁴⁺ , Calculated Mass= 4226.75	98.97 % (Method B1), RT=18.32 min.
5	m/z = 1057.88 (MH ₄ ⁴⁺), Calculated Mass= 4227.49	96.75 % (Method B1), RT=17.09 min.
6	m/z = 967.26 (MH ₄ ⁴⁺), Calculated Mass: 3865.01	98.68 % (Method B3), RT=44.04 min.
7	m/z= 968.53 (M-4H) ⁴⁺ , Calculated Mass= 3878.15	97.39 % (Method B3), RT=27.79 min.
8	m/z = 1057.72 (MH ₄ ⁴⁺), Calculated Mass= 4226.85	95.70% (Method B1), RT=16.62 min.
9	m/z = 1061.67 (MH ₄ ⁴⁺), Calculated Mass= 4242.65	95.15% (Method B1), RT=16.48 min.
10	m/z = 1058.18 (MH ₄ ⁴⁺), Calculated Mass= 4228.69	93.66 % (Method B1), RT=16.13 min.
11	m/z = 1075.09 (MH ₄ ⁴⁺), Calculated Mass= 4296.33	91.08 % (Method B2), RT=24.82 min.
12	m/z = 1056.95 (MH ₄ ⁴⁺), Calculated Mass: 4223.77	95.70 % (Method B2), RT=24.46 min.
13	m/z = 1405.12 (MH ₃ ³⁺), Calculated Mass: 4212.34	97.51% (Method B1), RT=19.06 min.
14	m/z = 1066.67 (MH ₄ ⁴⁺), Calculated Mass= 4262.65	95.73% (Method B1), RT=18.74 min.
15	m/z = 979.15 (MH ₄ ⁴⁺), Calculated Mass= 3912.6	98.09 % (Method B3), RT=44.69 min.
16	m/z = 1057.19 (MH ₄ ⁴⁺), Calculated Mass= 4224.76	92.10 % (Method B2), RT=25.05 min.
17	m/z = 1049.59 (MH ₄ ⁴⁺), Calculated Mass= 4194.33	96.01 % (Method B2), RT=25.16 min.
18	m/z = 1050.13 (MH ₄ ⁴⁺), Calculated Mass= 4196.49	92.06 % (Method B2), RT=24.55 min.
19	m/z = 1049.61 (MH ₄ ⁴⁺), Calculated Mass= 4194.41	94.41 % (Method B2), RT=24.82 min.
20	m/z = 1042.34 (MH ₄ ⁴⁺), Calculated Mass= 4165.32	94.56 % (Method B2), RT=25.26 min.
21	m/z = 1046.18 (MH ₄ ⁴⁺), Calculated Mass= 4180.72	94.33 % (Method B2), RT=25.17 min.
22	m/z = 1052.77 (MH ₄ ⁴⁺), Calculated Mass= 4207.08	93.12 % (Method B2), RT=24.92 min.
23	m/z = 1018.66 (M-4H) ⁴⁺ , Calculated Mass: 4078.67	92.00 % (Method B2), RT=25.84 min.

5

Biological Study:

Oral Glucose tolerance test (OGTT) in rats; Single Injection; 1mg/kg

Animals were divided into three groups – a normal control group, a test group and a third semaglutide group, with 4 animals in each group. The animals were fasted for 12 hours before initiation of OGTT. To the test group animals, the test compound was injected subcutaneously at 1 mg/kg dose. To the semaglutide group, a dose of 1 mg/kg was injected subcutaneously. To the normal control group, equivalent volume of vehicle was injected subcutaneously. After 166 hrs of subcutaneous injection of test drug or semaglutide or vehicle, blood glucose was measured with blood glucose meter (time 0 measurements). All the animals were then given 2 g/kg of glucose solution orally. Blood glucose was measured at 20min, 40 min, 60 min, 90 min and 120 min following glucose challenge.

It was surprisingly found compounds having X33 as Leu and Ile showed significant reduction of the blood glucose in the given studies whereas compounds with amino acids other than Leu and Ile had significantly less effect in lowering the blood glucose. The

polypeptide of present invention has shown significant reduction in blood glucose when compared to the control group. Compounds having amino acids other than Leu or Ile at X33 positions were also tested. For example, Leu at 32nd position in Compound 1 (Seq. ID 05) was replaced with Lys and Ser to obtain compounds Std-1 and Std-2, respectively. Std-1 and Std-2 showed only about 35 and 15 % reduction in blood glucose AUC_{0-120min} (Table 4).

Table 4: Percentage reduction in Blood Glucose AUC_{0-120min} in OGTT test @ 1 mg/Kg dose after 24 hrs.

Comp. #	% Glucose Reduction	Comp/std Semaglutide	Comp. #	% Glucose Reduction	Comp/std Liraglutide
Std-1	35.1	0.71	6	75.8	1.83
Std-2	14.8	0.30	7	71.9	1.73
Semaglutide	49.3		Liraglutide	41.5	

Similarly, the compounds 5 which differs from liraglutide in having an additional Leu at 32nd position and the compound 7 which differs in Liraglutide sequence and the compound 7 which differs from liraglutide by having 2nd amino acid Ala replaced with Aib and having Leu as an additional 32nd amino acid, showed blood glucose lowering effect at 24 hrs which was significantly higher than liraglutide (Table 4).

Provisional claims:

1. A polypeptide comprising the amino acid sequence:

H-X2-X3-X4-G-T-F-T-S-D-V-S-S-Y-L-X16-G-Q-A-A-X21-E-F-X24-A-W-L-V-R-
G-R-G-X33-X34

wherein X2 is Ser, Ser(OMe) , Aib, Ala, Gly, Val, Leu, Ile, (1-amino C₃₋₈ cycloalkyl) carboxylic acid, Trp or Thr;

X3 is absent or Gln;

X4 is Gln or Glu;

X16 is Glu or Asp;

X24 is Ile or Val or Leu;

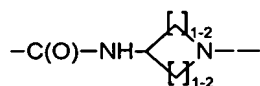
X33 is Leu, Ile or Tyr;

X34 is absent or Ala and

X21 is Lys wherein the side chain amino (ε amino) group of Lys is acylated with a moiety:

{-Q-T-U-W-Y-Z

wherein Q, T, U and W are absent or selected from a group consisting of -C(O)-M-NH-- wherein M is a C₅₋₁₀ alkyl chain interrupted with 1, 2 or 3 oxygen atoms, -C(O)-NH-C₂₋₅ alkyl-NH--, -C(O)-C(CH₃)₂-NH--and



Y is either absent or -C(O)-(CH₂)_m-CH(COOH)NH-- wherein m is an integer selected from 1 to 2 and -- is point of attachment with the group Z;

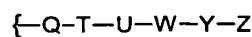
Z is -C(O)-(CH₂)_n-COOH or -C(O)-(CH₂)_n-CH₃ wherein n is an integer from 10 to 20.

2. The polypeptide as in provisional claim 1, wherein X2 is Ser, Ser(OMe) , Ala or Aib;
X3 is absent;
X4 is Glu;
X16 is Glu;
X24 is Ile;

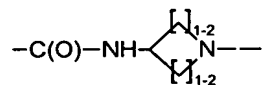
X33 is Leu, Ile or Tyr.

3. The polypeptide as in provisional claim 1, wherein X2 is Ser, Ser(OMe), Ala or Aib;
X3 is absent;
X4 is Gln;
X16 is Glu;
X24 is Ile;
X33 is Leu, Ile or Tyr.

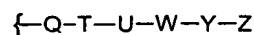
4. The polypeptide as in provisional claims 1, 2 or 3, wherein X21 is lipid modified Lys
wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is absent and T and W are $-C(O)-M-NH-$ and U is selected from a group
consisting of $-C(O)-M-NH-$, $-C(O)-NH-C_{2-5}$ alkyl- $NH-$, $-C(O)-C(CH_3)_2-NH-$ and

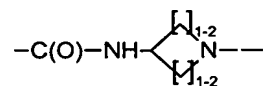


5. The polypeptide of provisional claims 1, 2 or 3, wherein X21 is lipid modified Lys
wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is absent; T is $-C(O)-M-NH-$;

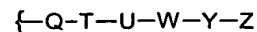
U is selected from a group consisting of $-C(O)-M-NH-$, $-C(O)-NH-C_{2-5}$ alkyl- $NH-$,
 $-C(O)-C(CH_3)_2-NH-$ and



and

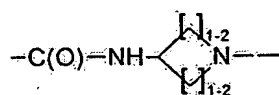
W is absent.

6. The polypeptide as in provisional claims 1, 2 or 3, wherein X21 is lipid modified Lys
wherein the side chain amino (ϵ amino) group of Lys is acylated with a moiety:



wherein Q is absent;

T is selected from a group consisting of $-C(O)-NH-C_{2-5}$ alkyl- $NH-$, $-C(O)-C(CH_3)_2-$
 $NH-$ and

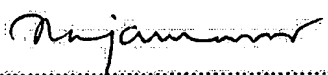


and

U and W are $-\text{C}(\text{O})-\text{M}-\text{NH}-$.

7. The polypeptide as in provisional claims 4 to 6, wherein Y is absent.
8. The polypeptide as in provisional claims 1 to 3 wherein Q, T, U and W are absent.
9. The polypeptide as in any preceding claims, wherein $-\text{C}(\text{O})-\text{M}-\text{NH}-$ group is ADO.
10. The polypeptide as in any preceding provisional claims, wherein X33 is Leu or Ile.

Dated 26th Day of October 2018

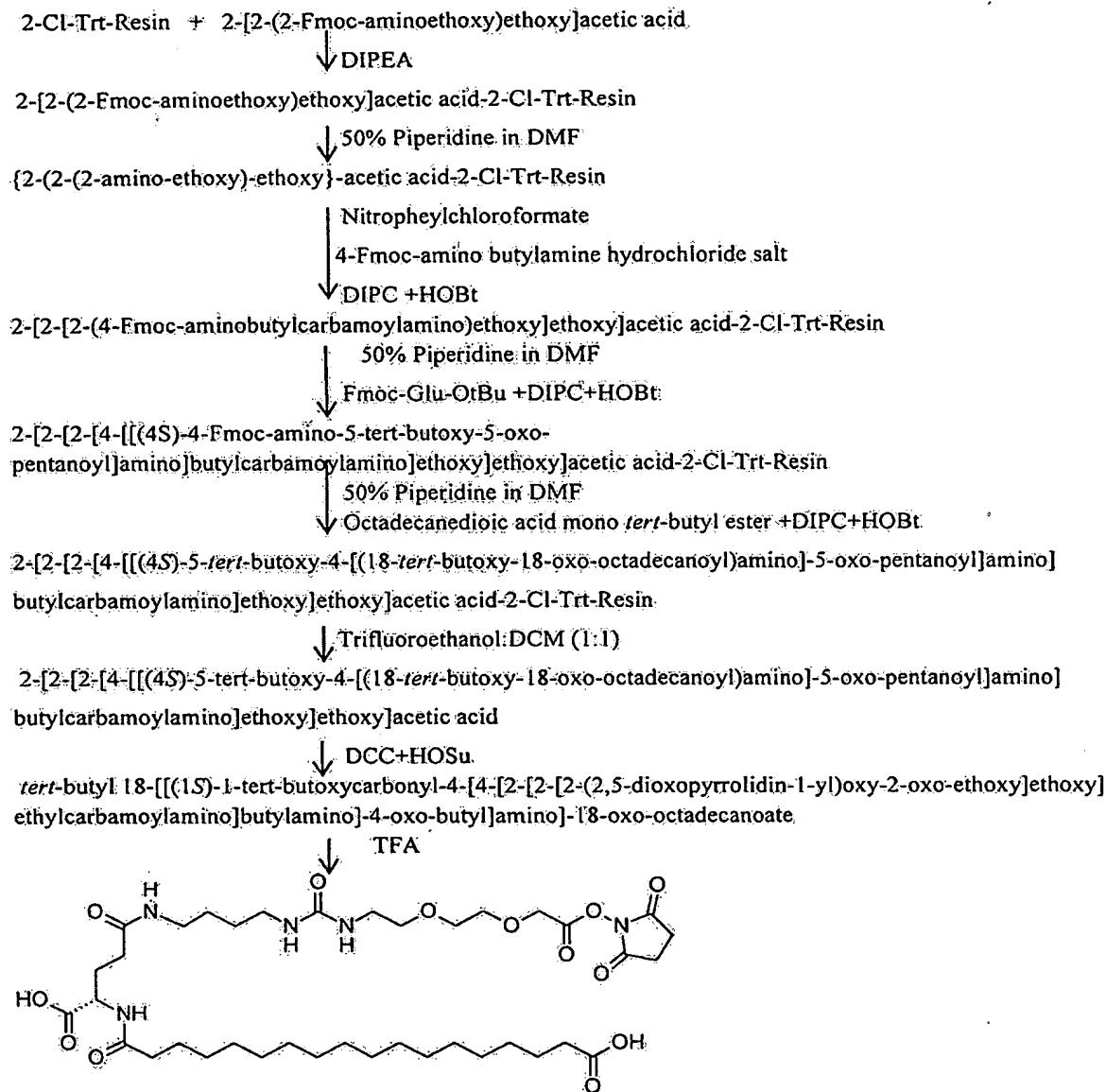
(Signature) 

Dr. RAJAMANNAR THENNATI
EXECUTIVE VICE PRESIDENT,
SUN PHARMACEUTICAL INDUSTRIES LTD.

Figure 1: Preparation of Moiety A-OSu

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33



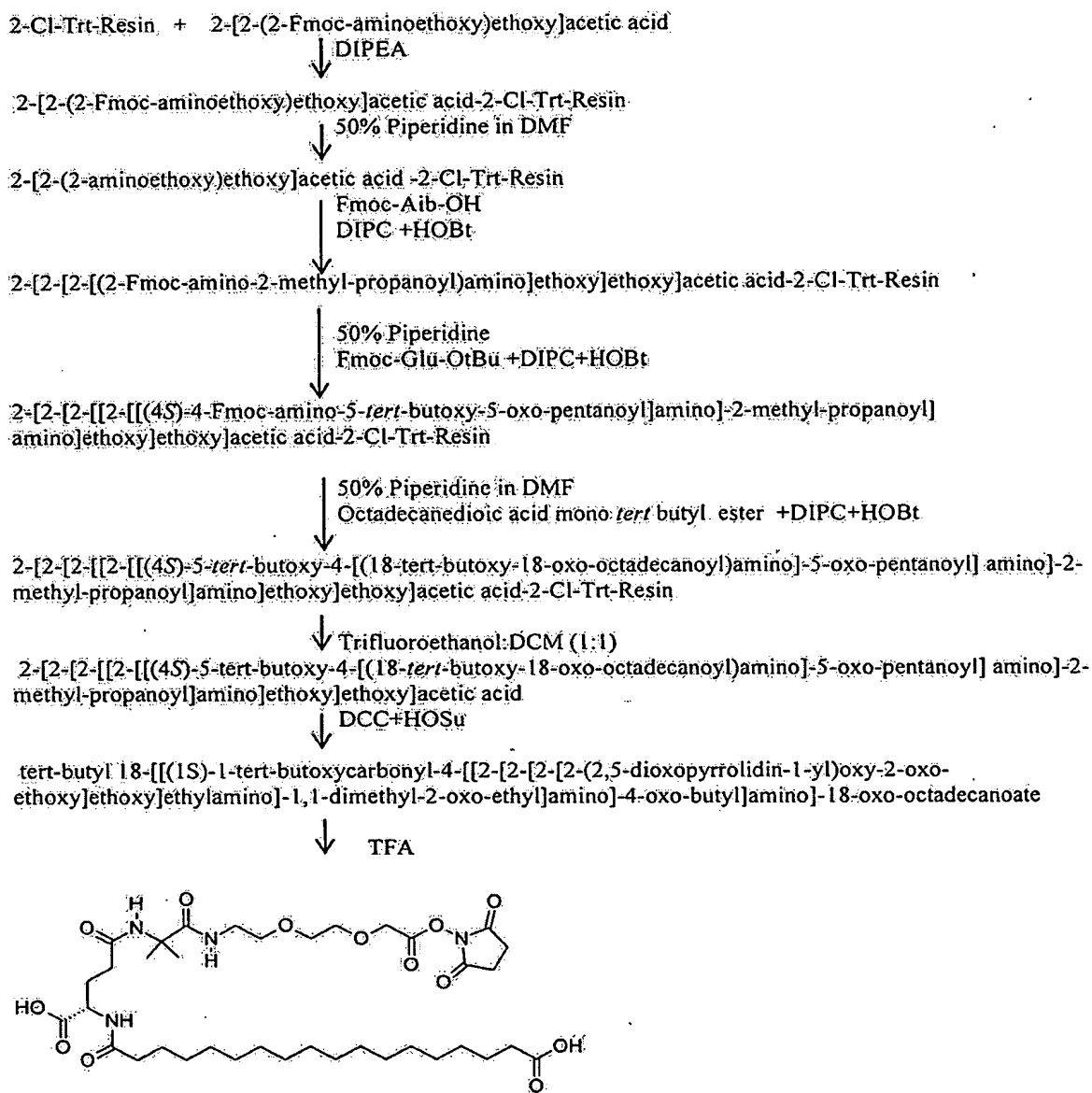
Moiety C-OSu

Figure 2: Preparation of Moiety C-OSu

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 SUN PHARMACEUTICAL INDUSTRIES LTD.



Moiety D-OSu

Figure 3: Preparation of Moiety D-OSu

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 SUN PHARMACEUTICAL INDUSTRIES LTD.

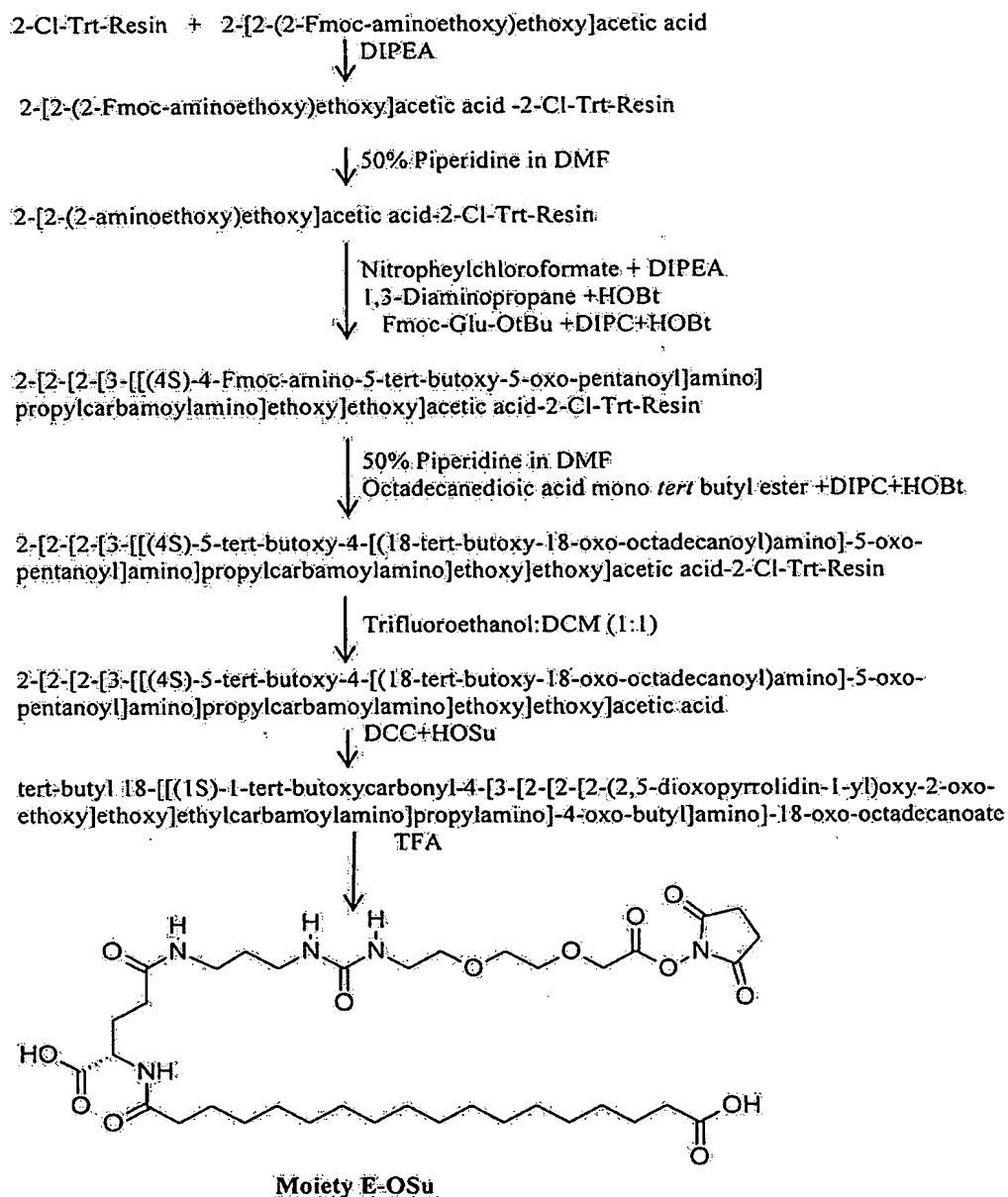
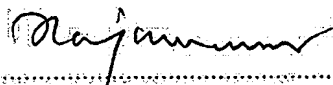


Figure 4: Preparation of Moiety E-OSu

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 SUN PHARMACEUTICAL INDUSTRIES LTD.

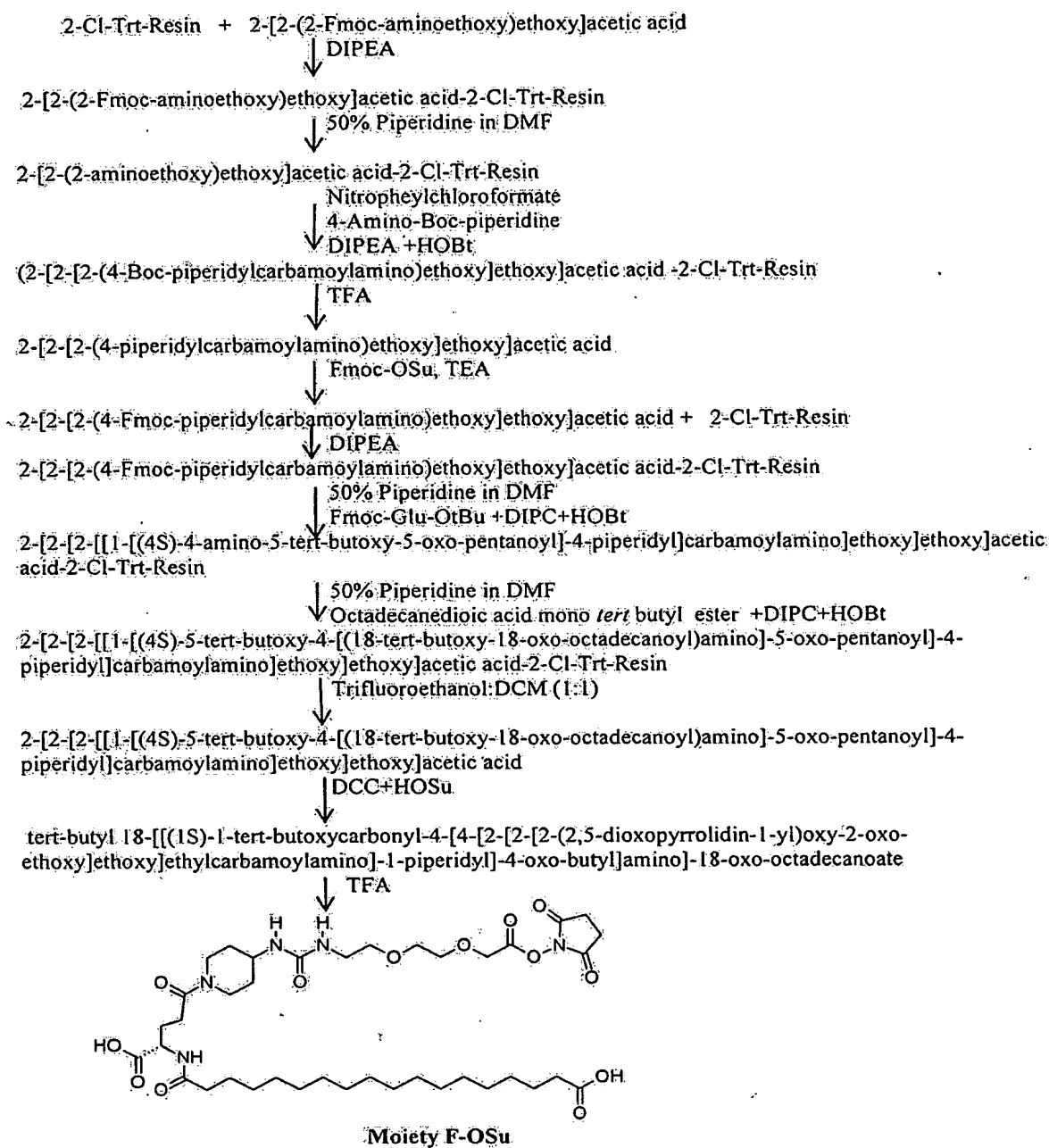
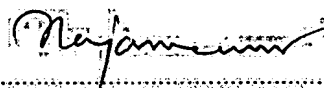


Figure 5: Preparation of Moiety F-OSu

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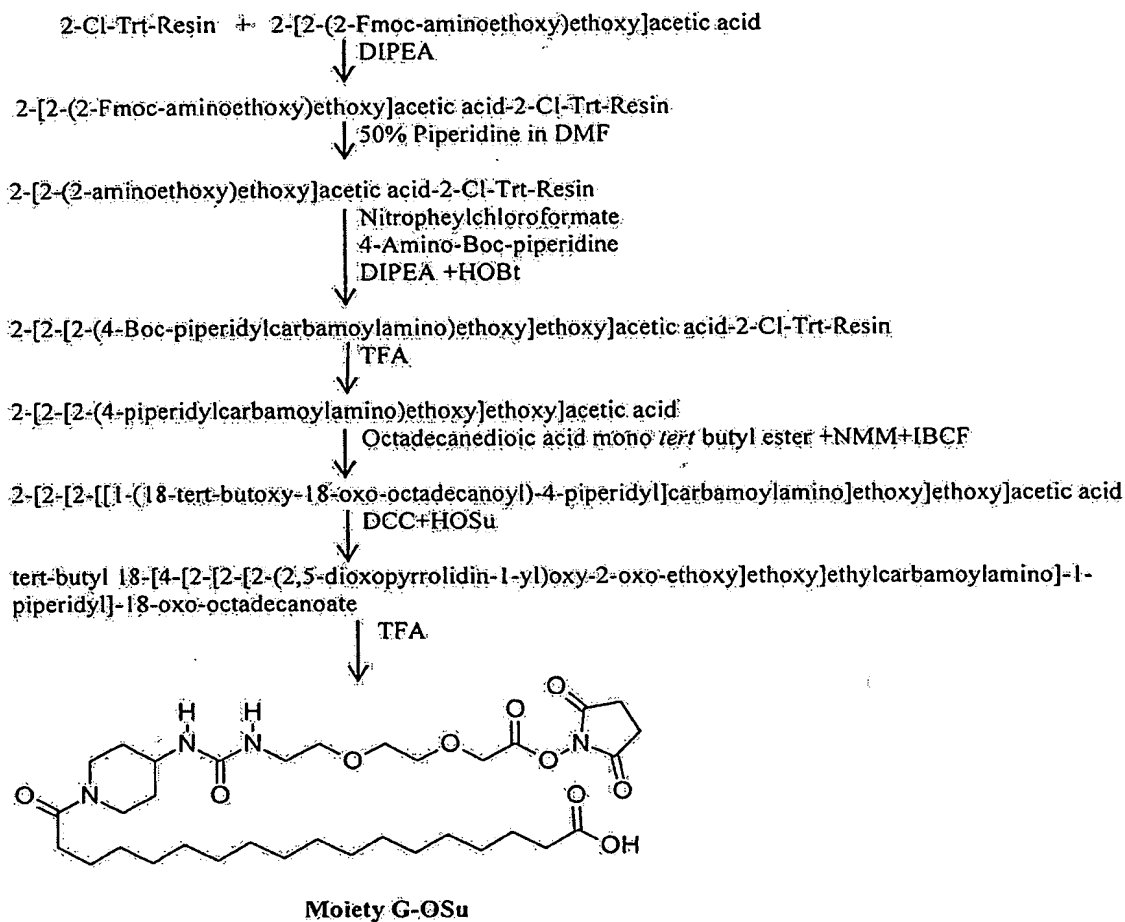
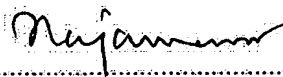


Figure 6: Preparation of Moiety G-OSu

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ABSTRACT

EXTENDED GLP-1 DERIVATIVES

The present invention pertains to a novel Glucagon like Peptide-1 (GLP-1) (7-38) analogs having an amino acid sequence with Leu or Ile or Leu/Ile-X at the C-terminal. The new analogs are potent GLP-1 agonist with reduced adverse effect and improved duration of action. The present invention further relates to acylated derivatives of the new analogs which have further improved potency and duration of action and are suitable for oral administration. The analogs of present invention may be useful in treatment of diabetes and obesity.

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<170> PatentIn version 3.5

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Xaa Xaa

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His Xaa Gln Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu
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Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly
20 25 30

Leu

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Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly Ile
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Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly Leu
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Xaa

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Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Arg Gly Arg Gly Leu
20 25 30

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