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(54) **Title:** THIOETHER CYCLIC PEPTIDE AMYLIN RECEPTOR MODULATORS

(57) **Abstract:** The present invention relates to amylinomimetic peptide analogues and derivatives thereof in which the N-terminus of each peptide is covalently linked to an internal amino acid side chain thiol functionality by way of a non-peptidyl cyclization bridging element. The thioether-cyclized amylinomimetic analogues and derivatives thereof may comprise one or more alterations including substitutions, insertions, deletions and modifications and may optionally comprise a serum albumin binding element, such as an alkyl chain of at least 14 carbon atoms optionally bearing an additional pendant carboxylate moiety, or a half-life extending biologic moiety, such as HSA, a non-targeting mAb or Fc. Moreover, the invention relates to compositions thereof and methods of treatment of conditions responsive to amylin receptor modulation.

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THIOETHER CYCLIC PEPTIDE AMYLIN RECEPTOR MODULATORS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/662,492, filed April 25, 2018, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 16, 2019, is named PRD3471WOPCT1_SL.txt and is 55,747 bytes in size.

FIELD OF THE INVENTION

[0001] This invention relates to thioether-cyclized analogues of amylin, pramlintide and davalintide and derivatives thereof (herein referred to as "amylinomimetic peptides"), which function as agonists of amylin receptors and as such are useful for the treatment of metabolic diseases and disorders, such as obesity, type 2 diabetes, metabolic syndrome, insulin resistance and dyslipidemia.

BACKGROUND OF THE INVENTION

[0002] Amylin is a naturally-occurring, 37-amino acid-containing peptide that is a structurally related member of the calcitonin family of peptides, which includes calcitonin (CT), calcitonin gene-related peptide (CGRP), adrenomedullin (AM) and intermedin (AM2). It is synthesized in and secreted by the pancreas in response to nutrient influx into the GI tract. Following release into circulation, amylin binds with high affinity to specific Class B GPCRs, located primarily in the hindbrain area postrema region of the central nervous system. As such, it is a centrally acting neuroendocrine hormone that serves to regulate glucose homeostasis by inhibiting gastric emptying, inhibiting the release of glucagon and inducing satiety (reviewed in Hay DL et al., Pharmacol Rev 2015;67:564-600). Amylin has been found to interact in vitro with three

target receptor subtypes - AMY1R, AMY2R and AMY3R, which are heterodimeric structures comprised of the calcitonin receptor (CTR) and a receptor modifying protein (RAMP1, RAMP2 and RAMP3, respectively) (Christopoulos G et al., Mol Pharmacol 1999;56:235-42). Association of the CTR with these RAMPs confers an increase in amylin affinity relative to that at CTR alone, and provides a basis for the selective receptor pharmacology of amylin (vs. CT) (Bower RL and Hay DL, Br J Pharmacol 2016;173:1883-98). While it has been generally accepted that amylin participates in high-affinity/potency interactions, particularly with AMY1R and AMY3R, simultaneous agonism at all receptor subtypes may not necessarily be required for pharmacological benefit. (Hay DL et al., Br J Pharmacol 2018;175:3-17; Hay DL, Headache 2017;57:89-96).

[0003] Human amylin, also known as islet amyloid polypeptide (IAPP), has several physicochemical properties that make it unsuitable as a pharmaceutical agent, including most notably, its low aqueous solubility and tendency to self-aggregate and adhere to surfaces. Pramlintide, an equipotent amylin analogue was developed by incorporating three specific residue mutations (A25P, S28P and S29P) into the amylin sequence (Young AA et al., Drug Dev Res 1996;37:231-48). These mutations confer improved physicochemical properties (reduced aggregation propensity) relative to amylin. Pramlintide reduces food intake (Smith SR et al., Am J Physiol Endocrinol Metab 2007;293:E620-7) and body weight (Aronne L et al., J Clin Endocrin Metab 2007;92:2977-83) in obese subjects. It is approved for the treatment of adult patients with type 1 diabetes as an adjunctive therapy to insulin, and for adult patients with type 2 diabetes as an adjunctive therapy to either insulin alone, or concurrently with metformin and/or sulfonylureas (Pullman J et al., Vasc Health Risk Manag 2006;2:203-12). Davalintide is a related synthetic peptide, 32 amino acids in length, whose structure is a chimera of the primary sequences of pramlintide and salmon calcitonin. As such, it is devoid of the amyloidogenic residues of human amylin (Westermark P et al., Proc Natl Acad Sci USA 1990;87:5036-40). It is a highly potent agonist at both amylin and calcitonin receptors, demonstrating enhanced pharmacological properties over native (rat) amylin at reducing food intake and body weight in rats (Mack CM et al., Int J Obes 2010; 34:385-95). Amylin, pramlintide and davalintide each have extremely short half-lives in

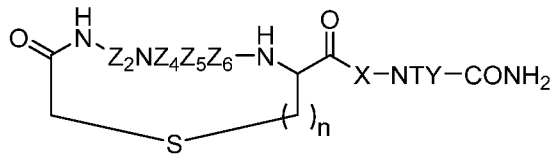
vivo (< 0.75 h), which limit their practical therapeutic utilities (Roth JD et al., Immun Endoc Metab Agents in Med Chem 2008;8:317-24; Mack CM et al., Diabetes Obes Metab 2011;13:1105-13). In the case of pramlintide, this short half-life necessitates a regimen of multiple daily administration for achieving clinical effectiveness. Thus, it is desirable to obtain amylin agonist peptides or derivatives thereof with improved metabolic stabilities and pharmacokinetic profiles.

[0004] One technique used for extending the half-lives of peptides involves conjugation to a biological carrier, such as albumin, a suitable mAb or antigen-binding fragment thereof, or a mAb-derived crystallization fragment domain (Fc) protein. Such bioconjugation chemistry is performed by the reaction of a selectively reactive sulfhydryl functionality on the biologic carrier molecule and a complementary electrophilic site engineered into the peptide of interest. While maleimide functionalities incorporated onto peptide molecules have served as suitable electrophiles for bioconjugation chemistry, the resultant bioconjugates may undergo a reverse-conjugation reaction (retro-Michael reaction) in vivo, leading to a loss of the peptide from the biologic carrier. More stable thioacetamide linkages, formed by way of coupling to a reactive haloacetamido-derivatized peptide are therefore used to avoid this reverse conjugation potential. However, the conditions required for bromoacetamide conjugation reaction cannot be used successfully with disulfide containing amylin analogues for their selective bioconjugation, due to the reactivity of the unhindered disulfide ring. Such attempted chemistry leads to concomitant ring opening and associated complicating side reactions. It has long been recognized that an intact disulfide loop is a critical molecular feature that is required for receptor activation and biological function (Roberts AN et al., Proc Natl Acad Sci USA 1989;86:9662-9666; Cornish J et al., Am. J. Physiol. 1998;274:E827-E833; Bower RL and Hay DL., Br. J. Pharmacol. 2016;173:1883-1898). Herein, an N-terminal cyclic thioether replacement for the cysteine disulfide connectivity of pramlintide and davalintide is identified which unexpectedly maintains amylin receptor agonist activity and is stable towards bioconjugation reaction chemistry.

SUMMARY OF THE INVENTION

[0005] In one general aspect, the invention relates to novel amylinomimetic compounds. Also provided herein are amylinomimetic derivatives of pramlintide or davalintide and their conjugates comprising a monoclonal antibody or an antigen binding fragment thereof coupled to an amylinomimetic peptide.

[0006] In one aspect, the invention is represented by a compound of Formula **I** or a derivative thereof (SEQ ID NO: 53)**:**

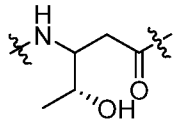


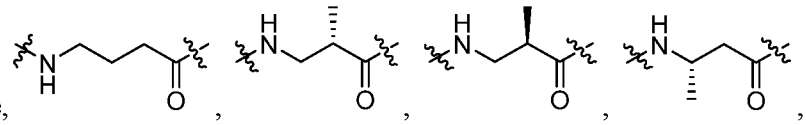
Formula **I**

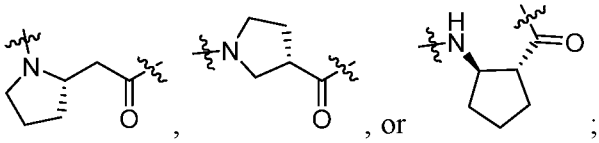
wherein

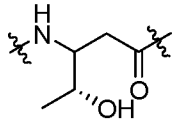
n is 1, or 2;

Z_2 is a direct bond, serine, or glycine;

Z_4 is T, or  ;

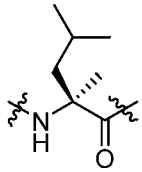
Z_5 is A, β-alanine, 

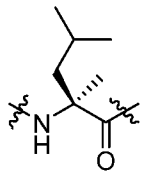
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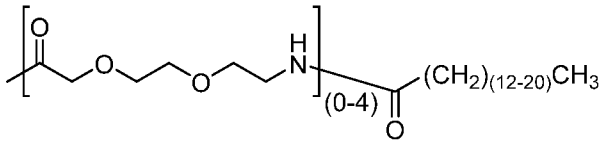
Z_6 is T, or  ;

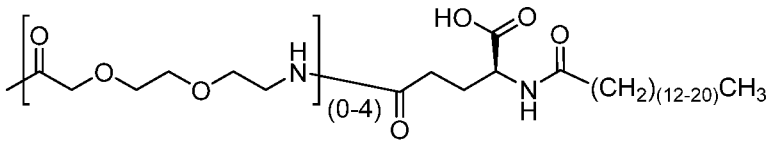
Z_{10} is Q, or E;

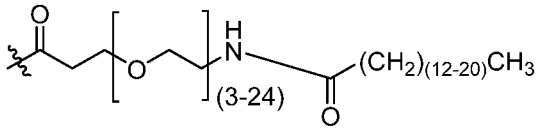
Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with $-C(=NH)NH_2$;

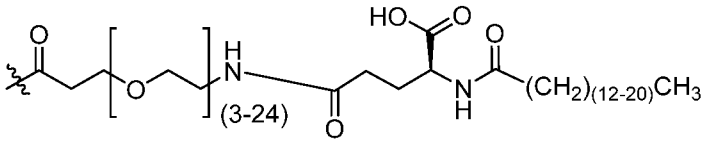
Z_{12} is L, or  ;

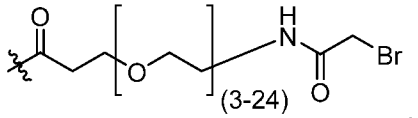
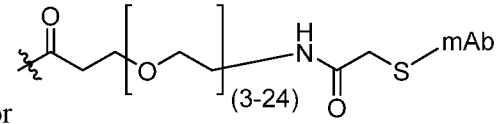
Z_{16} is L, or  ;

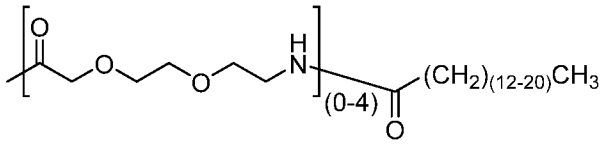
Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -$C(O)CH_2CH=CH_2$,  ,

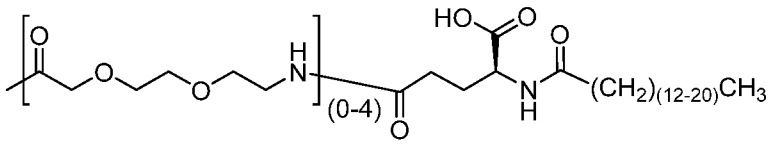
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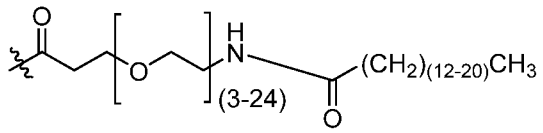
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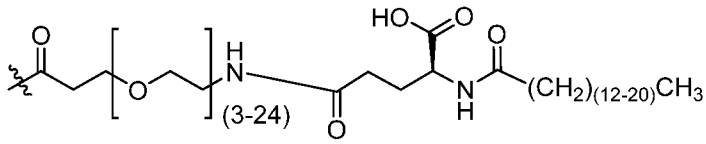
 , or  , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -$C(O)CH_2CH=CH_2$,  ,

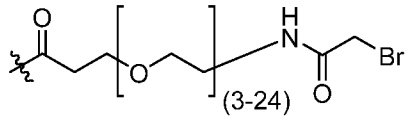
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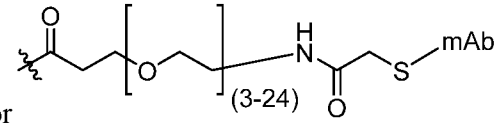
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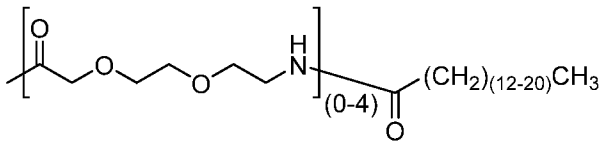
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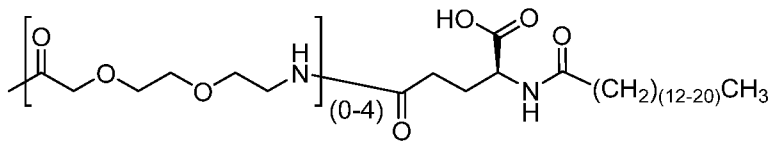
, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

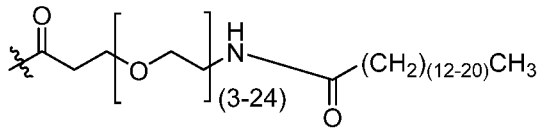
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$,



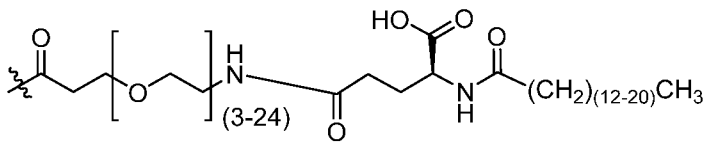
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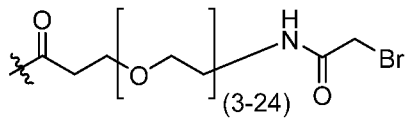
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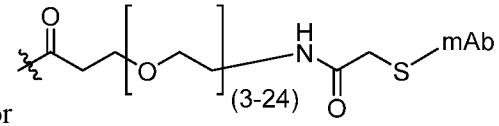
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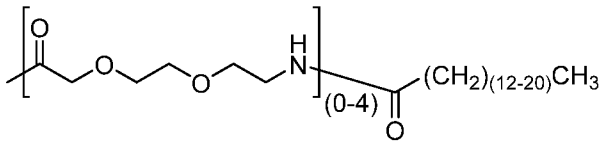
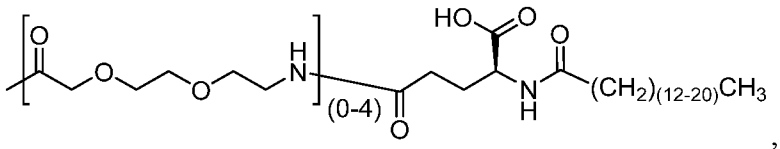
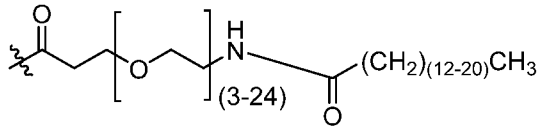
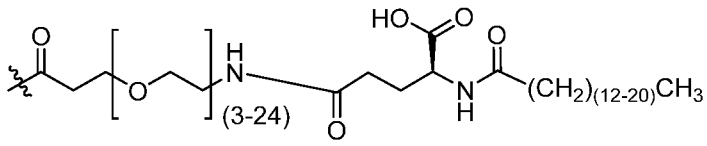
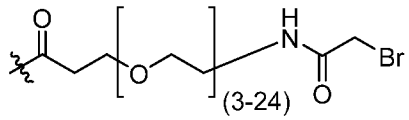
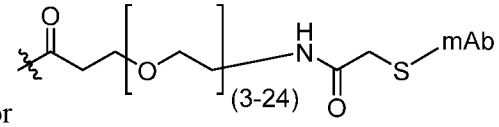
,



, or



, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

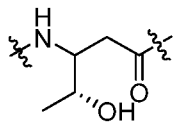
Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with–$C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, , , , , , , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

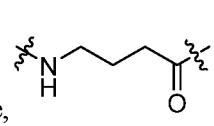
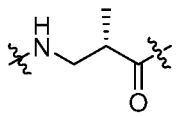
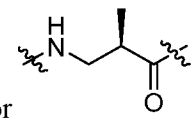
and pharmaceutically acceptable salts thereof.

[0007] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

Z_2 is a direct bond;

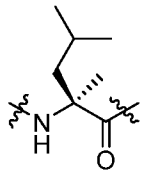
Z_4 is T, or ;

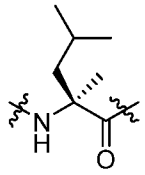
Z_5 is β-alanine, , , or ;

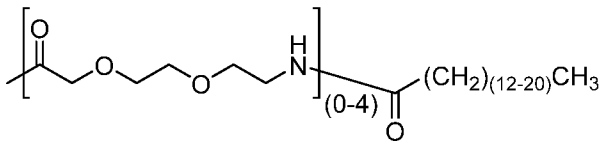
Z_6 is T;

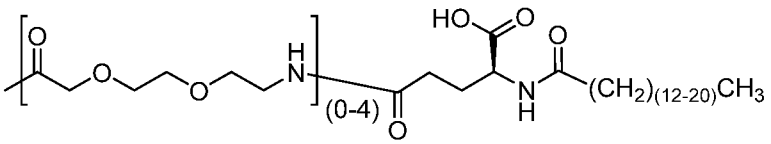
Z_{10} is Q, or E;

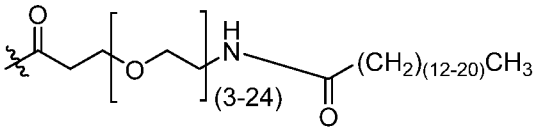
Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with –C(=NH)NH_2;

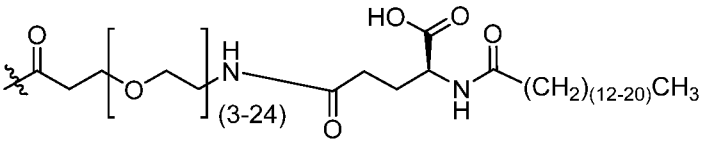
Z_{12} is L, or  ;

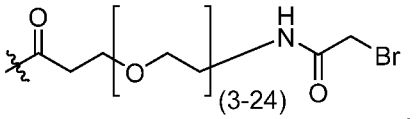
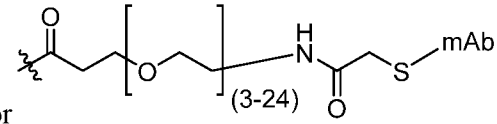
Z_{16} is L, or  ;

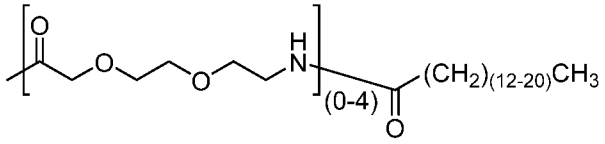
Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -C(O)CH_2CH=CH_2,  ,

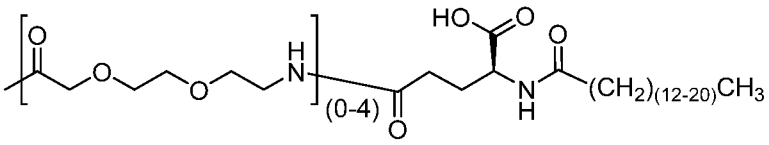
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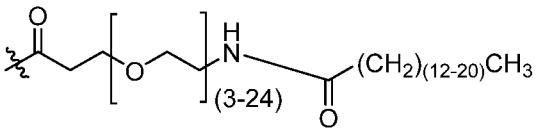
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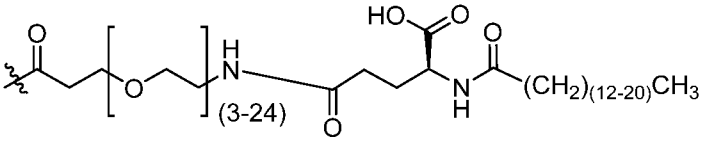
 ,

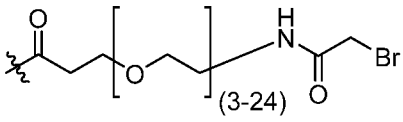
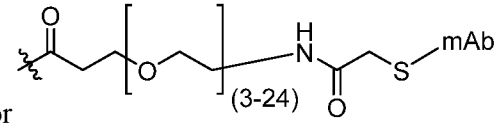
 , or  , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -C(O)CH_2CH=CH_2,  ,


,


,

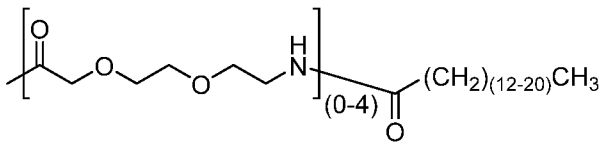

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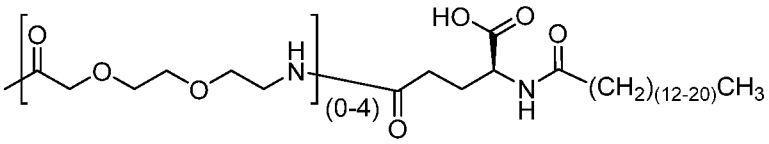

, or 
, wherein said

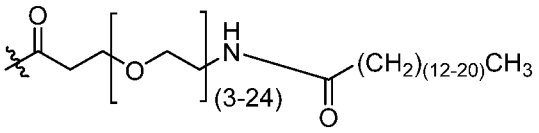
mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

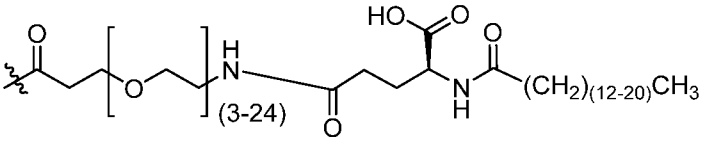
X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

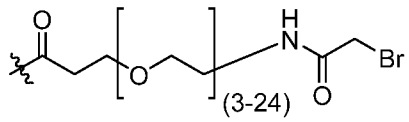
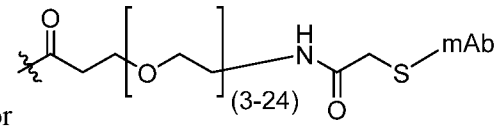
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$,

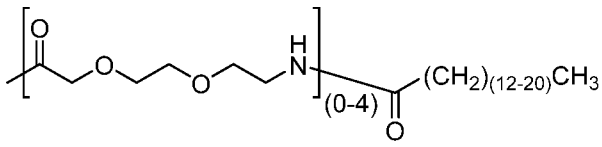

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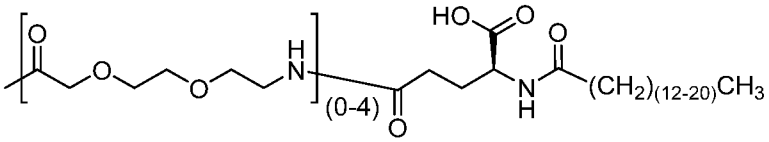

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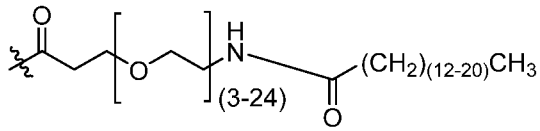

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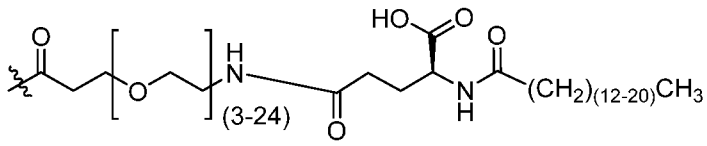

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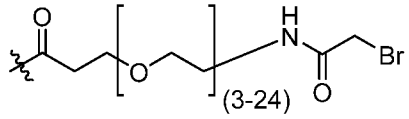
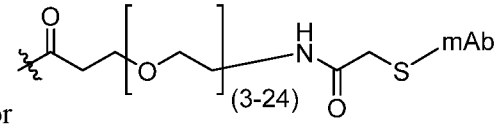

, or 
, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with–$C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, 
,


,


,


,

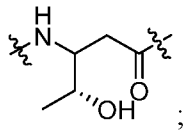

, or 
, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

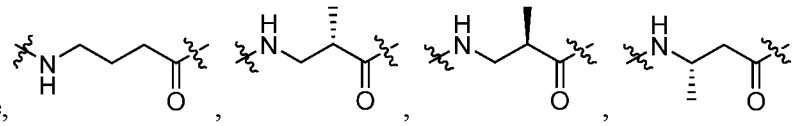
and pharmaceutically acceptable salts thereof.

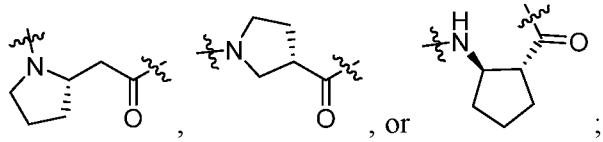
[0008] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

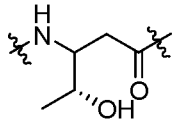
Z_2 is a direct bond, or serine;

Z_4 is T, or 
;

Z_5 is A, β-alanine, 

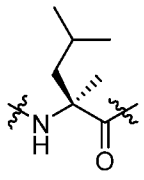


;

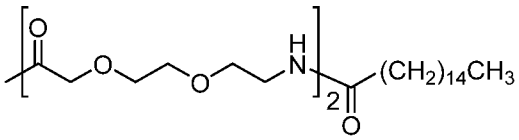
Z_6 is T, or  ;

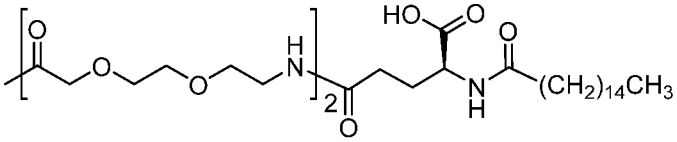
Z_{10} is Q, or E;

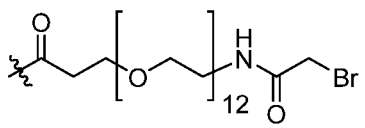
Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with $–C(=NH)NH_2$;

Z_{12} is L, or  ;

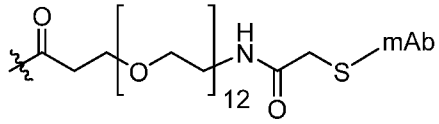
Z_{16} is L;

Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with $–C(=O)CH_3$, $-C(O)CH_2CH=CH_2$, 

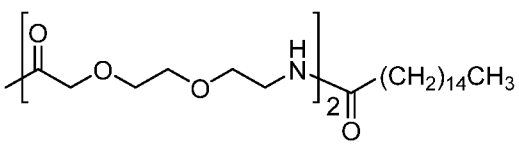


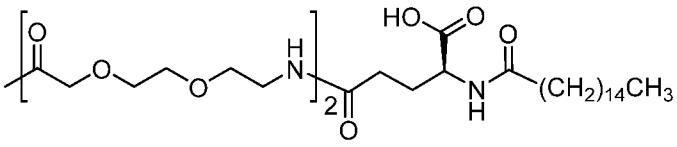
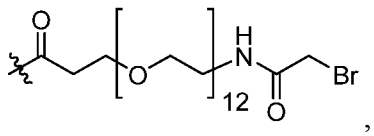
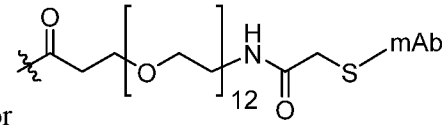


, or



, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

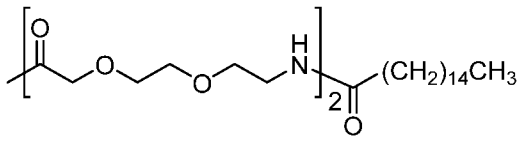
Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with $–C(=O)CH_3$, $-C(O)CH_2CH=CH_2$, 

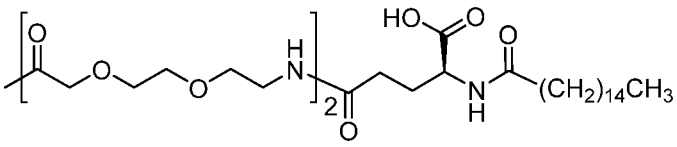
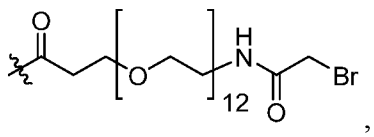
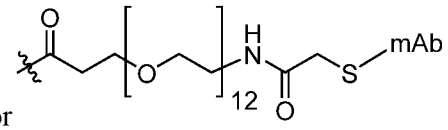




or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

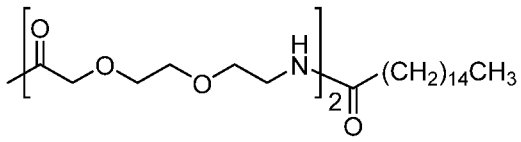
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$,

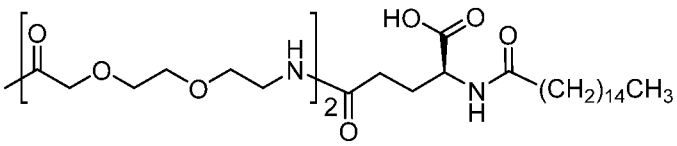
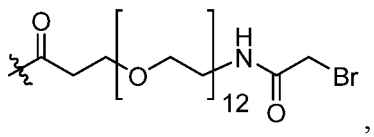


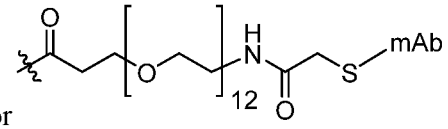




or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with$-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$,



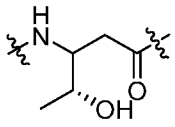
or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

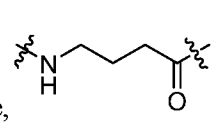
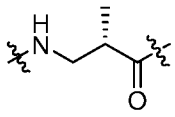
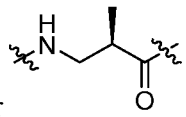
and pharmaceutically acceptable salts thereof.

[0009] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

Z_2 is a direct bond;

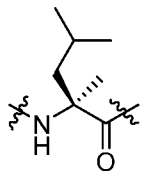
Z_4 is T, or ;

Z_5 is β-alanine, , , or ;

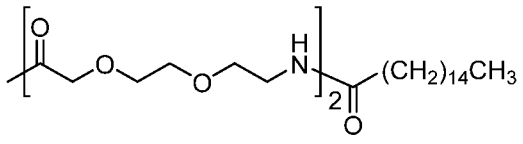
Z_6 is T;

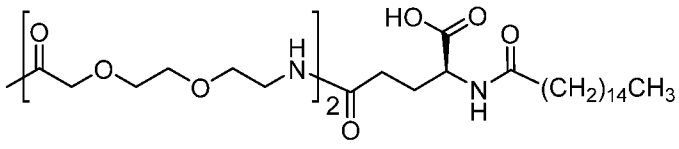
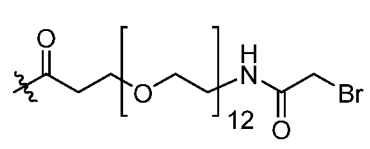
Z_{10} is Q, or E;

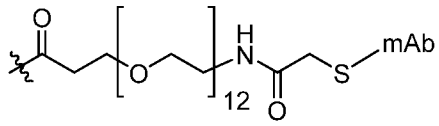
Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with $-C(=NH)NH_2$;

Z_{12} is L, or ;

Z_{16} is L;

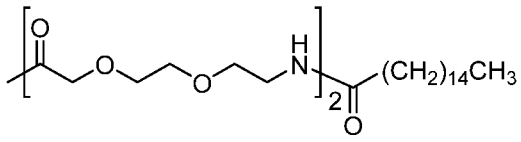
Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, $-C(O)CH_2CH=CH_2$, ,

, , or

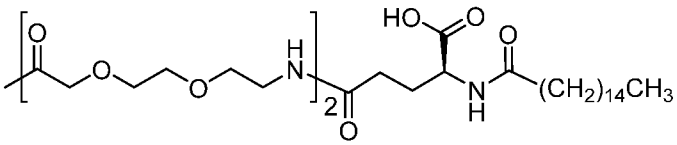


, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

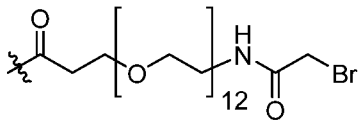
Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -C(O)$CH_2CH{=}CH_2$,



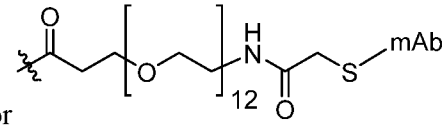
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,



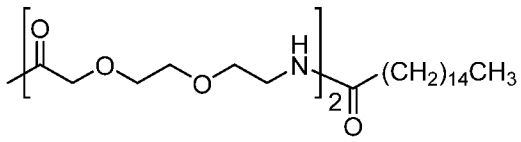
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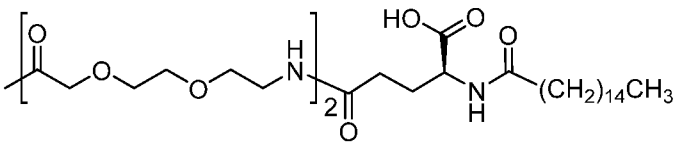
or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is ATZ$_{10}$Z$_{11}$Z$_{12}$ANFZ$_{16}$VHSSNNFGZ$_{25}$Z$_{26}$LPZ$_{29}$TNVGZ$_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

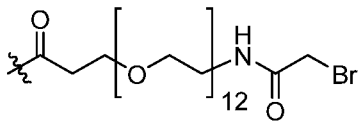
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with –C(=O)CH_3, -C(O)$CH_2CH{=}CH_2$,



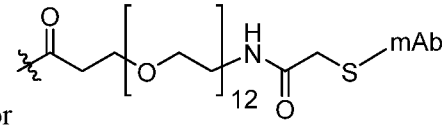
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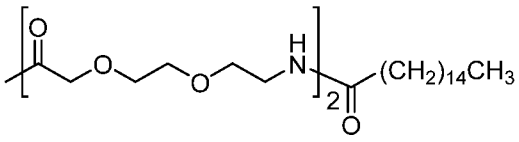
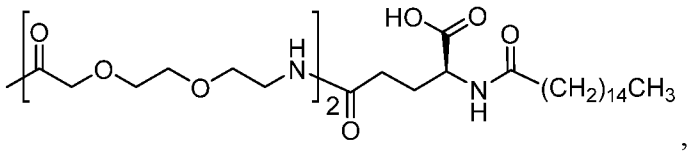
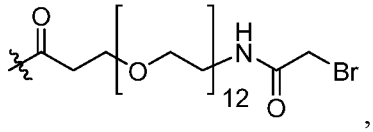
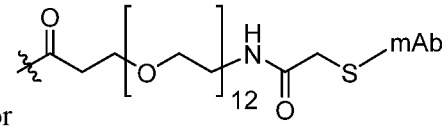
,



,



or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

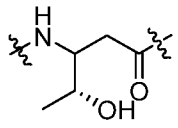
Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with–$C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, , , ,  or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

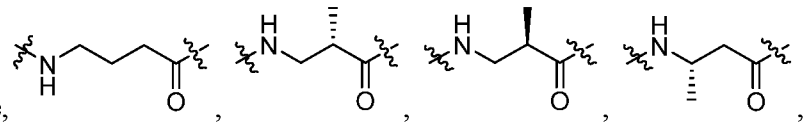
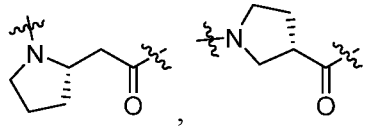
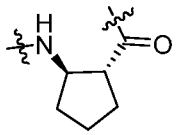
and pharmaceutically acceptable salts thereof.

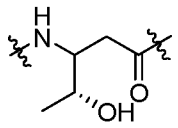
[0010] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

Z_2 is a direct bond, or serine;

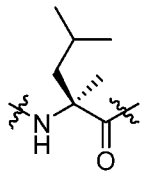
Z_4 is T, or ;

Z_5 is A, β-alanine, , , , or ;

Z_6 is T, or ;

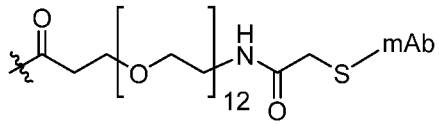
Z_{10} is Q, or E;

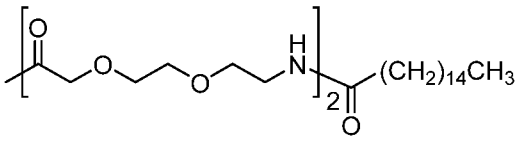
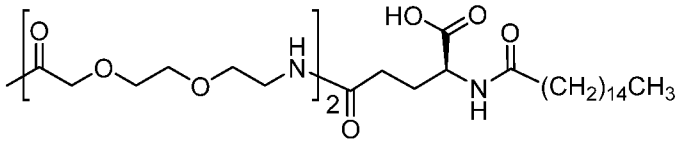
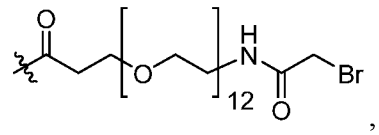
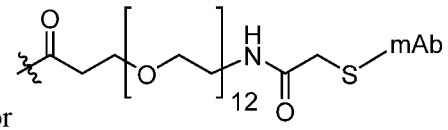
Z_{11} is R, or K, wherein the ε-amine of said K is substituted with $–C(=NH)NH_2$;

Z_{12} is L, or  ;

Z_{16} is L;

Z_{25} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, or

 , wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{26} is I, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, -C(O)$CH_2CH{=}CH_2$,  ,  ,  ,  or , wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

Z_{29} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

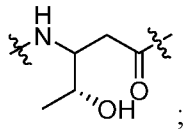
Z_{34} is S, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

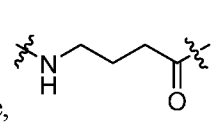
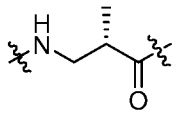
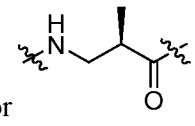
and pharmaceutically acceptable salts thereof.

[0011] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

Z_2 is a direct bond;

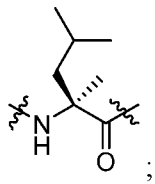
Z_4 is T, or  ;

Z_5 is β-alanine,  ,  , or  ;

Z_6 is T;

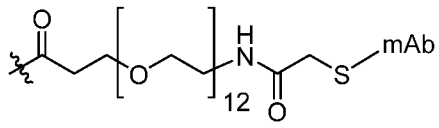
Z_{10} is Q, or E;

Z_{11} is R, or K, wherein the ε-amine of said K is substituted with $–C(=NH)NH_2$;

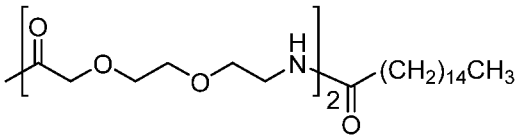
Z_{12} is L, or  ;

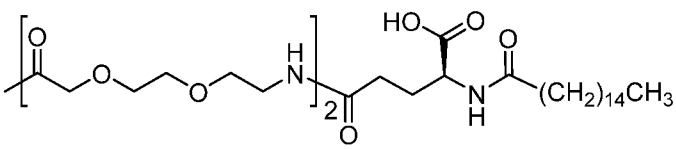
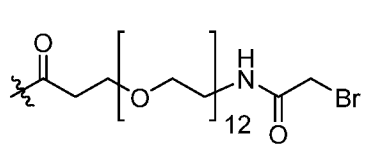
Z_{16} is L;

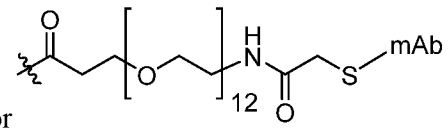
Z_{25} is P, or K, wherein the ε-amine of said K is substituted with $–C(=O)CH_3$, or

 , wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{26} is I, or K, wherein the ε-amine of said K is substituted with $–C(=O)CH_3$, -

$C(O)CH_2CH=CH_2$,  ,

 ,  ,

or  , wherein said mAb is substituted through another

thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is ATZ$_{10}$Z$_{11}$Z$_{12}$ANFZ$_{16}$VHSSNNFGZ$_{25}$Z$_{26}$LPZ$_{29}$TNVGZ$_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

Z$_{29}$ is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH$_3$;

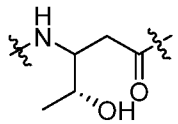
Z$_{34}$ is S, or K, wherein the ε-amine of said K is substituted with –C(=O)CH$_3$;

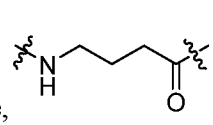
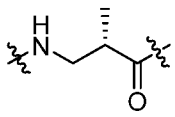
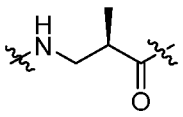
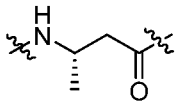
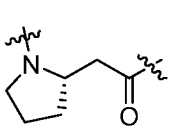
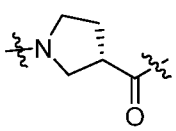
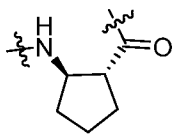
and pharmaceutically acceptable salts thereof.

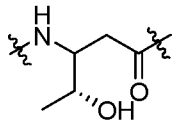
[0012] In certain embodiments, the invention is represented by a compound of Formula **I** or a derivative thereof, wherein:

n is 1, or 2;

Z$_2$ is a direct bond, or serine;

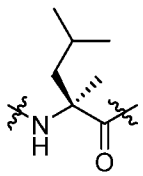
Z$_4$ is T, or ;

Z$_5$ is A, β-alanine, , , , , , , or ;

Z$_6$ is T, or ;

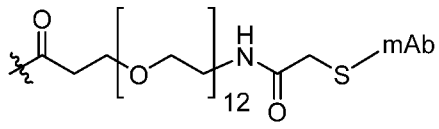
Z$_{10}$ is Q, or E;

Z$_{11}$ is R, or K, wherein the ε-amine of said K is substituted with –C(=NH)NH$_2$;

Z$_{12}$ is ;

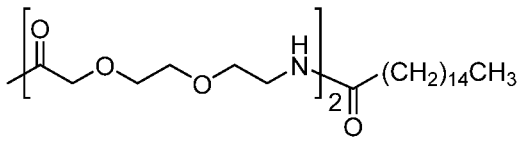
Z$_{16}$ is L;

Z_{25} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, or

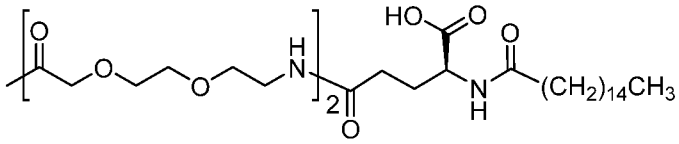


, wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

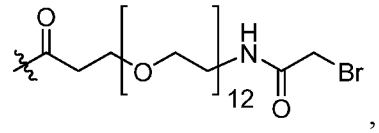
Z_{26} is I, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, -C(O)$CH_2CH{=}CH_2$,



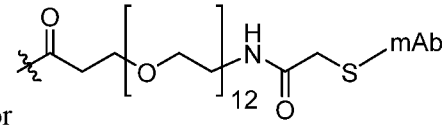
,



,



,



or , wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is ATZ10Z11Z12ANFZ16VHSSNNFGZ25Z26LPZ29TNVGZ34 (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

Z_{29} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

Z_{34} is S, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

and pharmaceutically acceptable salts thereof.

[0013] In certain embodiments, the compound is selected from the group consisting of SEQ ID NOs: 4-42, or a pharmaceutically acceptable salt thereof.

[0014] In certain embodiments, the monoclonal antibody or the antigen binding fragment thereof is covalently linked to the amylinomimetic peptide at a lysine residue of the amylinomimetic peptide via a linker. Non-limiting examples of the linker comprise aa PEG chain of 2-24 PEG units, ($OEG_{(0-4)}$-γ-Glu), ($OEG_{(1-4)}$), or an alkyl chain containing 2-10 carbon atoms, wherein said linker may include a group, such as but not limited to, acetyl.

[0015] In certain embodiments, only one of Z_{25}, Z_{26}, Z_{29} and Z_{34} in Formula **I** is lysine, and the lysine is covalently linked to an engineered cysteine residue of the monoclonal antibody or the antigen binding fragment thereof via the linker.

[0016] Another embodiment of the invention is a pharmaceutical composition comprising the compound of Formula **I**, or a compound selected from the group consisting of SEQ ID NOs:4-42, and a pharmaceutically acceptable carrier.

[0017] In certain embodiments, the monoclonal antibody or the antigen binding fragment thereof comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, and a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NO: 47, 48, 49, 50, 51, and 52, respectively. In certain embodiments, the isolated monoclonal antibody comprises a heavy chain variable domain (VH) having the polypeptide sequence of SEQ ID NO:43, and a light chain variable domain (VL) having the polypeptide sequence of SEQ ID NO:45. In certain embodiments, the isolated monoclonal antibody further comprises a Fc portion. In certain embodiments, the isolated monoclonal antibody comprises a heavy chain (HC) having the polypeptide sequence of SEQ ID NO:44 and a light chain (LC) having the polypeptide sequence of SEQ ID NO:46.

[0018] Also provided are conjugates comprising a monoclonal antibody or an antigen binding fragment thereof coupled to a amylinomimetic peptide, wherein the monoclonal antibody or the antigen binding fragment thereof comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, and a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NO: 47, 48, 49, 50, 51, and 52, respectively, preferably the monoclonal antibody or antigen binding fragment thereof comprises a heavy chain variable domain (VH) having the polypeptide sequence of SEQ ID NO:43, and a light chain variable domain (VL) having the polypeptide sequence of SEQ ID NO:45, and more preferably, the monoclonal antibody a heavy chain (HC) having the polypeptide sequence of SEQ ID NO:44 and a light chain (LC) having the polypeptide sequence of SEQ ID NO:46; the amylinomimetic peptide comprises a polypeptide sequence selected from the group consisting of SEQ ID NOs: 4-28, or a pharmaceutically acceptable salt thereof; and the monoclonal antibody or antigen binding fragment thereof is conjugated

to the amylinomimetic peptide at residue 25, 26, 29, or 34 of the amylinomimetic peptide, preferably at lysine residue 25 or 26 of the amylinomimetic peptide, directly or via a linker.

[0019] Also provided are methods of producing the conjugates of the invention. The methods comprise reacting an electrophile, preferably bromoacetamide introduced onto a sidechain of the amylinomimetic peptide, or a linker on said sidechain, preferably the sidechain of a lysine residue of the amylinomimetic peptide, with the sulfhydryl group of the cysteine residue of SEQ ID NO:49 of the monoclonal antibody or antigen-binding fragment thereof, thereby creating a covalent linkage between the amylinomimetic peptide and the monoclonal antibody or antigen-binding fragment thereof.

[0020] Also provided are pharmaceutical compositions comprising the conjugates of the invention and a pharmaceutically acceptable carrier.

[0021] Also provided are methods for treating or preventing a disease or disorder in a subject in need thereof, wherein said disease or disorder is selected from the group consisting of obesity, type I or type II diabetes, metabolic syndrome, insulin resistance, impaired glucose tolerance, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema. The methods comprise administering to the subject in need thereof an effective amount of the pharmaceutical compositions of the invention.

[0022] Also provided are methods of reducing food intake in a subject in need thereof. The methods comprise administering to the subject in need thereof an effective amount of the pharmaceutical composition of the invention.

[0023] Also provided are methods of modulating amylin receptor activity in a subject in need thereof. The methods comprise administering to the subject in need thereof an effective amount of the pharmaceutical composition of the invention.

[0024] Also provided are methods of modulating amylin receptor activity in a subject in need thereof, wherein said amylin receptor comprises AMY1R, and/or AMY2R and/or

AMY3R. The methods comprise administering to the subject in need thereof an effective amount of the pharmaceutical composition of the invention.

[0025] Also provided are methods of modulating amylin receptor activity in a subject in need thereof, wherein said amylin receptor is AMY1R.

[0026] Also provided are methods of modulating amylin receptor activity in a subject in need thereof, wherein said amylin receptor is AMY3R.

[0027] The methods comprise administering to the subject in need thereof an effective amount of the pharmaceutical composition of the invention.

[0028] In certain embodiments, the pharmaceutical composition is administered via an injection. In certain embodiments, the pharmaceutical composition is administered in a combination with at least one antidiabetic agent. The antidiabetic agent can, for example, be a glucagon-like-peptide-1 receptor modulator. In certain embodiments, the pharmaceutical composition is administered in combination with liraglutide.

[0029] Also provided are kits comprising the conjugates of the invention, preferably further comprising a liraglutide and a device for injection.

[0030] Also provided are methods of producing the pharmaceutical compositions of the invention. The methods comprise combining the conjugate with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[0031] Further aspects, features and advantages of the present invention will be better appreciated upon a reading of the following detailed description of the invention and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

DETAILED DESCRIPTION OF THE INVENTION

[0033] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein

incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0035] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

[0036] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0037] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0038] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those

elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0039] It should also be understood that the terms “about,” “approximately,” “generally,” “substantially” and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/ characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0040] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences (e.g., amylinomimetic$_{3\text{-}36}$ polypeptide sequences, antibody light chain or heavy chain sequences), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection using methods known in the art in view of the present disclosure.

[0041] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0042] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the

homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0043] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[0044] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0045] As used herein, "subject" means any animal, preferably a mammal, most preferably a human. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0046] The term "administering" with respect to the methods of the invention, means a method for therapeutically or prophylactically preventing, treating or ameliorating a syndrome, disorder or disease as described herein by using a conjugate of the invention or a form, composition or medicament thereof. Such methods include administering an effective amount of said conjugate, a form, composition or medicament thereof at different times during the course of a therapy or concurrently in a combination form. The

methods of the invention are to be understood as embracing all known therapeutic treatment regimens.

[0047] The term "effective amount" means that amount of active conjugate or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human, that is being sought by a researcher, veterinarian, medical doctor, or other clinician, which includes preventing, treating or ameliorating a syndrome, disorder, or disease being treated, or the symptoms of a syndrome, disorder or disease being treated.

[0048] As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts.

[0049] As used herein the term "coupled" refers to the joining or connection of two or more objects together. When referring to chemical or biological compounds, coupled can refer to a covalent connection between the two or more chemical or biological compounds. By way of a non-limiting example, an antibody of the invention can be coupled with a peptide of interest (e.g., amylinomimetic peptides of the invention) to form an antibody coupled peptide. In certain embodiments, an antibody of the invention can be covalently coupled with a peptide of the invention through a linker. The linker can, for example, be first covalently connected to the antibody or the peptide, then covalently connected to the peptide or the antibody. An antibody coupled peptide can be formed through specific chemical reactions designed to conjugate the antibody to the peptide. By way of an example, a mAb coupled amylinomimetic peptide conjugate can be formed through a conjugation reaction. The conjugation reaction can, for example, comprise reacting an electrophilic group (e.g., a bromoacetamide or a maleimide) with the sulfhydryl group of a cysteine residue on the mAb. The electrophilic group can, for example, be introduced onto a sidechain of an amino acid residue of an amylinomimetic peptide. The reaction of the electrophilic group with the sulfhydryl group results in the formation of a covalent thioether bond.

[0050] As used herein, the term "linker" refers to a chemical module comprising a covalent or atomic chain that covalently attaches an antibody to the peptide. The linker

can, for example, include, but is not limited to, a peptide linker, a hydrocarbon linker, a polyethylene glycol (PEG) linker, a polypropylene glycol (PPG) linker, a polysaccharide linker, a polyester linker, a hybrid linker consisting of PEG and an embedded heterocycle, and a hydrocarbon chain.

[0051] As used herein, the term "conjugate" refers to an antibody or a fragment thereof covalently coupled to a pharmaceutically active moiety. The term "conjugated to" refers to an antibody or a fragment thereof of invention covalently linked to or covalently connected to a pharmaceutically active moiety, preferably a therapeutic peptide, directly or indirectly via a linker. By way of a non-limiting example, the antibody can be a monoclonal antibody of the invention and the pharmaceutically active moiety can be a therapeutic peptide, such as a amylinomimetic peptide of interest.

[0052] Antibodies

[0053] As used herein, the term "non-targeting" in the context of an antibody refers to an antibody that does not specifically bind to any target *in vivo*. As used herein, an antibody that "specifically binds to a target" refers to an antibody that binds to a target antigen, with a KD of 1×10^{-8} M or less, preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as a Octet RED96 system. The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

[0054] Monoclonal antibodies, complete or a fragment thereof, can be used as a half-life extending moiety. Monoclonal antibodies are well-studied proteins that have been utilized and characterized for uses *in vivo*, and as such, the mechanisms that enable their protracted half-life *in vivo* and the mechanisms for their elimination *in vivo* are well understood. Additionally, the spatial separation and presentation of the two "arms" of the monoclonal antibody can be advantageous for effective bivalent presentation of a therapeutic moiety (i.e., a therapeutic peptide). Therapeutics in which toxins or other

small molecule drugs are chemically linked to a monoclonal antibody have been developed but typically utilize a monoclonal antibody that binds to a specific antigen and targets the antibody-drug conjugate to a tissue/cell of interest, which preferentially expressed the antigen, and typically the drug/small molecule is attached to the antibody in a manner that does not impact antigen binding of the antibody.

[0055] For therapeutic peptide-mAb conjugates, antigen specific binding by the half-life extending monoclonal antibody is not desired. Because of this, a heavy chain (HC) and light chain (LC) variable (V) domain pair not expected to specifically bind any target are used for preparing the coupling-enabled, non-targeting monoclonal antibody of the invention. To obtain a coupling-enabled, non-targeting monoclonal antibody, a cysteine residue is engineered into one of the complementarity determining regions (CDRs) of a selected non-targeting antibody. The pharmaceutically active moiety (e.g., therapeutic peptide/compound) can contain the appropriate chemical moiety to allow for the conjugation of the pharmaceutically active moiety to the engineered cysteine residue of the non-targeting monoclonal antibody.

[0056] The term "antibodies" as used herein is meant in a broad sense and includes non-human (e.g., murine, rat), human, human-adapted, humanized and chimeric monoclonal antibodies, antibody fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, and single chain antibodies.

[0057] Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the invention include heavy and/or light chain constant regions from mouse or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; (CDR1, CDR2, and CDR3)). The light chain variable region domains are alternatively referred to as LCDR1, LCDR2, and LCRD3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCRD2, and HCDR3.

[0058] Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgG is the most stable of the five types of immunoglobulins, having a serum half-life in humans of about 23 days. IgA and IgG are further sub-classified as the isotypes IgA_1, IgA_2, IgG_1, IgG_2, IgG_3 and IgG_4. Each of the four IgG subclasses has different biological functions known as effector functions. These effector functions are generally mediated through interaction with the Fc receptor (FcγR) or by binding C1q and fixing complement. Binding to FcγR can lead to antibody dependent cell mediated cytolysis, whereas binding to complement factors can lead to complement mediated cell lysis. An antibody of the invention utilized for its ability to extend half-life of a therapeutic peptide has no or minimal effector function, but retains its ability to bind FcRn, the binding of which can be a primary means by which antibodies have an extended *in vivo* half-life.

[0059] In certain embodiments, the invention relates to a conjugate comprising an isolated antibody or antigen binding fragment thereof comprising a light chain variable region having completely human Ig germline V gene sequences, and a heavy chain variable region having completely human Ig germline V gene sequences except HCDR3 having the amino acid sequence of SEQ ID NO:49 and a pharmaceutically active moiety (e.g., a amylinomimetic peptide of the invention) conjugated thereto, wherein the antibody or antigen binding fragment thereof does not specifically bind to any human antigen *in vivo*. In the present disclosure, with respect to an antibody or antigen binding fragment thereof according to an embodiment of the invention, the phrase "a conjugate comprising an antibody or antigen binding fragment thereof and a pharmaceutically active moiety conjugated thereto" is used interchangeably with the phrase "an antibody or antigen binding fragment thereof conjugated to a pharmaceutically active moiety."

[0060] As used herein, the term "antigen-binding fragment" refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any

other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. According to particular embodiments, the antigen-binding fragment comprises a light chain variable region, a light chain constant region, and an Fd segment (i.e., portion of the heavy chain which is included in the Fab fragment). According to other particular embodiments, the antigen-binding fragment comprises Fab and F(ab').

[0061] As used herein, the term "single-chain antibody" refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 15 to about 20 amino acids. As used herein, the term "single domain antibody" refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

[0062] The phrase "isolated antibody or antibody fragment" refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding a target antigen is substantially free of antibodies that specifically do not bind the target antigen). Moreover, an isolated antibody or antibody fragment can be substantially free of other cellular material and/or chemicals.

[0063] An antibody variable region consists of a "framework" region interrupted by three "antigen binding sites". The antigen binding sites are defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3), and three in the VL (LCDR1, LCDR2, LCDR3), are based on sequence variability (Wu and Kabat J Exp Med 132:211-50, 1970; Kabat et al Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) "Hypervariable regions," "HVR," or "HV," three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3), refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk Mol Biol 196:901-17, 1987). Other terms include "IMGT-CDRs" (Lefranc et al., Dev Comparat Immunol 27:55-77, 2003) and "Specificity Determining Residue Usage" (SDRU) (Almagro Mol Recognit 17:132-43, 2004). The International

ImMunoGeneTics (IMGT) database (http://www_mgt_org) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc et al., Dev Comparat Immunol 27:55-77, 2003.

[0064] "Framework" or "framework sequences" are the remaining sequences of a variable region other than those defined to be antigen binding sites. Because the antigen binding sites can be defined by various terms as described above, the exact amino acid sequence of a framework depends on how the antigen-binding site was defined.

[0065] In one embodiment of the invention, an isolated antibody or antigen binding fragment thereof comprises a light chain variable region having the LCDR1, LCDR2 and LCDR3 of the amino acid sequence of SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, respectively, and a heavy chain variable region having the HCDR1, HCDR2 and HCDR3 of the amino acid sequences of SEQ ID NO: 47, SEQ ID NO: 48 and SEQ ID NO: 49, respectively.

[0066] In another embodiment, the isolated antibody further comprises a Fc region derived from human IgG4 Fc region. Human IgG4 Fc region has reduced ability to bind FcγR and complement factors compared to other IgG sub-types. Preferably, the Fc region contains human IgG4 Fc region having substitutions that eliminate effector function. Thus, an isolated antibody further comprises a Fc region having a modified human IgG4 Fc region containing one or more of the following substitutions: substitution of proline for glutamate at residue 233, alanine or valine for phenylalanine at residue 234 and alanine or glutamate for leucine at residue 235 (EU numbering, Kabat, E. A. et al. (1991) *Sequences of Proteins of Immunological Interest,* 5th Ed. U.S. Dept. of Health and Human Services, Bethesda, Md., NIH Publication no. 91-3242). Removing the N-linked glycosylation site in the IgG4 Fc region by substituting Ala for Asn at residue 297 (EU numbering) is another way to ensure that residual effector activity is eliminated.

[0067] Preferably, an antibody of the invention can exist as a dimer joined together by disulfide bonds and various non-covalent interactions. Thus, the Fc portion useful for the antibody of the invention can be human IgG4 Fc region containing a substitution, such as serine to proline at position at 228 (EU numbering), that stabilizes heavy chain dimer formation and prevents the formation of half-IgG4 Fc chains.

[0068] In another embodiment, the C-terminal Lys residue in the heavy chain is removed, as commonly seen in recombinantly produced monoclonal antibodies.

[0069] "Human antibody" refers to an antibody having heavy and light chain variable regions in which both the framework and the antigen binding sites are derived from sequences of human origin. If the antibody contains a constant region, the constant region also is derived from sequences of human origin.

[0070] Human antibody comprises heavy or light chain variable regions that are "derived from" sequences of human origin if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such systems include human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice carrying human immunoglobulin loci as described herein. "Human antibody" may contain amino acid differences when compared to the human germline or rearranged immunoglobulin sequences due to for example naturally occurring somatic mutations or intentional introduction of substitutions in the framework or antigen binding sites. Typically, "human antibody" is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to an amino acid sequence encoded by a human germline or rearranged immunoglobulin gene. In some cases, "human antibody" may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik et al., J Mol Biol 296:57-86, 2000), or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi et al., J Mol Biol 397:385-96, 2010 and Intl. Pat. Publ. No. WO2009/085462). Antibodies in which antigen binding sites are derived from a non-human species are not included in the definition of "human antibody".

[0071] Isolated humanized antibodies may be synthetic. Human antibodies, while derived from human immunoglobulin sequences, may be generated using systems such as phage display incorporating synthetic CDRs and/or synthetic frameworks, or can be subjected to in vitro mutagenesis to improve antibody properties, resulting in antibodies that do not naturally exist within the human antibody germline repertoire in vivo.

[0072] The term "recombinant antibody" as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, antibodies isolated from a recombinant, combinatorial antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences, or antibodies that are generated in vitro using Fab arm exchange.

[0073] The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of a single molecular composition. The monoclonal antibodies of the invention can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

[0074] In certain embodiments, the term "mAb" refers to a monoclonal antibody having a variable heavy chain (VH) sequence comprising SEQ ID NO:43 and a variable light chain (VL) sequence comprising SEQ ID NO:45. In certain embodiments the mAb is a fully human monoclonal antibody having a heavy chain (HC) sequence comprising SEQ ID NO:44 and a light chain (LC) sequence comprising SEQ ID NO:46. In certain embodiments, the lysine residue at position 446 of SEQ ID NO:44 is optionally missing.

[0075] As used herein, the term "chimeric antibody" refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antibody derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antibody derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

[0076] As used herein, the term "multispecific antibody" refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a

first epitope or comprises germline sequences lacking any known binding specificity and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope or comprises germline sequences lacking any known binding specificity, and wherein the first and/or second immunoglobulin variable domain optionally include a conjugated pharmaceutically active moiety (e.g., a therapeutic peptide). In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, the first and second immunoglobulin variable domains include the same conjugated pharmaceutically active moiety. In an embodiment, the first and second immunoglobulin variable domains include different pharmaceutically active moieties. In an embodiment, only the first immunoglobulin variable domain includes a conjugated pharmaceutically active moiety. In an embodiment, only the second immunoglobulin variable domain includes a conjugated pharmaceutically active moiety. In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody, or a tetraspecific antibody molecule.

[0077] As used herein, the term "bispecific antibody" refers to a multispecific antibody that binds no more than two epitopes or two antigens and/or comprises two conjugated pharmaceutically active moieties (e.g., the same or different pharmaceutically active moiety). A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope or comprises germline sequences lacking any known binding specificity and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope or comprises germline sequences lacking any known binding specificity, and wherein the first and/or second immunoglobulin variable domain optionally include a conjugated pharmaceutically active moiety. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and

second epitopes overlap or substantially overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, the first and second immunoglobulin variable domains include the same conjugated pharmaceutically active moiety. In an embodiment, the first and second immunoglobulin variable domains include different pharmaceutically active moieties. In an embodiment, only the first immunoglobulin variable domain includes a conjugated pharmaceutically active moiety. In an embodiment, only the second immunoglobulin variable domain includes a conjugated pharmaceutically active moiety. In an embodiment a bispecific antibody comprises a first heavy chain variable domain sequence and light chain variable domain sequence which have binding specificity for a first epitope or comprise germline sequences lacking any known binding specificity and a second heavy chain variable domain sequence and light chain variable domain sequence which have binding specificity for a second epitope or comprise germline sequences lacking any known binding specificity, and wherein the first and/or second heavy chain variable domains optionally include a conjugated pharmaceutically active moiety. In an embodiment, the first and second heavy chain variable domains include the same conjugated pharmaceutically active moiety. In an embodiment, the first and second heavy chain variable domains include different conjugated pharmaceutically active moieties. In an embodiment, only the first heavy chain variable domain includes a conjugated pharmaceutically active moiety. In an embodiment, only the second heavy chain variable domain includes a conjugated pharmaceutically active moiety.

[0078] "Full length antibody" as used herein refers to an antibody having two full length antibody heavy chains and two full length antibody light chains. A full-length antibody heavy chain (HC) consists of well-known heavy chain variable and constant domains VH, CH1, CH2, and CH3. A full-length antibody light chain (LC) consists of well-known light chain variable and constant domains VL and CL. The full-length antibody may be lacking the C-terminal lysine (K) in either one or both heavy chains.

[0079] The term "Fab-arm" or "half molecule" refers to one heavy chain-light chain pair that specifically binds an antigen.

[0080] Full length bispecific antibodies can be generated for example using Fab arm exchange (or half molecule exchange) between two monospecific bivalent antibodies by introducing substitutions at the heavy chain CH3 interface in each half molecule to favor heterodimer formation of two antibody half molecules having distinct specificity either *in vitro* in cell-free environment or using co-expression. The Fab arm exchange reaction is the result of a disulfide-bond isomerization reaction and dissociation-association of CH3 domains. The heavy-chain disulfide bonds in the hinge regions of the parent monospecific antibodies are reduced. The resulting free cysteines of one of the parent monospecific antibodies form an inter heavy-chain disulfide bond with cysteine residues of a second parent monospecific antibody molecule and simultaneously CH3 domains of the parent antibodies release and reform by dissociation-association. The CH3 domains of the Fab arms may be engineered to favor heterodimerization over homodimerization. The resulting product is a bispecific antibody having two Fab arms or half molecules which each can bind a distinct epitope.

[0081] "Homodimerization" as used herein, with respect to the antibodies, refers to an interaction of two heavy chains having identical CH3 amino acid sequences. "Homodimer" as used herein, with respect to the antibodies, refers to an antibody having two heavy chains with identical CH3 amino acid sequences.

[0082] "Heterodimerization" as used herein, with respect to the antibodies, refers to an interaction of two heavy chains having non-identical CH3 amino acid sequences. "Heterodimer" as used herein, with respect to the antibodies, refers to an antibody having two heavy chains with non-identical CH3 amino acid sequences.

[0083] The "knob-in-hole" strategy (see, e.g., PCT Intl. Publ. No. WO 2006/028936) can be used to generate full length bispecific antibodies. Briefly, selected amino acids forming the interface of the CH3 domains in human IgG can be mutated at positions affecting CH3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically binding a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically binding a second antigen. After co-expression of the two antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a "hole" with the heavy chain with a "knob". Exemplary CH3

substitution pairs forming a knob and a hole are (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain): T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

[0084] Other strategies such as promoting heavy chain heterodimerization using electrostatic interactions by substituting positively charged residues at one CH3 surface and negatively charged residues at a second CH3 surface may be used, as described in US Pat. Publ. No. US2010/0015133; US Pat. Publ. No. US2009/0182127; US Pat. Publ. No. US2010/028637 or US Pat. Publ. No. US2011/0123532. In other strategies, heterodimerization may be promoted by following substitutions (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain): L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in U.S. Pat. Publ. No. US2012/0149876 or U.S. Pat. Publ. No. US2013/0195849.

[0085] In addition to methods described above, bispecific antibodies can be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Intl. Pat. Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promoter heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2-mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably

a reducing agent selected from the group consisting of: 2-mercaptoethylamine, dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20° C. in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

[0086] The numbering of amino acid residues in the antibody constant region throughout the specification is performed according to the EU index as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), unless otherwise explicitly stated.

[0087] Conjugates

[0088] In another general aspect, the invention relates to a conjugate comprising an antibody of the invention covalently conjugated to a pharmaceutically active moiety, such as a synthetic therapeutic peptide (e.g., an amylinomimetic peptide), in a site-specific manner, such that the antibody coupled peptide has an extended/increased half-life compared to the peptide alone. The invention also relates to pharmaceutical compositions and methods for use thereof. The conjugates are useful for preventing, treating, or ameliorating diseases or disorders, such as obesity, type 2 diabetes, metabolic syndrome (i.e., Syndrome X), insulin resistance, impaired glucose tolerance (e.g., glucose intolerance), hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema, among others.

[0089] In certain embodiments, the antibody of the invention is modified to comprise at least one cysteine residue substitution that is capable of being conjugated to the pharmaceutically active moiety to extend/increase the half-life of the pharmaceutically active moiety. In certain embodiments, the at least one cysteine residue substitution is comprised in a complementarity determining region of the antibody. In certain embodiments, the at least one cysteine residue substitution is in a heavy chain

complementarity determining region (HCDR). In certain embodiments, the at least one cysteine residue substitution is in an HCDR3, wherein the HCDR3 comprises an amino acid sequence of SEQ ID NO:49. In certain embodiments, the antibody comprising an HCDR3 comprising an amino acid sequence of SEQ ID NO:49 has at least one additional cysteine substitution that is capable of being conjugated to a pharmaceutically active moiety.

[0090] In certain embodiments the pharmaceutically active moiety can comprise a linker. The linker can be modified chemically to allow for the conjugation of the antibody to the pharmaceutically active moiety. The linker can, for example, include, but is not limited to, a peptide linker, a hydrocarbon linker, a polyethylene glycol (PEG) linker, a polypropylene glycol (PPG) linker, a polysaccharide linker, a polyester linker, a hybrid linker consisting of PEG and an embedded heterocycle, or a hydrocarbon chain. The PEG linkers can, for example, comprise 2-24 PEG units.

[0091] In certain embodiments, a monoclonal antibody of the invention is conjugated to one, two, three, four, five, or six pharmaceutically active moieties (e.g., therapeutic peptide(s)) of interest. In preferred embodiments, the non-targeting monoclonal antibody is conjugated to two pharmaceutically active moieties of interest. In certain embodiments where the monoclonal antibody is conjugated to at least two pharmaceutically active moieties of interest, the pharmaceutically active moieties of interest can be the same pharmaceutically active moiety or can be different pharmaceutically active moieties.

[0092] Methods of conjugating antibodies of the invention with the pharmaceutically active moieties of the invention are known in the art. Briefly, the antibodies of the invention can be reduced with a reducing agent (e.g., TCEP (tris(2-carboxyethyl) phosphine), purified (e.g., by protein A adsorption or gel filtration), and conjugated with the pharmaceutically active moiety (e.g., by providing a lyophilized peptide to the reduced antibody under conditions that allow for conjugation). After the conjugation reaction, the conjugate can be purified by ion exchange chromatography or hydrophobic interaction chromatography (HIC) with a final purification step of protein A adsorption. In certain embodiments, the antibodies of the invention can be purified prior to being reduced utilizing HIC methods. For more detailed description of the conjugation methods, see, e.g., Example 103 and Dennler et al., Antibodies 4:197-224 (2015).

[0093] In certain embodiments, the amylinomimetic peptide is a derivative of the amylinomimetic peptide of Formula **I** that is modified by one or more processes selected from the group consisting amidation, lipidation, and pegylation, or a pharmaceutically acceptable salt thereof.

[0094] In certain embodiments, a conjugate comprises a monoclonal antibody or a fragment thereof conjugated to an amylinomimetic peptide, wherein the amylinomimetic peptide is selected from the group consisting of SEQ ID NOs: 4-28.

[0095] In certain embodiments, a monoclonal antibody or the antigen binding fragment thereof is covalently linked to the amylinomimetic peptide at a lysine residue of the amylinomimetic peptide via a linker. The linker can, for example, comprise a linker selected from the group consisting of a PEG chain of 2-24 PEG units, an alkyl chain containing 2-10 carbon atoms, or a bond.

[0096] In certain embodiments, only one of Z_{25}, Z_{26}, Z_{29} and Z_{34} in Formula **I** is lysine, and the lysine is covalently linked to an engineered cysteine residue of the monoclonal antibody or the antigen binding fragment thereof via the linker. In a preferred embodiment, a monoclonal antibody or the antigen binding fragment thereof according to an embodiment of the invention is conjugated to an amylinomimetic peptide at residue 25 or 26 of the amylinomimetic. In another preferred embodiment, an electrophile, such as bromoacetamide is introduced onto a sidechain of a amylinomimetic, such as the amino side chain of a lysine at residue 25 or 26 of the amylinomimetic, and the electrophile reacts site specifically with the sulfhydryl group of the Cys residue engineered into a CDR, preferably HCDR3, of the monoclonal antibody or fragment thereof, thereby creating a covalent linkage between the amylinomimetic peptide and the monoclonal antibody or fragment thereof. More preferably, the amylinomimetic peptide is selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 19, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28. In one embodiment, the electrophile is introduced onto the sidechain of an amylinomimetic directly. In another embodiment, the electrophile is introduced onto the sidechain of an amylinomimetic indirectly via a linker.

[0097] Also provided are pharmaceutical compositions comprising the conjugates of the invention and further comprising a pharmaceutically acceptable carrier.

[0098] Also provided herein are amylinomimetic peptides exhibiting at least 70%, 75% 80%, 85%, 90%, 95%, or 99% sequence identity to pramlintide or davalintide. As an example of a method for determination of the sequence identity between two analogues the two peptides, pramlintide (SEQ ID NO: 2) and (SEQ ID NO: 4) are aligned.



Pramlintide (SEQ ID NO: 2)



(SEQ ID NO: 4)

[0099] The sequence identity of the analogue relative to pramlintide is given by the total number of aligned residues minus the number of different residues (i.e. the number of aligned identical residues) divided by the total number of residues in pramlintide. In this example the different residues are K1 and C2 which are absent. N3 is now substituted, but is retained in its original position. Accordingly, in said example the sequence identity is (37-2)/37 X 100.

[00100] Where the compounds according to this invention are amylinomimetic peptides coupled to a mAb, it is expected that said mAb will have two copies of said peptide coupled to it. It is understood by those skilled in the art that reaction products may include partial conjugation products, resulting in one amylinomimetic peptide coupled to the mAb. It is to be understood that all such mono-coupled compounds, di-coupled compounds and mixtures thereof are encompassed within the scope of the present invention.

Amylinomimetic peptides

[00101] Given its role in controlling appetite and food intake, pramlintide and/or davalintide may be effective in treating obesity. However, the therapeutic utility of pramlintide and/or davalintide as a treatment agent is limited by its rapid metabolism and short circulating half-life. Thus, the present invention is generally directed to modified

pramlintide and/or davalintide conjugates, which extend the half-life of the amylinomimetic peptide and reduce the metabolism of the peptide in vivo.

[00102] In certain embodiments of the invention, the modified pramlintide and/or davalintide peptides are amylinomimetic peptides. The terms "amylinomimetic peptide," "amylinomimetic analog," and "amylinomimetic peptide analog" can be used interchangeably.

[00103] The peptide sequences described herein are written according to the usual convention whereby the N-terminal region of the peptide is on the left and the C-terminal region is on the right. Although isomeric forms of the amino acids are known, it is the L-form of the amino acid that is represented unless otherwise expressly indicated. For convenience in describing the molecules of this invention, conventional and non-conventional abbreviations for various amino acids (both single and three-letter codes) and functional moieties are used. These abbreviations are familiar to those skilled in the art, but for clarity are listed as follows: A = Ala = alanine; R = Arg = arginine; N = Asn = asparagine; D = Asp = aspartic acid; βA = βAla = beta-alanine; C = Cys = cysteine; hC = hCys = homocysteine; E = Glu = glutamic acid; Q = Gln = glutamine; G = Gly = glycine; H = His = histidine; I = Ile = isoleucine; L = Leu = leucine; K = Lys = lysine; Nle = norleucine; F = Phe = phenylalanine; P = Pro = proline; S = Ser = serine; T = Thr = threonine; W = Trp = tryptophan; Y = Tyr = tyrosine and V = Val = valine.

[00104] Additional amino acid abbreviations used herein are listed as follows: ACPC = 2-aminocyclopentanecarboxylic acid; β-Aib = β-aminoisobutyric acid = 3-amino-2-methylpropionic acid; β-hA = β-hAla = β-homoalanine = 3-aminobutyric acid; α-MeL = α-MeLeu = α-methylleucine; β^3–hP = β^3–hPro = β^3–homoproline; β-hT = β–hThr = β-homothreonine;

[00105] For convenience, the amino acid residue numbering convention used in naming the amylinomimetic peptides of the present invention follows that of pramlintide and/or davalintide. Specific amino acid replacements that have been introduced into the peptides, relative to the native residues at the corresponding positions in pramlintide and/or davalintide, are indicated by the appropriate amino acid code, followed by the position of the substitution. Thus, "hC7" in the amylinomimetic peptide refers to a peptide in which homocysteine has replaced the corresponding native Cys7 residue of

pramlintide. Similarly, "K(Ac)26" in the amylinomimetic peptide refers to a peptide in which lysine substituted on the ε–amine with $CH_3C(O)$- has replaced the corresponding native Ile26 residue of pramlintide. Additional amino acid replacements occurring within amylinomimetic peptides are described according to this convention and will be recognized as such by one skilled in the art.

[00106] Also for convenience, the naming convention used for the amylinomimetic peptides of the present invention incorporates the amino residues involved in the cycle along with the linking group(s) between them in a left-to-right direction, starting from the N-terminal residue involved in the cycle. In all cases, the N-terminal amino acid residue of the cycle links by way of its α–amino functionality to an acetyl linking group, which in turn connects to the thiol side chain residue of the amino acid at position 7 of the amylinomimetic peptide. Thus, "cyclo-(N3-$COCH_2$- hC7)" is used to describe the cycle of an amylinomimetic peptide in which native Lys1 and Cys2 residues have been deleted from the sequence, and the α–amino functionality of Asn3 is acylated with an acetyl residue, whose methyl group is further linked by way of a thioether bond to the side chain of a hCys7 residue. Similarly, "cyclo-(S2- $COCH_2$- hC7)" is used to describe the cycle of an amylinomimetic peptide, in which the native Lys1 residue has been deleted, the native Cys2 residue has been replaced by Ser2 whose α-amino functionality is acylated by an acetyl group, which in turn, is linked by way of a thioether bond to the side chain of a hCys7 residue.

[00107] Lysine residues can be incorporated at various positions of the amylinomimetic sequence to provide a convenient functional handle for further derivatization. The lysine residues can be modified to be coupled to the monoclonal antibody either directly or indirectly. In an indirect coupling to the monoclonal antibody, the lysine residue can be modified to comprise a linker which will allow for the amylinomimetic peptide to be coupled to the monoclonal antibody. One skilled in the art will recognize that related orthologues could also be effectively employed as such and are contemplated herein.

[00108] The term, "K(γ-Glu)", appearing in the peptide sequence, represents a lysinyl residue whose side chain ε–amino group has been acylated by the γ-carboxyl group of glutamic acid.

[00109] The term, "K(Ac)" represents a lysinyl residue whose side chain ε-amino group has been substituted with an acetyl group.

[00110] The term, "K(Alloc)" represents a lysinyl residue whose side chain ε-amino group has been substituted with an allyloxycarbonyl group.

[00111] The term, "K(OEG_2-Pal)" represents a lysinyl residue whose side chain ε–amino group has been substituted with 17-amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanoic acid, wherein the 17-amino group is further substituted with palmitic acid, by means of an amide bond between the 17-amino group and the palmitic acid.

[00112] The term, "$(OEG)_2$" represents two OEG units linked together in succession via an amide linkage (i.e., 17-amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanoic acid).

[00113] The term, "K$(OEG)_2$" represents a lysinyl residue whose side chain ε-amino group has been acylated by 17-amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanoic acid.

[00114] The term, "K(OEG_2-γ-Glu-Pal)" represents a lysinyl residue whose side chain ε-amino group has been acylated by (22*S*)-22-amino-10,19-dioxo-3,6,12,15-tetraoxa-9,18-diazatricosanedioic acid via its 1-carboxylic acid functionality, and wherein said 22-amino group is further amidated with palmitic acid.

[00115] The term, "dPEGx" refers to a discrete oligomer containing x ethyleneglycol units linked to propanoic acid at one end and containing a terminal amino functionality on the distal end, which may be further functionalized.

[00116] The term, "K(dPEG12)" represents a lysinyl residue whose side chain ε-amino group has been acylated by 1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid via its 39-carboxylic acid functionality.

[00117] The term, "K(dPEG12-AcBr)" represents a lysinyl residue whose side chain ε-amino group has been acylated by 1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid via its 39-carboxylic acid functionality, and wherein said acid is amidated on its 1-amine group with a -$C(O)CH_2Br$ group.

[00118] **Half-life extending moieties**

[00119] In addition to the antibody of the present invention or an antigen binding fragment thereof, the conjugates of the invention can incorporate one or more other moieties for extending the half-life of the pharmaceutical active moiety (e.g., the

amylinomimetic peptide), for example via covalent interaction. Exemplary other half-life extending moieties include, but not limited to, albumin, albumin variants, albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof. Additional half-life extending moieties that can be incorporated into the conjugates of the invention include, for example, polyethylene glycol (PEG) molecules, such as PEG5000 or PEG20,000, fatty acids and fatty acid esters of different chain lengths, for example laurate, myristate, stearate, arachidate, behenate, oleate, arachidonate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like, polylysine, octane, carbohydrates (dextran, cellulose, oligo- or polysaccharides) for desired properties. These moieties can be direct fusions with the protein scaffold coding sequences and can be generated by standard cloning and expression techniques. Alternatively, well known chemical coupling methods can be used to attach the moieties to recombinantly and chemically produced conjugates of the invention.

[00120] A pegyl moiety can, for example, be added to the peptide molecules of the invention by incorporating a cysteine residue to the C-terminus of the molecule and attaching a pegyl group to the cysteine using well known methods.

[00121] Peptide molecules of the invention incorporating additional moieties can be compared for functionality by several well-known assays. For example, the biological or pharmacokinetic activities of a therapeutic peptide of interest, alone or in a conjugate according to the invention, can be assayed using known in vitro or in vivo assays and compared.

[00122] **Pharmaceutical Compositions**

[00123] In another general aspect, the invention relates to a pharmaceutical composition, comprising the conjugates and compounds of the invention and a pharmaceutically acceptable carrier. The term “pharmaceutical composition” as used herein means a product comprising a conjugate of the invention together with a pharmaceutically acceptable carrier. Conjugates and compounds of the invention and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[00124] As used herein, the term “carrier” refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal

encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term "pharmaceutically acceptable carrier" refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody pharmaceutical composition can be used in the invention.

[00125] Pharmaceutically acceptable acidic/anionic salts for use in the invention include, and are not limited to acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glyceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, pamoate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate and triethiodide. Organic or inorganic acids also include, and are not limited to, hydriodic, perchloric, sulfuric, phosphoric, propionic, glycolic, methanesulfonic, hydroxyethanesulfonic, oxalic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, saccharinic or trifluoroacetic acid.

[00126] Pharmaceutically acceptable basic/cationic salts include, and are not limited to aluminum, 2-amino-2-hydroxymethyl-propane-1,3-diol (also known as tris(hydroxymethyl)aminomethane, tromethane or "TRIS"), ammonia, benzathine, t-butylamine, calcium, chloroprocaine, choline, cyclohexylamine, diethanolamine, ethylenediamine, lithium, L-lysine, magnesium, meglumine, N-methyl-D-glucamine, piperidine, potassium, procaine, quinine, sodium, triethanolamine, or zinc.

[00127] In some embodiments of the invention, pharmaceutical formulations are provided comprising the conjugates of the invention in an amount from about 0.001 mg/ml to about 100 mg/ml, from about 0.01 mg/ml to about 50 mg/ml, or from about 0.1

mg/ml to about 25 mg/ml. The pharmaceutical formulation may have a pH from about 3.0 to about 10, for example from about 3 to about 7, or from about 5 to about 9. The formulation may further comprise at least one ingredient selected from the group consisting of a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizer(s) and surfactant(s).

[00128] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier may be used in formulating the pharmaceutical compositions of the invention.

[00129] In one embodiment of the invention, the pharmaceutical composition is a liquid formulation. A preferred example of a liquid formulation is an aqueous formulation, i.e., a formulation comprising water. The liquid formulation may comprise a solution, a suspension, an emulsion, a microemulsion, a gel, and the like. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 75%, 80%, 85%, 90%, or at least 95% w/w of water.

[00130] In one embodiment, the pharmaceutical composition may be formulated as an injectable which can be injected, for example, via an injection device (e.g., a syringe or an infusion pump). The injection may be delivered subcutaneously, intramuscularly, intraperitoneally, or intravenously, for example.

[00131] In another embodiment, the pharmaceutical composition is a solid formulation, e.g., a freeze-dried or spray-dried composition, which may be used as is, or whereto the physician or the patient adds solvents, and/or diluents prior to use. Solid dosage forms may include tablets, such as compressed tablets, and/or coated tablets, and capsules (e.g., hard or soft gelatin capsules). The pharmaceutical composition may also be in the form of sachets, dragees, powders, granules, lozenges, or powders for reconstitution, for example.

[00132] The dosage forms may be immediate release, in which case they may comprise a water-soluble or dispersible carrier, or they may be delayed release, sustained release,

or modified release, in which case they may comprise water-insoluble polymers that regulate the rate of dissolution of the dosage form in the gastrointestinal tract.

[00133] In other embodiments, the pharmaceutical composition may be delivered intranasally, intrabuccally, or sublingually.

[00134] The pH in an aqueous formulation can be between pH 3 and pH 10. In one embodiment of the invention, the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention, the pH of the formulation is from about 3.0 to about 7.0.

[00135] In another embodiment of the invention, the pharmaceutical composition comprises a buffer. Non-limiting examples of buffers include: arginine, aspartic acid, bicine, citrate, disodium hydrogen phosphate, fumaric acid, glycine, glycylglycine, histidine, lysine, maleic acid, malic acid, sodium acetate, sodium carbonate, sodium dihydrogen phosphate, sodium phosphate, succinate, tartaric acid, tricine, and tris(hydroxymethyl)-aminomethane, and mixtures thereof. The buffer may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific buffers constitute alternative embodiments of the invention.

[00136] In another embodiment of the invention, the pharmaceutical composition comprises a preservative. Non-limiting examples of buffers include: benzethonium chloride, benzoic acid, benzyl alcohol, bronopol, butyl 4-hydroxybenzoate, chlorobutanol, chlorocresol, chlorohexidine, chlorphenesin, o-cresol, m-cresol, p-cresol, ethyl 4-hydroxybenzoate, imidurea, methyl 4-hydroxybenzoate, phenol, 2-phenoxyethanol, 2-phenylethanol, propyl 4-hydroxybenzoate, sodium dehydroacetate, thiomerosal, and mixtures thereof. The preservative may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific preservatives constitute alternative embodiments of the invention.

[00137] In another embodiment of the invention, the pharmaceutical composition comprises an isotonic agent. Non-limiting examples of the embodiment include a salt (such as sodium chloride), an amino acid (such as glycine, histidine, arginine, lysine,

isoleucine, aspartic acid, tryptophan, and threonine), an alditol (such as glycerol, 1,2-propanediol propyleneglycol), 1,3-propanediol, and 1,3-butanediol), polyethyleneglycol (e.g. PEG400), and mixtures thereof. Another example of an isotonic agent includes a sugar. Non-limiting examples of sugars may be mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, alpha and beta- HPCD, soluble starch, hydroxyethyl starch, and sodium carboxymethylcellulose. Another example of an isotonic agent is a sugar alcohol, wherein the term "sugar alcohol" is defined as a C(4-8) hydrocarbon having at least one —OH group. Non-limiting examples of sugar alcohols include mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. Pharmaceutical compositions comprising each isotonic agent listed in this paragraph constitute alternative embodiments of the invention. The isotonic agent may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific isotonic agents constitute alternative embodiments of the invention.

[00138] In another embodiment of the invention, the pharmaceutical composition comprises a chelating agent. Non-limiting examples of chelating agents include citric acid, aspartic acid, salts of ethylenediaminetetraacetic acid (EDTA), and mixtures thereof. The chelating agent may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific chelating agents constitute alternative embodiments of the invention.

[00139] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer. Non-limiting examples of stabilizers include one or more aggregation inhibitors, one or more oxidation inhibitors, one or more surfactants, and/or one or more protease inhibitors.

[00140] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer, wherein said stabilizer is carboxy-/hydroxycellulose and derivates thereof (such as HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, 2-methylthioethanol, polyethylene glycol (such as PEG 3350), polyvinyl alcohol (PVA), polyvinyl

pyrrolidone, salts (such as sodium chloride), sulphur-containing substances such as monothioglycerol), or thioglycolic acid. The stabilizer may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific stabilizers constitute alternative embodiments of the invention.

[00141] In further embodiments of the invention, the pharmaceutical composition comprises one or more surfactants, preferably a surfactant, at least one surfactant, or two different surfactants. The term "surfactant" refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant may, for example, be selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants. The surfactant may be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific surfactants constitute alternative embodiments of the invention.

[00142] In a further embodiment of the invention, the pharmaceutical composition comprises one or more protease inhibitors, such as, e.g., EDTA, and/or benzamidine hydrochloric acid (HCl). The protease inhibitor may be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific protease inhibitors constitute alternative embodiments of the invention.

[00143] The pharmaceutical composition of the invention may comprise an amount of an amino acid base sufficient to decrease aggregate formation of the polypeptide during storage of the composition. The term "amino acid base" refers to one or more amino acids (such as methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), or analogues thereof. Any amino acid may be present either in its free base form or in its salt form. Any stereoisomer (i.e., L, D, or a mixture thereof) of the amino acid base may be present. The amino acid base may be present individually or in the combination with other amino acid bases, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific amino acid bases constitute alternative embodiments of the invention.

[00144] It is also apparent to one skilled in the art that the therapeutically effective dose for conjugates of the present invention or a pharmaceutical composition thereof will vary according to the desired effect. Therefore, optimal dosages to be administered may be readily determined by one skilled in the art and will vary with the particular conjugate used, the mode of administration, the strength of the preparation, and the advancement of the disease condition. In addition, factors associated with the particular subject being treated, including subject age, weight, diet and time of administration, will result in the need to adjust the dose to an appropriate therapeutic level.

[00145] For all indications, the conjugates of the invention are preferably administered peripherally at a dose of about 1 μg to about 50 mg per day in single or divided doses (e.g., a single dose can be divided into 2, 3, 4, 5, 6, 7, 8, 9, or 10 sub-doses), or at about 0.01 μg/kg to about 500 μg/kg per dose, more preferably about 0.05 μg/kg to about 250 μg/kg, most preferably below about 50 μg/kg. Dosages in these ranges will vary with the potency of each agonist, of course, and are readily determined by one of skill in the art. The above dosages are thus exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

[00146] In certain embodiments, the conjugates of the invention are administered at a dose of about 1 μg to about 5 mg, or at a dose of about 0.01 μg/kg to about 500 μg/kg, more preferably at a dose of about 0.05 μg/kg to about 250 μg/kg, most preferably at a dose below about 50 μg/kg with a dose of a second therapeutic agent (e.g., liraglutide) at a dose of about 1 μg to about 5 mg, or at a dose of about 0.01 μg/kg to about 500 μg/kg, more preferably at a dose of about 0.05 μg/kg to about 250 μg/kg, most preferably at a dose below about 50 μg/kg.

[00147] The pharmaceutically-acceptable salts of the conjugates of the invention include the conventional non-toxic salts or the quaternary ammonium salts which are formed from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, benzoate, benzenesulfonate, citrate, camphorate, dodecylsulfate, hydrochloride, hydrobromide, lactate, maleate, methanesulfonate, nitrate, oxalate, pivalate, propionate, succinate, sulfate and tartrate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as

calcium and magnesium salts, salts with organic bases such as dicyclohexylamino salts and salts with amino acids such as arginine. Also, the basic nitrogen-containing groups may be quaternized with, for example, alkyl halides.

[00148] The pharmaceutical compositions of the invention may be administered by any means that accomplish their intended purpose. Examples include administration by parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, buccal or ocular routes. Administration may be by the oral route. Suitable formulations for parenteral administration include aqueous solutions of the active conjugates in water-soluble form, for example, water-soluble salts, acidic solutions, alkaline solutions, dextrose-water solutions, isotonic carbohydrate solutions and cyclodextrin inclusion complexes.

[00149] The present invention also encompasses a method of making a pharmaceutical composition comprising mixing a pharmaceutically acceptable carrier with any of the conjugates of the present invention. Additionally, the present invention includes pharmaceutical compositions made by mixing one or more pharmaceutically acceptable carriers with any of the conjugates of the present invention.

[00150] Furthermore, the conjugates of the present invention may have one or more polymorph or amorphous crystalline forms and as such are intended to be included in the scope of the invention. In addition, the conjugates may form solvates, for example with water (i.e., hydrates) or common organic solvents. As used herein, the term "solvate" means a physical association of the conjugates of the present invention with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances, the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. The term "solvate" is intended to encompass both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like.

[00151] It is intended that the present invention include within its scope polymorphs and solvates of the conjugates of the present invention. Thus, in the methods of treatment of the present invention, the term “administering” shall encompass the means for treating, ameliorating or preventing a syndrome, disorder or disease described herein with the

conjugates of the present invention or a polymorph or solvate thereof, which would obviously be included within the scope of the invention albeit not specifically disclosed.

[00152] In another embodiment, the invention relates to the conjugates of the invention for use as a medicament.

[00153] The present invention includes within its scope prodrugs of the conjugates of this invention. In general, such prodrugs will be functional derivatives of the conjugates which are readily convertible *in vivo* into the required conjugate. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various disorders described with the conjugate specifically disclosed or with a conjugate which may not be specifically disclosed, but which converts to the specified conjugate *in vivo* after administration to the patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", Ed. H. Bundgaard, Elsevier, 1985.

[00154] Furthermore, it is intended that within the scope of the present invention, any element, in particular when mentioned in relation to the conjugates of the invention, shall comprise all isotopes and isotopic mixtures of said element, either naturally occurring or synthetically produced, either with natural abundance or in an isotopically enriched form. For example, a reference to hydrogen includes within its scope ^{1}H, ^{2}H (D), and ^{3}H (T). Similarly, references to carbon and oxygen include within their scope respectively 12C, ^{13}C and ^{14}C and ^{16}O and ^{18}O. The isotopes may be radioactive or non-radioactive. Radiolabeled conjugates of the invention may comprise a radioactive isotope selected from the group of ^{3}H, ^{11}C, ^{18}F, ^{122}I, ^{123}I, ^{125}I, ^{131}I, ^{75}Br, ^{76}Br, ^{77}Br and ^{82}Br. Preferably, the radioactive isotope is selected from the group of ^{3}H, ^{11}C and ^{18}F.

[00155] Some conjugates of the present invention may exist as atropisomers. Atropisomers are stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers. It is to be understood that all such conformers and mixtures thereof are encompassed within the scope of the present invention.

[00156] Where the conjugates according to this invention have at least one stereo center, they may accordingly exist as enantiomers or diastereomers. It is to be

understood that all such isomers and mixtures thereof are encompassed within the scope of the present invention.

[00157] Where the processes for the preparation of the conjugates according to the invention give rise to mixture of stereoisomers, these isomers may be separated by conventional techniques such as preparative chromatography. The conjugates may be prepared in racemic form, or individual enantiomers may be prepared either by enantiospecific synthesis or by resolution. The conjugates may, for example, be resolved into their component enantiomers by standard techniques, such as the formation of diastereomeric pairs by salt formation with an optically active acid, such as (-)-di-p-toluoyl-D-tartaric acid and/or (+)-di-p-toluoyl-L-tartaric acid followed by fractional crystallization and regeneration of the free base. The conjugates may also be resolved by formation of diastereomeric esters or amides, followed by chromatographic separation and removal of the chiral auxiliary. Alternatively, the conjugates may be resolved using a chiral column via high performance liquid chromatography (HPLC) or SFC. In some instances, rotamers of conjugates may exist which are observable by ^{1}H NMR leading to complex multiplets and peak integration in the ^{1}H NMR spectrum.

[00158] During any of the processes for preparation of the conjugates of the present invention, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups, such as those described in Protective Groups in Organic Chemistry, ed. J.F.W. McOmie, Plenum Press, 1973; and T.W. Greene & P.G.M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, 1991, each of which is herein incorporated by reference in its entirety for all purposes. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

[00159] **Methods of Use**

[00160] The present invention is directed to a method for preventing, treating or ameliorating an amylin receptor mediated syndrome, disorder or disease in a subject in need thereof comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention.

[00161] The present invention also provides a method for preventing, treating, delaying the onset of, or ameliorating a disorder, disease, or condition or any one or more

symptoms of said disorder, disease, or condition in a subject in need thereof, comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention.

[00162] According to particular embodiments, the disease disorder, or condition is selected from the group consisting of obesity, type I or II diabetes, metabolic syndrome (i.e., Syndrome X), insulin resistance, impaired glucose tolerance (e.g., glucose intolerance), hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and/or eczema.

[00163] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder, or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[00164] The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00165] As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related the disease, disorder, or condition, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of the disease, disorder, or condition in the subject.

[00166] In one embodiment, the invention provides a method for preventing, treating, delaying the onset of, or ameliorating obesity, or any one or more symptoms of obesity in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention. In some embodiments, the body weight of a subject is reduced, for example, by between about 0.01% to about 0.1%, between about 0.1% to about 0.5%, between about 0.5% to about 1%, between about 1% to about 5%, between about 2% to about 3%, between about 5% to about 10%, between about 10% to about 15%, between about 15% to about 20%, between about 20% to about 25%, between about 25% to about 30%, between about 30% to about 35%, between about 35% to about 40%, between about 40% to about 45%, or between about 45% to about 50%, relative to the body

weight of a subject prior to administration of any of the conjugates, compounds, pharmaceutical compositions, forms, or medicaments of the invention described herein, or compared to control subjects not receiving any of the conjugates, compositions, forms, medicaments, or combinations of the invention described herein.

[00167] In some embodiments, the reduction in body weight is maintained for about 1 week, for about 2 weeks, for about 3 weeks, for about 1 month, for about 2 months, for about 3 months, for about 4 months, for about 5 months, for about 6 months, for about 7 months, for about 8 months, for about 9 months, for about 10 months, for about 11 months, for about 1 year, for about 1.5 years, for about 2 years, for about 2.5 years, for about 3 years, for about 3.5 years, for about 4 years, for about 4.5 years, for about 5 years, for about 6 years, for about 7 years, for about 8 years, for about 9 years, for about 10 years, for about 15 years, or for about 20 years, for example.

[00168] The present invention provides a method of preventing, treating, delaying the onset of, or ameliorating a syndrome, disorder or disease, or any one or more symptoms of said syndrome, disorder, or disease in a subject in need thereof, wherein said syndrome, disorder or disease is selected from the group consisting of obesity, type I or type II diabetes, metabolic syndrome (i.e., Syndrome X), insulin resistance, impaired glucose tolerance (e.g., glucose intolerance), hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema, comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention.

[00169] As used herein, metabolic syndrome refers to a subject having any one or more of the following: high blood sugar (e.g., high fasting blood sugar), high blood pressure, abnormal cholesterol levels (e.g., low HDL levels), abnormal triglyceride levels (e.g., high triglycerides), a large waistline (i.e., waist circumference), increased fat in the abdominal area, insulin resistance, glucose intolerance, elevated C-reactive protein levels

(i.e., a proinflammatory state), and increased plasma plasminogen activator inhibitor-1 and fibrinogen levels (i.e., a prothrombotic state).

[00170] The present invention provides a method of reducing food intake in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention. In some embodiments, food intake of a subject is reduced, for example, by between about 0.01% to about 0.1%, between about 0.1% to about 0.5%, between about 0.5% to about 1%, between about 1% to about 5%, between about 2% to about 3%, between about 5% to about 10%, between about 10% to about 15%, between about 15% to about 20%, between about 20% to about 25%, between about 25% to about 30%, between about 30% to about 35%, between about 35% to about 40%, between about 40% to about 45%, or between about 45% to about 50%, relative to food intake of a subject prior to administration of any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein, or compared to control subjects not receiving any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein.

[00171] In some embodiments, the reduction in food intake is maintained for about 1 week, for about 2 weeks, for about 3 weeks, for about 1 month, for about 2 months, for about 3 months, for about 4 months, for about 5 months, for about 6 months, for about 7 months, for about 8 months, for about 9 months, for about 10 months, for about 11 months, for about 1 year, for about 1.5 years, for about 2 years, for about 2.5 years, for about 3 years, for about 3.5 years, for about 4 years, for about 4.5 years, for about 5 years, for about 6 years, for about 7 years, for about 8 years, for about 9 years, for about 10 years, for about 15 years, or for about 20 years, for example.

[00172] The present invention provides a method of reducing glycated hemoglobin (A1C) in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention. In some embodiments, A1C of a subject is reduced, for example, by between about 0.001% and about 0.01%, between about 0.01% and about 0.1%, between about 0.1% and about 0.2%, between about 0.2% and about 0.3%, between about 0.3% and about 0.4%, between about 0.4% and about 0.5%, between

about 0.5% and about 1%, between about 1% and about 1.5%, between about 1.5% and about 2%, between about 2% and about 2.5%, between about 2.5% and about 3%, between about 3% and about 4%, between about 4% and about 5%, between about 5% and about 6%, between about 6% and about 7%, between about 7% and about 8%, between about 8% and about 9%, or between about 9% and about 10% relative to the A1C of a subject prior to administration of any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein, or compared to control subjects not receiving any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein.

[00173] In other embodiments, methods are provided for reducing fasting blood glucose levels in a subject in need thereof, the methods comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention. Fasting blood glucose levels may be reduced to less than about 140 to about 150 mg/dL, less than about 140 to about 130 mg/dL, less than about 130 to about 120 mg/dL, less than about 120 to about 110 mg/dL, less than about 110 to about 100 mg/dL, less than about 100 to about 90 mg/dL, or less than about 90 to about 80 mg/dL, relative to the fasting blood glucose levels of a subject prior to administration of any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein, or compared to control subjects not receiving any of the conjugates, compounds, compositions, forms, medicaments, or combinations of the invention described herein.

[00174] The present invention provides a method of modulating amylin receptor activity in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention. As used herein, "modulating" refers to increasing or decreasing receptor activity.

[00175] In some embodiments, an effective amount of a conjugate or compound of the invention or a form, composition or medicament thereof is administered to a subject in need thereof once daily, twice daily, three times daily, four times daily, five times daily, six times daily, seven times daily, or eight times daily. In other embodiments, an effective amount of a conjugate or compound of the invention or a form, composition or

medicament thereof is administered to a subject in need thereof once every other day, once per week, twice per week, three times per week, four times per week, five times per week, six times per week, two times per month, three times per month, or four times per month.

[00176] Another embodiment of the invention comprises a method of preventing, treating, delaying the onset of, or ameliorating a disease, disorder or syndrome, or one or more symptoms of any of said diseases, disorders, or syndromes in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention in a combination therapy. In certain embodiments, the combination therapy is a second therapeutic agent. In certain embodiments, the combination therapy is a surgical therapy.

[00177] As used herein, the term "in combination," in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy.

[00178] As used herein, combination therapy refers to administering to a subject in need thereof one or more additional therapeutic agents, or one or more surgical therapies, concurrently with an effective amount of a conjugate or compound of the invention or a form, composition or medicament thereof. In some embodiments, the one or more additional therapeutic agents or surgical therapies can be administered on the same day as an effective amount of a conjugate of the invention, and in other embodiments, the one or more additional therapeutic agents or surgical therapies may be administered in the same week or the same month as an effective amount of a conjugate or compound of the invention.

[00179] In certain embodiments, wherein the disease or disorder is selected from the group consisting of obesity, type II diabetes, metabolic syndrome, insulin resistance and dyslipidemia, the second therapeutic agent can be an antidiabetic agent. In certain embodiments, the antidiabetic agent can be a glucagon-like peptide-1 (GLP-1) receptor modulator.

[00180] The present invention also contemplates preventing, treating, delaying the onset of, or ameliorating any of the diseases, disorders, syndromes, or symptoms described herein in a subject in need thereof with a combination therapy that comprises administering to the subject in need thereof an effective amount of a conjugate,

compound, or pharmaceutical composition of the invention, in combination with any one or more of the following therapeutic agents: a dipeptidyl peptidase-4 (DPP-4) inhibitor (e.g., sitagliptin, saxagliptin, linagliptin, alogliptin, etc.); a GLP-1 receptor agonist (e.g., short-acting GLP-1 receptor agonists such as exenatide and lixisenatide; intermediate-acting GLP-1 receptor agonists such as liraglutide; long-acting GLP-1 receptor agonists such as exenatide extended-release, albiglutide, dulaglutide); a sodium-glucose co-transporter-2 (SGLT-2) inhibitors (e.g., canaglifozin, dapaglifozin, empaglifozin, etc.); bile acid sequestrants (e.g., colesevelam, etc.); dopamine receptor agonists (e.g., bromocriptine quick-release); biguanides (e.g., metformin, etc.); insulin; oxyntomodulin; sulfonylureas (e.g., chlorpropamide, glimepiride, glipizide, glyburide, glibenclamide, glibornuride, glisoxepide, glyclopyramide, tolazamide, tolbutamide, acetohexamide, carbutamide, etc.); and thiazolidinediones (e.g; pioglitazone, rosiglitazone, lobeglitazone, ciglitazone, darglitazone, englitazone, netoglitazone, rivoglitazone, troglitazone, etc.). In some embodiments, the dose of the additional therapeutic agent(s) is reduced when given in combination with a conjugate or compound of the invention. In some embodiments, when used in combination with a conjugate or compound of the invention, the additional therapeutic agent(s) may be used in lower doses than when each is used singly.

[00181] In certain embodiments, wherein the disease or disorder is selected from the group consisting of obesity, type I or type II diabetes, metabolic syndrome (i.e., Syndrome X), insulin resistance, impaired glucose tolerance (e.g., glucose intolerance), hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema, the second therapeutic agent can be liraglutide.

[00182] The present invention contemplates preventing, treating, delaying the onset of, or ameliorating any of the diseases, disorders, syndromes, or symptoms described herein in a subject in need thereof, with a combination therapy that comprises administering to the subject in need thereof an effective amount of a conjugate, compound, or pharmaceutical composition of the invention in combination with a surgical therapy. In

certain embodiments, the surgical therapy can be bariatric surgery (e.g., gastric bypass surgery, such as Roux-en-Y gastric bypass surgery; sleeve gastrectomy; adjustable gastric band surgery; biliopancreatic diversion with duodenal switch; intragastric balloon; gastric plication; and combinations thereof).

[00183] In embodiments in which the one or more additional therapeutic agents or surgical therapies is administered on the same day as an effective amount of a conjugate or compound of the invention, the conjugate or compound of the invention may be administered prior to, after, or simultaneously with the additional therapeutic agent or surgical therapy. The use of the term "in combination" does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

[00184] Further embodiments of the present invention include the incorporation of additional proteolysis-stabilizing modifications of amylin agonist peptides, preferably in the peptide region between residues 10 and 17. Such proteolysis–stabilizing modifications include, but are not limited to amino acid substitution by one or more non-proteinogenic amino acids, C-alpha-alkylated amino acids, homologous amino acids or synthetic amino acids, and the like. Combinations of more than one such proteolysis-stabilizing modification are contemplated herein.

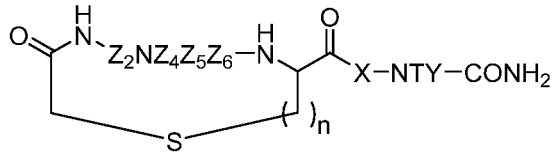
[00185] In further embodiments of the present invention, the thioether-cyclized amylinomimetic peptides or derivatives thereof contain at least one amino acid residue which is derivatized with a half-life extension moiety. Such half-life extension moieties are introduced at suitable (tolerant) sites via conjugation onto appropriately mutated residues at these positions. Examples of half-life extension moieties include, but are not limited to albumin-binding lipids, such as palmitate or similar fatty acids, and protein bioconjugates, such as HSA, mAb, or Fc conjugates.

[00186] Further embodiments of the present invention include amino acid substitutions and/or peptide modifications that are introduced to improve upon the physicochemical properties of the thioether-cyclized amylin peptides. In certain instances, native amylin amino acid residues may be mutated to residues that reduce the pI of the peptide, thereby making it more readily formulatable for administration, while at the same time maintaining amylin receptor potency. In other instances, derivatization with water-soluble functional groups, such as, but not limited to polyethylene glycols is contemplated as a means of improving the solubility of the thioether-cyclized amylin analogues in suitable formulation vehicles.

EMBODIMENTS

[00187] The invention provides also the following non-limiting embodiments.

[00188] Embodiment 1 is a conjugate comprising a monoclonal antibody or an antigen binding fragment thereof coupled to an amylinomimetic peptide, wherein the amylinomimetic peptide is represented by Formula **I** or a derivative or pharmaceutically acceptable salt thereof (SEQ ID NO: 53):

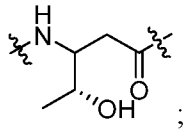


Formula **I**

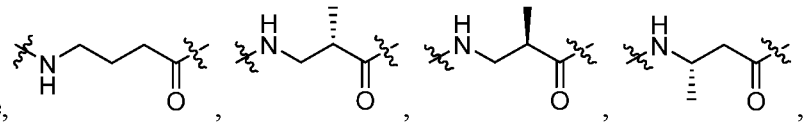
wherein

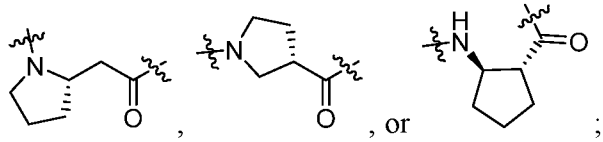
n is 1, or 2;

Z_2 is a direct bond, serine, or glycine;

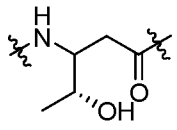
Z_4 is T, or 

;

Z_5 is A, β-alanine, 

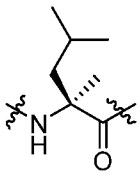


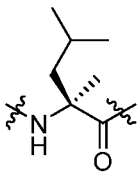
;

Z_6 is T, or ;

Z_{10} is Q, or E;

Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with $-C(=NH)NH_2$;

Z_{12} is L, or ;

Z_{16} is L, or ;

Z_{25} is P, or K;

Z_{26} is I, or K;

X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

Z_{29} is P, or K;

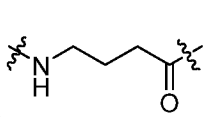
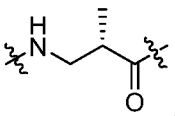
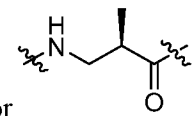
Z_{34} is S, or K;

wherein the derivative is the compound of Formula **I** that is modified by one or more processes selected from the group consisting of amidation, glycosylation, carbamylation, sulfation, phosphorylation, cyclization, lipidation, and pegylation.

[00189] Embodiment 2 is the conjugate of embodiment 1, wherein the amylinomimetic peptide is a derivative of the amylinomimetic peptide of Formula **I** that is modified by one or more processes selected from the group consisting amidation, lipidation, and pegylation, or a pharmaceutically acceptable salt thereof.

[00190] Embodiment 3 is the conjugate of embodiment 1, wherein the amylinomimetic peptide is represented by Formula **I** or the derivative or pharmaceutically acceptable salt thereof, wherein:

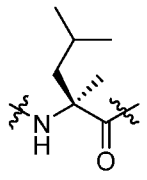
Z_2 is a direct bond;

Z_5 is β-alanine,  ,  , or  ;

Z_6 is T.

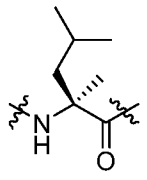
[00191] Embodiment 4 is the conjugate of embodiment 1, wherein the amylinomimetic peptide is represented by Formula **I** or the derivative or pharmaceutically acceptable salt thereof, wherein:

Z_{16} is L;

Z_{12} is  .

[00192] Embodiment 5 is the conjugate of embodiment 1, wherein the amylinomimetic peptide is represented by Formula **I** or the derivative or pharmaceutically acceptable salt thereof, wherein:

Z_{11} is R;

Z_{12} is  .

[00193] Embodiment 6 is the conjugate of embodiment 1, wherein the amylinomimetic peptide is selected from the group consisting of SEQ ID NOs:4-28.

[00194] Embodiment 7 is the conjugate of any one of embodiments 1-6, wherein the monoclonal antibody or the antigen binding fragment thereof is covalently linked to the amylinomimetic peptide at a lysine residue of the amylinomimetic peptide via a linker.

[00195] Embodiment 8 is the conjugate of any one of embodiments 1-7, wherein the monoclonal antibody or the antigen binding fragment thereof comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, and a light chain

complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NO: 47, 48, 49, 50, 51, and 52, respectively

[00196] Embodiment 9 is the conjugate of embodiment 8, wherein the isolated monoclonal antibody comprises a heavy chain variable domain (VH) having the polypeptide sequence of SEQ ID NO:43, and a light chain variable domain (VL) having the polypeptide sequence of SEQ ID NO:45.

[00197] Embodiment 10 is the conjugate of embodiment 9, further comprising a Fc portion.

[00198] Embodiment 11 is the conjugate of embodiment 10, comprising a heavy chain (HC) having the polypeptide sequence of SEQ ID NO:44 and a light chain (LC) having the polypeptide sequence of SEQ ID NO:46.

[00199] Embodiment 12 is a method of producing the conjugate of any one of embodiments 1-11, comprising reacting an electrophile, preferably a bromoacetamide derivatized linker on a sidechain of the amylinomimetic peptide, preferably the amino sidechain of a lysine residue of the amylinomimetic peptide, with the sulfhydryl group of the cysteine residue of SEQ ID NO:49 of the monoclonal antibody or antigen-binding fragment thereof, thereby creating a covalent linkage between the amylinomimetic peptide and the monoclonal antibody or antigen-binding fragment thereof.

[00200] Embodiment 13 is a pharmaceutical composition comprising the conjugate of any one of embodiments 1-11 and a pharmaceutically acceptable carrier.

[00201] Embodiment 14 is a method for treating or preventing obesity in a subject in need thereof, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 13.

[00202] Embodiment 15 is the method of embodiment 14, wherein administration of the effective amount of the pharmaceutical composition to the subject in need thereof results in a reduction in body weight of about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, or about 20% to about 25% as compared to the body weight of the subject prior to administration of the pharmaceutical composition.

[00203] Embodiment 16 is a method for treating or preventing a disease or disorder in a subject in need thereof, wherein said disease or disorder is selected from the group consisting of obesity, type I or type II diabetes, metabolic syndrome, insulin resistance,

impaired glucose tolerance, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 13.

[00204] Embodiment 17 is the method of embodiment 16, wherein said disease or disorder is obesity.

[00205] Embodiment 18 is the method of embodiment 16, wherein said disease or disorder is type I diabetes.

[00206] Embodiment 19 is the method of embodiment 16, wherein said disease or disorder is type II diabetes

[00207] Embodiment 20 is the method of embodiment 16, wherein said disease or disorder is metabolic syndrome.

[00208] Embodiment 21 is the method of embodiment 16, wherein said disease or disorder is a renal disease.

[00209] Embodiment 22 is the method of embodiment 16, wherein said disease or disorder is non-alcoholic steatohepatitis (NASH).

[00210] Embodiment 23 is the method of embodiment 16, wherein said disease or disorder is non-alcoholic fatty liver disease (NAFLD).

[00211] Embodiment 24 is a method of reducing food intake in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 13.

[00212] Embodiment 25 is the method of embodiment 24, wherein administration of the effective amount of the pharmaceutical composition to the subject in need thereof results in a reduction in food intake of about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, about 35% to about 40%, about 40% to about 45%, or about 45% to about

50% as compared to the food intake of the subject prior to administration of the pharmaceutical composition.

[00213] Embodiment 26 is a method of modulating amylin receptor activity in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 13.

[00214] Embodiment 27 is the method of any one of embodiments 14-26, wherein the pharmaceutical composition is administered via an injection.

[00215] Embodiment 28 is the method of embodiment 27, wherein the injection is delivered subcutaneously, intramuscularly, intraperitoneally, or intravenously.

[00216] Embodiment 29 is the method of any one of embodiments 14-28, wherein the pharmaceutical composition is administered in a combination with a second therapeutic agent.

[00217] Embodiment 30 is the method of embodiment 29, wherein the disease or disorder is selected from the group consisting of obesity, type 2 diabetes, metabolic syndrome, insulin resistance and dyslipidemia and the second therapeutic agent is at least one antidiabetic agent.

[00218] Embodiment 31 is the method of embodiment 30, wherein said antidiabetic agent is a glucagon-like-peptide-1 receptor modulator.

[00219] Embodiment 32 is the method of embodiment 29, wherein the second therapeutic agent is liraglutide.

[00220] Embodiment 33 is the method of any one of embodiments 14-32, wherein the pharmaceutical composition is administered daily, weekly, or monthly to the subject in need thereof.

[00221] Embodiment 34 is the method of embodiment 33, wherein the pharmaceutical composition is administered once, twice, three, four, five, or six times per day.

[00222] Embodiment 35 is the method of embodiment 33, wherein the pharmaceutical composition is administered once, twice, three, four, five, or six times per week.

[00223] Embodiment 36 is the method of embodiment 33, wherein the pharmaceutical composition is administered once, twice, three, or four times per month.

[00224] Embodiment 37 is a kit comprising the conjugate of any one of embodiments 1-14 or a pharmaceutical composition of embodiment 13, preferably the kit further

comprising an effective amount of a second therapeutic agent, more preferably, the kit further comprising an effective amount of liraglutide.

[00225] Embodiment 38 is the kit of embodiment 37, wherein the kit further comprises an injection device.

[00226] Embodiment 39 is a method of producing a pharmaceutical composition comprising a compound selected from the group consisting of SEQ ID NOs:4-42, and a pharmaceutically acceptable carrier.

SYNTHESIS

[00227] Compounds or conjugates of the present invention can be synthesized in accordance with the general synthetic methods known to those who are skilled in the art. The following description of the synthesis is for exemplary purposes and is in no way meant to be a limit of the invention.

[00228] The thioether-cyclized amylinomimetic peptides or derivatives of this invention may be synthesized by a variety of known, conventional procedures for the formation of successive peptide linkages between amino acids, and are preferentially carried out by solid phase peptide synthesis (SPPS), as generally described by Merrifield (J. Am. Chem. Soc., 1963, 85, 2149-2154), using an automated peptide synthesizer, traditional bench synthesis, or a combination of both approaches. Conventional procedures for peptide synthesis involve the condensation between the free amino group of one amino acid residue, whose other reactive functionalities have been suitably protected, and the free carboxyl group of another amino acid, whose reactive functionalities have also been suitably protected. Examples of condensation agents typically utilized for peptide bond formation include diisopropylcarbodiimide (DIC) with or without 1-hydroxybenztriazole (HOBT) or ethyl cyano(hydroxyimino)acetate (Oxyma Pure), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU), 2-(1*H*-7-azabenztriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HATU), 2-(6-chloro-1*H*-benztriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), 1-Cyano-2-ethoxy-2-oxoethylideneaminooxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyOxim), 2-(1*H*-benzotriazole-1-yl)-

1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), and the like.

[00229] The automated peptide synthetic methodology may be carried out at room temperature (rt), or at elevated temperatures, preferably through the application of microwave heating, as described by Yu (J. Org. Chem., 1992, 57, 4781-4784) and as more recently refined by Palasek (J. Pept. Sci., 2007, 13, 143-148).

[00230] Compounds of the present invention (C-terminal amides) can be conveniently prepared using N-α-FMOC protected amino acid methodology, whereby the carboxy terminus of a suitably protected N-α-FMOC protected amino acid is coupled onto a conventional solid phase resin using a suitable coupling agent. Suitable conventional, commercially-available solid phase resins include Rink amide MBHA resin, Rink amide AM resin, Tentagel S RAM Resin, FMOC-PAL-PEG PS resin, SpheriTide Rink amide resin, ChemMatrix Rink resin, Sieber amide resin, TG Sieber resin and the like. The resin-bound FMOC-amino acid may then be deprotected by exposure to 20% piperidine in either DMF or NMP, treatment of which serves to selectively remove the FMOC protecting group. Additional FMOC-protected amino acids are then subsequently coupled and deprotected sequentially, thereby generating the desired resin-bound protected peptide. In certain instances, it may be necessary to utilize an orthogonally reactive protecting group for another amine in the peptide sequence that would withstand the FMOC deprotection conditions. Protecting groups such 4-methyltrityl (Mtt) or 4-methoxytrityl (Mmt), both removable by 1% TFA/DCM treatments, or preferably allyloxycarbonyl (alloc; removable by $Pd(PPh_3)_4/PhSiH_3$ treatment), 1-(4,4-dimethyl-2,6-dioxocyclohex-1-yliden)ethyl (Dde; removable by treatment with 2-3 % hydrazine/DMF) and 1-(4,4-dimethyl-2,6-dioxocyclohex-1-yliden)-3-methylbutyl (ivDde; removable by treatment with 2-3 % hydrazine/DMF) can be used effectively in such instances.

[00231] In conventional peptide synthetic methodologies, reactive side chains of alpha amino acids are generally protected throughout the synthesis with suitable protecting groups to render them inert to the coupling and deprotection protocols. While multiple protecting groups for amino acid side chains are known in the art, herein the following protecting groups are most preferred: *tert*-butyl (t-Bu) for serine, threonine, glutamic acid, aspartic acid and tyrosine; trityl (Trt) for asparagine, glutamine, cysteine,

homocysteine and histidine; *tert*-butyloxycarbonyl (Boc) for tryptophan and the ε-amino group of lysine; and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine. These protecting groups are removed upon strong acid treatment, such as with high concentrations of trifluoroacetic acid (TFA).

[00232] Upon completion of the SPPS, the resin-bound, side chain-protected peptide is deprotected and concomitantly cleaved from the resin using a cleavage cocktail that consists predominantly of (TFA) along with various combinations of carbocation scavengers, such as triisopropylsilane (TIPS), water, phenol and anisole. The crude solid peptide is then isolated by precipitation of the peptide/cocktail filtrate with cold ether. The crude peptide thus obtained is then dissolved at low concentration (ca., < 5 mg/mL) in a largely aqueous solvent system containing an organic co-solvent such as acetonitrile or ethanol. Upon raising the pH of the solution to > 7, the peptide then undergoes an intramolecular cyclization reaction to form the corresponding crude thioether-cyclized amylin analogue of the present invention. Thioether-cyclized amylin analogues thus formed may be purified using purification techniques generally known in the art. A preferable method of peptide purification used herein is reverse phase high performance liquid chromatography (HPLC). Purified peptides are then characterized by liquid chromatography/mass spectrometry (LC/MS).

[00233] It is understood that the following examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggestive to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporate by reference in their entirety for all purposes.

ABBREVIATIONS

Herein and throughout the specification, the following abbreviations may be used:

aq	aqueous
alloc	allyloxycarbonyl
Boc	*tert*-butoxycarbonyl
BSA	bovine serum albumin
CDI	1,1'-carbonyldiimidazole
CT	calcitonin

CTR	calcitonin receptor
DCM	dichloromethane
Dde	1-(4,4-dimethyl-2,6-dioxocyclohex-1-yliden)ethyl
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMA	N, N-dimethylacetamide
DMEM	Dulbecco's modified eagle's medium
DMF	N,N-dimethylformamide
EDC	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
EDTA	ethylenediaminetetraacetic acid
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethyl alcohol
FBS	fetal bovine serum
FMOC	9-fluorenylmethyloxycarbonyl
g	gram(s)
h	hour(s)
HATU	2-(1*H*-7-azabenztriazol-1-yl)-1,1,3,3-tetramethylaminium-hexafluorophosphate
HBSS	Hank's balanced salt solution
HBTU	2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate)
HCTU	2-(6-chloro-1*H*-benztriazol-1-yl)-1,1,3,3-tetramethylaminium-hexafluorophosphate
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIC	hydrophobic interaction chromatography
HOBT	1-hydroxybenztriazole
HPLC	high performance liquid chromatography
HTRF	homogeneous time resolved fluorescence
IBMX	3-isobutyl-1-methylxanthine
ivDde	1-(4,4-dimethyl-2,6-dioxocyclohex-1-yliden)-3-methylbutyl
LCMS	high performance liquid chromatography with mass spectrometer
Me	methyl
MeCN	acetonitrile
mg	milligram
min	minute(s)
mL	milliliter
Mmt	4-methoxytrityl
Mtt	4-methyltrityl
NMP	1-methyl-2-pyrrolidone
OEG	8-amino-3,6-dioxaoctanoyl
ORF	open reading frame
Oxyma	ethyl cyano(hydroxyimino)acetate
Pal	palmitoyl
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl

PBS	phosphate buffered saline
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium(0)
$PhSiH_3$	phenylsilane
PyBroP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
Pyoxim	1-cyano-2-ethoxy-2-oxoethylideneaminooxy-tris-pyrrolidino-phosphonium hexafluorophosphate
RAMP	receptor activity-modifying protein
rt	room temperature
RT	retention time
sat'd	saturated
SPPS	solid phase peptide synthesis
t-Bu	*tert*-butyl
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TFA	trifluoroacetic acid
TIPS	triisopropylsilane
Tris	tris(hydroxymethyl)aminomethane
Trt	triphenylmethyl

EXAMPLES

Example 1: Synthesis of Thioether-cyclized Amylinomimetic Peptides (Scheme 1)

[00234] Step A: Synthesis of Resin-bound C-terminal Amide Peptide

[00235] The protected peptidyl resin was synthesized using FMOC strategy as described above on a CEM Liberty Blue Microwave peptide synthesizer using low loading Rink amide resins, preferably, FMOC-PAL-PEG PS resin (ca., 0.16 – 0.2 meq/g, supplied by Applied Biosystems) on a scale of 0.1 mmol, as depicted in Scheme 1. Standard FMOC-protected amino acids (supplied by Novabiochem (EMD Millipore), Bachem, Peptides International, Sigma-Aldrich or Chem-Impex) were coupled in 5-fold excess relative to resin loading using DIC/Oxyma as the coupling agents and a reaction temperature of ca., 90 °C for 4 min. FMOC-Arg(Pbf)-OH was double coupled at 90 °C for 4 min each and FMOC-His(Trt)-OH was coupled using a two-stage protocol: 4 min at rt followed by 8 min at 50 °C. Single FMOC deprotections were carried out using 20% piperidine in DMF (deprotection solution) at 90 °C for 1.5 min.

[00236] Step B: Procedure for Bromoacetylation of Resin-bound Peptide (Scheme 2)

[00237] The FMOC-deprotected peptide-resin (0.1 mmol) was treated with a solution of bromoacetic anhydride (6-20 eq.) in DMF (5 mL) in a microwave reactor at 50 °C for

5 min, by which time the reaction was generally determined to be complete as per a Kaiser ninhydrin test. In cases where the coupling was determined to be incomplete, the coupling was repeated with fresh reagents.

[00238] Step C: Procedure for Peptide Cleavage from Resin

[00239] Upon completion of the SPPS, the resin was washed extensively with DMF and then with DCM and dried. The resin was then treated with a cleavage cocktail (10 mL / 0.1 mmol scale) consisting of either TFA/water/TIPS (95:2.5:2.5) (Cleavage Cocktail A) or more preferably with TFA/water/phenol/TIPS (88:5:5:2) (Cleavage Cocktail B) and heated in a microwave reactor at 38 °C for 40 min, then filtered. The resin was washed with TFA and the combined filtrates were concentrated under a stream of nitrogen to a volume of ca. 2.5 mL and the peptide was precipitated by the addition of cold diethyl ether (40 mL). The peptide/ether suspension was centrifuged and the ether layer was decanted. The peptide pellet was re-suspended in ether, centrifuged and decanted, and this process was repeated a third time. The crude peptide thus obtained was dried under a mild nitrogen stream.

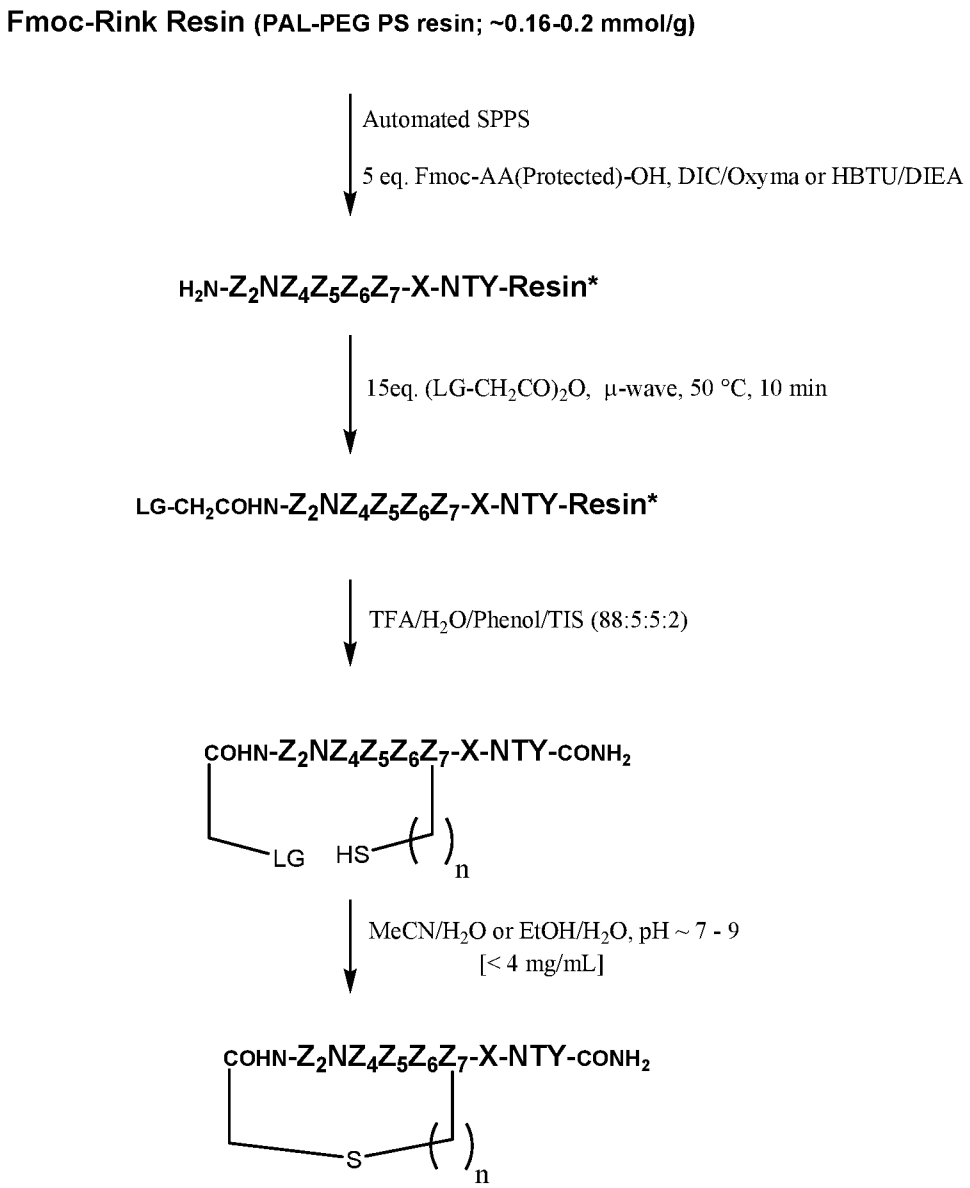
[00240] Step D: Procedure for Peptide Cyclization (Thioether Formation)

[00241] The crude cysteine- or homocysteine-containing peptide was dissolved in deoxygenated MeCN/water (50-60% MeCN) or EtOH/water (50-60% EtOH) at a concentration of < 4 mg/mL. The pH of the peptide solution was then raised to ca. 7 – 9 through the addition of either solid $NaHCO_3$, sat'd aq. $NaHCO_3$ or 1M aq. Tris buffer (pH 7.5) and the resulting solution was stirred at rt for 3-16 h. Typically, the cyclizations were complete within 1 h, as determined by analytical LC/MS.

[00242] Step E: Procedure for Peptide Purification

[00243] The cyclization reaction mixture was acidified to pH 1.5 – 3 by the addition of TFA, and the solution was concentrated to remove most of the organic co-solvent (MeCN or EtOH) to a point where slight clouding occurred. A minimal amount of the co-solvent was added back as necessary to render the mixture homogeneous and the resultant solution was then purified directly by preparative HPLC in multiple injections. Purifications were performed on either an Agilent PrepStar HPLC system or a Gilson HPLC 2020 Personal Purification System using a reverse phase C18 or C8 column selected from the following: Varian Pursuit XRs C18 (21x250 mm, 100 Å, 5 μm);

Varian Pursuit XRs Diphenyl (30x100 mm, 100 Å, 5 μm); Zorbax 300 SB-C8 (21x250 mm, 300 Å, 5 μm); Waters Atlantis T3 C18 (19x250 mm, 100 Å, 5 μm); Agilent Polaris 5 C18-A (30x250 mm, 180 Å, 5 μm). The mobile phase consisted of gradient elutions of buffer A (0.1% TFA in water) and Buffer B (0.1% TFA in MeCN) ranging in initial concentration of 10 - 20 % B to final concentrations of 40 - 90 % B with run times ranging between 36 – 80 min. UV detection was monitored at 220 and 254 nm. Product-containing fractions were analyzed by analytical HPLC on an Agilent 1100 HPLC system using an appropriate column type from above (4.6x250 mm, 300 Å, 5 μm). Pure fractions were combined, concentrated to remove most of the organic phase, and then lyophilized. TFA/HCl salt exchange was subsequently carried out by triple lyophilization from 2 mM HCl, according to the procedure described by Andrushchenko, et al., (J. Pept. Sci., 2006, 13, 37-43).



wherein LG is a leaving group

X is **ATZ$_{10}$Z$_{11}$Z$_{12}$ANFLVHSSNNFGZ$_{25}$Z$_{26}$LPZ$_{29}$TNVGZ$_{34}$**, or **VLGRLSQELHRLQTYPRTNTGS**

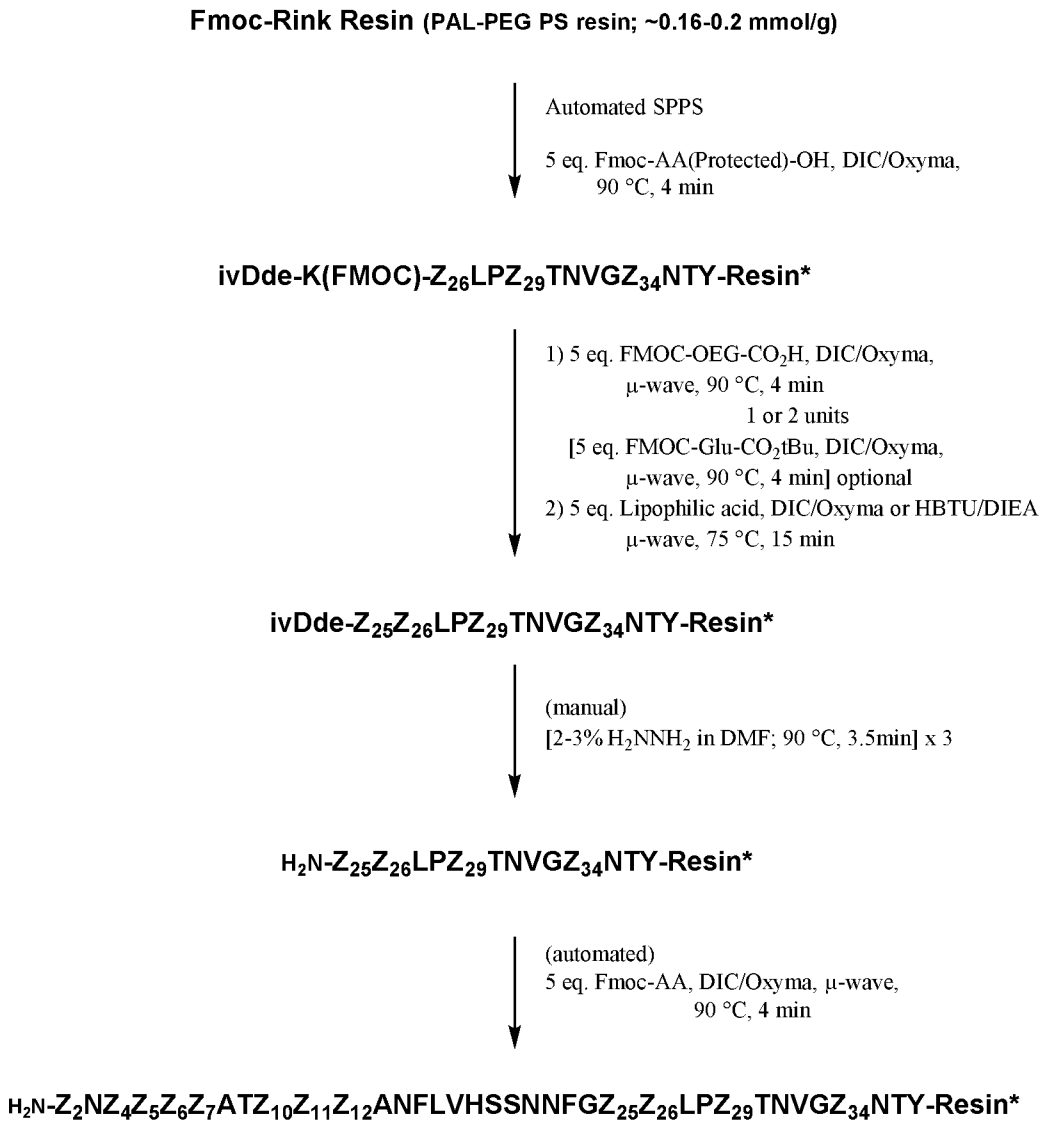
*Amino acids are protected

Scheme 1: Synthesis of Thioether-cyclized Amylinomimetic Peptides: Amylin/Pramlintide/Davalintide Analogues (Scheme 1 discloses SEQ ID NOS 56, 56, 56-58, and 55, respectively, in order of appearance)

Example 2: Synthesis of Lipidated Amylinomimetic Peptides (Scheme 2)

[00244] Step A: Procedure for Introducing Derivatized Lysine Residues into Peptide Sequences Built on Standard Rink Amide Resin

[00245] To a resin-bound C-terminal amide peptide, elaborated to the point preceding the desired point of derivatization and prepared as described in Example 1A, were sequentially coupled either Dde-Lys(FMOC)-OH or ivDde-Lys(FMOC)-OH and then FMOC-OEG-OH (one or two units coupled in tandem), optionally followed by FMOC-Glu-OtBu under microwave conditions (either manually or on the Liberty Blue Peptide Synthesizer) using DIC/Oxyma coupling methods as described in Example 1A. Following FMOC deprotection, the resin was treated with a solution of the lipophilic acid [ex., palmitic acid] (5-10 eq.), DIC (5-10 eq.) and either HOBT or Oxyma (5-10 eq.) in DMF under microwave conditions at 90 °C for 10 min. The reaction was then drained and the resin was washed with DMF. Scheme 2 shows the introduction of lipidated lysine at position Z_{25}. Those skilled in the art will recognize that a similar approach will yield lipidated lysine at Z_{26}. Z_{29}, or Z_{34}.



* Amino acids are protected

Scheme 2: Procedure for Introducing Derivatized Lysine Residues into Amylinomimetic Peptides (Scheme 2 discloses SEQ ID NOS 59, 60, 60, and 61, respectively, in order of appearance)

[00246]

[00247] Step B: Procedure for Deprotecting Dde- or ivDde-protected Lysinyl Peptide

[00248] The derivatized lysinyl peptide resin was treated with a solution of 3 % hydrazine in DMF (6 mL / 0.1 mmol resin) under microwave conditions at 90 °C for 3.5

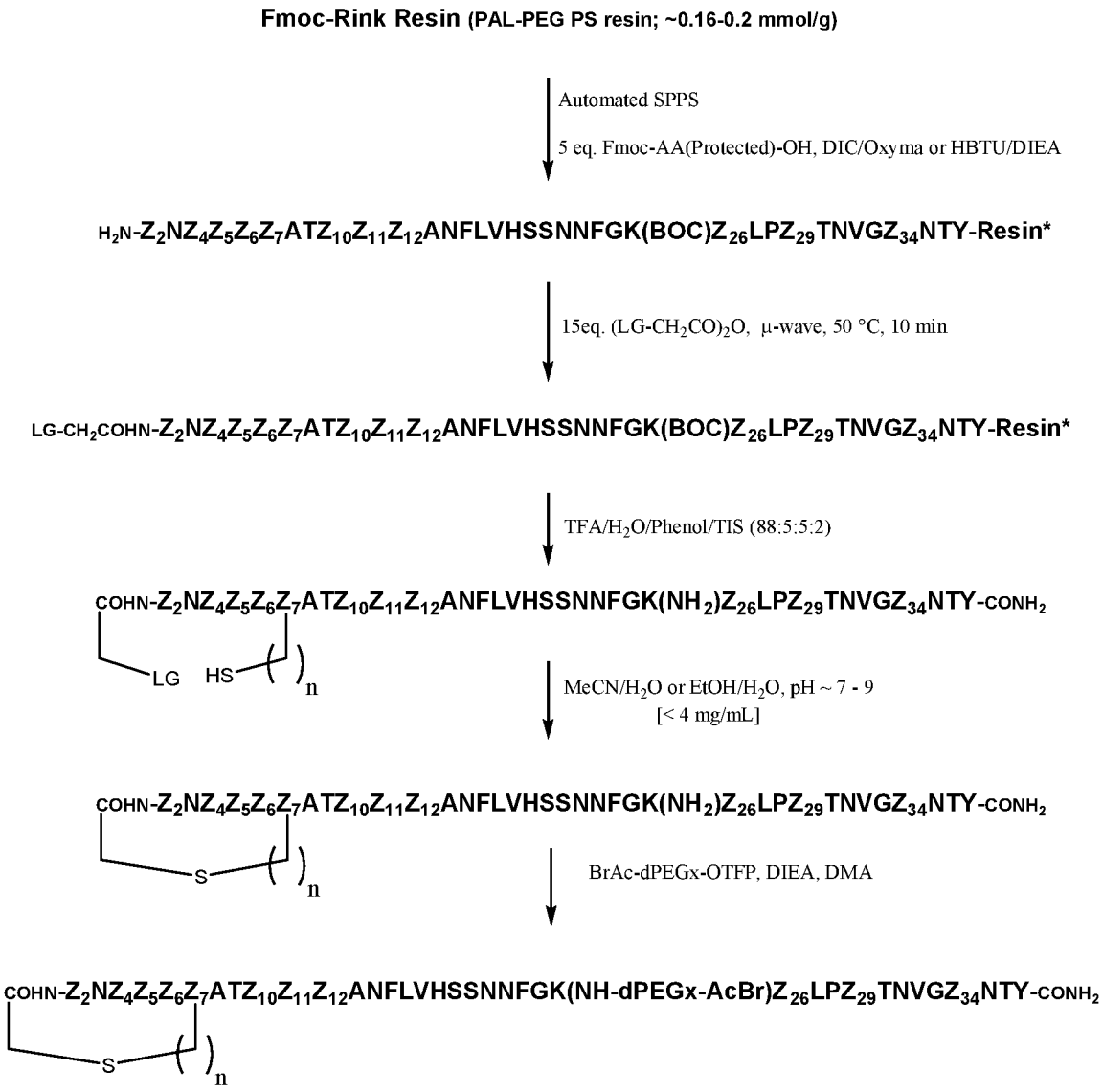
min. The reaction was drained and this procedure was repeated two additional times. The reaction was drained and the resin was washed extensively with DMF and then with DCM.

[00249] Step C: Procedure for Direct Incorporation of FMOC-Lys(Pal-Glu-OtBu)-OH Residue

[00250] In cases where the palmitoylated-γ-Glu-Lysinyl residue is to be incorporated into the sequence, FMOC-Lys(Pal-Glu-OtBu)-OH (available from Peptides International or ActivePeptide) may be used directly in the procedure described in Example 1A.

Example 3: Synthesis of BrAc-dPEGx-Derivatized Amylinomimetic Peptides (Scheme 3)

[00251] A solution of the thioether-cyclized peptide obtained from Example 1E, in which one of Z_{25}, Z_{26}, Z_{29} or Z_{34} was K(NH_2) (7 μmol), and BrAc-dPEGx-OTFP (3 eq.) in DMA (0.3 mL) was treated with DIEA (7 eq) and the resultant solution was stirred at rt. Upon completion of the reaction (ca., 1h), the mixture was acidified with TFA, diluted with water (0.1% TFA) and purified by reversed phase chromatography, as described in Example 1E. Scheme 3 shows the introduction of lipidated lysine at position Z_{25}. Those skilled in the art will recognize that a similar approach will yield BrAc-dPEGx-derivatized lysine at Z_{26}. Z_{29}, or Z_{34}.



*Amino acids are protected
LG is a leaving group

Scheme 3: Procedure for Synthesizing BrAc-dPEGx-Derivatized Amylinomimetic Peptides (Scheme 3 discloses SEQ ID NOS 62 and 62-65, respectively, in order of appearance)

Example 4: Synthesis of Thioether-Cyclized Amylinomimetic Peptide-mAb Conjugate

[00252] Expression and Purification of the mAb

[00253] The fully human monoclonal antibody (mAb) can be recombinantly expressed in a mammalian expression host and purified from the cell culture supernatant using standard methods that are known in the field. For example, a cDNA sequence encoding the light (LC) and heavy chains (HC) of the mAb, each including an appropriate signal peptide to enable secretion, can be cloned into separate mammalian expression vectors or into a single expression vector using standard molecular biology methods. Expression vectors used can be any of those commercially available such as pEE12.4, pcDNA™3.1(+) or pIRESpuro3 or any custom expression vector with similar functionalities. In such vectors transcription of the heavy and light chains of the mAb are each driven by any of the known effective promoters such as the hCMV-MIE promoter. Transfection grade plasmid DNA is prepared for separate LC and HC expression constructs or a single construct expressing both LC and HC using standard methods such as a QIAGEN Plasmid Midi Kit.

[00254] Purified plasmid DNA is prepared for transfection with a lipid-based transfection reagent such as Freestyle™ Max transfection reagent, following manufacturer's instructions, and is then transfected into a standard mammalian expression host cell line, such as CHO-S or HEK 293-F. If the mAb LC and HC are encoded by separate expression constructs, the two constructs are simultaneously transfected. Prior to and after transfection, mammalian cells are cultured for maintenance or for mAb expression following standard cell culture methods whereby the cell density ranges to maintain, the culture media to use, and the other cell culture conditions followed are determined by the specific mammalian host cell line utilized. These parameters are typically documented by the vendor from which the cell line was obtained or in the scientific literature. For example, CHO-S cells are maintained in CHO Freestyle™ media in suspension, shaking at 125 RPM in a humidified incubator set at 37 °C and 8% CO_2, and split when the cell concentration is between 1.5 and 2.0 x 10^6 cells per ml.

[00255] Cell culture supernatants from the transiently transfected mammalian cells expressing the mAb are harvested several days after transfection, clarified by centrifugation and filtered. Duration of expression for CHO-S cells is typically four days but can be adjusted and can differ for different mammalian host cell lines. Large scale

transfections (>10 liters) are concentrated 10-fold using a concentrator such as a Centramate. The mAb is purified from the clarified supernatant using a Protein A affinity column such as the HiTrap MabSelect Sure utilizing standard methods for binding mAb to Protein A resin, washing the resin and eluting the protein using low pH buffer. The protein fractions are neutralized immediately by elution into tubes containing pH 7 buffer and peak fractions are pooled, filtered and dialyzed against phosphate buffered saline (PBS), pH 7.2 overnight at 4 °C. After dialysis the mAb is filtered again (0.2μ filter) and the protein concentration is determined by absorbance at 280nm. Quality of the purified mAb protein is assessed by SDS-polyacrylamide gel electrophoresis (PAGE) and analytical size exclusion HPLC and endotoxin levels are measured using a limulus amebocyte lysate (LAL) assay. Purified mAb is stored at 4 °C.

[00256] Expression and Purification of MSCB97 from transiently transfected CHO cells

[00257] MSCB97 was expressed in ExpiCHO-S™ cells (ThermoFisher Scientific, Waltham, MA; Cat # A29127) by transient transfection of the cells with purified plasmid DNA of a MSCB97 expression construct following manufacturer's recommendations. Briefly, ExpiCHO-S™ cells were maintained in suspension in ExpiCHO™ expression medium (ThermoFisher Scientific, Cat # A29100) in a shaking incubator set at 37 °C, 8% CO_2 and 125 RPM. The cells were passaged so that on the day of transfection, dilution down to 6.0×10^6 cells per ml could be achieved, maintaining cell viability at 98% or better. Transient transfections were done using the ExpiFectamine™ CHO transfection kit (ThermoFisher Scientific Cat # A29131). For each ml of diluted cells to be transfected, one microgram of plasmid DNA is used and diluted into OptiPRO™ SFM complexation medium. ExpiFectamine™ CHO reagent is used at a 1:3 ratio (v/v, DNA:reagent) and also diluted into OptiPRO™. The diluted DNA and transfection reagent were combined for one minute, allowing DNA/lipid complex formation, and then added to the cells. After overnight incubation, ExpiCHO™ feed and ExpiFectamine™ CHO enhancer were added to the cells. Cells were cultured with shaking at 32 °C for five days prior to harvesting the culture supernatants.

[00258] Culture supernatants from the transiently transfected ExpiCHO-S™ cells were harvested by clarifying through centrifugation (30 min, 6000 rpm) followed by filtration

(0.2μ PES membrane, Corning). Large scale transfections (5 to 20 liters) were first concentrated 10-fold using a Pall Centramate Tangential Flow Filtration system. 10x DPBS (Dulbecco's phosphate buffered saline), pH7.2 was added to the supernatant to 1x final concentration prior to loading onto an equilibrated (DPBS, pH 7.2) HiTrap MabSelect Sure Protein A column (GE Healthcare; Little Chalfont, United Kingdom) at a relative concentration of ~20 mg protein per ml of resin, using an AKTA FPLC chromatography system. After loading, the column was washed with 10 column volumes of DPBS, pH7.2. The protein was eluted with 10 column volumes of 0.1 M Na-Acetate, pH 3.5. Protein fractions were neutralized immediately by elution into tubes containing 2.0 M Tris, pH 7 at 20% the elution fraction volume. Peak fractions were pooled and the pH adjusted to ~5.5 with additional Tris, if necessary. The purified protein was filtered (0.2μ) and the concentration was determined by absorbance at 280nm on a BioTek SynergyHTTM spectrophotometer. The quality of the purified protein was assessed by SDS-PAGE and analytical size exclusion HPLC (Dionex HPLC system). The endotoxin level was measured using a turbidometric LAL assay (Pyrotell®-T, Associates of Cape Cod).

[00259] Conjugation of MSCB97 to amylinomimetic peptides

[00260] To the mAb (1.2 mL, 19 mg/mL) was added 4 eq. TCEP followed by EDTA (100mM; 12 μL). After 2 h at rt, LCMS analysis indicated that the disulfide adducts at position C102 had been completely reduced. The reduced mAb was treated with Zebra desalting spin column (7x10mL, 7K MWCO, pre-equilibrated with Tris-acetate 100mM pH 5.6) to remove the liberated cysteines/GSH. To this reduced mAb was added a solution of the bromoacetylated amylinomimetic peptide from Example 3 in Milli Q grade water (7 eq vs mAb, 30-35 mg/mL) followed by EDTA (100mM; 13.5 μL). The pH of the reaction was adjusted to 7.9 by dropwise addition of 1M Tris-acetate buffer (pH ~ 9). The reaction was allowed to proceed overnight at rt with gentle agitation. The reaction was then diluted with sat'd $(NH_4)_2SO_4$ (10% v/v) and the crude conjugate was purified by hydrophobic interaction chromatography (TOSOH TSKgel Phenyl HIC), eluting with a linear gradient (40-100% B/A, solvent A: 5% i-PrOH, 1M $(NH_4)_2SO_4$, 100mM phosphate buffer, pH 6.0; solvent B: 20% i-PrOH, 100mM phosphate buffer, pH 6.0). Final purification was achieved by protein A adsorption (PBS buffer) and elution

(NaOAc, pH 3.5). The pH of the product was adjusted to 6 with 2.5M Tris (pH 7.7; 10 v%) and dialyzed against PBS to give the final sample.

Example 5: Peptide and Peptide-Bioconjugate Analysis and Characterization

[00261] Method A: Purified peptides were analyzed by LC/MS on an Hewlett Packard Series 1100 MSD system configured with an HP 1100 series HPLC using a Waters Atlantis T3 C18 (4.6x250 mm, 300 Å, 5 μm) column. Depending on the polar/non-polar nature of the peptide, one of two linear gradients was used (buffer A: Water + 0.1% TFA; buffer B: MeCN + 0.1% TFA) at a flow rate of 1 mL/min and a column temperature of 35 °C [Method A1: 15 – 60 %B over 22 min; Method A2: 40 – 90 %B over 22 min]. Electrospray analysis (ES-API, positive ion scan) provided mass analysis for each peptide. In all cases, multiple charged species were observed with 1/3[M+3]+ and 1/4[M+4]+ ions being the characteristic, most prominently observed ions. All products yielded their expected multi-charged ions within acceptable limits. Results of the mass spectral analyses of the peptides and observed LC retention times (RT) are shown in Table 1.

[00262] Method B: Purified peptides were analyzed by HPLC on a Shimadzu 10AVP system using a YMC-Pack-ODS-A (4.6x250 mm, 200Å, 5 μm) column. A linear solvent gradient (20 – 80 %B over 30 min) was used (buffer A: Water + 0.05% TFA; buffer B: MeCN + 0.05% TFA) at a flow rate of 1 mL/min. Mass spectra were obtained on a Waters Xevo G2 ToF spectrometer (TOF MS ES, positive ion scan). In all cases, multiple charged species were observed with 1/3[M+3]+ and 1/4[M+4]+ ions being the characteristic, most prominently observed ions. All products yielded their expected multi-charged ions within acceptable limits. Results of the mass spectral analyses of the peptides and observed LC retention times (RT) are shown in Table 1.

[00263] Method C: Purified amylinomimetic peptide-mAb conjugates were analyzed by hydrophobic interaction chromatography (HIC) on an Hewlett Packard Series 1100 MSD system configured with an HP 1100 series HPLC using a MAbPac HIC-10 (4.6x1000 mm, 1000 Å, 5 μm) column. A linear solvent gradient (0 – 100 %B over 30 min) was used (buffer A: 5% i-PrOH, 1.5M $(NH_4)_2SO_4$, 100mM phosphate buffer, pH 6.0; buffer B20% i-PrOH, 100mM phosphate buffer, pH 6.0) at a flow rate of 0.5 mL/min. Intact mass measurements were obtained on a Waters Xevo G2-XS QToF

spectrometer (TOF MS ES, positive ion scan). Results of the analytical characterization of the amylinomimetic peptide-mAb conjugates are shown in Table 1.

Table 1: Analytical data for Thioether-cyclized Amylinomimetic Compounds.

Seq. I.D. No.	MW	MW Found	$1/3[M+3]^+$ (Calc'd)	$1/3[M+3]^+$ (Found)	$1/4[M+4]^+$ (Calc'd)	$1/4[M+4]^+$ (Found)	HPLC Method	RT (min)
4	3760.2		1254.4	1254.3	941.1	941.0	B	15.3
5	3831.2		1278.1	1277.8	958.8	958.7	A1	15.5
6	3774.2		1259.1	1258.9	944.6	944.4	B	14.2
7	3831.3		1278.1	1278.0	958.8	958.7	B	11.4
8	3873.3		1292.1	1291.7	969.3	969.2	A1	16.0
9	3817.2		1273.4	1273.2	955.3	955.2	B	13.7
10	3859.3		1287.4	1287.0	965.8	965.7	A1	16.3
11	3831.3		1278.1	1278.0	958.8	958.8	B	11.4
12	3845.3		1282.8	1282.7	962.3	962.3	B	13.0
13	3845.3		1282.8	1282.7	962.3	962.2	B	12.3
14	3845.3		1282.8	1282.7	962.3	962.3	B	11.8
15	3845.3		1282.8	1282.5	962.3	962.2	B	14.4
16	3871.3		1291.4	1291.3	968.8	968.8	B	14.9
17	3857.3		1286.8	1286.7	965.3	965.3	B	12.7
18	3871.3		1291.4	1291.3	968.8	968.7	B	13.8
19	3845.3		1282.8	1282.5	962.3	962.1	B	13.2
20	3831.3		1278.1	1278.0	958.8	958.7	B	13.2
21	3845.3		1282.8	1282.7	962.3	962.2	B	13.1
22	3831.3		1278.1	1278.0	958.8	958.7	B	14.6
23	3918.4		1307.1	1306.9	980.6	980.4	B	12.7
24	3904.3		1302.4	1302.3	977.1	977.0	B	15.3
25	3847.3		1283.4	1283.4	962.8	962.8	B	12.4
26	3847.3		1283.4	1283.4	962.8	962.8	B	12.2
27	3857.4		1286.8	1286.7	965.4	965.3	B	12.4
28	3448.9		1150.6	1150.2	863.2	862.9	B	12.9
29	4317.9		1440.3	1440.1	1080.5	1080.5	A2	11.1
30	4447.1		1483.4	1483.2	1112.8	1112.6	A2	10.3
31	4523.9		1509.0	1508.8	1132.0	1131.8	A1	16.4
32	4523.9		1509.0	1508.8	1132.0	1131.9	A1	15.9
33	4510.8		1504.6	1504.4	1128.7	1128.6	A1	16.2
34	4539.9		1514.3	1514.1	1136.0	1135.8	A1	17.1
35	4539.9		1514.3	1514.2	1136.0	1135.9	A1	16.6
36	4526.9		1510.0	1509.9	1132.7	1132.7	A1	16.9
37	155396	155394					C	25.1
38	155396	155393					C	23.4
39	155369	155363					C	22.4
40	155428	155418					C	27.4

41	155428	155418					C	27.2
42	155402	155388					C	26.2

Example 6: Human Calcitonin/RAMP3 Receptor cAMP Assay (AMY3R Assay)

[00264] The method used to test the potency of amylinomimetic analogs in vitro was a cell based assay designed to measure cAMP produced by adenylate cyclase through modulation of the human calcitonin G-protein coupled receptor through its interaction with receptor activity-modifying protein 3 (CTR/RAMP3). Production of cAMP in human AMY3R-transfected 1321N1 astrocytoma cells (DiscoverX) was induced by amylinomimetic analogs and controls in a dose-dependent manner, and measured in the LANCE FRET-based competitive cAMP immunoassay (PerkinElmer).

[00265] Cells were cultured in DMEM, 10% FBS, 2.5 μg/ml puromycin and 800 μg/μl of G418. For assay, the cells were collected by removing the media, washing with PBS and versene to lift the cells (Life Technologies). Cells were centrifuged at 450 x g for 5 min, and supernatants were aspirated. Cells were resuspended in 1x HBSS (Life Technologies), 5 mM HEPES (Life Technologies), 0.1% BSA (Perkin Elmer), 1.0 mM 3-Isobutyl-1-methylxanthine (IBMX) (Sigma) at 0.5 x 10^6 cells/ml, and 10 μL of suspended cells was added to each well of a 384-well white opti-plate (PerkinElmer) to a final density of 5000 cells/well. Dilutions of amylin analogs and controls were prepared in 1x HBSS, 5 mM HEPES, 0.1% BSA, and 10 μL/well of each sample were added to designated wells. Plates were incubated with shaking at rt for 30 min. Then 20 μL/well LANCE cAMP detection reagent mix (PerkinElmer) was added to each assay plate, which was incubated with shaking at rt for 2 h to 24 h. Plates were read on a Perkin Elmer Envision plate reader using a protocol based on the manufacturer recommendations included in the LANCE Ultra cAMP Kit. All samples were measured in quadruplicate. Data were analyzed using the Crucible in-house data analysis software, designed by Eudean Shaw, to derive parameters such as EC50, LogEC50, HillSlope (nH), top, and bottom, by plotting raw LANCE cAMP values versus log compound concentrations. Data were fitted with 4-P model using a non-linear weighted least squares application within R environment (Open Source http://cran.us.r-project.org/) implemented by the NonClinical Statistics & Computing department at Janssen R&D.

[00266] The potencies of the amylinomimetic analogues of the present invention relative to that of pramlintide, which was used as a control in the same assay, are presented in Table 2 below:

Table 2: AMY3 Receptor Potencies of Thioether-cyclized Amylinomimetic Compounds and Pramlintide (Seq. 2)

Seq. I.D. No.	**AMY3R EC_{50} (pM) Seq 4-42**	**AMY3R EC_{50} (pM) Seq 2**	**Relative potency (fold change rel. to Seq 2)**
4	2.9	0.49	-6.0
5	33	2.4	-13.8
6	0.28	0.36	1.3
7	0.43	0.36	-1.2
8	1.12	0.38	-2.9
9	447	0.56	-794
10	198	0.38	-521
11	78	0.56	-139
12	11	0.49	-22
13	0.16	0.40	2.4
14	0.42	0.43	1.0
15	39	0.49	-79
16	22	0.49	-45
17	1800	0.45	-4027
18	33	0.49	-68
19	0.23	0.41	1.8
20	4.9	0.45	-11
21	18	0.45	-39
22	471	0.56	-845
23	14	0.45	-32
24	183	0.56	-326
25	0.37	0.41	1.1
26	2.2	0.40	-5.5
27	0.32	0.36	1.1
28	0.059	0.36	6.1
29	0.064	0.41	6.4
30	0.028	0.41	14.6
31	ND		
32	ND		
33	ND		
34	ND		
35	ND		
36	ND		

37	8.6	0.19	-45
38	313	0.19	-1647
39	1485	0.19	-7816
40	11.4	0.19	-60
41	430	0.19	-2263
42	1840	0.19	-9684

Example 7: Human Calcitonin/RAMP1 Complex cAMP Assay (AMY1R Assay)

[00267] The potency and selectivity of amylinomimetic analogs in vitro was assessed using a cell based assay designed to measure cAMP production following modulation of the human CTR or CTR/RAMP1 complex (AMY1R). Production of cAMP in human CTR or AMY1R transiently transfected COS7 cells was induced by amylinomimetic analogs and controls in a dose-dependent manner, and measured using a HTRF cAMP kit (CisBio cAMP Dynamic kit kit, Cat # 62AM4PEC).

[00268] The plasmid encoding the HA-tagged human calcitonin receptor was generated by sub-cloning human CTR ORF (ENST00000426151.5), tagged at its N-terminus immediately after the signal peptide with 3xHA, into pcDNA3.1(+) using EcoRV and XhoI. The plasmid encoding Flag-tagged human RAMP1 was generated by sub-cloning human RAMP1 ORF (ENST00000254661.4), tagged at the N-terminus immediately after the signal peptide with a Flag-tag (DYKDDDDK (SEQ ID NO: 66)), into pcDNA3.1(+) using EcoRV and XhoI.

[00269] COS-7 cells were cultured in DMEM (ThermoFisher Scientific # 11965092) containing 10% FBS (Hyclone # SH30070.03) and 1% Penicillin-Streptomycin (ThermoFisher Scientific # 15140122) and transfected using Fugene HD (Promega # E2312) in 384-well white poly-D-lysine coated plates (Corning # 356663). For each condition, the DNA (µg):Fugene HD (µL) mix ratio was 1:3 and 10,000 cells/well were added in 40 µL on top of 10 µL DNA:Fugene mix. Plates were incubated at 37 °C in a CO_2 incubator for 48 h. The CTR:RAMP1 cDNA transfection ratio (2:9) was optimized to favor the formation of the amylin-1 receptor (AMY1R) and the amount of CTR cDNA transfected was optimized such that the expression of the calcitonin and amylin receptors at the cell surface were not significantly different, as assessed by ELISA against the HA tag.

[00270] The day of the assay, the culture media was replaced with assay buffer containing HBSS with calcium and magnesium, 20 mM HEPES and 0.1% Fatty acid free BSA, pH 7.4, and cells were starved for 1 h at 37 °C. The assay buffer was then replaced with fresh assay buffer containing 500 μM IBMX, and compounds were added in assay buffer (no IBMX). Plates were incubated with shaking at rt for 30 min. cAMP was detected according to the manufacturer's protocol (CisBio cAMP Dynamic kit, Cat # 62AM4PEC). Fluorescence was read with a PHERAstar plate reader using an excitation of 337 nm and emissions of 620 and 665 nm. Data were normalized on the maximal response of pramlintide. Emax and EC50 determinations were made from an agonist-response curve analyzed with a curve fitting program using a 4-parameter logistic dose response equation in Graphpad Prism 7.0. Data presented are representative of three independent experiments performed in quadruplicate for each compound. Data are represented as averages. AMY1R potencies of compounds relative to that of pramlintide (seq 2) are represented as the fold change. Potencies of compounds at the AMY1R relative to their potencies at the CTR are also presented as fold differences (Table 3).

[00271] For cell surface receptor expression determinations, cells from the same transfection as that used for the cAMP assay were plated into 96-well plates, fixed with 4% paraformaldehyde and blocked with PBS + 1% FBS. Rat anti-HA-Peroxidase (clone 3F10, Roche Bioscience # 12013819001) was applied for 30 min at 0.5 mg/L. After washes with blocking buffer and PBS, chemoluminescence was detected using SuperSignal substrate (Pierce, Rockford, IL, USA) and a PHERAstar plate reader.

Table 3: AMY1 and CT Receptor Potencies of Thioether-cyclized Amylinomimetic Compounds and Pramlintide (Seq. 2)

Seq. I.D. No.	**AMY1R EC_{50} (pM) Seq 37-42**	**AMY1R Relative potency (fold change rel. to Seq 2)**	**CTR EC_{50} (nM) Seq 37-42**	**Relative potency AMY1R vs CTR (fold difference)**
2	2.3	1	0.061	27
37	64	-27	3.5	55
38	82	-35	36.8	448

39	197	-85	> 500	> 1000
40	55	-24	1.6	29
41	85	-37	31.2	367
42	287	-123	> 500	> 1000

Example 8: Efficacy Studies *In Vivo*

[00272] Gastric Emptying: Acetaminophen (AAP) Absorption in Lean C57Bl/6N Mice

[00273] Male lean C57BL/6 mice (6-8 weeks of age) were obtained from Taconic Laboratory. Mice were housed one mouse per cage with AlphaDri bedding in a temperature-controlled room with 12-h light/dark cycle. Mice were allowed ad libitum access to water and maintained on a regular diet (Lab Diet Cat: 5K75). Animals were acclimated to the facility for at least one week prior to the start of the experiment.

[00274] The day prior to dosing, mice were grouped into cohorts of ten animals based on individual body weights. At 5:00 - 6:00 pm the following day, animals were deprived of food and treated with either vehicle (PBS, pH 7.4) or test compound at a dose of 30 nmol/kg (3 nmol/mL) via subcutaneous administration. After 18 h, an acetaminophen (AAP) suspension mixture [AAP (10 mg/mL); HPMC (5 mg/mL); acacia gum (50 mg/mL)] was administered to the animals (10 mL/kg) by oral gavage. Whole blood samples (tail snip; ~ 25 μL) were collected at 5, 10, 15, 30 and 60 min time points into DMPK-C Dry Blood Spot Cards. Blood dot cards were dried completely and placed into individual bags with desiccant pending LC/MS analysis by standard techniques. Statistical analyses were performed using one-way ANOVA with Dunnett's post-test in Prism. All data are presented as the mean.

[00275] **Food Intake in Fasted Lean C57BL6N Mice: Acute Dosing**

[00276] Male C57BL/6 mice (6-8 weeks of age) were obtained from Taconic Laboratory. Mice were housed one mouse per cage with AlphaDri bedding in a temperature-controlled room with 12-h light/dark cycle. Mice were allowed ad libitum access to water and maintained on a regular diet (Lab Diet Cat: 5K75). Animals were acclimated in BioDAQ cages (Research Diets, Inc., New Brunswick, NJ) no less than 72 h prior to the start of the experiment.

[00277] Once acclimated in the BioDAQ cages, mice were grouped into cohorts of ten animals based on their individual body weights and food intake over the previous 24 h. At 4:00-5:00 pm, animals were weighed and treated with either vehicle (PBS, pH 7.4) or test compound at a dose of 30 nmol/kg (3 nmol/mL) via subcutaneous administration. Following a subsequent overnight fasting period (16-18 h), changes in food weight for each cage were recorded continuously by the BioDAQ automated monitoring system for the next 48 h. Crumbs were removed daily from hoppers and the areas around the cages with a vacuum. Food was replenished as necessary. The percentage of mean cumulative food intake relative to vehicle over the 12-48 h period following dosing was calculated and is reported in Table 4. Statistical analyses were performed using two-way ANOVA with Dunnett's post-test in Prism. All data are presented as the mean.

Table 4: In Vivo Efficacy Studies of Thioether-cyclized Amylinomimetic Compounds

Seq. I.D. No.	AAP Absorption C57BL6 Mice [AAP] @ 15 min (μM)	AAP Absorption C57BL6 Mice AUC_{0-90} (μM•min)	Food Intake in Fasted Lean Mice % Change (12-36h)	Food Intake in Fasted Lean Mice % Change (12-48h)
Vehicle	489	20835		
40	320[§]	13292[§]	-20.2***	-25.3***
41	437*	18904	-2.7	-8.8
42	444*	18325	ND	ND

ND = not determined

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; [§] $p < 0.0001$

Example 9: PK Studies in Male C57BL6N Mice

[00278] Male C57BL/6N mice (6-8 weeks of age, Taconic) were single-housed with AlphaDri bedding in a temperature-controlled room with 12-hour light/dark cycle and given free access to LabDiet5K75 rodent diet and drinking water. Mice were grouped (N = 3) based upon fed body weight; compounds were formulated in PBS (1 nmol/mL) and administered subcutaneously at a dose of 10 nmol/kg. Whole blood samples (tail snip; ~ 50 μL) were collected at 4h, 24h, 72h, 96h and 7d time points into EDTA-coated Sarstedt Microvette® tubes containing a protease inhibitor cocktail (Roche complete protease inhibitors and Millipore DPPIV inhibitors; 2.5 μL) and placed on ice. The final bleed (7 d) was a terminal bleed with a target volume of 500 μL (25 μL of protease inhibitor

cocktail). Samples were then centrifuged (10,000 rpm) at 4 °C for 10 minutes; plasma was then transferred to a 96-well plate (~25µL/well of plasma) which was stored at -80 °C pending bioanalysis.

[00279] Plasma samples were analyzed using an LC-MS/MS assay of surrogate peptides for quantitation. In this assay, the analytes were extracted from plasma using immuno-affinity capture by an anti-human IgG Fc antibody, followed by protease digestion (trypsin or pepsin) and reversed phase LC-MS/MS analysis. The multiple reaction monitoring (MRM) MS analysis was conducted on an API5000 triple quadruple mass spectrometer operated in positive electrospray mode. The peptide derived from N-terminal region of the amylinomimetic sequence was monitored as a surrogate for quantitation of active conjugate, while a peptide located on Fc region of the mAb was monitored as a surrogate for the total level of mAb. Standard curve and quality control samples were prepared by spiking the reference standards of the amylinomimetic conjugates in plasma and were processed simultaneously using the same procedure as the study samples. Data are shown in Table 5.

Table 5: Pharmacokinetic Parameters of Thioether-cyclized Amylinomimetic Compounds in Male C57BL/6N mice

Seq. I.D. No.	**$t_{1/2}$ (days)**	**C_{max} (nmol/L)**	**AUC_{0-168} (h•nmol/L)**
37	4.5	86.5	10033
38	2.7	73.5	5743
39	2.4	64.8	4701
40	1.9*	137.3	15765
41	2.4	81.3	6656
42	2.7	83.2	6544

*estimated from limited time-point sampling taken during elimination phase

[00280] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the

particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

[00281] All documents cited herein are incorporated by reference.

[00282] Exemplary amylinomimetic sequences or conjugates thereof of the invention include:

SEQ ID NO: 1
Name: Amylin(1-37)
Structure:



SEQ ID NO: 2
Name: Pramlintide(1-37)
Structure:



SEQ ID NO: 3
Name: Davalintide(1-32)
Structure:



SEQ ID NO: 4
Name: [cyclo-(N3- $COCH_2$- C7)]-Pramlintide3-37
Structure:



SEQ ID NO: 5
Name: [cyclo-(N3- $COCH_2$- hC7), K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 6
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5]-Pramlintide3-37
Structure:



SEQ ID NO: 7
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 8
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(Alloc)26]-Pramlintide3-37
Structure:



SEQ ID NO: 9
Name: [cyclo-(N3- $COCH_2$- C7), β-A5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 10
Name: [cyclo-(N3- $COCH_2$- C7), β-A5, K(Alloc)26]-Pramlintide3-37
Structure:



SEQ ID NO: 11
Name: [cyclo-(N3- $COCH_2$- C7), Abu5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 12
Name: [cyclo-(N3- $COCH_2$- hC7), Abu5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 13
Name: [cyclo-(N3- $COCH_2$- hC7), (*S*)-β-Aib5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 14
Name: [cyclo-(N3- $COCH_2$- hC7), (*R*)-β-Aib5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 15
Name: [cyclo-(N3- $COCH_2$- hC7), β-hA5, K(Ac)26]-Pramlintide3-37
Structure:



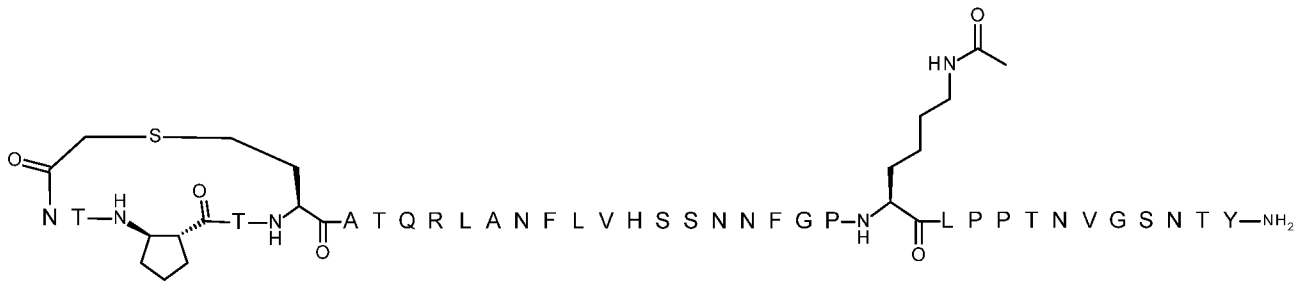
SEQ ID NO: 16
Name: [cyclo-(N3- $COCH_2$- hC7), β-hP5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 17
Name: [cyclo-(N3- $COCH_2$- hC7), {pyrrolidinyl-(3*S*)-carboxyl}5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 18
Name: [cyclo-(N3- $COCH_2$- hC7), {(1*R*,2*R*)-ACPC}5, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 19
Name: [cyclo-(N3- $COCH_2$- hC7), β-hT4, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 20
Name: [cyclo-(N3- $COCH_2$- C7), β-hT4, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 21
Name: [cyclo-(N3- $COCH_2$- hC7), β-hT6, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 22
Name: [cyclo-(N3- $COCH_2$- C7), β-hT6, K(Ac)26]-Pramlintide3-37
Structure:



SEQ ID NO: 23
Name: [cyclo-(S2- $COCH_2$- hC7), β-A5, K(Ac)26]-Pramlintide2-37
Structure:



SEQ ID NO: 24
Name: [cyclo-(S2- $COCH_2$- C7), β-A5, K(Ac)26]-Pramlintide2-37
Structure:



SEQ ID NO: 25
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(Ac)25]-Pramlintide3-37
Structure:



SEQ ID NO: 26
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(Ac)29]-Pramlintide3-37
Structure:



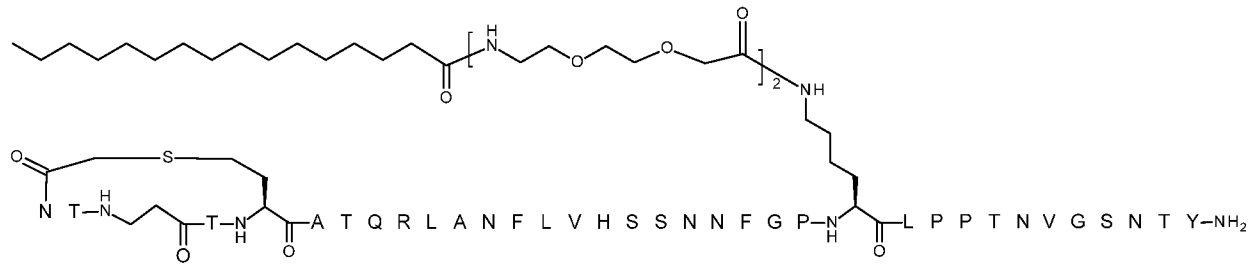
SEQ ID NO: 27
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(Ac)34]-Pramlintide3-37
Structure:



SEQ ID NO: 28
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5]-Davalintide3-32
Structure:



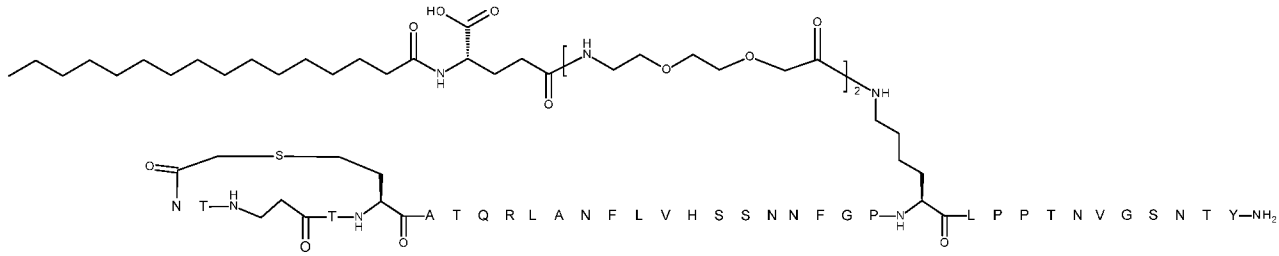
SEQ ID NO: 29
Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(OEG_2-Pal)26]-Pramlintide3-37
Structure:



SEQ ID NO: 30

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, K(OEG_2-γ-Glu-Pal)26]-Pramlintide3-37

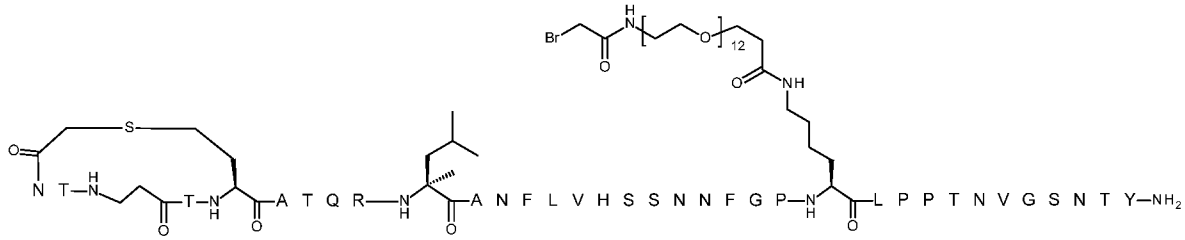
Structure:



SEQ ID NO: 31

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, α-MeL12, K(dPEG12-AcBr)26]-Pramlintide3-37

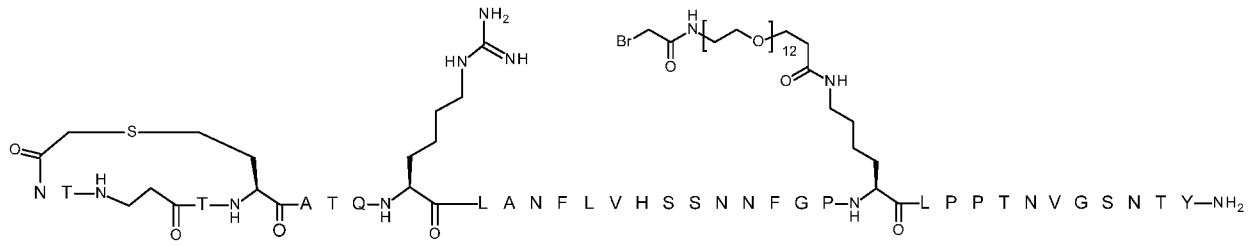
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SEQ ID NO: 32

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, hR11, K(dPEG12-AcBr)26]-Pramlintide3-37

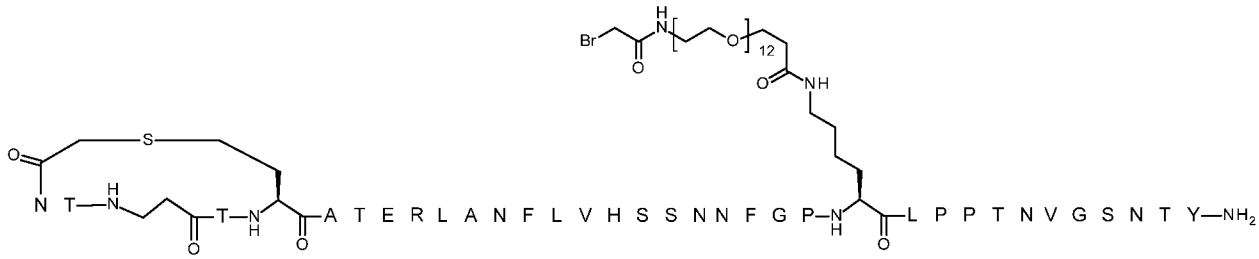
Structure:



SEQ ID NO: 33

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, E10, K(dPEG12-AcBr)26]-Pramlintide3-37

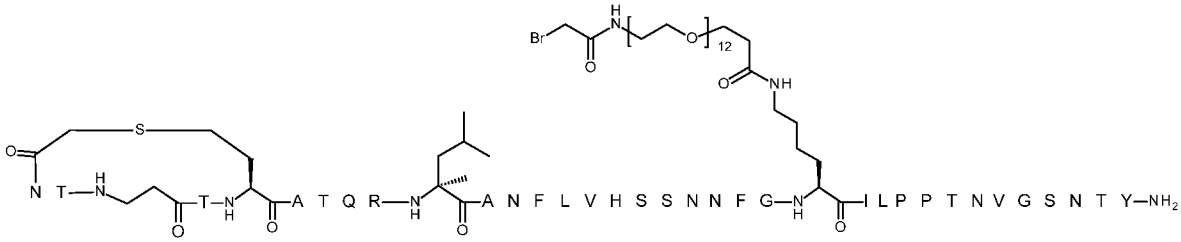
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SEQ ID NO: 34

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, α-MeL12, K(dPEG12-AcBr)25]-Pramlintide3-37

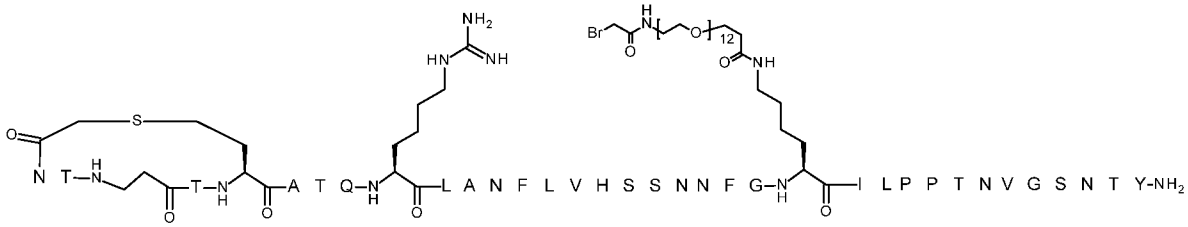
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SEQ ID NO: 35

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, hR11, K(dPEG12-AcBr)25]-Pramlintide3-37

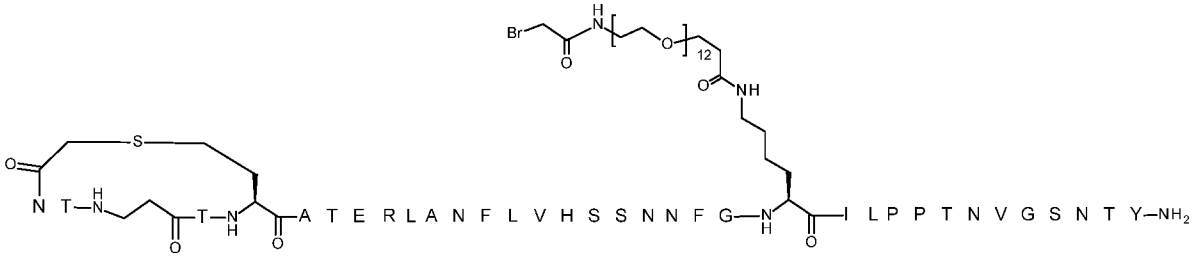
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SEQ ID NO: 36

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, E10, K(dPEG12-AcBr)25]-Pramlintide3-37

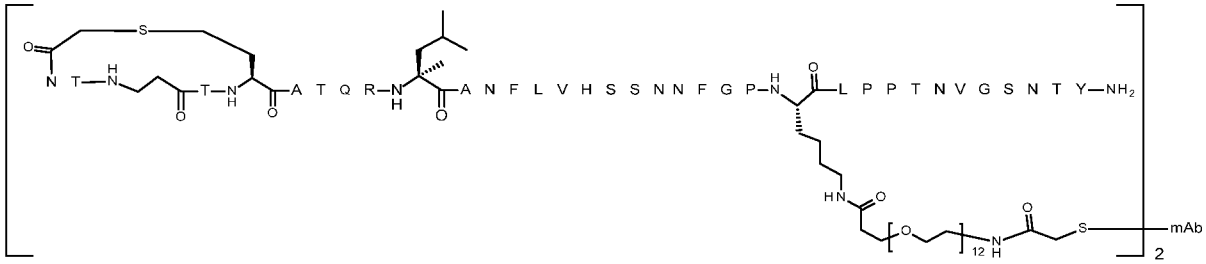
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SEQ ID NO: 37

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, α-MeL12, K(dPEG12)26]-Pramlintide3-37 mAb homodimer conjugate

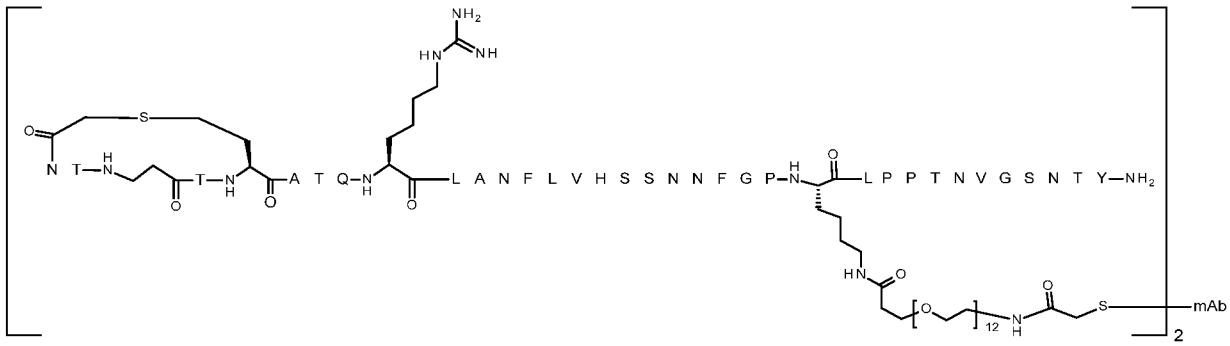
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SEQ ID NO: 38

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, hR11, K(dPEG12)26]-Pramlintide3-37 mAb homodimer conjugate

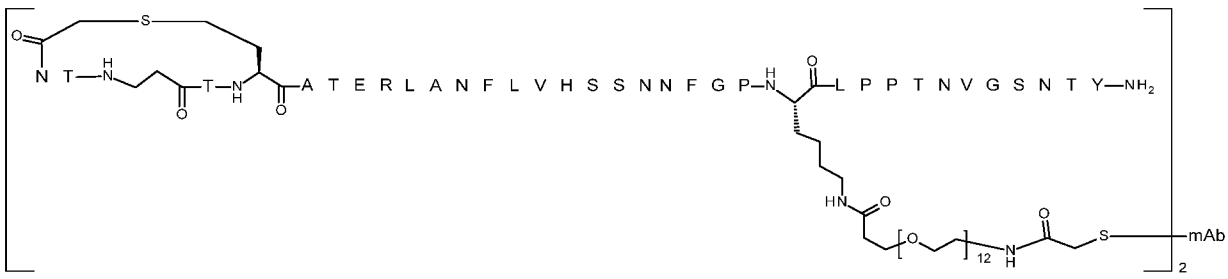
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SEQ ID NO: 39

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, E10, K(dPEG12)26]-Pramlintide3-37 mAb homodimer conjugate

Structure:



SEQ ID NO: 40

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, α-MeL12, K(dPEG12)25]-Pramlintide3-37 mAb homodimer conjugate

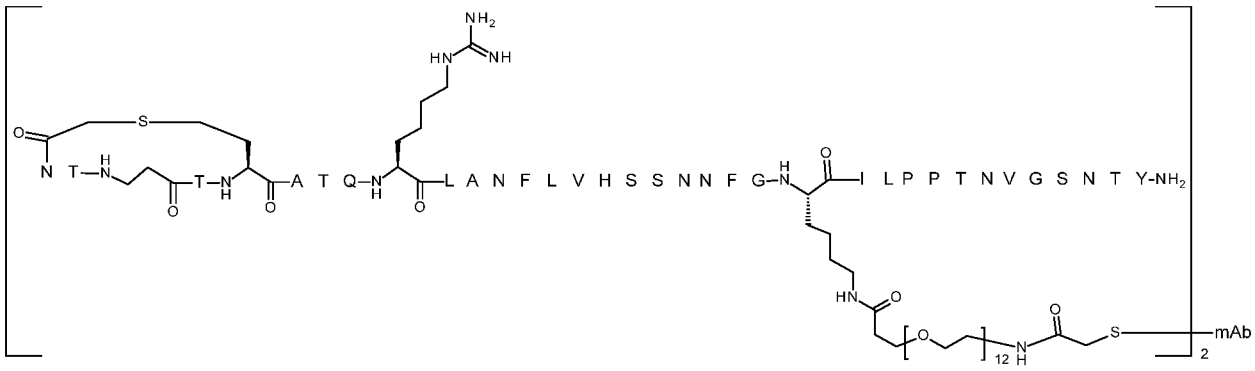
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SEQ ID NO: 41

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, hR11, K(dPEG12)25]-Pramlintide3-37 mAb homodimer conjugate

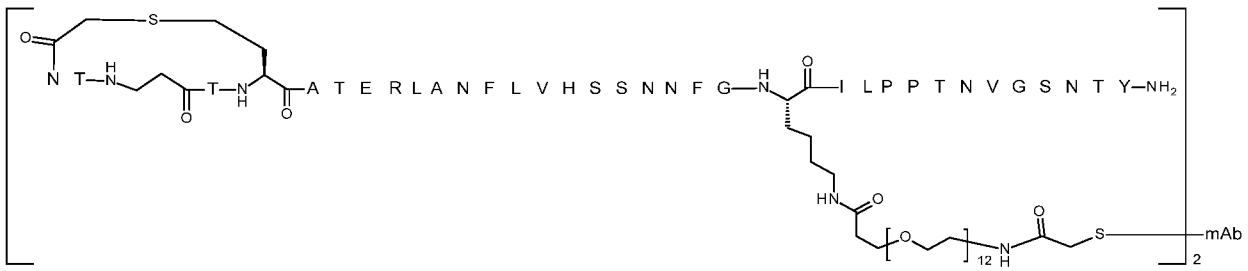
Structure:



SEQ ID NO: 42

Name: [cyclo-(N3- $COCH_2$- hC7), β-A5, E10, K(dPEG12)25]-Pramlintide3-37 mAb homodimer conjugate

Structure:



SEQ ID NO: 43

Name: MSCB97 VH (heavy chain variable region)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAKYDGCYGELDFWGQGTLVTVSS

SEQ ID NO: 44

Name: MSCB97 HC (heavy chain)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAKYDGCYGELDFWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV
DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 45

Name: MSCB97 VL (light chain variable region)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDF
TLTISSLEPEDFAVYYCQQRSNWPLTFGQGTKVEIK

SEQ ID NO: 46

Name: MSCB97 LC (light chain)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDF
TLTISSLEPEDFAVYYCQQRSNWPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

SEQ ID NO: 47

Name: MSCB97 HCDR1

SYAMS

SEQ ID NO: 48

Name: MSCB97 HCDR2

AISGSGGSTYYADSVKG

SEQ ID NO: 49

Name: MSCB97 HCDR3

YDGCYGELDF

SEQ ID NO: 50

Name: MSCB97 LCDR1

RASQSVSSYLA

SEQ ID NO: 51

Name: MSCB97 LCDR2

DASNRAT

SEQ ID NO: 52

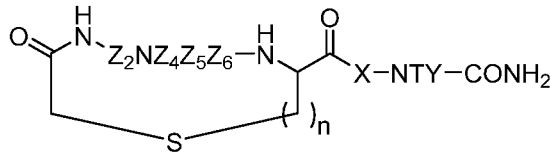
Name: MSCB97 LCDR3

QQRSNWPLT

CLAIMS

We Claim:

1. A compound of Formula **I** (SEQ ID NO: 53)

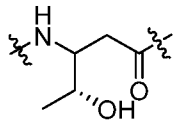


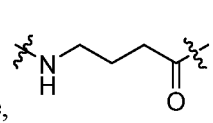
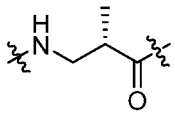
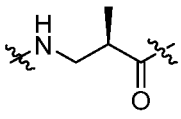
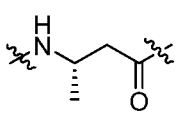
Formula **I**

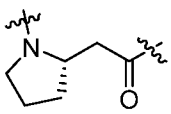
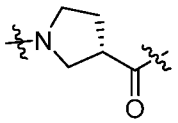
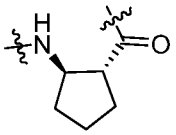
wherein

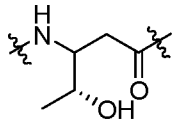
n is 1, or 2;

Z_2 is a direct bond, serine, or glycine;

Z_4 is T, or  ;

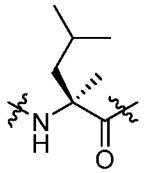
Z_5 is A, β-alanine, , , , ,

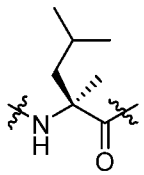
, , or  ;

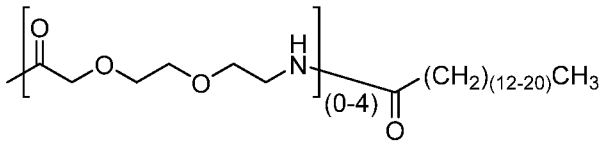
Z_6 is T, or  ;

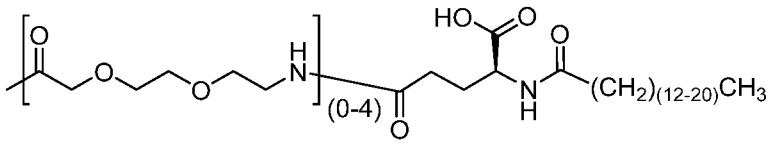
Z_{10} is Q, or E;

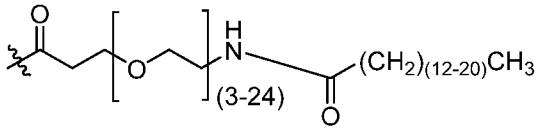
Z_{11} is R, or K, wherein the ε-amine of said K is optionally substituted with –C(=NH)NH_2;

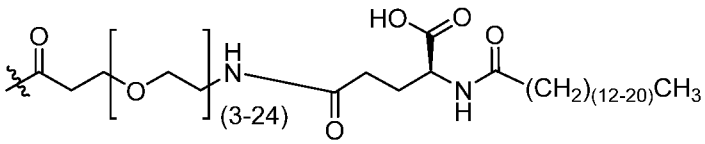
Z_{12} is L, or  ;

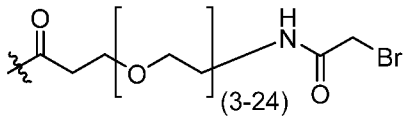
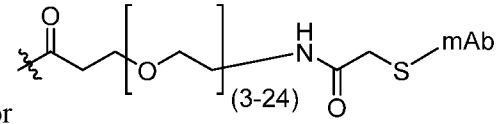
Z_{16} is L, or  ;

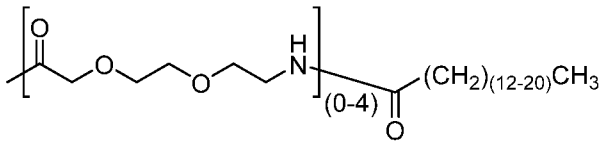
Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

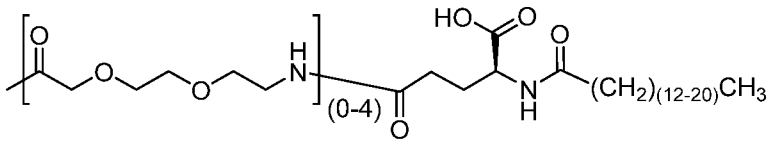
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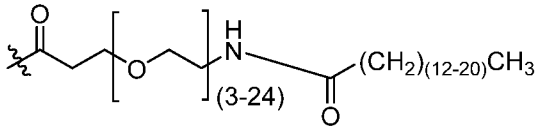
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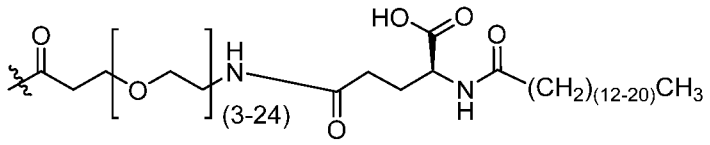
,

, or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

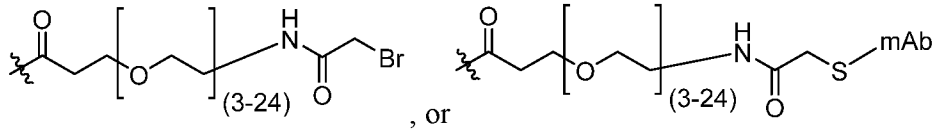
Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

,

,



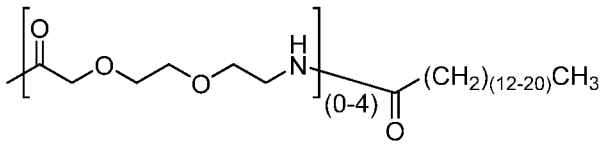
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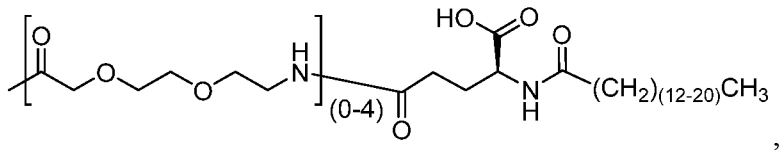
, or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

X is $ATZ_{10}Z_{11}Z_{12}ANFZ_{16}VHSSNNFGZ_{25}Z_{26}LPZ_{29}TNVGZ_{34}$ (SEQ ID NO: 54), or VLGRLSQELHRLQTYPRTNTGS (SEQ ID NO: 55);

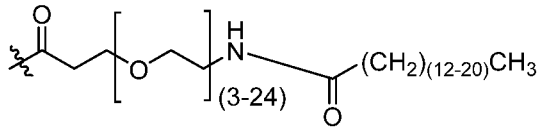
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$,



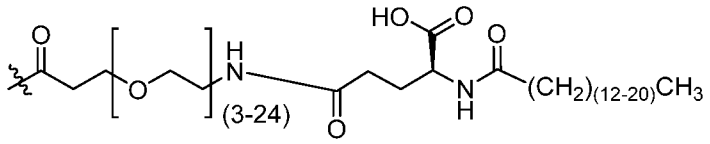
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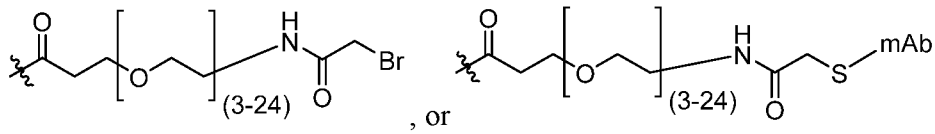
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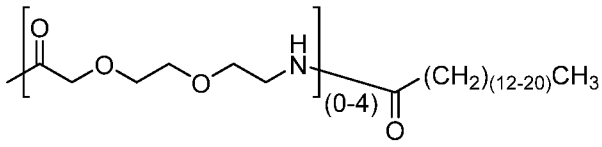
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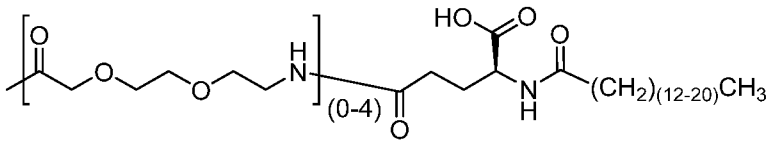


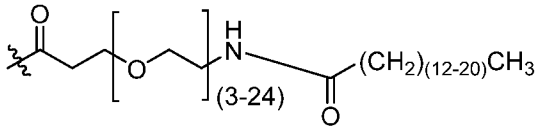
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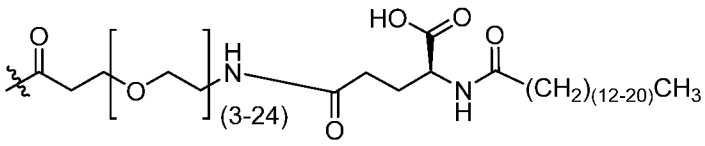


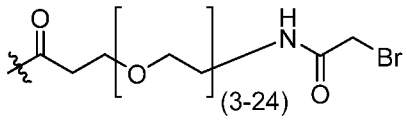
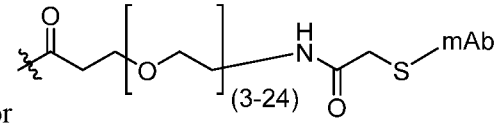
, or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with–$C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

,

,

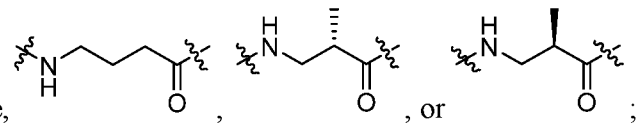
,

, or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

and pharmaceutically acceptable salts thereof.

2. The compound of claim 1 wherein:

Z_2 is a direct bond;

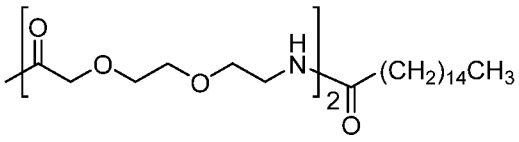
Z_5 is β-alanine, ;

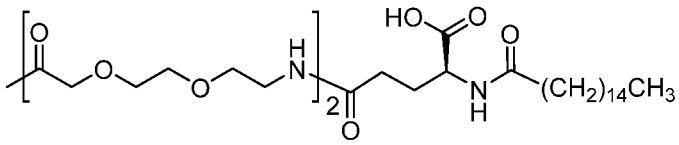
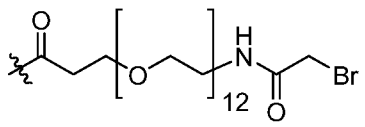
Z_6 is T;

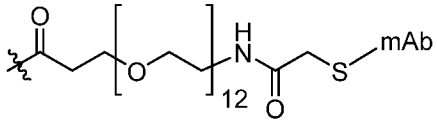
and pharmaceutically acceptable salts thereof.

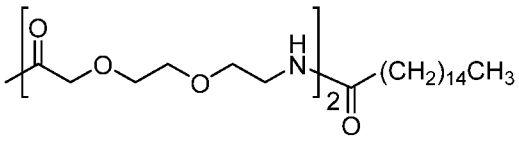
3. The compound of claim 1 wherein:

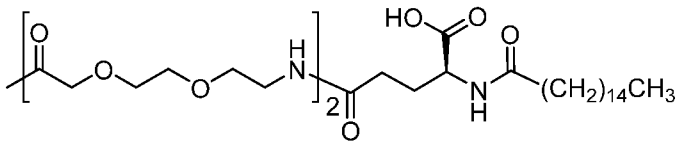
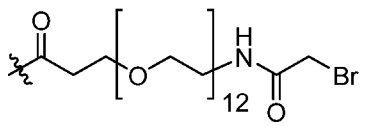
Z_{16} is L;

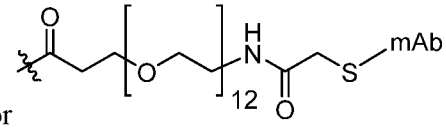
Z_{25} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

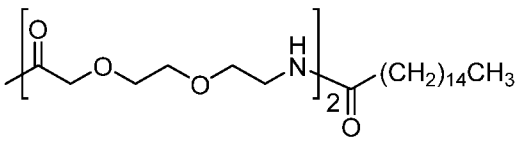
, , or

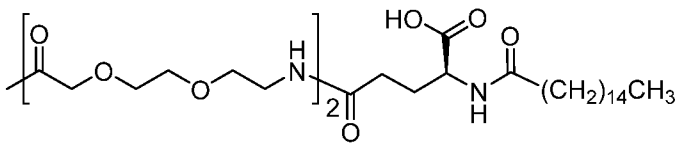
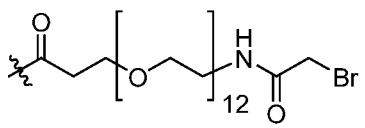
, wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

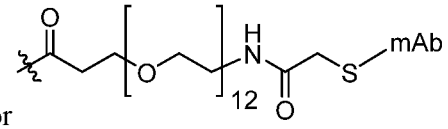
Z_{26} is I, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

, ,

 or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

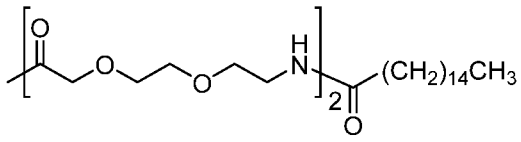
Z_{29} is P, or K, wherein the ε-amine of said K is optionally substituted with $-C(=O)CH_3$, -$C(O)CH_2CH=CH_2$, ,

, ,

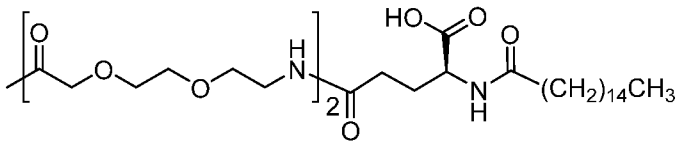


or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

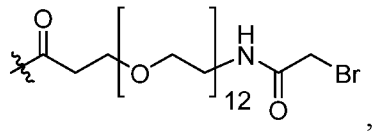
Z_{34} is S, or K, wherein the ε-amine of said K is optionally substituted with–C(=O)CH_3, -



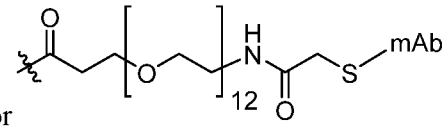
C(O)CH_2CH=CH_2, ,



,



,

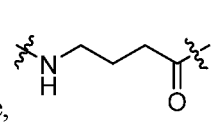
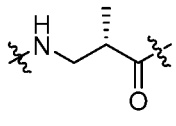
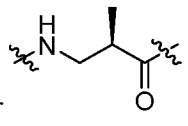


or , wherein said mAb is optionally substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

and pharmaceutically acceptable salts thereof.

4. The compound of claim 3 wherein:

Z_2 is a direct bond;

Z_5 is β-alanine,  ,  , or  ;

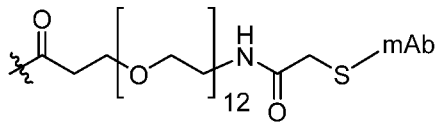
Z_6 is T;

and pharmaceutically acceptable salts thereof.

5. The compound of claim 3 wherein:

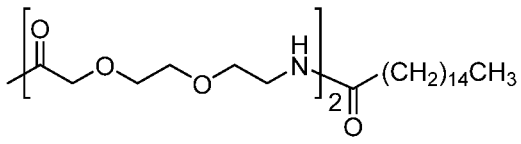
Z_{11} is R, or K, wherein the ε-amine of said K is substituted with –C(=NH)NH_2;

Z_{25} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, or

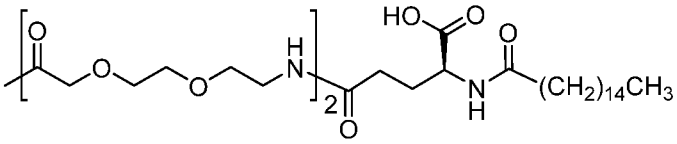


, wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

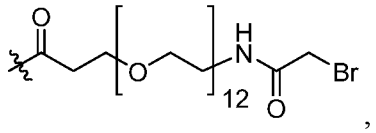
Z_{26} is I, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3, -C(O)$CH_2CH{=}CH_2$,



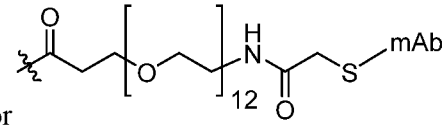
,



,



,



or , wherein said mAb is substituted through another thioether bond to a second compound of Formula **I**, so that there are two identical compounds of Formula **I** on the mAb;

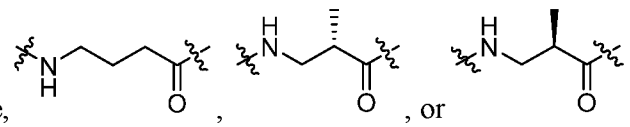
Z_{29} is P, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

Z_{34} is S, or K, wherein the ε-amine of said K is substituted with –C(=O)CH_3;

and pharmaceutically acceptable salts thereof.

6. The compound of claim 5 wherein:

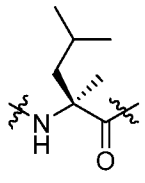
Z_2 is a direct bond;

Z_5 is β-alanine, , or ;

Z_6 is T;

and pharmaceutically acceptable salts thereof.

7. The compound of claim 5 wherein:

Z_{12} is  ;

and pharmaceutically acceptable salts thereof.

8. The compound of claim 5 selected from the group consisting of (SEQ ID NOS 4-42, respectively, in order of appearance):







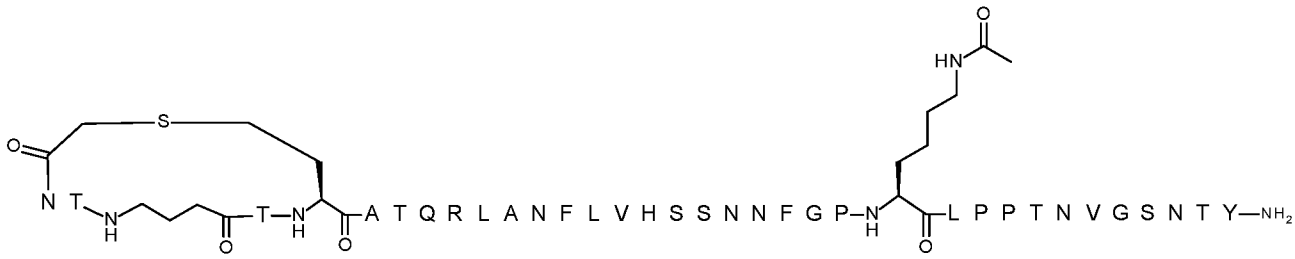


































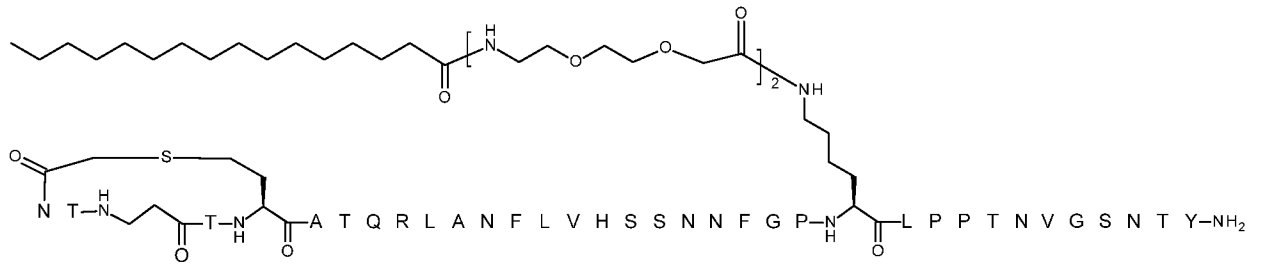


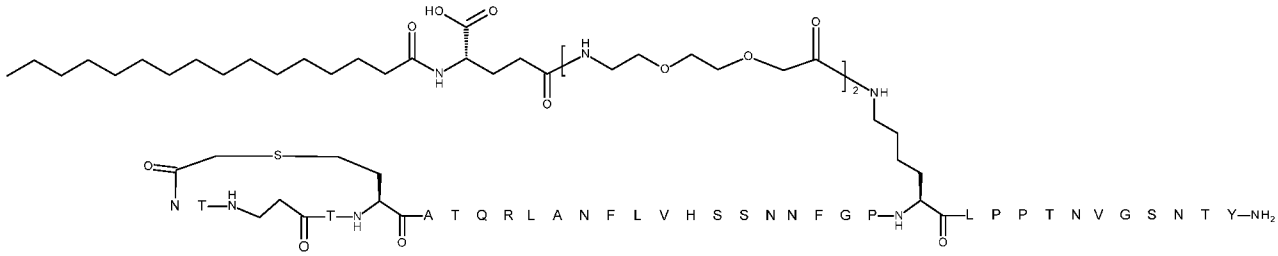


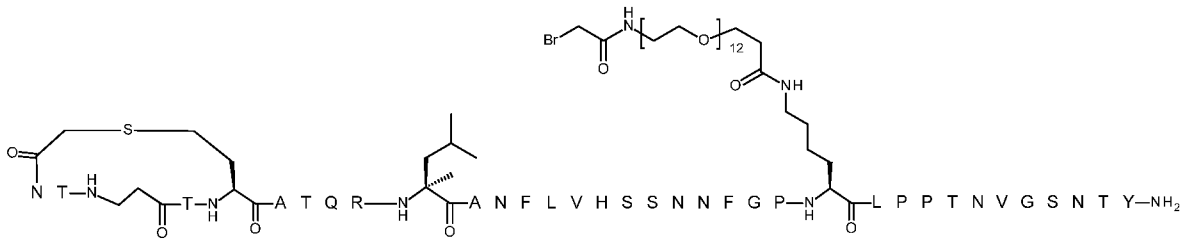


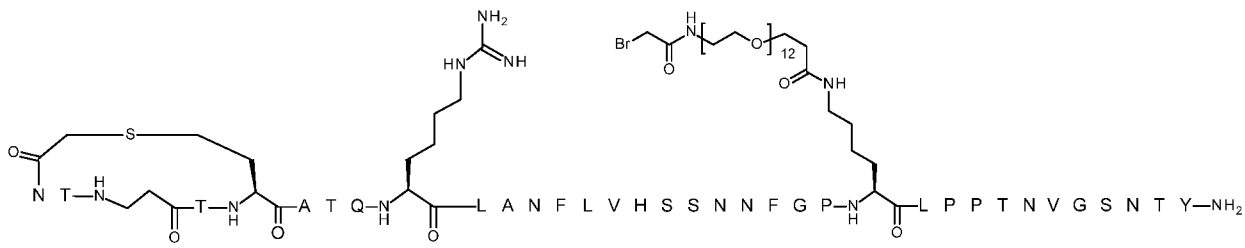


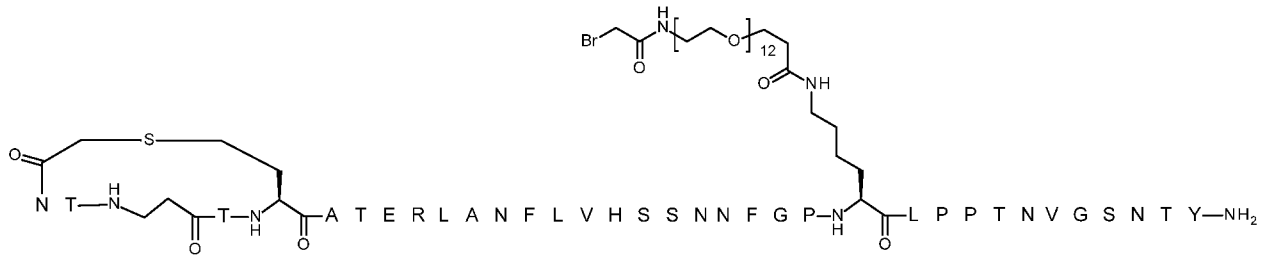


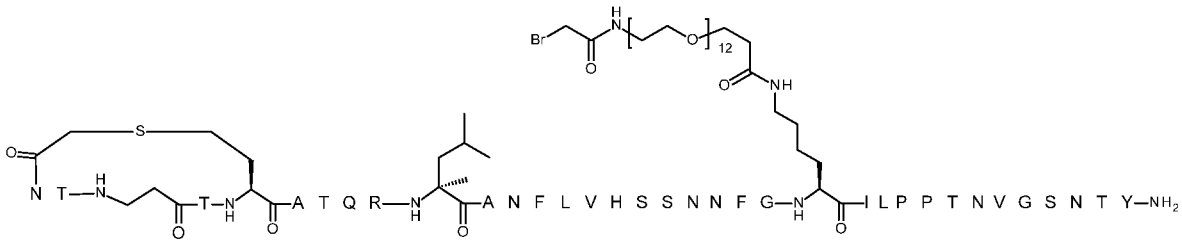


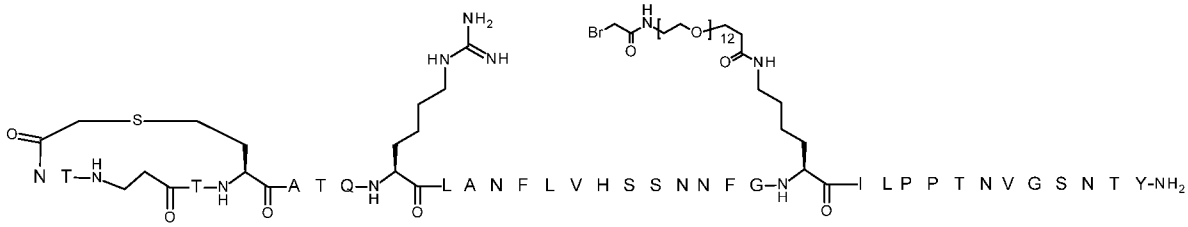


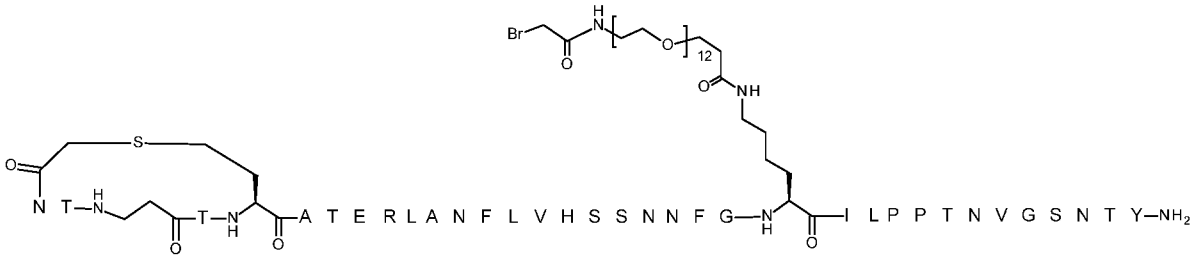




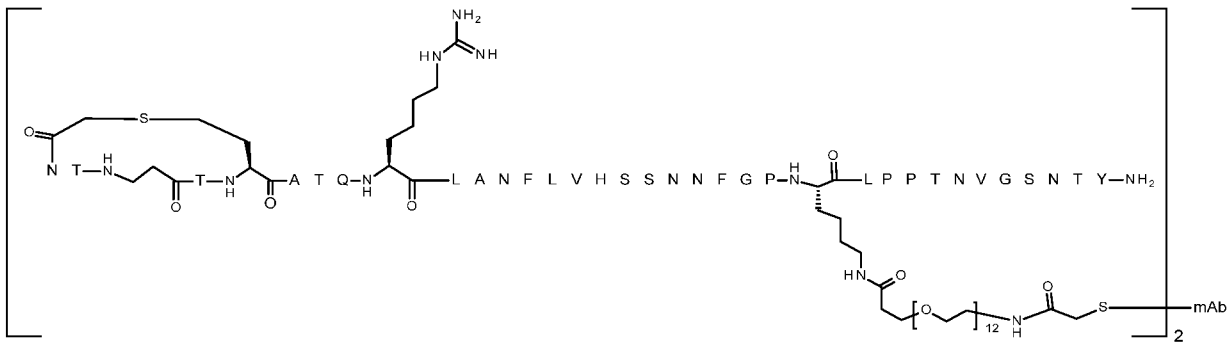


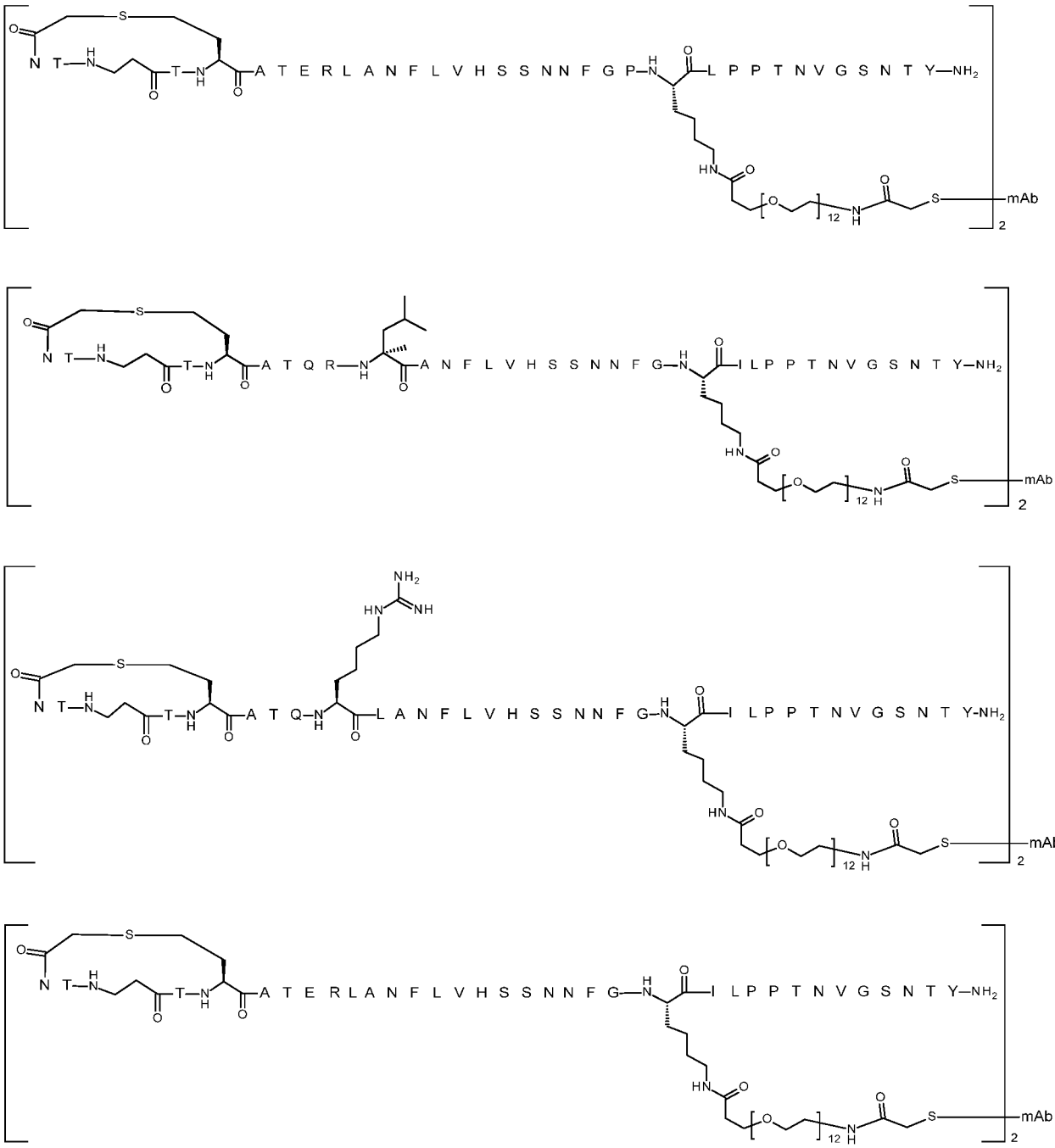












and pharmaceutically acceptable salts thereof.

9. The compound of claim 1, wherein the compound is selected from the group consisting of SEQ ID NOs:4-42, or a pharmaceutically acceptable salt thereof.

10. A pharmaceutical composition comprising the compound of any one of claims 1-9 and a pharmaceutically acceptable carrier.

11. A method for treating or preventing a disease or disorder in a subject in need thereof, wherein said disease or disorder is selected from the group consisting of obesity, type I or type II diabetes, metabolic syndrome, insulin resistance, impaired glucose tolerance, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypoglycemia due to congenital hyperinsulinism (CHI), dyslipidemia, atherosclerosis, diabetic nephropathy, and other cardiovascular risk factors such as hypertension and cardiovascular risk factors related to unmanaged cholesterol and/or lipid levels, osteoporosis, inflammation, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), renal disease, and eczema, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 10.

12. A method of reducing food intake in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 10.

13. A method of modulating amylin receptor activity in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 10.

14. The method of claim 13, wherein said amylin receptor comprises AMY1R, and/or AMY2R and/or AMY3R.

15. The method of claim 14, wherein said amylin receptor is AMY1R.

16. The method of claim 14, wherein said amylin receptor is AMY3R.

17. The method of any one of claims 11-14, wherein the pharmaceutical composition is administered via an injection.

18. The method of any one of claims 11-16, wherein the pharmaceutical composition is administered in a combination with at least one antidiabetic agent.

19. The method of claim 18, wherein said antidiabetic agent is a glucagon-like-peptide-1 receptor modulator.

20. The method of claim 18, wherein the pharmaceutical composition is administered in combination with liraglutide.

21. A kit comprising the conjugate of any one of claims 1-8, preferably further comprising liraglutide and a device for injection.

22. A method of producing the pharmaceutical composition of claim 10