

## GLP-1/GIP DUAL AGONISTS

### FIELD OF THE INVENTION

The present invention relates to long acting glucagon-like peptide-1 (GLP-1) and  
5 human glucose-dependent insulinotropic polypeptide/ Gastro Intestinal Peptide (GIP) dual  
agonist polypeptides which may be useful for treating type 2 diabetes mellitus (T2D),  
diabetes with obesity, obesity and hyperlipidemia.

### BACKGROUND OF THE INVENTION

Treatment of type 2 diabetes mellitus (T2DM) with glucagon-like peptide-1 receptor  
10 agonists (GLP-1RAs) leads to improved glycaemic control, reduced body weight, and  
improvement in several cardiovascular risk factors. These benefits are mediated by the  
glucagon-like peptide-1 receptor (GLP-1R), a member of the class B family of G protein-  
coupled receptors, that is expressed in pancreatic beta-cells, various cell types of the  
gastrointestinal tract and neurons throughout both the central (CNS) and the peripheral  
15 nervous systems. Activation of GLP-1R signaling by GLP-1RAs improves glucose  
homeostasis by enhancing glucose-stimulated insulin secretion, delaying gastric emptying  
and decreasing plasma glucagon levels, and reduces body weight by activating anorexigenic  
pathways in the brain. Due to the glucose-dependence of beta-cell activation, GLP-1RAs  
are not associated with increased risk of hypoglycaemia. While the broad metabolic benefits  
20 of GLP-1RAs have established this class in the T2DM treatment paradigm, many patients  
do not reach their HbA1c/glycaemic targets and weight loss achieved with these agents,  
thus, requiring a higher dose, which also increases GI adverse events, and remains well  
below what can be attained with bariatric surgery, the most potent clinical intervention for  
obesity. Thus, there are significant opportunities to improve upon the existing GLP-1RA  
25 class.

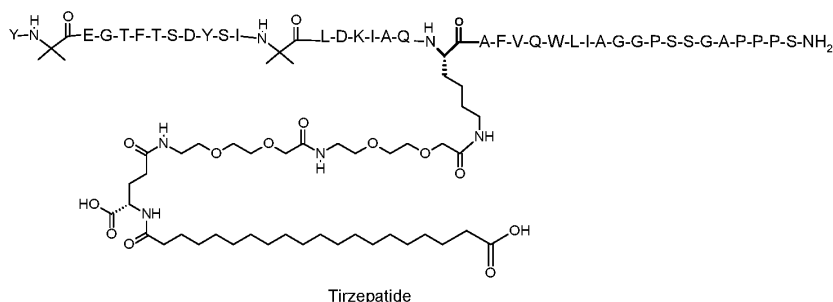
One emerging approach is to combine foundational GLP-1RA therapy with  
pharmacological strategies targeting additional pathways implicated in nutrient and energy  
metabolism, such as the glucose-dependent insulinotropic polypeptide (GIP) pathway. GIP  
is an incretin that is secreted from K cells in the upper small intestine, duodenum, in  
30 response to food. Postprandial GIP levels are approximately 4-fold higher compared to  
GLP-1 under normal physiological conditions. GIP is responsible for the majority of the  
insulinotropic incretin effect in man, and has important additional functions that are distinct

from GLP-1. Unlike GLP-1, GIP is both glucagonotropic and insulinotropic in a glycaemic-dependent manner, dose-dependently stimulating glucagon secretion under hypoglycaemic conditions and insulin under hyperglycaemic conditions, glucagon released does facilitate insulin secretion. Although both GIP-receptor (GIPR) and GLP-1R are present in beta-cells,

5 GIPR expression is distributed differently in extra-pancreatic tissues as GIPR is abundant in adipose tissue and is found in many non-overlapping areas of the CNS. GIP is implicated in adipose tissue carbohydrate and lipid metabolism by its actions to regulate glucose uptake, lipolysis and lipoprotein lipase activity. The findings suggest that pharmacological activation of GIPR may have a therapeutic benefit on peripheral energy metabolism.

10 Recently, uni-molecular, multi-functional peptides that combine GLP-1RA activity with GIP activity have been suggested as new therapeutic agents for glycaemic and weight control.

United States Patent No. 9474780 discloses dual GLP-1 and GIP receptor agonists including tirzepatide.



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Tirzepatide is under Phase-III clinical studies for T2DM and obesity.

WIPO publication numbers WO2017/74714A1, WO2020/23386A1, WO2020/023388A1, WO2015/067715A2, WO2016/111971A1 and WO2013/164483A1 disclose GLP-1 R and GIP R dual agonist compounds.

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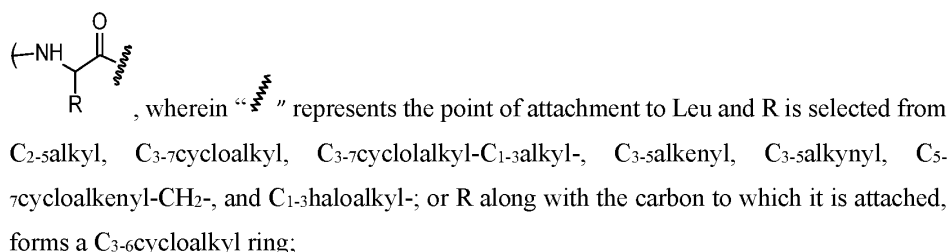
## SUMMARY OF THE INVENTION

The present invention provides a polypeptide or a pharmaceutically acceptable salt thereof, comprising an amino acid sequence:

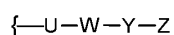
Y-X1-E-G-T-F-T-S-D-Y-S-I-X2-L-Xaa15-K-I-A-Xaa19-X3-Xaa21-F-V-Xaa24-W-L-X4-A-G-G-P-S-S-G-A-P-P-S-X5-X6-X7-X8-X9-X10-X11 (Seq. ID 1)

25 wherein X1 is Aib, (L)-norvaline or (D)-norvaline;

X2 is selected from Aib, Leu, (D)-Leu, Val, (D)-Val, Ile, (D)-Ile, and L or D isomer of an amino acid of Formula



X3 is Gln or Lys; wherein, when X3 is Lys, the side chain amino (ε amino) group of Lys is acylated with a moiety:



10 wherein U is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-\}$  wherein } is point of attachment with group W;

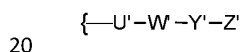
W is selected from a group consisting of  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_p-\text{NH}-$ ],  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ ] and  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ ], wherein p is 3 or 4 and wherein ] is point of attachment with group Y;

15 Y is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH}-$  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 14 to 20;

X4 is Leu, Ile or Glu;

X5 is absent, Arg or Lys; wherein when X5 is Lys, the side chain amino (ε amino) group of Lys is acylated with a moiety:



wherein U' is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-\}$  wherein } is point of attachment with group W';

W' is selected from a group consisting of  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_q-\text{NH}-$ ],  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ ] and  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ ], wherein q is 3 or 4 and wherein ] is point of attachment with group Y';

25

Y' is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH}-$  and -- is point of attachment with the group Z';

$Z'$  is  $-C(O)-(CH_2)_n-COOH$  or  $-C(O)-(CH_2)_m-CH_3$  wherein  $m$  is an integer from 14 to 20;

X6 is absent or Lys;

X7 is absent or Lys;

X8 is absent or Lys;

5 X9 is absent or Lys;

X10 is absent or Lys;

X11 is absent or Lys;

Xaa15 is Asp or Glu;

Xaa19 is Gln or Ala;

10 Xaa21 is Ala or Glu;

Xaa24 is Gln or Asn;

wherein the acid group of the C terminal amino acid is a free carboxylic acid group or is amidated as a C-terminal primary amide and at least one of X3 and X5 is Lys; and

with the proviso that when X1 is Aib, X2 is not Aib.

## 15 ABBREVIATIONS

Aib: 2-Aminoisobutyric acid

DIPEA: *N,N*-Di-isopropylethylamine

HOBt: 1-Hydroxybenzotriazole

DIPC: *N,N*-Di-isopropylcarbodiimide

20 THF: Tetrahydrofuran

DCM: Dichloromethane

## DETAILED DESCRIPTION OF THE INVENTION

“Pharmaceutically acceptable salt” according to the invention include acid addition salts formed with either organic or inorganic acids. Suitable pharmaceutically acceptable salts of the compounds of the invention include acid addition salts which may be salts of inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, and the like or of organic acids such as, for example, acetic acid, benzenesulfonic acid, methanesulfonic

acid, benzoic acid, citric acid, lactic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, amino acids such as glutamic acid or aspartic acid, and the like. The pharmaceutically acceptable acid addition salt of the compounds of the present invention includes salts formed with the addition of one or more  
5 equivalents of acids, for example, monohydrochloride, dihydrochloride salts, etc. Salts can be prepared by any process under the purview of an ordinary person skilled in the art. (See Berge et al., J. Pharm. Sci. 1977, 66, 1-19; and Handbook of Pharmaceutical Salts, Properties, and Use; Stahl and Wermuth, Ed.; Wiley-VCH and VHCA: Zurich, Switzerland, 2002.).

10 The term “alkyl” as used herein refers to a saturated hydrocarbon chain radical that includes solely carbon and hydrogen atoms in the backbone, either linear or branched, having from 1 to 6 carbon atoms, both inclusive unless defined otherwise and which is attached to the rest of the molecule by a single bond. Suitable non-limiting examples of alkyl groups include, e.g., methyl, ethyl, n-propyl, 1-methylethyl (isopropyl), n-pentyl, n-  
15 hexyl, etc.

The term “haloalkyl” as used herein refers to any “alkyl” having one or more hydrogen atom(s) replaced by a halogen atom, wherein halogen atom may be selected from fluorine, chlorine, bromine or iodine.

The numerical in phrases like “C<sub>2-5</sub>”, refers to the number of carbon atoms in the chain. For example, the phrase “C<sub>2-5</sub> alkyl” refers to an alkyl chain having 2 to 5 carbon  
20 atoms.

The term “alkenyl” as used herein refers to a hydrocarbon chain containing at least one carbon-carbon double bond, and may have (*E*) or (*Z*) configuration. An alkenyl group may contain 2 to 8 carbon atoms unless specified otherwise. Unless set forth or recited to  
25 the contrary, all alkenyl groups described herein may form part of a straight or branched chain. Suitable non-limiting examples of alkenyl groups include, e.g., ethylene, 2-propenyl (allyl), 2-methyl-2-propenyl and 2-butenyl.

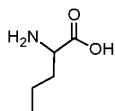
The term “alkynyl” refers to a hydrocarbon chain having at least one carbon-carbon triple bond. An alkynyl group may contain 2 to 8 carbon atoms unless specified otherwise.  
30 Unless set forth or recited to the contrary all alkynyl groups described or claimed herein may form part of a straight or branched chains. The non-limiting examples of alkynyl groups include 2-propynyl, 3-butyne and propargyl.

The term “*cycloalkyl*” as used herein refers to a non-aromatic monocyclic ring system of 3 to 7 carbon atoms unless specified otherwise. Cycloalkyl ring include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

5 The term “*cycloalkenyl*” refers to a non-aromatic monocyclic 5 to 7 membered cycloalkyl ring system, with at least one carbon-carbon double bond. The non-limiting examples of cycloalkenylmethyl group includes cyclopentenylmethyl and cyclohexenylmethyl.

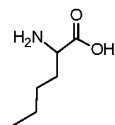
The term “*effective amount or amount effective*” as used herein refers to an amount of the compound which is sufficient, upon single or multiple dose administration(s) to a subject, in curing, alleviating, relieving or partially addressing the clinical manifestation of given disease or state and its complications beyond that expected in the absence of such treatment. Thus, the result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. It is understood that “a therapeutically effective amount” can vary from subject to subject depending on age, weight, general condition of the subject, the condition being treated, the severity of the condition being treated, and the judgment of the prescribing physician.

The amino acid “norvaline” as used herein can be represented by structure



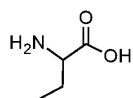
and can also be defined by the chemical name as “2-aminopentanoic acid”. The term (L)-norvaline and (D)-norvaline refers to “L” and “D” isomer of norvaline, respectively.

The amino acid “norleucine” as used herein can be represented by structure



and can also be defined by the chemical name as “2-Aminohexanoic acid”. The term (L)-norleucine and (D)-norleucine refers to “L” and “D” isomer of norleucine, respectively.

25 The amino acid “homoalanine” as used herein can be represented by structure



and can also be defined by the chemical name as “2-aminobutyric acid”. The term (L)-homoalanine and (D)-homoalanine refers to “L” and “D” isomer of homoalanine, respectively.

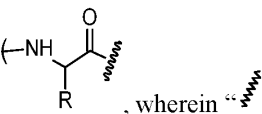
The present invention provides stable long acting GLP-1/GIP agonist polypeptides which may be useful for treating type 2 diabetes mellitus (T2D), diabetes with obesity, obesity and hyperlipidemia. The polypeptides of present invention are long acting which may not require frequent administration to a patient in need thereof.

Accordingly, in one aspect, the present invention provides a polypeptide or a pharmaceutically acceptable salt thereof, comprising an amino acid sequence:

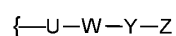
Y-X1-E-G-T-F-T-S-D-Y-S-I-X2-L-Xaa15-K-I-A-Xaa19-X3-Xaa21-F-V-Xaa24-W-L-X4-A-G-G-P-S-S-G-A-P-P-S-X5-X6-X7-X8-X9-X10-X11 (Seq. ID 1)

wherein X1 is Aib, (L)-norvaline or (D)-norvaline;

X2 is selected from Aib, Leu, (D)-Leu, Val, (D)-Val, Ile, (D)-Ile, and L or D isomer of an amino acid of Formula

, wherein “” represents the point of attachment to Leu, R is selected from C<sub>2</sub>-salkyl, C<sub>3</sub>-cycloalkyl, C<sub>3</sub>-cycloalkyl-C<sub>1-3</sub>alkyl-, C<sub>3</sub>-salkenyl, C<sub>3</sub>-salkynyl, C<sub>5</sub>-cycloalkenyl-CH<sub>2</sub>-, and C<sub>1-3</sub>haloalkyl; or R along with the carbon to which it is attached to, forms C<sub>3-6</sub>cycloalkyl ring;

X3 is Gln or Lys; wherein, when X3 is Lys, the side chain amino (ε amino) group of Lys is acylated with a moiety:



wherein U is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  wherein } is point of attachment with group W;

W is selected from a group consisting of  $-C(O)-NH-(CH_2)_p-NH-$ ,  $-C(O)-C(CH_3)_2-NH-$  and  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ , wherein p is 3 or 4 and wherein ] is point of attachment with group Y;

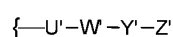
Y is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH}-$  and  $-$  is point of attachment with the group Z;

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 14 to 20;

X4 is Leu, Ile or Glu;

X5 is absent, Arg or Lys; wherein when X5 is Lys, the side chain amino ( $\epsilon$  amino) group of

5 Lys is acylated with a moiety:



wherein U' is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-\}$  wherein  $\}$  is point of attachment with group W';

W' is selected from a group consisting of  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_q-\text{NH}-$ ,  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  and  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ , wherein q is 3 or 4 and wherein  $\}$  is point of attachment with group Y';

Y' is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH}-$  and  $-$  is point of attachment with the group Z';

Z' is  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  or  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{CH}_3$  wherein m is an integer from 14 to 20;

X6 is absent or Lys;

15 X7 is absent or Lys;

X8 is absent or Lys;

X9 is absent or Lys;

X10 is absent or Lys;

X11 is absent or Lys;

20 Xaa15 is Asp or Glu;

Xaa19 is Gln or Ala;

Xaa21 is Ala or Glu;

Xaa24 is Gln or Asn;

wherein the acid group of the C terminal amino acid is a free carboxylic acid group or is  
25 amidated as a C-terminal primary amide and at least one of X3 and X5 is Lys; and

with the proviso that when X1 is Aib, X2 is not Aib.

In one embodiment of the present invention, X1 is Aib.

In another embodiment of the present invention, X2 is Aib.

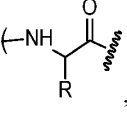
In another embodiment of present invention, X4 is Ile.

In another embodiment of the present invention, X1 is (L)-norvaline.

In another embodiment of the present invention, X2 is Leu.

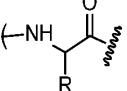
5 In another embodiment of the present invention, X2 is Ile.

In another embodiment of the present invention, X2 is selected from a L or D isomer of an amino acid of the Formula

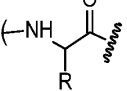
, wherein “” represents the point of attachment to Leu, and R is selected from C<sub>2</sub>-salkyl, C<sub>3</sub>-7cycloalkyl, C<sub>3</sub>-7cycloalkyl-C<sub>1</sub>-3alkyl-, C<sub>3</sub>-salkenyl, C<sub>3</sub>-salkynyl, C<sub>5</sub>-7cycloalkenyl-CH<sub>2</sub>-, and C<sub>1</sub>-3haloalkyl; or R along with the carbon to which it is attached to, forms C<sub>3</sub>-6cycloalkyl ring.

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In another embodiment of the present invention, X2 is an amino acid of the Formula

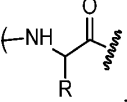
, which is present in “L” configuration.

In another embodiment, of the present invention, X2 is an amino acid of the Formula

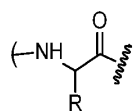
, which is present in “D” configuration.

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In another embodiment of the present invention, X2 is an amino acid of the Formula

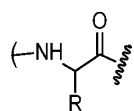
, wherein, R is C<sub>2</sub>-salkyl. In another embodiment, R is selected from ethyl, n-propyl, isopropyl and n-butyl.

In yet another embodiment of the present invention, X2 is an amino acid of the  
20 Formula



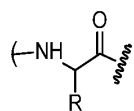
, wherein, R is n-propyl, thus forming X2 as norvaline. In another embodiment, X2 is (L)-norvaline. In another embodiment, X2 is (D)-norvaline.

In yet another embodiment of the present invention, X2 is an amino acid of the Formula



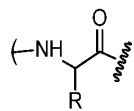
5 , wherein, R is n-butyl, thus forming X2 as norleucine. In another embodiment, X2 is (L)-norleucine. In another embodiment, X2 is (D)-norleucine.

In yet another embodiment of the present invention, X2 is an amino acid of the Formula



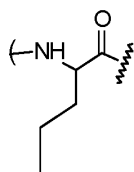
10 , wherein, R is ethyl, thus forming X2 as homoalanine. In another embodiment, X2 is (L)-homoalanine. In another embodiment, X2 is (D)-homoalanine.

In another embodiment of the present invention, X2 is an amino acid of the Formula



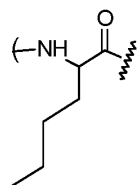
, wherein, R is C<sub>3-7</sub>cyclolalkyl-C<sub>1-3</sub>alkyl-. In another embodiment, R is selected from cyclopropylmethyl-, cyclopentylmethyl- and cyclohexylmethyl-.

In another embodiment of the present invention, X2 is an amino acid of the Formula

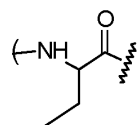


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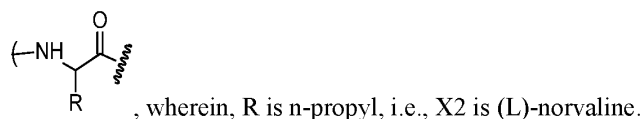
In another embodiment of the present invention, X2 is an amino acid of the Formula



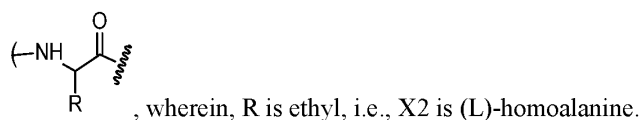
In another embodiment of the present invention, X2 is an amino acid of the Formula



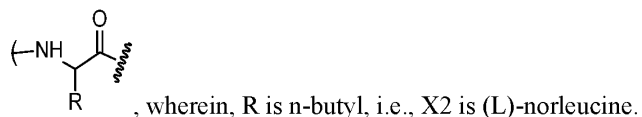
In another embodiment of the present invention, X1 is Aib and X2 is L-isomer of an amino acid of the Formula



In another embodiment of the present invention, X1 is Aib and X2 is L-isomer of an amino acid of the Formula



In another embodiment of the present invention, X1 is Aib and X2 is L-isomer of an amino acid of the Formula

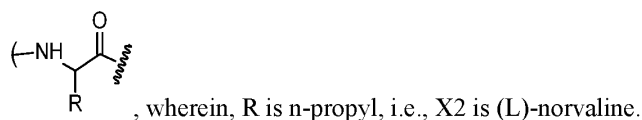


In another embodiment of the present invention, X1 is Aib and X2 is Leu.

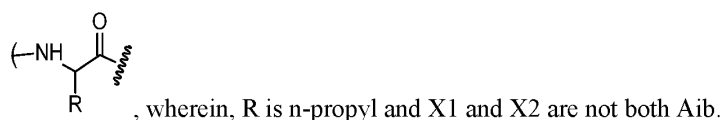
In another embodiment of the present invention, X1 is Aib and X2 is Ile.

In another embodiment of the present invention, X1 is (L)-norvaline and X2 is Aib.

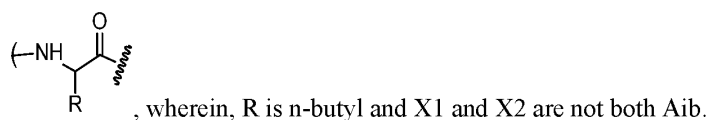
In another embodiment of the present invention, wherein X1 is (L)-norvaline and X2 is L-isomer of an amino acid of the Formula



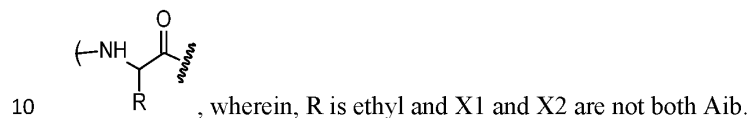
In one embodiment of the present invention, X1 is Aib or (L)-norvaline; and X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



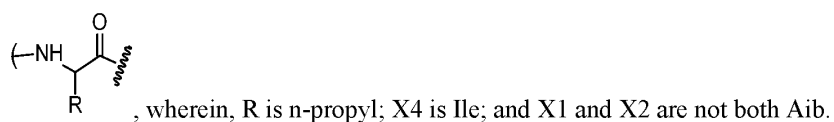
- 5 In one embodiment of the present invention, X1 is Aib or (L)-norvaline; and X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



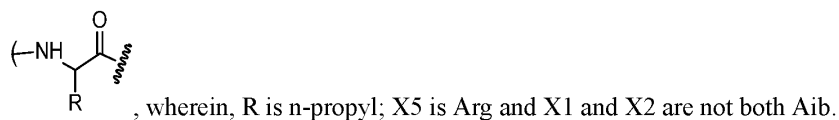
In one embodiment of the present invention, X1 is Aib or (L)-norvaline; and X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



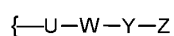
In one embodiment of the present invention, X1 is Aib or (L)-norvaline; X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



- 15 In one embodiment of the present invention, X1 is Aib or (L)-norvaline; X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



In one embodiment of the present invention, X3 is Lys, wherein the side chain amino (ε amino) group of Lys is acylated with a moiety:



In another embodiment of present invention, W is  $-C(O)-C(CH_3)_2-NH-$ .

In another embodiment of the present invention, W is  $-C(O)-NH-(CH_2)_p-NH-$ , wherein p is 3 or 4.

5 In another embodiment of the present invention, W is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ .

In another embodiment of the present invention, Z is  $-C(O)-(CH_2)_n-COOH$  and n is 16, 17, 18, 19 or 20. In a preferred embodiment n is 16, 18 or 20. In yet another preferred embodiment n is 18 or 20.

10 In another preferred embodiment of the present invention, Z is  $-C(O)-(CH_2)_n-COOH$  and n is 16 or 18. In yet another preferred embodiment n is 18.

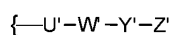
In another embodiment of the present invention, W is  $-C(O)-C(CH_3)_2-NH-$  and Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 18.

In another embodiment of the present invention, W is  $-C(O)-C(CH_3)_2-NH-$  and Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 16.

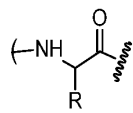
In another embodiment of the present invention, W is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  and Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 16.

In another embodiment of the present invention, W is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  and Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 18.

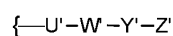
20 In one embodiment of the present invention, X3 is Gln; and X5 is Lys, wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:



In one embodiment of the present invention, X1 is Aib or (L)-norvaline; X2 is Aib, Leu, Ile or L isomer of an amino acid of the Formula



25 , wherein, R is n-propyl; X3 is Gln; and X5 is Lys; and X1 and X2 are not both Aib; wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:



In another embodiment of present invention, W' is  $-C(O)-C(CH_3)_2-NH-$ .

In another embodiment of the present invention, W' is  $-C(O)-NH-(CH_2)_q-NH-$ , wherein q is 3 or 4.

5 In another embodiment of the present invention, W' is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ .

In another embodiment of the present invention, Z' is  $-C(O)-(CH_2)_m-COOH$  and m is 16, 17, 18, 19 or 20. In a preferred embodiment, m is 16, 18 or 20. In yet another preferred embodiment m is 18 or 20.

10 In another preferred embodiment of the present invention, Z' is  $-C(O)-(CH_2)_m-COOH$  and m is 16 or 18. In yet another preferred embodiment m is 18.

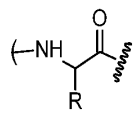
In another embodiment of the present invention, W' is  $-C(O)-C(CH_3)_2-NH-$  and Z' is  $-C(O)-(CH_2)_m-COOH$ , wherein m is 18.

15 In another embodiment of the present invention, W' is  $-C(O)-C(CH_3)_2-NH-$  and Z' is  $-C(O)-(CH_2)_m-COOH$ , wherein m is 16.

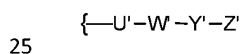
In another embodiment of the present invention, W' is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  and Z' is  $-C(O)-(CH_2)_m-COOH$ , wherein m is 16.

In another embodiment of the present invention, W' is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  and Z' is  $-C(O)-(CH_2)_m-COOH$ , wherein m is 18.

20 In one embodiment of the present invention, X1 is Aib; X2 is L isomer of an amino acid of the Formula



, wherein, R is n-propyl; Xaa15 is Glu; Xaa19 is Ala; X3 is Gln; Xaa21 is Glu; Xaa24 is Asn; X4 is Leu; and X5 is Lys, wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:



wherein W' is  $-C(O)-C(CH_3)_2-NH-$ ; and

Z' is  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  wherein m is 18.

In another embodiment of the present invention, the C terminal amino acid is amidated as a C-terminal primary amide.

In another embodiment of the present invention, X5, X6, X7, X8, X9, X10 and X11  
5 are absent.

In another aspect, the present invention provides a polypeptide or a pharmaceutically acceptable salt thereof, comprising an amino acid sequence:

Y-Aib-E-G-T-F-T-S-D-Y-S-I-X2-L-D-K-I-A-Q-X3-A-F-V-Q-W-L-X4-A-G-G-P-S-S-G-  
A-P-P-P-S-X5-X6-X7-X8-X9-X10-X11 (Seq. ID 2)

10 wherein X2 is Leu, Ile, (L)-norvaline, (L)-homoalanine or (L)-norleucine;

X4 is Ile;

X5 is absent or Arg;

X6 is absent or Lys;

X7 is absent or Lys;

15 X8 is absent or Lys;

X9 is absent or Lys;

X10 is absent or Lys;

X11 is absent or Lys;

X3 is Lys wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:

20  $\{\text{---U---W---Y---Z}$

wherein U is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-\}$  wherein } is point of attachment with group W;

W is selected from a group consisting of  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_p-\text{NH}-$ ],  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ ]  
and  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ ], wherein p is 3 or 4 and wherein ] is point of  
25 attachment with group Y;

Y is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH--}$  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 14 to 20; and wherein the acid group of the C terminal amino acid is a free carboxylic acid group or is amidated as a C-terminal primary amide.

In one embodiment of the present invention, X2 is (L)-norvaline.

5 In one embodiment of the present invention, X2 is (L)-homoalanine.

In one embodiment of the present invention, X2 is (L)-norleucine.

In another embodiment of the present invention, X2 is Leu.

In another embodiment of the present invention, X2 is Ile.

In another embodiment of the present invention, X2 is (L)-norvaline and X5 is Arg.

10 In another embodiment of present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ .

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_{3-4}-\text{NH}-$ .

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ .

In another embodiment of the present invention, Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and n is  
15 16, 17, 18, 19 or 20. In a preferred embodiment n is 16, 18 or 20. In yet another preferred embodiment n is 18 or 20.

In another preferred embodiment of the present invention, Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and n is 16 or 18. In yet another preferred embodiment n is 18.

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  and Z  
20 is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 18.

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 16.

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 16.

25 In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 18.

In another embodiment of the present invention, the C terminal amino acid is amidated as a C-terminal primary amide.

In another embodiment of the present invention, X5, X6, X7, X8, X9, X10 and X11 are absent.

In a preferred embodiment X2 is (L)-norvaline, X4 is Ile; X5, X6, X7, X8, X9, X10 and X11 are absent; W is  $-C(O)-C(CH_3)_2-NH-$ ; and Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 18.

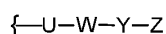
In another aspect, the present invention provides a polypeptide or a pharmaceutically acceptable salt thereof, comprising an amino acid sequence:

Y-X1-E-G-T-F-T-S-D-Y-S-I-X2-L-D-K-I-A-Q-X3-A-F-V-Q-W-L-X4-A-G-G-P-S-S-G-A-P-P-P-S (Seq. ID 3)

wherein X1 is Aib or (L)-norvaline; X2 is Aib, Leu, Ile, (L)-norvaline, (L)-homoalanine or (L)-norleucine;

X4 is Ile;

X3 is Lys wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:



wherein U is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  wherein } is point of attachment with group W;

W is selected from a group consisting of  $-C(O)-NH-(CH_2)_p-NH-$ ,  $-C(O)-C(CH_3)_2-NH-$  and  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ , wherein p is 3 or 4 and wherein ] is point of attachment with group Y;

Y is  $-C(O)-(CH_2)_2-CH(COOH)NH--$  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 14 to 20;

and wherein the acid group of the C terminal amino acid is a free carboxylic acid group or is amidated as a C-terminal primary amide,

with a proviso that when X1 is Aib, X2 is not Aib.

In one embodiment of the present invention, X1 is Aib.

In another embodiment of the present invention, X1 is (L)-norvaline.

In another embodiment of the present invention, X2 is Aib.

In another embodiment of the present invention, X2 is (L)-norvaline.

In another embodiment of the present invention, X2 is (L)-norleucine.

In another embodiment of the present invention, X2 is (L)-homoalanine.

In another embodiment of the present invention, X2 is Leu.

In another embodiment of the present invention, X2 is Ile.

5 In another embodiment of the present invention, X1 is Aib and X2 is (L)-norvaline.

In another embodiment of the present invention, X1 is Aib and X2 is (L)-norleucine.

In another embodiment of the present invention, X1 is Aib and X2 is (L)-homoalanine.

In another embodiment of the present invention, X1 is (L)-norvaline and X2 is Aib.

10 In another embodiment of the present invention, X1 is Aib and X2 is Leu.

In another embodiment of the present invention, X1 is Aib and X2 is Ile.

In another embodiment of the present invention, X1 is (L)-norvaline and X2 is (L)-norvaline.

In another embodiment of present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ .

15 In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_{3-4}-\text{NH}-$ .

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ .

In another embodiment of the present invention, Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and n is 16, 17, 18, 19 or 20. In a preferred embodiment n is 16, 18 or 20. In yet another preferred  
20 embodiment n is 18 or 20.

In another preferred embodiment of the present invention, Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and n is 16 or 18. In yet another preferred embodiment n is 18.

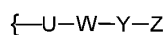
In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 18.

25 In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 16.

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 16.

In another embodiment of the present invention, W is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$  and Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 18.

In another embodiment of the present invention, X1 is Aib; X2 is (L)-norvaline; and X3 is Lys wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a moiety:



5

wherein W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ ; Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and n is 18.

In another embodiment of the present invention, the C terminal amino acid is amidated as a C-terminal primary amide.

In another aspect, the present invention provides a polypeptide or pharmaceutically acceptable salt thereof comprising an amino acid sequence selected from the group consisting of:

- i.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 04)
- 15 ii.) Tyr L-norvaline Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Aib Leu Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 05)
- iii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Leu Leu Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 06)
- 20 iv.) Tyr L-norvaline Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 07)
- v.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Glu Lys Ile Ala Ala Gln Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser Lys-NH<sub>2</sub> (Seq ID: 08)
- 25 vi.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser Arg (Seq ID: 09)

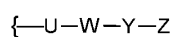
vii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-homoalanine Leu Asp  
Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser  
Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 10)

5 viii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norleucine Leu Asp Lys  
Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly  
Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 11)

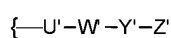
ix.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Ile Leu Asp Lys Ile Ala  
Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro  
Pro Pro Ser-NH<sub>2</sub> (Seq ID: 12)

10 In another aspect, the present invention provides a polypeptide or pharmaceutically  
acceptable salt thereof, selected from the representative compounds as disclosed in the Table  
1.

In the embodiments of the present invention, the groups U, W, Y and Z in the moiety



15 or the groups U', W', Y' and Z' in the moiety



have meaning as defined in this specification and should not be interpreted as or mixed with  
the single letter code of the amino acids;

In embodiments of the present invention, the group {-U-W-Y-Z} and/or {-U'-W'-Y'-Z'} is  
20 selected from the representative structures of Moiety A, B, C and D as disclosed in Table 2.

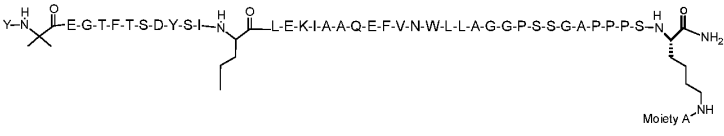
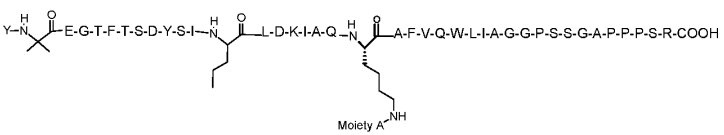
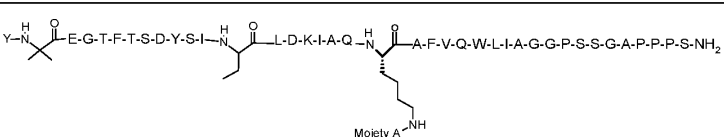
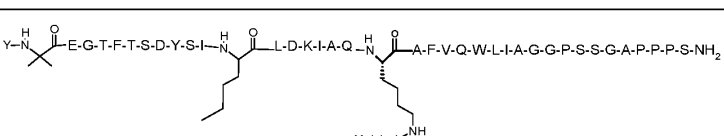
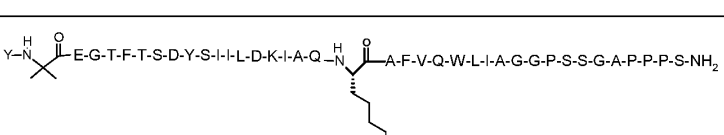
The polypeptide sequences mentioned in the specification are represented by the  
single letter code of the amino acids as approved by IUPAC.

Unless stated otherwise, the specification intends to cover both L and D isomers of  
the amino acids in the sequence. However, in preferred embodiments, all the amino acids  
25 are in "L" configuration unless indicated otherwise.

Table 1 and 2 provides some of the representative compounds of the present  
invention.

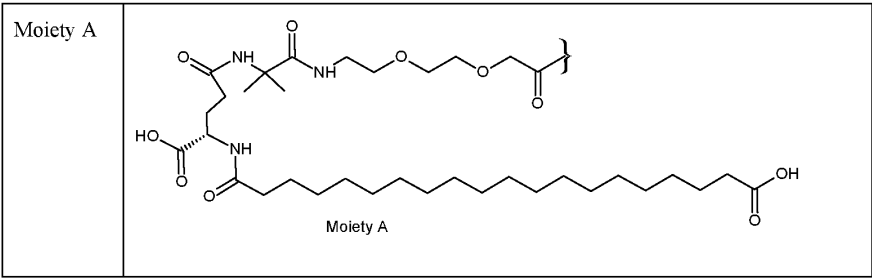
**Table 1: Representative polypeptide compounds of present disclosure**

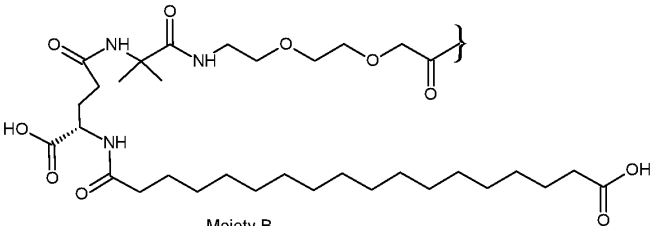
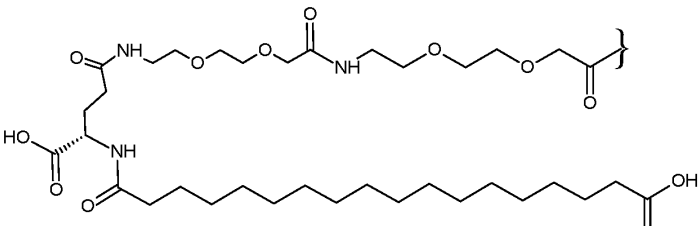
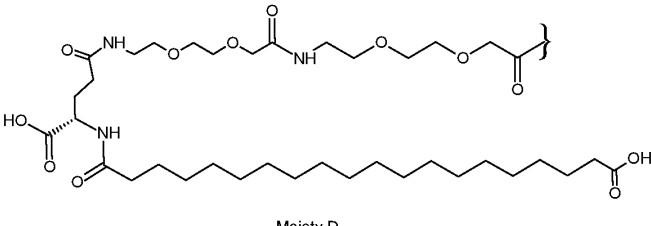
| Compd No. | Structure                     | SEQ ID |
|-----------|-------------------------------|--------|
| 1         | <p>Moietly A<sup>NH</sup></p> | 04     |
| 2         | <p>Moietly A<sup>NH</sup></p> | 05     |
| 3         | <p>Moietly A<sup>NH</sup></p> | 06     |
| 4         | <p>Moietly A<sup>NH</sup></p> | 07     |
| 5         | <p>Moietly C<sup>NH</sup></p> | 04     |
| 6         | <p>Moietly D<sup>NH</sup></p> | 04     |
| 7         | <p>Moietly B<sup>NH</sup></p> | 04     |

|    |                                                                                     |    |
|----|-------------------------------------------------------------------------------------|----|
| 8  |   | 08 |
| 9  |   | 09 |
| 10 |   | 10 |
| 11 |   | 11 |
| 12 |  | 12 |

\*Unless stated otherwise all the amino acids mentioned are in “L” configuration.

Table 2: Structure of Moiety A, Moiety B, Moiety C and Moiety D



|          |                                                                                                                                 |
|----------|---------------------------------------------------------------------------------------------------------------------------------|
| Moiety B |  <p style="text-align: center;">Moiety B</p>  |
| Moiety C |  <p style="text-align: center;">Moiety C</p>  |
| Moiety D |  <p style="text-align: center;">Moiety D</p> |

In another aspect, the present invention provides a method of treating or preventing hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers in a patient, comprising administering to a patient in need thereof, an effective amount of a polypeptide of the present invention or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a method of treatment of type 2 diabetes in a patient comprising administering to a patient in need of such treatment an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a method of treatment of obesity in a patient comprising administering to a patient in need of such treatment an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

5 In another aspect, the present invention provides a method of treatment of hyperlipidemia in a patient comprising administering to a patient in need of such treatment an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

10 In an embodiment, the present invention provides a pharmaceutical composition comprising a polypeptide of the present invention or a pharmaceutically acceptable salt thereof with one or more of a pharmaceutically acceptable carrier, diluent, or excipient.

The compounds of the invention are preferably formulated as pharmaceutical compositions administered by parenteral routes (e.g., subcutaneous, intravenous, intraperitoneal, intramuscular or transdermal). Such pharmaceutical compositions and processes for preparing same are well known in the art. (See, e.g., Remington: The Science and 50 Practice of Pharmacy (D. B. Troy, Editor, 21st Edition, Lippincott, Williams & Wilkins, 2006).

In another aspect, the polypeptides of the present invention or the pharmaceutically acceptable salts thereof for use as a medicament.

20 In another aspect, the polypeptides of the present invention or the pharmaceutically acceptable salts thereof for use in the treatment or prevention of a disease in a patient, wherein said disease is optionally selected from the group consisting of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, 25 alcoholism and gastric ulcers.

In another aspect, the polypeptide of the present invention or the pharmaceutically acceptable salts thereof may be provided simultaneously, separately, or sequentially in combination with an effective amount of one or more additional therapeutic agents.

30 In another aspect, the pharmaceutical composition according to the present invention comprise a polypeptide of the present invention or a pharmaceutically acceptable salt thereof for use as a medicament.

In another aspect, the pharmaceutical composition according to the present invention comprise a polypeptide of the present invention or a pharmaceutically acceptable salt thereof, for use in the treatment or prevention of a disease in a patient, wherein said disease is optionally selected from the group consisting of hyperglycemia, type 2 diabetes, impaired  
5 glucose tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers.

In another aspect, the pharmaceutical composition according to the present invention comprise a polypeptide of the present invention or a pharmaceutically acceptable salt thereof  
10 is provided simultaneously, separately, or sequentially in combination with an effective amount of one or more additional therapeutic agents.

The present invention may involve one or more embodiments. It is to be understood that the embodiments below are illustrative of the present invention and are not intended to limit the claims to the specific embodiments exemplified. It is also to be understood that the  
15 embodiments defined herein may be used independently or in conjunction with any definition, any other embodiment defined herein. Thus the invention contemplates all possible combinations and permutations of the various independently described embodiments.

Other features of the present invention will become apparent from the following  
20 examples. Generally speaking, the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including the accompanying claims and drawings). Thus, features, integers, characteristics, compounds or chemical moieties described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example  
25 described herein, unless incompatible therewith.

Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

#### **EXAMPLES:**

**Instruments and analytical methods:** Instruments used for characterization and analysis  
30 of the compounds of the present invention are HPLC (Waters e2695 Alliance; Detector Waters (2489 UV/Visible)).

Mass instrument: HPLC: Waters e2695 Alliance; Detector: Acquity- QDa.

The final compounds of the present disclosure 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: 30 Mpa), Column: Phenyl, 10 $\mu$  Flow: 75mL/min

Mobile Phase:

|                | For first purification              | For second purification                           | For third purification              |
|----------------|-------------------------------------|---------------------------------------------------|-------------------------------------|
| Mobile Phase A | pH 8.0 Phosphate buffer             | 1% Acetic acid in water                           | pH 8.2 Ammonium formate buffer      |
| Mobile Phase B | Acetonitrile                        | 1% Acetic acid in Acetonitrile:n-Propanol (50:50) | Acetonitrile                        |
| Gradient       | 15 to 45% Mobile Phase-B in 300 min | 20 to 50 % Mobile Phase-B in 250 min              | 20 to 50% Mobile Phase-B in 250 min |

The purity of the compounds of the present disclosure were analyzed by one of the RP-HPLC methods as outlined below:

#### HPLC Method A:

- 10 Column: Xbridge Peptide BEH C18 (4.6 mm x 250 mm, 3.5 $\mu$ )

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

- 15 Flow rate: 0.8 mL/min

Detection: UV detection at 210 nm

Column Temperature: 65 °C

Sample Tray temperature: 5 °C

Run Time: 90 min.

| Time (min) | Mobile Phase A % | Mobile Phase B % |
|------------|------------------|------------------|
| 0          | 55               | 45               |
| 3          | 55               | 45               |
| 5          | 40               | 60               |
| 60         | 39               | 61               |
| 65         | 0                | 100              |
| 75         | 0                | 100              |

|       |    |    |
|-------|----|----|
| 75.01 | 55 | 45 |
| 90    | 55 | 45 |

**HPLC Method B:**

Column: XSelect CSH C18 (4.6 mm x 150 mm, 2.5 $\mu$ )

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

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

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

Flow rate: 0.8 mL/min

Detection: UV detection at 210 nm

Column Temperature: 65 °C

- 10 Sample Tray temperature: 5 °C

Run Time: 90 min.

| Time (min) | Mobile Phase A % | Mobile Phase B % |
|------------|------------------|------------------|
| 0          | 55               | 45               |
| 3          | 55               | 45               |
| 5          | 40               | 60               |
| 60         | 39               | 61               |
| 65         | 0                | 100              |
| 75         | 0                | 100              |
| 75.01      | 55               | 45               |
| 90         | 55               | 45               |

**HPLC Method C:**

Column: Xbridge Peptide BEH C18 (4.6 mm x 250 mm, 3.5 $\mu$ )

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

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

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

- 20 Column Temperature: 65 °C

Sample Tray temperature: 5 °C

Run Time: 60 min.

| Time (min) | Mobile Phase A % | Mobile Phase B % |
|------------|------------------|------------------|
| 0          | 55               | 45               |
| 2          | 41               | 59               |
| 50         | 40               | 60               |
| 51         | 55               | 45               |
| 60         | 55               | 45               |

#### HPLC Method D:

Column: XSelect CSH C18 (4.6 mm x 150 mm, 2.5μ)

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

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

Buffer: Potassium dihydrogen orthophosphate in water, Added Triethylamine and pH adjusted to 2.5±0.1 with orthophosphoric acid

Flow rate: 0.5 mL/min

- 10 Detection: UV detection at 214 nm

Column Temperature: 60 °C

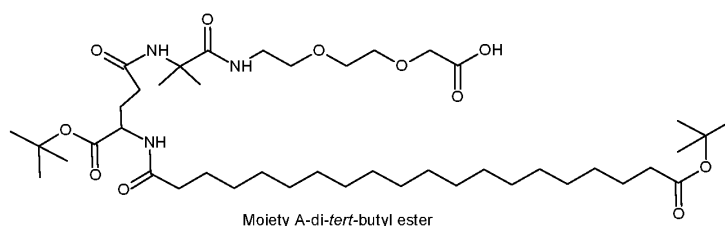
Sample Tray temperature: 10 °C

Run Time: 90 min.

| Time (min) | Mobile Phase A % | Mobile Phase B % |
|------------|------------------|------------------|
| 0          | 55               | 45               |
| 6          | 55               | 45               |
| 10         | 40               | 60               |
| 80         | 39               | 61               |
| 80.1       | 0                | 100              |
| 85         | 0                | 100              |
| 85.1       | 55               | 45               |
| 90         | 55               | 45               |

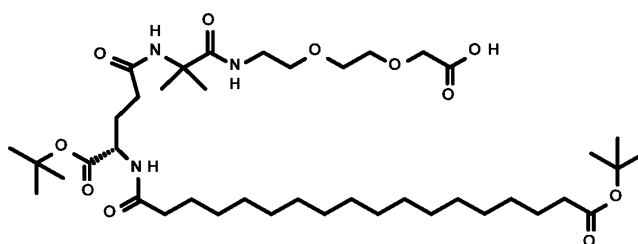
#### 15 METHOD OF PREPARATION:

**Example 1: Preparation of 2-[2-[2-[[2-[(4S)-5-tert-butoxy-4-[(20-tert-butoxy-20-oxo-icosanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid (Moiety A-di-*tert*-butyl ester)**



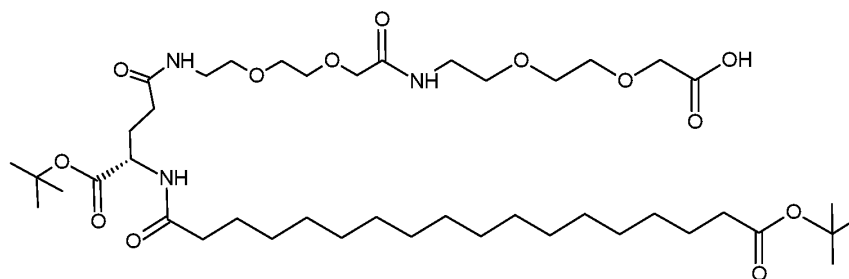
Moiety A-di-*tert*-butyl ester was prepared using solid phase synthesis using 2-chlorotrityl chloride resin. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine followed by coupling with Fmoc-Aib-OH in THF 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 then 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 20-(*tert*-butoxy)-20-oxoicosanoic acid to give 2-[2-[2-[[2-[(4*S*)-5-*tert*-butoxy-4-[(20-*tert*-butoxy-20-oxo-icosanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin. This 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-[(20-*tert*-butoxy-20-oxo-icosanoyl)amino]-5-oxo-pentanoyl]amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid (**Moiety A-di-*tert*-butyl ester**). (LCMS=  $m/z$ : 814.10 ( $M+H^+$ )).

**Example 2: Preparation of 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**

Moiety B-di-*tert*-butyl ester

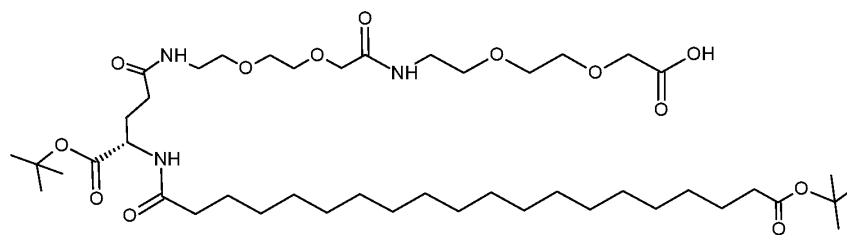
Moiety B-di-*tert*-butyl ester was prepared using solid phase synthesis using 2-chlorotrityl chloride resin. 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid was attached to 2-chlorotrityl chloride resin in presence of DIPEA to yield 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin. The Fmoc protecting group was removed by selective de-blocking of amino group using piperidine followed by coupling with Fmoc-Aib-OH in THF using DIPC and HOBt which yielded 2-[2-[2-(2-Fmoc-amino-2-methylpropanoyl)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 (Moiety B-di-*tert*-butyl ester). (LCMS= m/z: 786.39 (M+H<sup>+</sup>)).

**Example 3: Preparation of 2-[2-[2-[[2-[2-[2-[[5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (Moiety C-di-*tert*-butyl ester)**

Moiety C-di-*tert*-butyl ester

Moiety C-di-*tert*-butyl ester was prepared using solid phase synthesis using 2-chlorotrityl chloride resin. 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 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid in THF using DIPC and HOBt which yielded {(Fmoc-amino-ethoxy)-ethoxy}-acetyl-{-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 Fmoc-Glu({(amino-ethoxy)-ethoxy}-acetyl-{-amino-ethoxy}-ethoxy}-acetic acid-2-Cl-Trt-Resin)-OtBu. 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-[2-[2-[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) to obtain 2-[2-[2-[2-[2-[2-[5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (**Moiety C-di-*tert*-butyl ester**) (LCMS= m/z: 846.10 (M+H<sup>+</sup>)).

**Example 4: Preparation of 2-[2-[2-[2-[2-[2-[5-*tert*-butoxy-4-[(20-*tert*-butoxy-20-oxo-icosanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (**Moiety D-di-*tert*-butyl ester**)**

Moiety D- di-*tert*-butyl ester

Moiety D-di-*tert*-butyl ester was prepared using solid phase synthesis using 2-chlorotrityl chloride resin as schematically represented below. 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 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid in THF using DIPC and HOBt which yielded {(Fmoc-amino-ethoxy)-ethoxy}-acetyl-{-(-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 Fmoc-Glu({(amino-ethoxy)-ethoxy}-acetyl-{-(-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin)-OtBu. The Fmoc group of the resultant compound was selectively de-blocked using piperidine and the free amino group was then coupled with 20- (tert-Butoxy)-20-oxoicosanoic acid to give

2-[2-[2-[2-[2-[2-[5-tert-butoxy-4-[(20-tert-butoxy-20-oxo-icosanoyl)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) to obtain

2-[2-[2-[2-[2-[2-[5-tert-butoxy-4-[(20-tert-butoxy-20-oxo-icosanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (**Moiety D-di-*tert*-butyl ester**) (LCMS= m/z: 874.15 (M+H<sup>+</sup>)).

#### Example 5: Preparation of Compound 1

The parent peptide was synthesized by solid-phase method. The starting resin used for synthesis was Fmoc-Rink amide resin. Selective de-blocking of Fmoc protected amino group of rink amide resin was carried out using piperidine to yield Rink amide resin which was then coupled with Fmoc-Ser(tBu)-OH to yield Fmoc-Ser(tBu)-Rink amide Resin. This

coupling reaction was performed by using diisopropylcarbodiimide, *N*-hydroxybenzotriazole (DIPC-HOBt) as coupling reagent. This completed one cycle. Acetic anhydride and diisopropylethyl amine/pyridine was used to terminate/cap the uncoupled amino groups at every amino acid coupling. Selective de-blocking of the amino group of Fmoc-Ser(tBu)-Rink amide Resin was done using piperidine. Then coupling with Fmoc-Pro-OH using HOBt and DIPC yielded Fmoc-Pro-Ser(tBu)-rink amide Resin. This completed the second cycle. Acetic anhydride and diisopropylethyl amine/pyridine were used to terminate the uncoupled amino groups at every amino acid coupling.

The above three steps, i.e., selective Capping, 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 the remaining 37 amino acid residues. The selective deblocking, i.e., capping of uncoupled amino group done by using acetic anhydride and diisopropylethylamine/pyridine, 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, e.g., hydroxyl group of Serine, Tyrosine or Threonine were protected with *tert*-butyl(-tBu) group, amino group of Lysine was protected with *tert*-butyloxycarbonyl (-Boc) and (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (IVDde) group respectively and carboxylic acid groups of aspartic acid or glutamic acid were protected with -tBu group and amide group of glutamine was protected with trityl (-Trt) group. The above mentioned three steps, i.e., selective capping, deblocking and then coupling with next Fmoc protected amino acid, were performed to get Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-[L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.

De-blocking of Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-[L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin was carried out using piperidine followed by Boc protection of peptide resin using Boc anhydride to yield Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-[L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin. De-

- protection of the IVDde group of the peptide resin was carried out using hydrazine hydrate and then it was coupled with Moiety A-di-*tert*-butyl ester using diisopropylcarbodiimide, *N*-hydroxybenzotriazole (DIPC-HOBt) as coupling reagent to yield an intermediate compound resin, Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-
- 5 Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-[L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(NH-Moiety A di-*tert*-butyl ester)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin, which on cleavage and de-protection using trifluoroacetic acid with ethane-1,2-dithiol and triisopropylsilane followed by purification through preparative HPLC resulted in Compound 1.
- 10 **Mass (LCMS):**  $m/z = 1192.7$  ( $MH_4^{4+}$ ), Calculated Mass= 4766.77; **HPLC Purity (Method B):** 98.60 %, RT=33.8 min.

#### Example 6: Synthesis of Compound 2

- Compound 2 was prepared by solid phase method as per the analogous process given for Example 5, wherein, Fmoc-[L-norvaline]-OH was used at position 2 instead of Fmoc-Aib-
- 15 OH and Fmoc-Aib-OH was used at position 13<sup>th</sup> instead of Fmoc-[L-norvaline]-OH to get Boc-Tyr(tBu)-[L-norvaline]-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.
- 20 Then coupling with Moiety A-di-*tert*-butyl ester followed by cleavage, de- protection and preparative HPLC purification as per Example 5 resulted in Compound 2.
- Mass (LCMS):**  $m/z = 1192.6$  ( $MH_4^{4+}$ ), Calculated Mass= 4766.4; **HPLC Purity (Method B):** 96.09 %, RT=25.6 min.

#### Example 7: Synthesis of Compound 3

- Compound 3 was prepared by solid phase method as per the analogous process given for Example 5, wherein Fmoc-Leu-OH was used at position 13 instead of Fmoc-[L-norvaline]-
- 25 OH to get Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-Leu-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-
- 30 Ser(tBu)-resin.

Then coupling with Moiety A-di-*tert*-butyl ester followed by cleavage, de- protection and preparative HPLC purification as per Example 5 resulted in Compound 3.

**Mass (LCMS):**  $m/z = 1196.1$  ( $MH_4^{4+}$ ), Calculated Mass=4780.4; **HPLC Purity (Method A):** 95.27 %, RT=39.1 min.

#### 5 Example 8: Synthesis of Compound 4

Compound 4 was prepared by solid phase method as per the analogous process given for Example 5, wherein Fmoc-[L-norvaline]-OH was used at position 2 instead of Fmoc-Aib-OH to get Boc-Tyr(tBu)-[L-norvaline]-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile- [L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-  
10 Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.

Then coupling with Moiety A-di-*tert*-butyl ester followed by cleavage, de-protection and preparative HPLC purification as per Example 5 resulted in compound 4.

**Mass (LCMS):**  $m/z = 1196.32$  ( $MH_4^{4+}$ ), Calculated Mass= 4781.25; **HPLC Purity (Method A):** 94.21 %, RT= 29.8 min.  
15

#### Example 9: Synthesis of Compound 5

De-protection of IVDde group of peptide resin: Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-[L-norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-  
20 Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin (prepared as per Example 5) was carried out using hydrazine hydrate and then it was coupled with Moiety C-di-*tert*-butyl ester using diisopropylcarbodiimide, *N*-hydroxybenzotriazole (DIPC-HOBt) as coupling reagent to yield an intermediate compound resin, Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile- [L-  
25 norvaline]-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(NH-Moiety C di-*tert*-butyl ester)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin, which on cleavage and de-protection using trifluoroacetic acid with ethane-1,2-dithiol and triisopropylsilane followed by purification through preparative HPLC resulted in Compound 5.

**Mass (LCMS):**  $m/z = 1600.80$  ( $MH_3^{3+}$ ), Calculated Mass= 4799.376; **HPLC Purity (Method A):** 98.64 %, RT=15.9 min.  
30

**Example 10: Synthesis of Compound 6**

Compound 6 was prepared by solid phase method as per the analogous process given for Example 9, wherein coupling with Moiety D-di-*tert*-butyl ester was carried out, followed by cleavage, de protection and preparative HPLC purification as per Example 9 resulted in compound 6.

**Mass (LCMS):**  $m/z = 1609.98$  ( $MH_3^{3+}$ ), Calculated Mass= 4826.916;

**HPLC Purity (Method A):** 96.31 %, RT=26.7 min.

**Example 11: Synthesis of Compound 7**

Compound 7 was prepared by solid phase method as per the analogous process given for Example 9, wherein coupling with Moiety B-di-*tert*-butyl ester carried out, followed by cleavage, de protection and preparative HPLC purification as per Example 9 resulted in compound 7.

**Mass (LCMS):**  $m/z = 1580.64$  ( $MH_3^{3+}$ ), Calculated Mass= 4738.896;

**HPLC Purity (Method A):** 98.43 %, RT=18.5 min.

**Example 12: Synthesis of Compound 8**

The parent peptide was synthesized by solid-phase method. The starting resin used for the synthesis was Fmoc-Rink amide resin. Selectively de-blocking of Fmoc protected amino group of rink amide resin using piperidine followed by coupling with Fmoc-Lys(IVDde)-OH with the Rink amide resin. The coupling was performed by using DIPC-HOBt to yield Fmoc- Lys(IVDde)-Rink amide Resin, this completed one cycle. Acetic anhydride and diisopropylethyl amine/pyridine was used to terminate/cap the uncoupled amino groups at the end of every amino acid coupling. Selective de-blocking Fmoc of amino group of Fmoc-Lys(IVDde)-Rink amide Resin using piperidine, Then coupling with second amino acid i.e., Fmoc-Ser(tBu)-OH using HOBt and DIPC yielded Fmoc-Ser(tBu)- Lys(IVDde)-rink amide Resin. This completed the second cycle. As stated earlier acetic anhydride and diisopropylethyl amine/pyridine was used to terminate the uncoupled amino groups [Capping] after each amino acid coupling.

The above three steps, i.e., deblocking of Fmoc- protection of amino acid attached to the resin, coupling of next amino acid residue in sequence with Fmoc-protected amino group and selective Capping, were repeated for the remaining 38 amino acid residues. The side chain of the Fmoc-protected amino acids used were protected orthogonally, e.g., hydroxyl

group of Serine, Tyrosine or Threonine were protected with tert-butyl(-tBu) group, amino group of Lysine was protected with tert-butyloxycarbonyl (-Boc) and (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (IVDde) group respectively and carboxylic acid groups of aspartic acid or glutamic acid were protected with -tBu group, amide group of  
 5 glutamine and asparagine was protected with trityl (-Trt) group. The above mentioned three steps, i.e., selective capping, deblocking and then coupling with next Fmoc protected amino acid were performed to get Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Glu(OtBu)-Lys(Boc)-Ile-Ala-Ala-Gln(Trt)-Glu(OtBu)-Phe-Val-Asn(Trt)-Trp-Leu-Leu-Ala-Gly-Gly-Pro-Ser(tBu)-  
 10 Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(IVDde)-Rink amide resin.

De-blocking of Fmoc group from Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Glu(OtBu)-Lys(Boc)-Ile-Ala-Ala-Gln(Trt)-Glu(OtBu)-Phe-Val-Asn(Trt)-Trp-Leu-Leu-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(IVDde)-Rink amide resin was  
 15 done using piperidine followed by Boc protection of Peptide resin using Boc anhydride to yield Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Glu(OtBu)-Lys(Boc)-Ile-Ala-Ala-Gln(Trt)-Glu(OtBu)-Phe-Val-Asn(Trt)-Trp-Leu-Leu-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(IVDde)-Rink amide resin.

20 De-protection of the IVDde group of peptide resin using Hydrazine hydrate followed by coupling of moiety-A-di-*tert* butyl ester was performed using diisopropylcarbodiimide, N-hydroxybenzotriazole (DIPC-HOBt) as the coupling reagent to yield protected compound 8-resin.

Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Glu(OtBu)-Lys(Boc)-Ile-Ala-Ala-Gln(Trt)-Glu(OtBu)-Phe-Val-Asn(Trt)-Trp-Leu-Leu-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(NH moiety A-di-*tert* butyl ester)-Rink amide resin cleavage and de-protection  
 25 using trifluoroacetic acid with ethane-1,2-dithiol and triisopropylsilane followed by purification through preparative HPLC resulted in Compound 8. The HPLC purity of Compound 8 was assessed by Method given below **Mass (LCMS):** m/z = 980.42 (MH<sup>5+</sup>), Calculated Mass = 4897.06 **HPLC Purity (Method D):** 94.55 %, RT=44.9 min.

### Example 13: Synthesis of Compound 9

The parent peptide was synthesized by solid-phase method. The starting resin used for synthesis was Wang resin. Fmoc protected Arg(Pbf) 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-Arg(Pbf)-Wang Resin. Selective de-  
 5 blocking of amino group of Fmoc-Arg(Pbf)-Wang Resin using piperidine followed by coupling with Fmoc-Ser(tBu)-OH using HOBt/DIPC yielded Fmoc-Ser(tBu)-Arg(Pbf)-Wang Resin. This completed one cycle. Acetic anhydride and diisopropylethyl amine/pyridine were used to terminate the uncoupled amino groups at every amino acid  
 10 coupling.

The above two 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 the remaining 38 amino acid residues. The side chain of the Fmoc-protected amino acids were protected orthogonally, e.g., hydroxyl group of Serine, Tyrosine  
 15 or Threonine were protected with tert-butyl(-tBu) group, amino group of Lysine was protected with tert-butyloxycarbonyl (-Boc) and (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (IVDde) group respectively and carboxylic acid groups of aspartic acid or glutamic acid were protected with -tBu group. The above mentioned three steps, i.e., selective capping, deblocking and then coupling with next Fmoc protected amino acid were  
 20 performed to get Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Arg(Pbf)-Wang resin.

De-blocking of Fmoc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-  
 25 Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Arg(Pbf)-Wang resin, using piperidine followed by Boc protection of Peptide resin using Boc anhydride to yield Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-  
 30 Norvaline-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Arg(Pbf)-Wang resin. De-protection of IVDde group of peptide resin using Hydrazine hydrate followed by coupling of moiety A-di-*tert* butyl ester was performed by using

diisopropylcarbodiimide, N-hydroxybenzotriazole (DIPC-HOBt) as coupling reagent to yield Compound 9 -Wang resin.

Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-L-Norvaline-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)- Lys(NH moiety A-  
 5 di-*tert* butyl ester)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Arg(Pbf)-Wang resin

Cleavage and de-protection from resin using trifluoroacetic acid with ethane-1,2-dithiol , triisopropylsilane followed by purification through preparative HPLC resulted in Compound 9. **Mass (LCMS):** m/z = 985.88 (MH<sup>5+</sup>), Calculated Mass= 4924.36 **HPLC**  
 10 **Purity (Method C):** 93.52 %, RT=27.8 min.

#### Example 14: Synthesis of Compound 10

Compound 10 was prepared by solid phase method as per the analogous process given for Example 5, wherein, Fmoc-[2-amino butyric acid] was used at position 13 instead of Fmoc-[L-norvaline]-OH to get Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-  
 15 Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-2-amino butyric acid-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.

Then coupling with Moiety A-di-*tert*-butyl ester followed by cleavage, de- protection and preparative HPLC purification as per Example 5 resulted in Compound 10.

20 **Mass (LCMS):** m/z = 1189.57 (MH<sup>4+</sup>), Calculated Mass=4754.248; **HPLC Purity (Method D):** 96.79 %, RT=42.6 min.

#### Example 15: Synthesis of Compound 11

Compound 11 was prepared by solid phase method as per the analogous process given for Example 5, wherein, Fmoc-norleucine was used at position 13 instead of Fmoc-[L-norvaline]-OH to get Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-  
 25 Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-Norleucine-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.

Then coupling with Moiety A-di-*tert*-butyl ester followed by cleavage, de- protection and  
 30 preparative HPLC purification as per Example 5 resulted in Compound 11. **Mass (LCMS):**

$m/z = 1196.66$  ( $MH4^{4+}$ ), Calculated Mass=4782.608; **HPLC Purity (Method D):** 95.43 %,  $RT=58.7$  min.

#### Example 16: Synthesis of Compound 12

- Compound 12 was prepared by solid phase method as per the analogous process given for
- 5 Example 5, wherein, Fmoc-Ile-OH was used at position 13 instead of Fmoc-[L-norvaline]-OH to get Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)-Phe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Ile-Ile-Leu-Asp(OtBu)-Lys(Boc)-Ile-Ala-Gln(Trt)-Lys(IVDde)-Ala-Phe-Val-Gln(Trt)-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-resin.
- 10 Then coupling with Moiety A-di-tert-butyl ester followed by cleavage, de- protection and preparative HPLC purification as per Example 5 resulted in Compound 12.

**Mass (LCMS):**  $m/z = 1196.32$  ( $MH4^{4+}$ ), Calculated Mass = 4781.25; **HPLC Purity (Method D):** 94.38 %,  $RT=47.7$  min.

#### 15 BIOLOGICAL STUDIES:

##### Example 12: Efficacy study in db/db mice at 10nM/kg dose

- The effect of compounds of present invention on blood glucose, food intake and body weight was studied in mice. This study was performed in a type 2 diabetic mouse (db/db) model. The animals were divided into 4 treatment groups (n=6)- a diabetic control group,
- 20 Compound 1 (10nM/kg), Compound 2 (10nM/kg) and Tirzepatide (10nM/kg). Baseline blood glucose was measured from all the animals. All the animals were administered with test items subcutaneously. Blood glucose was measured at 4hr, 8hr, 12hr, 24hr, 48hr, 72hr and 96hr post treatment. Delta blood glucose (mM) was calculated. The results are provided in Table 3. Similarly, body weight changes and cumulative food consumption was measured
- 25 at 48hr and 96hr post treatment. The results of body weight changes are provided in Table 4 and cumulative food consumption are provided in Table 5. Similarly, the efficacy of Compounds 3, 4, 5, 6 and 7 in db/db mice at 10nM/kg dose was carried out in separate study. The animals were divided into 7 treatment groups (n=6) - a diabetic control group, Compound 1, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
- 30 Baseline blood glucose was measured from all the animals. All the animals were administered with test items subcutaneously. Blood glucose was measured at 4hr, 8hr, 12hr,

24hr, 48hr, 72hr and 96hr post treatment. Delta blood glucose (mM) was calculated. The results are also provided in Table 3. Similarly, body weight changes and cumulative food consumption was measured at 48hr and 96hr post treatment. The results of body weight changes are also provided in Table 4 and cumulative food consumption are also provided in Table 5. Similarly, the efficacy of Compound 8 and 9 in db/db mice at 10nM/kg dose were carried out in a separate study. The animals were divided into 3 treatment groups (n=5) - a diabetic control group, Compound 8 and Compound 9. Baseline blood glucose was measured from all the animals. All the animals were administered with test items subcutaneously. Blood glucose was measured at 4hr, 8hr, 12hr, 24hr, 48hr, 72hr and 96hr post treatment. Delta blood glucose (mM) was calculated. The results are provided in Table 3. Similarly, body weight changes and cumulative food consumption was measured at 48hr and 96hr post treatment. The results of body weight changes are provided in Table 4 and cumulative food consumption are provided in Table 5. Another separate efficacy study for Compound 10, 11 and 12 in db/db mice at 10nM/kg dose were carried out. The animals were divided into 4 treatment groups (n=5) - a diabetic control group, Compound 10, Compound 11 and Compound 12. Baseline blood glucose was measured from all the animals. All the animals were administered with test items subcutaneously. Blood glucose was measured at 4hr, 8hr, 12hr, 24hr, 48hr, 72hr and 96hr post treatment. Delta blood glucose (mM) was calculated. The results are provided in Table 3. Similarly, body weight changes and cumulative food consumption was measured at 48hr and 96hr post treatment. The results of body weight changes are provided in Table 4 and cumulative food consumption are provided in Table 5.

Table 3. Effect on blood glucose

| Delta Blood Glucose (mM) Mean ( $\pm$ SD) |      |                       |                       |                       |                       |                      |                      |                      |
|-------------------------------------------|------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|----------------------|----------------------|
| Study 1                                   |      |                       |                       |                       |                       |                      |                      |                      |
| Treatment Groups (n=6)                    | 0 hr | 4hr                   | 8hr                   | 12hr                  | 24hr                  | 48hr                 | 72hr                 | 96hr                 |
| Diabetic Control                          | 0    | 1.0 ( $\pm$ 3.3)      | 1.4 ( $\pm$ 3.3)      | 4.6 ( $\pm$ 3.7)      | 2.8 ( $\pm$ 2.0)      | 5.3 ( $\pm$ 3.6)     | 4.5 ( $\pm$ 5.4)     | 5.6 ( $\pm$ 3.5)     |
| Compound 1, 10nM/kg/s.c                   | 0    | -12.7*** ( $\pm$ 3.2) | -12.0*** ( $\pm$ 3.9) | -10.5*** ( $\pm$ 3.1) | -15.5*** ( $\pm$ 4.0) | -6.7*** ( $\pm$ 2.0) | -4.3*** ( $\pm$ 3.0) | -2.2*** ( $\pm$ 3.0) |
| Compound 2, 10nM/kg/s.c                   | 0    | -7.4*** ( $\pm$ 4.0)  | -7.6*** ( $\pm$ 1.8)  | -9.7*** ( $\pm$ 4.3)  | -9.5*** ( $\pm$ 4.0)  | -3.6*** ( $\pm$ 2.2) | -2.1** ( $\pm$ 2.0)  | -1.1** ( $\pm$ 2.6)  |
| Tirzepatide, 10nM/kg/s.c                  | 0    | -9.9*** ( $\pm$ 5.1)  | -8.7*** ( $\pm$ 1.2)  | -8.6*** ( $\pm$ 3.2)  | -11.4*** ( $\pm$ 3.9) | -5.3*** ( $\pm$ 2.2) | -3.7*** ( $\pm$ 1.4) | 3.7 ( $\pm$ 1.8)     |
| Study 2                                   |      |                       |                       |                       |                       |                      |                      |                      |
| Treatment Groups (n=6)                    | 0 hr | 4hr                   | 8hr                   | 12hr                  | 24hr                  | 48hr                 | 72hr                 | 96hr                 |

|                                                      |             |                    |                    |                    |                    |                   |                  |                |
|------------------------------------------------------|-------------|--------------------|--------------------|--------------------|--------------------|-------------------|------------------|----------------|
| <b>Diabetic Control</b>                              | 0           | 1.1<br>(±3.0)      | 0.8<br>(±2.5)      | 1.2<br>(±1.8)      | 0.8<br>(±2.3)      | 2.2<br>(±2.0)     | 2.7<br>(±0.8)    | 2.7<br>(±2.3)  |
| <b>Compound 3,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -10.3***<br>(±3.2) | -13.9***<br>(±3.5) | -15.8***<br>(±3.7) | -10.9***<br>(±5.7) | -6.6***<br>(±8.0) | 0.1<br>(±2.1)    | -0.7<br>(±2.9) |
| <b>Compound 4,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -10.4***<br>(±6.0) | -13.3***<br>(±4.5) | -13.8***<br>(±6.4) | -4.9*<br>(±3.7)    | -3.7*<br>(±4.7)   | 0.2<br>(±2.5)    | -1.9<br>(±5.4) |
| <b>Compound 5,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -10.7***<br>(±4.8) | -11.9***<br>(±6.5) | -11.6***<br>(±7.2) | -7.2**<br>(±7.4)   | -2.8<br>(±4.8)    | -0.8<br>(±2.0)   | 0.9<br>(±1.8)  |
| <b>Compound 6,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -15.6***<br>(±4.7) | -15.9***<br>(±5.6) | -16.3***<br>(4.4)  | -11.8***<br>(±4.4) | -9.5***<br>(±6.6) | -2.6*<br>(±2.9)  | -1.6<br>(±2.3) |
| <b>Compound 7,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -8.5***<br>(±2.4)  | -8.0***<br>(±5.7)  | -4.1<br>(3.7)      | -1.8<br>(±3.6)     | -0.7<br>(±1.3)    | -1.0<br>(±1.5)   | -0.7<br>(±0.9) |
| <b>Compound 1,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -10.0***<br>(±3.0) | -13.5***<br>(±1.4) | -12.3***<br>(±1.8) | -11.5***<br>(±5.0) | -7.9***<br>(±3.8) | -4.3**<br>(±1.4) | -1.4<br>(±3.1) |
| <b>Study 3</b>                                       |             |                    |                    |                    |                    |                   |                  |                |
| <b>Treatment Groups (n=5)</b>                        | <b>0 hr</b> | <b>4hr</b>         | <b>8hr</b>         | <b>12hr</b>        | <b>24hr</b>        | <b>48hr</b>       | <b>72hr</b>      | <b>96hr</b>    |
| <b>Diabetic Control</b>                              | 0           | -0.1<br>(±1.2)     | 0.1<br>(±2.8)      | 2.1<br>(±1.7)      | 0.7<br>(±4.1)      | 1.2<br>(±1.8)     | 1.0<br>(±2.2)    | 1.1<br>(±2.3)  |
| <b>Compound 8,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -13.6***<br>(±2.5) | -13.6***<br>(±3)   | -13.6***<br>(±3.5) | -8.5***<br>(±2.7)  | -5.6*<br>(±2.1)   | -1.8<br>(±2.4)   | -0.6<br>(±2.3) |
| <b>Compound 9,<br/>10nM/kg/s.c/si<br/>ngle dose</b>  | 0           | -13.1***<br>(±3.6) | -12.7***<br>(±1.8) | -12.1***<br>(±2.5) | -8.7***<br>(±2.2)  | -7.1***<br>(±2)   | -5.6*<br>(±2.6)  | 0<br>(±2.9)    |
| <b>Study 4</b>                                       |             |                    |                    |                    |                    |                   |                  |                |
| <b>Treatment Groups (n=5)</b>                        | <b>0 hr</b> | <b>4hr</b>         | <b>8hr</b>         | <b>12hr</b>        | <b>24hr</b>        | <b>48hr</b>       | <b>72hr</b>      | <b>96hr</b>    |
| <b>Diabetic Control</b>                              | 0           | 0.2<br>(±2.7)      | 1.2<br>(±2.4)      | 2.2<br>(±3.3)      | 1.7<br>(±3.1)      | 2.5<br>(±2.4)     | 3.6<br>(±4)      | 7<br>(±1.7)    |
| <b>Compound 10,<br/>10nM/kg/s.c/si<br/>ngle dose</b> | 0           | -12.1***<br>(±3.1) | -13.6***<br>(±3.2) | -14.7***<br>(±2.9) | -15.6***<br>(±3.5) | -4.0**<br>(±3.8)  | 2.3<br>(±1.8)    | 1.7<br>(±2.7)  |
| <b>Compound 11,<br/>10nM/kg/s.c/si<br/>ngle dose</b> | 0           | -13.3***<br>(±4.4) | -13.3***<br>(±3.8) | -13.6***<br>(±3.2) | -15.4***<br>(±3.8) | -6.1***<br>(±4.5) | 3.6<br>(±3.6)    | 6<br>(±2.7)    |
| <b>Compound 12,<br/>10nM/kg/s.c/si<br/>ngle dose</b> | 0           | -14.6***<br>(±0.7) | -14.8***<br>(±1.3) | -14.9***<br>(±1.9) | -4.8**<br>(±2.3)   | 2.5<br>(±3.6)     | 3.9<br>(±3.2)    | 6.8<br>(±1.8)  |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs Diabetic Control; Two way ANOVA followed by Bonferroni's post-test.

**Table 4. Effect on body weight**

|                        | Body Wt. Change (%)<br>48hr. vs. Baseline |    | Body Wt. Change (%) 96hr.<br>vs. Baseline |    |
|------------------------|-------------------------------------------|----|-------------------------------------------|----|
| Study 1                |                                           |    |                                           |    |
| Treatment Groups (n=6) | Mean                                      | SD | Mean                                      | SD |

|                                      |             |           |             |           |
|--------------------------------------|-------------|-----------|-------------|-----------|
| Diabetic Control                     | 1.0         | 0.5       | 1.4         | 0.5       |
| Compound 1, 10nM/kg/s.c              | -5.1***     | 0.3       | -4.2***     | 0.9       |
| Compound 2, 10nM/kg/s.c              | -4.3***     | 0.7       | -3.3***     | 1.3       |
| Tirzepatide, 10nM/kg/s.c             | -4.2***     | 0.8       | -2.8***     | 0.9       |
| <b>Study 2</b>                       |             |           |             |           |
| Treatment Groups (n=6)               | <b>Mean</b> | <b>SD</b> | <b>Mean</b> | <b>SD</b> |
| Diabetic Control                     | 1.8         | 1.6       | 4.1         | 3.5       |
| Compound 3, 10nM/kg/s.c/single dose  | -5.6***     | 0.8       | -4.9***     | 1.4       |
| Compound 4, 10nM/kg/s.c/single dose  | -3.8***     | 1.0       | -1.9**      | 1.3       |
| Compound 5, 10nM/kg/s.c/single dose  | -4.6***     | 4.0       | -3.3***     | 6.0       |
| Compound 6, 10nM/kg/s.c/single dose  | -5.5***     | 2.0       | -2.2**      | 1.3       |
| Compound 7, 10nM/kg/s.c/single dose  | -4.8***     | 0.5       | -2.9***     | 1.1       |
| Compound 1, 10nM/kg/s.c/single dose  | -5.1***     | 1.2       | -3.5***     | 0.7       |
| <b>Study 3</b>                       |             |           |             |           |
| Treatment Groups (n=5)               | <b>Mean</b> | <b>SD</b> | <b>Mean</b> | <b>SD</b> |
| Diabetic Control                     | 1.1         | 0.7       | 1.8         | 1.0       |
| Compound 8, 10nM/kg/s.c/single dose  | -3.1***     | 1.0       | -2.8***     | 1.3       |
| Compound 9, 10nM/kg/s.c/single dose  | -3.4***     | 0.6       | -3.8***     | 0.5       |
| <b>Study 4</b>                       |             |           |             |           |
| Treatment Groups (n=5)               | <b>Mean</b> | <b>SD</b> | <b>Mean</b> | <b>SD</b> |
| Diabetic Control                     | 0.9         | 1.7       | 0.9         | 1.0       |
| Compound 10, 10nM/kg/s.c/single dose | -4.5***     | 0.6       | -3.1***     | 0.9       |
| Compound 11, 10nM/kg/s.c/single dose | -4.7***     | 1.8       | -2.0***     | 1.1       |
| Compound 12, 10nM/kg/s.c/single dose | -3.0***     | 1.7       | -0.4        | 2.5       |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Diabetic Control; One way ANOVA followed by Bonferroni's post test.

**Table 5. Effect on food consumption**

|                         | Cumulative food Intake (g)<br>0-48hr. vs. Baseline |     | Cumulative food Intake (g)<br>0-96hr. vs. Baseline |     |
|-------------------------|----------------------------------------------------|-----|----------------------------------------------------|-----|
| Study 1                 |                                                    |     |                                                    |     |
| Treatment Groups (n=6)  | Mean                                               | SD  | Mean                                               | SD  |
| Diabetic Control        | 11.7                                               | 1.6 | 24.6                                               | 4.0 |
| Compound 1, 10nM/kg/s.c | 7.5***                                             | 2.0 | 13.9***                                            | 2.4 |
| Compound 2, 10nM/kg/s.c | 6.5***                                             | 0.1 | 21.6                                               | 1.1 |

|                                                 |               |            |                |            |
|-------------------------------------------------|---------------|------------|----------------|------------|
| <b>Tirzepatide, 10nM/kg/s.c</b>                 | <b>4.9***</b> | <b>1.4</b> | <b>13.0***</b> | <b>1.3</b> |
| <b>Study 2</b>                                  |               |            |                |            |
| <b>Treatment Groups (n=6)</b>                   | <b>Mean</b>   | <b>SD</b>  | <b>Mean</b>    | <b>SD</b>  |
| <b>Diabetic Control</b>                         | 12.0          | 0.1        | 25.8           | 4.9        |
| <b>Compound 3,<br/>10nM/kg/s.c/single dose</b>  | 6.0***        | 4.8        | 16.0***        | 3.8        |
| <b>Compound 4,<br/>10nM/kg/s.c/single dose</b>  | 4.6***        | 2.4        | 14.4***        | 4.3        |
| <b>Compound 5,<br/>10nM/kg/s.c/single dose</b>  | 6.2***        | 1.6        | 18.4**         | 4.5        |
| <b>Compound 6,<br/>10nM/kg/s.c/single dose</b>  | 4.2***        | 1.5        | 16.0***        | 3.6        |
| <b>Compound 7,<br/>10nM/kg/s.c/single dose</b>  | 5.6***        | 0.7        | 19.6*          | 0.3        |
| <b>Compound 1,<br/>10nM/kg/s.c/single dose</b>  | 3.8***        | 0.7        | 12.5***        | 2.5        |
| <b>Study 3</b>                                  |               |            |                |            |
| <b>Treatment Groups (n=5)</b>                   | <b>Mean</b>   | <b>SD</b>  | <b>Mean</b>    | <b>SD</b>  |
| <b>Diabetic Control</b>                         | 14.9          | 0.5        | 29.8           | 1.2        |
| <b>Compound 8,<br/>10nM/kg/s.c/single dose</b>  | 4.0***        | 1.0        | 13.9***        | 1.6        |
| <b>Compound 9,<br/>10nM/kg/s.c/single dose</b>  | 4.2***        | 0.9        | 16.7***        | 3.4        |
| <b>Study 4</b>                                  |               |            |                |            |
| <b>Treatment Groups (n=5)</b>                   | <b>Mean</b>   | <b>SD</b>  | <b>Mean</b>    | <b>SD</b>  |
| <b>Diabetic Control</b>                         | 14.4          | 0.8        | 25.7           | 0.4        |
| <b>Compound 10,<br/>10nM/kg/s.c/single dose</b> | 8.2***        | 2.2        | 20.5**         | 3.9        |
| <b>Compound 11,<br/>10nM/kg/s.c/single dose</b> | 8.2***        | 1.1        | 21.7*          | 1.0        |
| <b>Compound 12,<br/>10nM/kg/s.c/single dose</b> | 10.2*         | 0.7        | 26.0           | 0.5        |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Diabetic Control; One way ANOVA followed by Bonferroni's post test.

The results demonstrate that Compound 1 and Compound 2 showed statistically significant blood glucose reduction upto 96hr post treatment. The effect on blood glucose reduction for the compounds was superior to tirzepatide when tested at the same concentration.

Compound 1 and Compound 2 also showed statistically significant body weight reduction which was comparable to tirzepatide. Compound 1 showed a significant reduction in food consumption which was comparable to tirzepatide. No significant reduction in food consumption was observed for Compound 2 as compared to diabetic control.

Similarly the results demonstrate that Compounds 3, 4, 5, 6 and 7 of present invention showed statistically significant blood glucose reduction upto 96hr post treatment. Also statistically significant reduction in food intake and body weight was observed for these compounds compared to diabetic control.

#### 5 Example 13: Efficacy study in db/db mice at 3 and 20nM/kg dose

The effect of compounds of present invention on blood glucose, food intake and body weight was studied on mice. This study was performed in type 2 diabetic mouse (db/db) model. The animals were divided into 5 treatment groups (n=8)- a diabetic control group, Compound 1 (3nM/kg and 20nM/kg) and Tirzepatide (3nM/kg and 20nM/kg). Baseline blood glucose was measured from all the animals. All animals were administered with test items subcutaneously. Blood glucose was measured at 4hr, 24hr, 48hr, 72hr post treatment. Delta blood glucose (mM) was calculated. Body weight changes and cumulative food consumption was measured at 72hr post treatment. The results of delta blood glucose are provided in Table 6. Similarly the results of body weight changes are provided in Table 7 and food consumption are provided in Table 8.

Table 6. Effect of Compound 1 on blood glucose

| Treatment Groups (n=8)   | Delta Blood Glucose (mM), Mean ( $\pm$ SD) |                          |                          |                          |                         |
|--------------------------|--------------------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
|                          | 0hr                                        | 4hr                      | 24hr                     | 48hr                     | 72hr                    |
| Diabetic Control         | 0.0                                        | -0.1<br>( $\pm$ 1.9)     | 0.9<br>( $\pm$ 1.6)      | 0.1<br>( $\pm$ 1.2)      | 0.3<br>( $\pm$ 1.8)     |
| Compound 1, 3nM/kg/s.c   | 0.0                                        | -8.9***<br>( $\pm$ 3.1)  | -9.9***<br>( $\pm$ 4.7)  | -5.8**<br>( $\pm$ 3.4)   | -3.2<br>( $\pm$ 2.3)    |
| Compound 1, 20nM/kg/s.c  | 0.0                                        | -13.7***<br>( $\pm$ 3.1) | -13.6***<br>( $\pm$ 5.9) | -14.0***<br>( $\pm$ 6.6) | -7.9***<br>( $\pm$ 4.9) |
| Tirzepatide, 3nM/kg/s.c  | 0.0                                        | -4.4*<br>( $\pm$ 3.2)    | -1.6<br>( $\pm$ 2.9)     | 0.7<br>( $\pm$ 1.8)      | 1.4<br>( $\pm$ 1.8)     |
| Tirzepatide, 20nM/kg/s.c | 0.0                                        | -8.2***<br>( $\pm$ 3.6)  | -11.1***<br>( $\pm$ 4.0) | -7.6***<br>( $\pm$ 4.7)  | -1.3<br>( $\pm$ 2.1)    |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Diabetic Control; Two way ANOVA followed by Bonferroni's post-test.

Table 7. Effect of Compound 1 on body weight

| Treatment Groups (n=8)  | Body weight change (%) 72hr vs. Baseline |     |
|-------------------------|------------------------------------------|-----|
|                         | Mean                                     | SD  |
| Diabetic Control        | 1.3                                      | 1.1 |
| Compound 1, 3nM/kg/s.c  | -4.0***                                  | 0.7 |
| Compound 1, 20nM/kg/s.c | -6.2***                                  | 1.4 |
| Tirzepatide, 3nM/kg/s.c | -3.4***                                  | 2.2 |

|                                 |                |            |
|---------------------------------|----------------|------------|
| <b>Tirzepatide, 20nM/kg/s.c</b> | <b>-4.9***</b> | <b>0.8</b> |
|---------------------------------|----------------|------------|

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Diabetic Control; One way ANOVA followed by Bonferroni's post test

**Table 8. Effect of Compound 1 on food consumption**

| Treatment Groups (n=8)          | Cumulative Food Intake (g), 0-72hr |      |
|---------------------------------|------------------------------------|------|
|                                 | Mean                               | SD   |
| <b>Diabetic Control</b>         | 16.4                               | 1.40 |
| <b>Compound 1, 3nM/kg/s.c</b>   | 10.2***                            | 1.66 |
| <b>Compound 1, 20nM/kg/s.c</b>  | 6.3***                             | 1.03 |
| <b>Tirzepatide, 3nM/kg/s.c</b>  | 9.6***                             | 1.71 |
| <b>Tirzepatide, 20nM/kg/s.c</b> | 8.4***                             | 2.16 |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Diabetic Control; one way ANOVA followed by Bonferroni's post test

The results demonstrate that Compound 1 at 3nM/kg and 20nM/kg showed dose dependent improvement in glucose lowering effect upto 72 hours. The effect was superior to tirzepatide at similar dosage.

The effect compound 1 on food intake and body weight at 3 and 20nM/kg dose was significant, dose dependent and comparable to tirzepatide.

The results presented above demonstrate that the compounds of present invention are potent inhibitors of GLP-1 and GIP receptors and can be effective in treatment of type 2 diabetes, diabetes with obesity, obesity and hyperlipidemia.

#### **Example 16: Cellular cAMP assay**

*In-vitro* potency determination was performed using a cAMP assay. G protein coupled receptor (GPCR) activation following ligand binding initiates a series of second messenger cascades that results in a cellular response. Signaling by the GLP-1R and GIP-R involves activation of adenylate cyclase and cAMP production. Cellular cAMP production was determined using the cAMP Hunter™ eXpress GPCR Assay (Eurofins DiscoverX).

Cellular cAMP Assay of tirzepatide, Compound 1, Compound 3, Compound 6, Compound 10 and Compound 11 was performed and the half effective concentrations on GLP-1R-expressing cells and GIPR-expressing cells was as mentioned in below Table 9:

**Table 9: Half-effective concentrations on GLP-1R-expressing cells and GIPR-expressing cells**

| Compound           | Half effective Concentration (EC50) |       | Half effective Concentration (EC50) |            |
|--------------------|-------------------------------------|-------|-------------------------------------|------------|
|                    | GLP-1R                              | GIPR  | GLP-1R (Exenatide)                  | GIPR (GIP) |
| <b>Tirzepatide</b> | 6.8nM                               | 1.9nM | 78.9pM                              | 306.1pM    |

|             | GLP-1R | GIPR   | GLP-1R<br>(Exenatide) | GIPR<br>(GIP) |
|-------------|--------|--------|-----------------------|---------------|
| Compound 1  | 12.4pM | 27.6pM | 35.9pM                | 179.2pM       |
|             |        |        |                       |               |
|             | GLP-1R | GIPR   | GLP-1R<br>(Exenatide) | GIPR<br>(GIP) |
| Compound 3  | 32.7pM | 12.8pM | 87.3pM                | 41.1pM        |
| Compound 6  | 19.6pM | 17.7pM |                       |               |
|             |        |        |                       |               |
|             | GLP-1R | GIPR   | GLP-1R<br>(Exenatide) | GIPR<br>(GIP) |
| Compound 10 | 3.6pM  | 7.3pM  | 15pM                  | 50.4pM        |
| Compound 11 | 5.9pM  | 6.4pM  |                       |               |

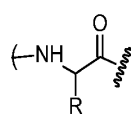
**Claims:**

1. A polypeptide or a pharmaceutically acceptable salt thereof comprising the amino acid sequence:

Y-X1-E-G-T-F-T-S-D-Y-S-I-X2-L-Xaa15-K-I-A-Xaa19-X3-Xaa21-F-V-Xaa24-W-L-X4-A-G-G-P-S-S-G-A-P-P-S-X5-X6-X7-X8-X9-X10-X11 (Seq. ID 1)

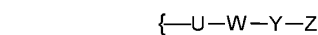
wherein X1 is Aib, (L)-norvaline or (D)-norvaline;

X2 is selected from Aib, Leu, (D)-Leu, Val, (D)-Val, Ile, (D)-Ile, and L or D isomer of an amino acid of the Formula



, wherein “” represents the point of attachment to Leu and R is selected from C<sub>2</sub>-alkyl, C<sub>3</sub>-cycloalkyl, C<sub>3</sub>-cycloalkyl-C<sub>1-3</sub>alkyl-, C<sub>3</sub>-alkenyl, C<sub>3</sub>-alkynyl, C<sub>5</sub>-cycloalkenyl-CH<sub>2</sub>- and C<sub>1-3</sub>haloalkyl; or R along with the carbon to which it is attached, forms a C<sub>3-6</sub>cycloalkyl ring;

X3 is Gln or Lys; wherein, when X3 is Lys, the side chain amino (ε amino) group of Lys is acylated with a moiety:



wherein U is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  wherein } is point of attachment with group W;

W is selected from a group consisting of  $-C(O)-NH-(CH_2)_p-NH-$ ,  $-C(O)-C(CH_3)_2-NH-$  and  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ , wherein p is 3 or 4 and wherein ] is point of attachment with group Y;

Y is  $-C(O)-(CH_2)_2-CH(COOH)NH-$  and -- is point of attachment with the group Z;

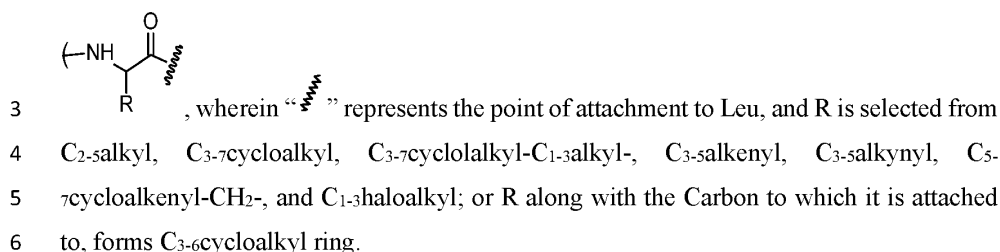
Z is  $-C(O)-(CH_2)_n-COOH$  or  $-C(O)-(CH_2)_n-CH_3$  wherein n is an integer from 14 to 20;

X4 is Leu, Ile or Glu;

X5 is absent, Arg or Lys; wherein when X5 is Lys, the side chain amino (ε amino) group of Lys is acylated with a moiety:

- 26  $\{\text{---U'---W'---Y'---Z'}\}$
- 27 wherein U' is  $\text{---C(O)---CH}_2\text{---O---(CH}_2\text{)}_2\text{---O---(CH}_2\text{)}_2\text{---NH---}$  wherein } is point of  
28 attachment with group W';
- 29 W' is selected from a group consisting of  $\text{---C(O)---NH---(CH}_2\text{)}_q\text{---NH---}$ ,  $\text{---C(O)---C(CH}_3\text{)}_2\text{---}$   
30  $\text{NH---}$  and  $\text{---C(O)---CH}_2\text{---O---(CH}_2\text{)}_2\text{---O---(CH}_2\text{)}_2\text{---NH---}$ , wherein q is 3 or 4 and wherein ]  
31 is point of attachment with group Y';
- 32 Y' is  $\text{---C(O)---(CH}_2\text{)}_2\text{---CH(COOH)NH---}$  and --- is point of attachment with the group  
33 Z';
- 34 Z' is  $\text{---C(O)---(CH}_2\text{)}_m\text{---COOH}$  or  $\text{---C(O)---(CH}_2\text{)}_m\text{---CH}_3$  wherein m is an integer from 14  
35 to 20;
- 36 X6 is absent or Lys;
- 37 X7 is absent or Lys;
- 38 X8 is absent or Lys;
- 39 X9 is absent or Lys;
- 40 X10 is absent or Lys;
- 41 X11 is absent or Lys;
- 42 Xaa15 is Asp or Glu;
- 43 Xaa19 is Gln or Ala;
- 44 Xaa21 is Ala or Glu;
- 45 Xaa24 is Gln or Asn;
- 46 wherein the acid group of the C terminal amino acid is a free carboxylic acid group  
47 or is amidated as a C-terminal primary amide and at least one of X3 and X5 is Lys;  
48 and
- 49 with the proviso that when X1 is Aib, X2 is not Aib.
- 1 2. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 wherein X1 is Aib.
- 1 3. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 wherein X1 is (L)-norvaline.

- 1 4. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 wherein X2 is Aib, Leu, Ile or L or D isomer of an amino acid of the Formula:



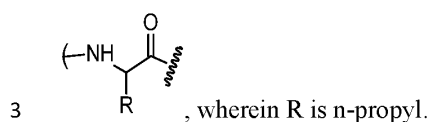
- 1 5. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 4,  
2 wherein R is C<sub>2</sub>-alkyl.

- 1 6. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 5,  
2 wherein R is ethyl.

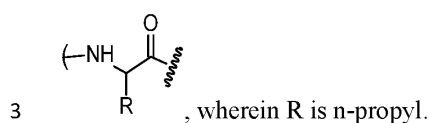
- 1 7. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 5,  
2 wherein R is n-propyl.

- 1 8. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 5,  
2 wherein R is n-butyl.

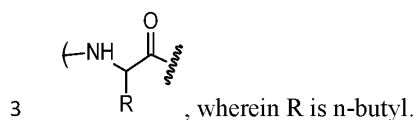
- 1 9. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is L or D isomer of an amino acid of the Formula:



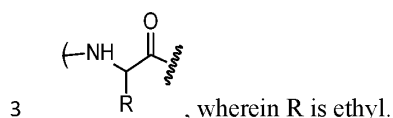
- 1 10. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 3,  
2 wherein X2 is L or D isomer of an amino acid of the Formula:



- 1 11. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is L or D isomer of an amino acid of the Formula:



- 1 12. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is L or D isomer of an amino acid of the Formula:



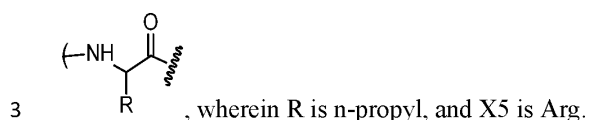
- 1 13. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 3,  
2 wherein X2 is Aib.

- 1 14. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is Leu.

- 1 15. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is Ile.

- 1 16. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-15, wherein X5, X6, X7, X8, X9, X10 and X11 are absent.

- 1 17. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 2,  
2 wherein X2 is L or D isomer of an amino acid of the Formula:

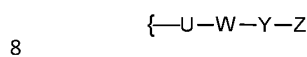


- 1 18. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 comprising an amino acid sequence:

3 Y-Aib-E-G-T-F-T-S-D-Y-S-I-X2-L-D-K-I-A-Q-X3-A-F-V-Q-W-L-X4-A-G-G-P-  
4 S-S-G-A-P-P-P-S-X5-X6-X7-X8-X9-X10-X11 (Seq. ID 2)

5 wherein X2 is Leu, Ile, (L)-norvaline, (L)-homoalanine or (L)-norleucine;

6 X3 is Lys, wherein the side chain amino (ε amino) group of Lys is acylated with a  
7 moiety:



9 wherein U is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  wherein } is point of attachment  
10 with group W;

11 W is selected from a group consisting of  $-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_p-\text{NH}-$ ,  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-$   
 12  $\text{NH}-$  and  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$ , wherein p is 3 or 4 and wherein ]  
 13 is point of attachment with group Y;

14 Y is  $-\text{C}(\text{O})-(\text{CH}_2)_2-\text{CH}(\text{COOH})\text{NH}-$  and -- is point of attachment with the group Z;

15 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 14 to  
 16 20;

17 X4 is Ile;

18 X5 is absent or Arg;

19 X6 is absent or Lys;

20 X7 is absent or Lys;

21 X8 is absent or Lys;

22 X9 is absent or Lys;

23 X10 is absent or Lys;

24 X11 is absent or Lys;

25 wherein the acid group of the C terminal amino acid is a free carboxylic acid group  
 26 or is amidated as a C-terminal primary amide.

1 19. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 18,  
 2 wherein X2 is (L)-norvaline.

1 20. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 18,  
 2 wherein X2 is (L)-norleucine.

1 21. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 18,  
 2 wherein X2 is (L)-homoalanine.

1 22. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 18,  
 2 wherein X2 is Leu.

1 23. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 18,  
 2 wherein X2 is Ile.

1 24. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
 2 of the claims 18-23, wherein X5, X6, X7, X8, X9, X10 and X11 are absent.

1 25. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 19,  
2 wherein X5 is Arg.

1 26. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 19,  
2 wherein X4 is Ile;

3 X5, X6, X7, X8, X9, X10 and X11 are absent;

4 W is  $-C(O)-C(CH_3)_2-NH-$ ; and

5 Z is  $-C(O)-(CH_2)_n-COOH$ , wherein n is 18.

1 27. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 comprising an amino acid sequence:

3 Y-X1-E-G-T-F-T-S-D-Y-S-I-X2-L-D-K-I-A-Q-X3-A-F-V-Q-W-L-X4-A-G-G-P-  
4 S-S-G-A-P-P-P-S (Seq. ID 3)

5 wherein X1 is Aib or (L)-norvaline; X2 is Aib, Leu, Ile, (L)-norvaline, (L)-  
6 homoalanine or (L)-norleucine;

7 X4 is Ile;

8 X3 is Lys wherein the side chain amino ( $\epsilon$  amino) group of Lys is acylated with a  
9 moiety:

10  $\{ -U-W-Y-Z$

11 wherein U is  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$  wherein } is point of attachment  
12 with group W;

13 W is selected from a group consisting of  $-C(O)-NH-(CH_2)_p-NH-$ ,  $-C(O)-C(CH_3)_2-$   
14  $NH-$  and  $-C(O)-CH_2-O-(CH_2)_2-O-(CH_2)_2-NH-$ , wherein p is 3 or 4 and wherein ]  
15 is point of attachment with group Y;

16 Y is  $-C(O)-(CH_2)_2-CH(COOH)NH--$  and -- is point of attachment with the group Z;

17 Z is  $-C(O)-(CH_2)_n-COOH$  or  $-C(O)-(CH_2)_n-CH_3$  wherein n is an integer from 14 to  
18 20;

19 and wherein the acid group of the C terminal amino acid is a free carboxylic acid  
20 group or is amidated as a C-terminal primary amide;

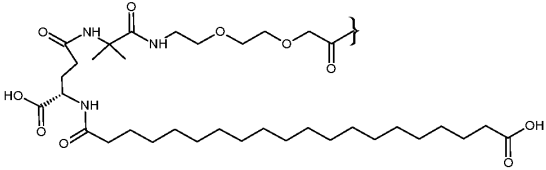
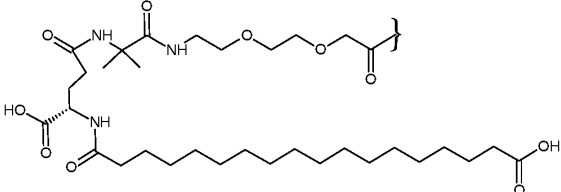
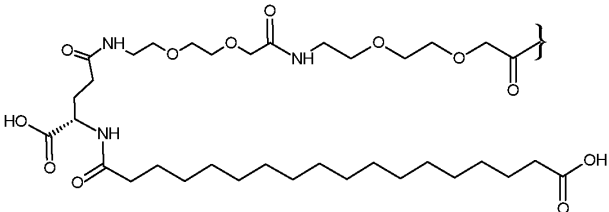
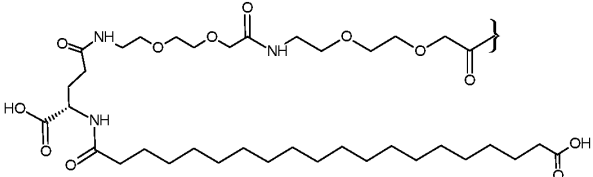
21 with a proviso that when X1 is Aib, X2 is not Aib.

- 1 28. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib.
- 1 29. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is (L)-norvaline.
- 1 30. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is Aib.
- 1 31. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is Leu.
- 1 32. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is Ile.
- 1 33. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is (L)-norvaline.
- 1 34. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is (L)-norleucine.
- 1 35. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X2 is (L)-homoalanine.
- 1 36. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib and X2 is (L)-norvaline.
- 1 37. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 and X2 are (L)-norvaline.
- 1 38. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib and X2 is (L)-norleucine.
- 1 39. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib and X2 is (L)-homoalanine.
- 1 40. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is (L)-norvaline and X2 is Aib.
- 1 41. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib and X2 is Leu.

- 1 42. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib and X2 is Ile.
- 1 43. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 27,  
2 wherein X1 is Aib;  
3 X2 is (L)-norvaline;  
4 W is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ ; and  
5 Z is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$ , wherein n is 18.
- 1 44. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-43, wherein W and/or W' is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$  or  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-$   
3  $\text{O}-(\text{CH}_2)_2-\text{NH}-$ .
- 1 45. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-43, wherein Z and/or Z' is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and/or  $-\text{C}(\text{O})-(\text{CH}_2)_m-$   
3  $\text{COOH}$  and n or m is 16 or 18.
- 1 46. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-43, wherein W and/or W' is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ , Z and/or Z' is  $-\text{C}(\text{O})-$   
3  $(\text{CH}_2)_n-\text{COOH}$  and/or  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  and n or m is 18.
- 1 47. The polypeptide or pharmaceutically acceptable salt thereof according to any one of  
2 the claims 1-43, wherein W and/or W' is  $-\text{C}(\text{O})-\text{C}(\text{CH}_3)_2-\text{NH}-$ , Z and/or Z' is  $-\text{C}(\text{O})-$   
3  $(\text{CH}_2)_n-\text{COOH}$  and/or  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  and n or m is 16.
- 1 48. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-25 and 27 to 42, wherein W and/or W' is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-$   
3  $\text{NH}-$ , Z and/or Z' is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and/or  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  and n or m is 16.
- 1 49. The polypeptide or pharmaceutically acceptable salt thereof according to any one of  
2 the claims 1-25 and 27 to 42, wherein W and/or W' is  $-\text{C}(\text{O})-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{NH}-$   
3  $]$ , Z and/or Z' is  $-\text{C}(\text{O})-(\text{CH}_2)_n-\text{COOH}$  and/or  $-\text{C}(\text{O})-(\text{CH}_2)_m-\text{COOH}$  and n or m is 18.
- 1 50. The polypeptide or a pharmaceutically acceptable salt thereof according to claim 1,  
2 comprising an amino acid sequence selected from the group consisting of:
- 3 i.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Asp Lys  
4 Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly  
5 Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 04)

- 6 ii.) Tyr L-norvaline Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Aib Leu Asp Lys  
 7 Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly  
 8 Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 05)
- 9 iii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Leu Leu Asp Lys Ile Ala  
 10 Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro  
 11 Pro Pro Ser-NH<sub>2</sub> (Seq ID: 06)
- 12 iv.) Tyr L-norvaline Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu  
 13 Asp Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser  
 14 Ser Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 07)
- 15 v.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Glu Lys  
 16 Ile Ala Ala Gln Glu Phe Val Asn Trp Leu Leu Ala Gly Gly Pro Ser Ser Gly  
 17 Ala Pro Pro Pro Ser Lys-NH<sub>2</sub> (Seq ID: 08)
- 18 vi.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norvaline Leu Asp Lys  
 19 Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly  
 20 Ala Pro Pro Pro Ser Arg (Seq ID: 09)
- 21 vii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-homoalanine Leu Asp  
 22 Lys Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser  
 23 Gly Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 10)
- 24 viii.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile L-norleucine Leu Asp Lys  
 25 Ile Ala Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly  
 26 Ala Pro Pro Pro Ser-NH<sub>2</sub> (Seq ID: 11)
- 27 ix.) Tyr Aib Glu Gly Thr Phe Thr Ser Asp Tyr Ser Ile Ile Leu Asp Lys Ile Ala  
 28 Gln Lys Ala Phe Val Gln Trp Leu Ile Ala Gly Gly Pro Ser Ser Gly Ala Pro  
 29 Pro Pro Ser-NH<sub>2</sub> (Seq ID: 12)

1 51. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
 2 of the claims 1-50, wherein -U-W-Y-Z and/or -U'-W'-Y'-Z' is selected from the group  
 3 consisting of:

|                                                                                     |                  |
|-------------------------------------------------------------------------------------|------------------|
|    | Moiety A;        |
|    | Moiety B;        |
|  | Moiety C;<br>and |
|  | Moiety D         |

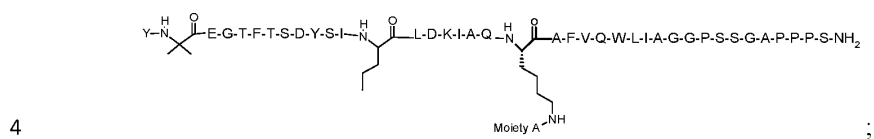
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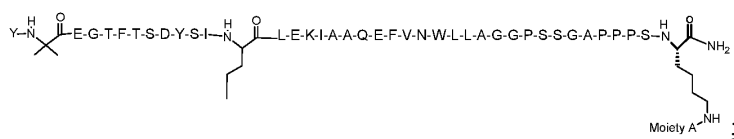
1 52. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-51, wherein the C terminal amino acid is amidated as a C-terminal primary  
3 amide.

1 53. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of the claims 1-51, wherein the C terminal amino acid is a free carboxylic acid.

1 54. A polypeptide or a pharmaceutically acceptable salt thereof, selected from the group  
2 consisting of:

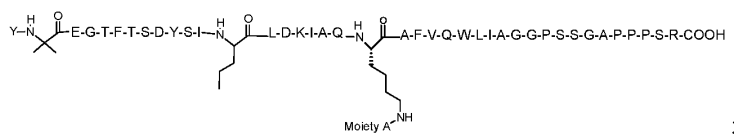
3 Compound 1:





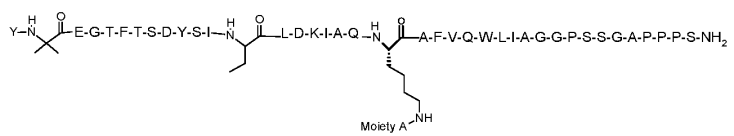
18

19                      Compound 9:



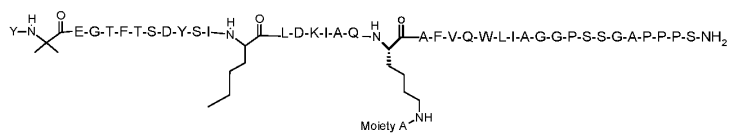
20

21                      Compound 10:



22

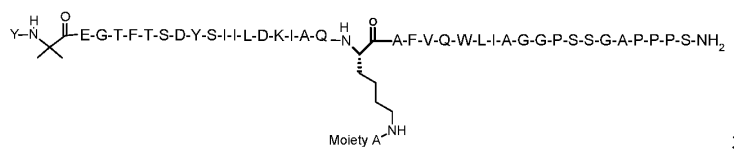
23                      Compound 11:



24

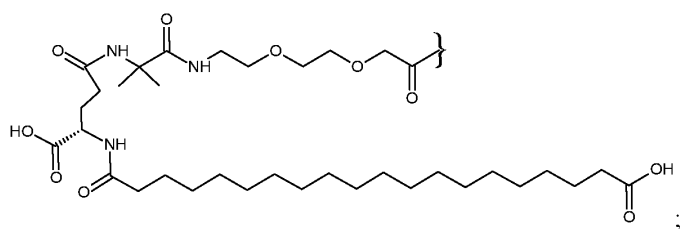
; and

25                      Compound 12:



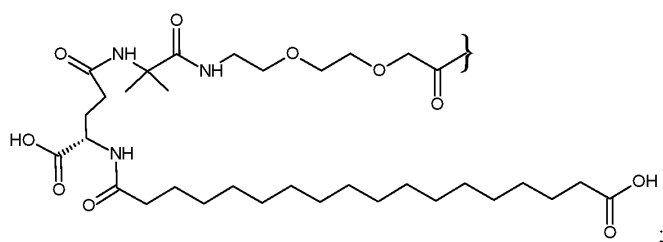
26

27            wherein, Moiety A is

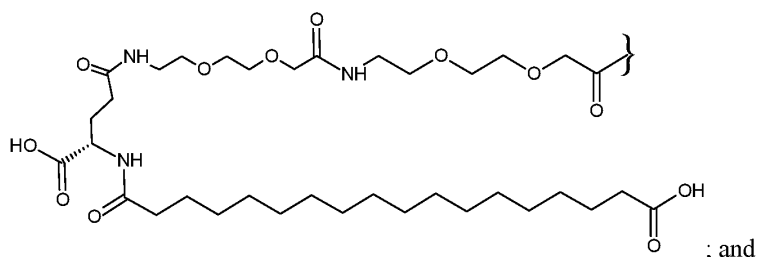


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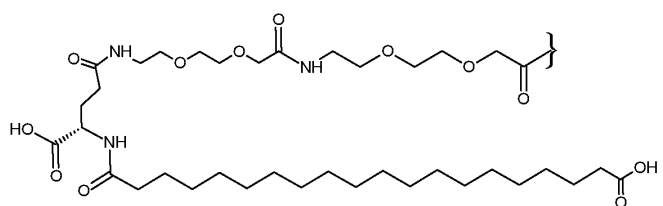
29                      Moiety B is



Moiety C is



Moiety D is



- 1 55. A pharmaceutical composition comprising a polypeptide or a pharmaceutically
- 2 acceptable salt thereof according to any one of the claims 1-54, and one or more of
- 3 pharmaceutically acceptable excipient.
- 1 56. A pharmaceutical composition according to claim 55 for use as a medicament.
- 1 57. A pharmaceutical composition according to claim 55 for use in the treatment or
- 2 prevention of a disease in a patient.
- 1 58. A pharmaceutical composition for use according to claim 57, wherein said disease
- 2 is selected from the group consisting of hyperglycemia, type 2 diabetes, impaired glucose
- 3 tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X,
- 4 dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart
- 5 disease, stroke, inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers.

1 59. A pharmaceutical composition for use according to claims 56-58, wherein said  
2 pharmaceutical composition is provided simultaneously, separately, or sequentially in  
3 combination with an effective amount of one or more additional therapeutic agents.

1 60. A method of treating or preventing hyperglycemia, type 2 diabetes, impaired glucose  
2 tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X,  
3 dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart  
4 disease, stroke, inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers in  
5 a patient, wherein said method comprises administering to a patient in need thereof, an  
6 effective amount of a pharmaceutical composition according to claim 55.

1 61. The method according to claim 60, further comprising administering  
2 simultaneously, separately, or sequentially in combination with an effective amount of one  
3 or more therapeutic agents.

1 62. A pharmaceutical composition according to claim 55, for use in the preparation of a  
2 medicament for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired  
3 glucose tolerance, type 1 diabetes, obesity, hypertension, syndrome X, dyslipidemia,  
4 cognitive disorders, atherosclerosis, myocardial infarction, coronary heart disease, stroke,  
5 inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers.

1 63. A polypeptide or a pharmaceutically acceptable salt thereof according to any one of  
2 claims 1-54 for use as a medicament.

1 64. A polypeptide or a pharmaceutically acceptable salt thereof according to any one of  
2 claims 1-54 for use in the treatment or prevention of a disease in a patient.

1 65. A polypeptide or a pharmaceutically acceptable salt thereof for use according to  
2 claim 64, wherein said disease is selected from the group consisting of hyperglycemia, type  
3 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity, hypertension,  
4 hyperlipidemia, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial  
5 infarction, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia,  
6 alcoholism and gastric ulcers.

1 66. The polypeptide or a pharmaceutically acceptable salt thereof according to claims  
2 63-65, wherein said polypeptide or pharmaceutically acceptable salt thereof is provided  
3 simultaneously, separately, or sequentially in combination with an effective amount of one  
4 or more additional therapeutic agents.

1 67. A method of treating or preventing hyperglycemia, type 2 diabetes, impaired glucose  
2 tolerance, type 1 diabetes, obesity, hypertension, hyperlipidemia, syndrome X,  
3 dyslipidemia, cognitive disorders, atherosclerosis, myocardial infarction, coronary heart  
4 disease, stroke, inflammatory bowel syndrome, dyspepsia, alcoholism and gastric ulcers in  
5 a patient, comprising administering to a patient in need thereof, an effective amount of the  
6 polypeptide or pharmaceutically acceptable salt thereof according to any one of claims 1-  
7 54.

1 68. The method according to any one of claims 67, further comprising administering  
2 simultaneously, separately, or sequentially in combination with an effective amount of one  
3 or more therapeutic agents.

1 69. The polypeptide or a pharmaceutically acceptable salt thereof according to any one  
2 of claims 1-54, for use in the preparation of a medicament for the treatment or prevention  
3 of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity,  
4 hypertension, syndrome X, dyslipidemia, cognitive disorders, atherosclerosis, myocardial  
5 infarction, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia,  
6 alcoholism and gastric ulcers.